ORIGINAL PAPER

Investigating PGPR bacteria for their competence to protect hybrid maize from the factor drought stress

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Abstract

Drought and heat are severe environmental stresses that constantly affect plant growth and development. Maize (*Zea mays* L.) is known for its sensitivity to abiotic stresses, which often causes significant yield losses. With plant growth-promoting rhizobacteria (PGPR), the harmful effects of drought and heat stress on plants can be alleviated. The aim of the present study was to investigate the physiological traits of plants affected by drought stress in a treatment including four PGPR bacteria, two regimes of irrigation and two stages of phenophases, and the analysis of the influence of the individual factors and their combined effects, respectively. In addition, the investigations covered the testing of the nutrient acquisition ability and nutrient use efficiency, the plant growth stimulating effect of PGPR bacteria, and the analysis of the performance trials. The largest amount of soluble orthophosphate was produced by *Pseudomonas putida* (51.636 µg/cm^3). This is significantly more than the amount of phosphate solubilized by *Bacillus pumilus* (15.601 µg/cm3), *Pseudomonas fluorescens* (14.817 µg/cm3) and *Bacillus megaterium* (14.621 µg/cm3). The germination vigour of the seedlings in the treatment with *Pseudomonas putida* was 55% higher, represented by the value of 15,237.125, in comparison with the control, with the value of 9792.188. The *Pseudomonas putida* treatment resulted in the most outstanding fresh/dry weight ratio (7.312) compared to the control (7.780), when the interaction between all factors was taken into account under stressed conditions at 50% female flowering stage. The highest dry matter content of the plants (18.344%) compared to the control (17.344%) was measured in the case of the *Pseudomonas putida* treatment during the milk stage of development under stressed conditions. In the field trial, the plants treated with KD2 (*Bacillus pumilus* and *Pseudomonas putida*) presented significantly higher results in the aspect of grain yield (6.278 t/ha) compared to the control (5.468 t/ha).

Keywords *Zea mays* L. · PGPR · Phosphate solubilizing · Drought stress · Yield

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Introduction

Drought stress in maize plant culture

Drought stress is considered in plant biology to have such complex and devastating effect, which can be compared only to cancer in mammalian biology (Pennisi 2008). Drought influences the plant growth and development, but the severity of the impact depends on the timing and intensity of the stress (Nakashima et al. 2012). It has negative influence on various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrate uptake, and nutrient metabolism (Aslam et al. 2015). The drought stress causes reduction in moisture content and water use efficiency, modifies the turgor pressure, induces the closing of stomata and inhibits plant growth. Improving the adaptability of plants to drought stress had been an

important goal of plant breeders for a long time, but the difficulty is that this trait belongs to the qualitative traits, which are mostly coordinated by multiple gene interactions (Lopes et al. 2011). Maize, along with wheat and rice, provides at least 30% of the calories from food for more than 4.5 billion people in 94 developing countries, where one-third of the child population suffers from malnutrition (Hoisington et al. 1996). According to forecasts, the demand for maize in developing countries will be almost the double of the current quantity by 2050 (Chaudhary et al. 2014). According to yield estimates, 15–20% of the grain yield of maize is lost annually due to drought damage. The yield loss may become more substantial as drought periods may become more frequent and more severe due to climate change (FAOSTAT 2010; Adewale et al. 2018). Irrigation is not available as an option to many farmers, and its extension is limited in the developing countries (World Bank 2005). The solutions offered by genetics and the choice of the proper biological base to improve drought tolerance and yield stability are important tools of stabilizing global production. Therefore, the breeding of maize varieties with improved tolerance to drought stress and higher water use efficiency (WUE) has become a priority in breeding programmes, both in the private and the public sectors (Bruce et al. 2002). In the breeding programmes, various strategies are used to improve drought tolerance, such as recurrent selection and evaluation of segregating populations under stressed conditions in the network of multi-location performance trials, genome-wide selection methods, and transgenic technology (Mickelbart et al. 2015). Maize plants respond very sensitively to drought at 2–4 leaf stage due to their underdeveloped root system. On the influence of stress, the plant stand may thin out and the plant development is impeded. Typical visual symptoms of drought stress on maize plants are the change in the colour of the leaf tissue from green to greenish-grey, as well as the rolling of the lower and then the upper leaves. Stomata close, photosynthesis is dramatically reduced and the plant growth slows down (Edmeades 2013). Drought causes general physiological deterioration and stuntedness in the development of the plants as typical symptoms at 6–14–leaf stage. Maize has the maximum sensitivity to drought during flowering. A frequent symptom caused by drought stress can be the asynchrony between pollen shedding and silking during the anthesis-silking interval, which leads to the reduction of the harvestable yield. Drought stress causes delay and asynchrony in tassel development and ear growth. The tassels produce pollen with low fertility and viability, or the pollen may turn totally sterile. The silks become less receptive or eventually totally barren. The main reason of yield loss is the ovary or grain abortion after flowering in a dry season. Due to drought, the grain number per ear substantially reduces (Edmeades et al. 2000). In certain maize growing regions, the severity of drought stress often increases at the end of the

growing season. In this situation, the assimilated substance accumulated in the maize stalk is mobilized again (Jurgens et al. 1978). From the root up to aerial tissues, the abscisic acid interacts with other growth regulators, especially ethylene and reactive forms of oxygen (Ribaut et al. 2009). There is convincing evidence, that the development of the root system capable of reaching water in the deeper layers of the soil is a very valuable trait in drought-stressed environments (Robertson et al. 1993). Many plant species, including maize, respond to water deficit by redirecting growth and dry matter accumulation from the shoot to the root (Hsiao et al. 2000). Another potentially valuable stress tolerance mechanism is osmotic adjustment (OA), which involves the active accumulation of solutes in the cell. This accumulation enables moisture retention at low external water potential, thus limiting turgor loss and cell shrinkage damage. In case of permanent or severe lack of moisture, these dissolved substances also play a role in the stabilization of various macromolecular structures. (Tardieu et al. 1998). Stomata respond dynamically to changes in the environment and play a vital role in limiting moisture loss during drought. The signalling and response system of maize is sensitive to the reduction of water supply. It limits water loss in the early stages of water deficit, so leaf moisture can be maintained on the well-watered level until the more advanced stages of drought (Cochard 2002). The year effect, genotype and nutrient supply have the greatest influence on the yield of maize. Water supply is essential for maize and the success of maize cultivation highly depends on it. It has been evidenced, that in favourable years fertilization increased the yield by 40–50%, however, in extremely dry, drought-stressed years, fertilization did not have any yield-increasing effect. Drought during the period of tasseling can reduce the yield by 40–50% (Claassen and Shaw 1970). Great attention must be paid to choosing the correct biological base (Pepó et Pepó 1993). Due to the frequency of drought-stressed seasons, the importance of genetic base has become of utmost importance (Badr et al. 2020).

The role of soil microorganisms in crop production

Phosphorus and phosphorus mobilization

Phosphorus (P) is an important nutrient for living organisms. It supports a number of physiological processes, such as respiration, photosynthesis and energy-transmitting and converting metabolic processes. As a component of DNA and RNA, it contributes to the regulation of physiological processes and compiles the architecture of phospholipids. As a component of ATP and ADP coenzymes, it participates in phosphorylation processes and in the energy balance of plants. The total phosphorus content of the soil generally ranges 500–800 mg/kg on dry soil basis (Mengel

et Kirkby 1987). It means that the soil solution may contain totally 10^{-2}–10^{-3} mol m^{-3} phosphorus, which equals to 0.14–0.014 kg/ha P calculated on the level of 60% water capacity of the soil (Hossner et al. 1973). The phosphorus content of the domestic soils varies from 0.02 to 0.10%, in the form of approximately 50% organic and 50% inorganic bonds. The organically bound phosphorus is present in the soil in the forms of phytin (60%), nucleic acids, phospholipids, and sugar phosphates, and the inorganic phosphorus in the forms of fluoro-, chloro-, and hydroxyapatites, calcium phosphates, variscite, strengite and vivanite. Their ratio is influenced by the changes in the pH range and depends on the $CaCO_3$ content (Hsu et Jackson 1960). Plants are able to uptake the phosphorus in the form of $H_2PO_4^-$ and HPO_4^{2-} ions. The preference is influenced by the pH of the soil. In acidic environment, the absorption of $H_2PO_4^-$, and in alkaline environment, the uptake of HPO_4^{2-} is characteristic. Phosphorus occurs in soils mainly in the upper layers, and due to adsorption, it is bound on the surface of soil colloids, which makes it difficult or impossible for the plants to absorb it. As a result, phosphorus cannot be leached from the soil, so it becomes available for the plants as a reserve nutrient during slow processes of mobilization. The nitrogen content of the soil also affects its uptake, because due to anion antagonism, if the nitrogen is present in great amount, it inhibits the phosphorus uptake (Crossman 2012). Organic phosphorus compounds are decomposed in the soil by an enzymatic hydrolytic process, during which phosphoric acid is produced. The favourable nitrogen and carbon ratio advances the binding and transformation of phosphorus. The most favourable ratio is around C/N/P = 100/10/1. Certain species of bacteria and fungi are able to release inorganic forms of phosphorus as an outcome of their organic acid production, thereby transferring inorganic phosphates into solution (Richard et al. 2018). Certain bacteria species, such as *Azotobacter* and *Azospirillum*, enhance the mobilization of phosphorus. *Bacillus megaterium var. phosphaticum, Bacillus polymyxa, Pseudomonas fluorescens* and *Streptomyces albus* species have a noticeable role in phosphate transformation. The role of arbuscular mycorrhizal fungi is also remarkable in facilitating phosphate uptake. The symbiotic interaction between the fungal hyphae and the plant root system enables a sort of metabolism. The hyphae supply the host plants with water and nutrients dissolved in it (in particular phosphorus and microelements), while the plant delivers carbohydrates produced during the photosynthesis to the fungus (Deacon 2006).

Secretion of plant growth regulating substances by microorganisms

A great number of microbes living in the rhizosphere produce plant growth-regulating substances, some of which have hormonal effects, such as auxins (indole-3-acetic acid), gibberellins, cytokinins and ethylene. So far, most strains that had been identified, belonged to the group, which produces indole-3-acetic acid, just to mention *Rhizobium leguminosarum* (Biswas et al. 2000), *Azospirillum brasilense, Bacillus circulans, Bacillus megaterium, Streptomyces anthocysnicus* (Thankuria et al. 2004), *Azospirillum lipoferum, Mesorhizobium loti* (Chandra et al. 2007). On the other hand, *Bacillus subtilis* (Liu et al. 2013) and *Bacillus megaterium* (Ortíz-Castro et al. 2008; Ali et al. 2009) produce cytokinin. In addition, *Bacillus megaterium* can produce ethylene and auxin as well. (López-Bucio et al. 2007). Gibberellins can be produced by a wide range of microbes, just to mention *Pseudomonas monteilii* as an example (Pandya et al. 2014). *Pseudomonas fluorescens,* belonging to another *Pseudomonas* species and *Bacillus subtilis* have also the potential to synthesize gibberellins (Sivasakthi et al. 2013).

Mode of action of PGPR to mitigate the stress caused by drought

The biological, physical and chemical properties of the soil change considerably on the influence of the drought and heat stress, and as an outcome, the soil becomes unsuitable for microbial and plant growth (Meseka et al. 2018; Tesfaye et al. 2018). However, the interaction between the PGPR and the plant may alleviate the damages caused by the mentioned stresses. The PGPRs are rhizosphere bacteria and they are estimated to have the potential to induce drought resistance in plants by various mechanisms, including the increase of drought tolerance and resilience resulting in physiological and biochemical changes. These changes lead to the modification of phytohormonal content of plants (Khalid et al. 2006; Ali et al. 2011; García et al. 2017) and the protection with antioxidants. PGPRs also produce osmolytes and bacterial exopolysaccharides (EPS) to ensure the survival of plants under drought stress conditions (Vanderlinde et al. 2010). The production of heat shock proteins (HSPs), (Berjak 2006), dehydrins (Timmusk and Wagner 1999) and volatile organic compounds (VOCs) (Ryu 2004) induces drought tolerance in plants (Kaushal and Wani 2016; Elshafie et al. 2017). Moreover, PGPR can initiate chemical and physical changes in maize plants that improve resistance to environmental stresses through systemic tolerance (Kannojia et al. 2019). These protection mechanisms can facilitate significant changes in the structural and functional traits of the plants, resulting in abiotic stress resistance (Misra et al. 2017; Sarkar et al. 2018). Plant growth-promoting rhizobacteria are able to achieve it mainly because they respond to stress in a similar way to heat-stressed plants in case of water-deficit or in high-temperature periods. Microorganisms identify

the environmental changes, such as elevated temperature and salt concentration in the soil in the form of changes in the osmosis and are able to give proper answer to them (García et al. 2017).

Materials and methods

Plant material and PGPR

The maize genotype in the trial was provided by the Maize Breeding Department of the Cereal Research Non-profit Ltd., Szeged. The bacterial species were supplied by Toximent LP, Szolnok and the Hungarian University of Agriculture and Life Sciences (MATE). Table 1 shows the maize genotype and the bacteria studied in the trials.

Pure cultures

Bacillus megaterium (BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), *Pseudomonas putida* (PP) and *Escherichia coli* (K) were tested on pure cultures. Liquid and solid media were used to create the cultures. The culture was prepared based on Luria Bertani (LB) medium (5 g/l yeast extract, 10 g/l NaCl, pH 7.5 ± 0.2). To the solid version 15 g/l bacteriological agar was added. The accomplished media were subjected to sterilization at high temperature and pressure for 45 min before use. 1000 ml of liquid LB medium was poured into Erlenmeyer flasks in a laminar box under sterile conditions. 200 µl of bacterial suspension was pipetted into the liquid culture medium, and then the flasks were sealed. After inoculation, they were shaken for 24 h at 28 °C at 180 rpm. Samples were taken from the pure cultures made from the tested bacterial species and a glycerol strain was prepared for later use. The samples were stored at -70 °C. It is possible to start pure cultures from the frozen cells at any time.

Table 1 Maize genotype and bacterial species involved in the experiments

Hybrid maize		Bacterium	
Maturity group	Genotype	Type	Species
FAO 300	GKT 3385	Control	*Escherichia coli*
		PGPRs	*Bacillus megaterium*
			Bacillus pumilus
			Pseudomonas fluorescens
			Pseudomonas putida

Testing the phosphate solubilizing ability of bacteria

To test the phosphate solubilizing ability, modified solid Pikovskaya medium (0.5 g/l yeast extract, 10 g/l sucrose, 5 g/l $Ca_3(PO_4)_2$, 0,5 g/l $(NH_4)_2SO_4$, 0.2 g/l KCl, 0.1 g/l $MgSO_4$, 0.0001 $MnSO_4$, 0.0001 $FeSO_4$, 15 g bacteriological agar and pH 7.0 ± 0.2) was used. The culture medium was poured into Petri dishes with a diameter of 9 cm in a laminar box under sterile conditions and then allowed to solidify. The bacteria were inoculated at 4 points from the prepared liquid pure bacterial cultures into the Petri dishes by point inoculation with an inoculation loop. The experiment included 5 bacterial species, such as *Bacillus megaterium* (BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), *Pseudomonas putida* (PP) and *Escherichia coli* (K), with 8 replicates per bacterium. The bacterium *Echerichia coli* (K) was used as control. Cultures were incubated at 30 °C for 7 days. The cultures were photographed and analysed by the Fiji ImageJ photo analysis program. The recorded properties were the diameter of the bacterial colonies (CD) and the diameter of the clearing zones (HD). Knowing the diameter of the colonies and clearing zones, the phosphate solubilization index (SI) was calculated for each bacterial species.

$$SI = (CD + HD) / CD$$

For the quantitative analysis of tricalcium phosphate $(Ca_3(PO_4)_2)$, modified liquid Pikovskaya medium was used. The culture medium was poured into 100 ml Erlenmeyer flasks in a laminar box under sterile conditions. The bacteria were inoculated from the prepared liquid pure bacterial cultures into the Erlenmeyer flasks with an inoculation loop. Uninoculated modified liquid Pikovskaya medium was used as a control. The cultures were incubated in a shaking thermostat at 28 °C for 7 days at 180 rpm. After incubation, the cultures were centrifuged at 13,000 rpm for 10 min. The supernatant was collected from the samples, and the amount of available phosphate was determined by using the molybdenum blue method with a spectrophotometer at a wavelength of 600 nm (OD_{600}). The absorbance of the samples was measured at the same wavelength, and the concentrations of the transformed P standard curve were expressed in µg/cm³.

Testing the plant growth promoting effect of bacteria on maize seedlings

The experiment was conducted in eight replications. The model crop was GKT 3385, an early grain maize hybrid belonging to FAO 390 maturity group bred in Szeged. The bacterial species involved in the experiment (*Bacillus*

megaterium (BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), and *Pseudomonas putida* (PP) were multiplied on liquid Luria Bertani (LB) medium (5 g/l yeast extract, 10 g/l NaCl, and pH 7.5 ± 0.2). The liquid culture medium was poured into 100 ml Erlenmeyer flasks and inoculated with the investigated bacterial species, then incubated in a shaking thermostat for 5 days at 28 °C at a speed of 180 rpm, then the supernatant was poured off and the pellet was dissolved up to 100 ml in water cleaned by sterile reverse osmosis system (RO). The optical density of the bacterial solution was set to one at 600 nm (OD_{600}) with a spectrophotometer. 100 seeds were sterilized in 70% alcohol for 15 min, then washed in NEOMAGNOL 1000 mg (chlorogen) solution (1 tablet/500 ml water) for 30 min, then washed $3 \times$ with water cleaned with sterile reverse osmosis (RO). Sterile filter paper wetted with 10 ml of RO water was placed in Petri dishes with a diameter of 9 cm and 10 seeds were placed in each dish for germination. The seeds germinated in darkness at 25 °C. After 48 h of incubation, the nongerminating seeds and those with phytosanitary problems were removed and from the remaining healthy seeds, 8 seeds per treatment were selected, which were placed in Petri dishes on filter paper. On the first day, in each treatment, 5 ml of bacterial suspension (10^8 CFU/ml) (*Bacillus megaterium* (BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), and *Pseudomonas putida* (PP)) was pipetted on the germs. The germs were taken under observation on the fourth day. First, photographic documentation was made, then the seedlings were separated from the seeds. The coleoptiles and radicles were dissected and collected separately, and then the fresh weight was measured. The isolated coleoptiles and radicles were photographed, and then their length (CL, RL) was determined using the Fiji ImageJ photo analysis program. With respect to the fact, that only healthy seedlings were included into the experiment, the germination percentage (GP) was considered 100%. By determining the length of the coleoptiles and radicles, the germination vigour index (SVI, Seedling Vigour Index) was calculated.

$$SVI = (CL + RL)/GP$$

Testing the hybrid maize protection against the factor drought stress

Several phenological parameters were observed and recorded during the experiment. The experiment was set up with four replications under irrigated (70% of soil water capacity) (WC) and non-irrigated (20% of soil water capacity) (WC) conditions. 4 kg of soil was used (1:1 mixture of sand and Galli ground black peat) + 20 g/pot Compo NPK 12:8:16, a 90-day slow-release fertilizer per each 5-L growing pot. The tested bacteria species (*Bacillus megaterium*

(BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), and *Pseudomonas putida* (PP) were grown in liquid Luria Bertani (LB) medium. The liquid medium was poured into 100 ml Erlenmeyer flasks and inoculated with the tested bacterial species, then incubated in a shaking thermostat for 5 days at 28 °C at a speed of 180 rpm. Following that, the supernatant was poured off and the pellet was dissolved up to 100 ml in water purified by sterile reverse osmosis (RO) system. The optical density of the bacterial solution was set to one at 600 nm (OD_{600}) with a spectrophotometer. Our model plant was GKT 3385, an early grain maize hybrid belonging to FAO 390 maturity group. The trial was carried out in the greenhouse of Cereal Research Non-profit Ltd. in the light condition of 14 h light/10 h dark. The temperature in the greenhouse was 25 °C during the day and 20 °C at night. Wet weight (WW) of the soil was measured, then it was dried to constant weight in an oven at 70 °C and its dry weight (DW) was also recorded. Knowing the wet and dry weight of the soil, the moisture content of the soil was determined in mass weight percentage (MWP).

$$MWP = (WW - DW)/DW * 100$$

When planting the seeds, the bacterial suspensions (10 ml/pot, 10^8 CFU/ml) were inoculated into the pots directly into the seed environment. At planting, the water capacity of the soil was set to 70% (4840 g) in all the pots. In the case of the optimal water supply treatment, the soil moisture was reinstated to this value with irrigation. For the stressed treatment, the 70% water capacity was dried to 20% (4240 g). In the case of stressed treatment, the soil moisture was adjusted to this value with irrigation. Irrigation was carried out three times a week and the data were recorded in an irrigation diary. The first sampling was done in the R1 phenophase of the plants (50% female flowering). The second sampling was done in the R3 phenophase of the plants, at the milk stage of development. The recorded parameters included water demand (W), plant height (PH), root fresh weight (RFW), above-ground biomass fresh weight (BFW), above-ground biomass dry weight (BDW). The calculated parameters were the water use efficiency (WUE), fresh/dry weight ratio (FW/DW) and dry matter content (DMC).

$$WUE = DW/W$$

$$FW/DW \text{ ratio} = FW/DW$$

$$DMC = (DW/FW) * 100$$

Field trial

The trial was set up in the maize breeding nursery of Cereal Research Non-profit Ltd. in Kiszombor

(46°11′16″N20°23′43″N) in 2021. During the trial, 3 treatments were applied, namely, the untreated control (K), and the bacterial combinations of *Bacillus megaterium* and *Pseudomonas fluorescens* (KD1), and *Bacillus pumilus* and *Pseudomonas putida* (KD2) with 3 replicates in random block design. In the experiment, we used 4 rows of plots with an area of 18 m². They are 3 m wide and 6 m long. The plants were sown at a row spacing of 70 cm and a plant spacing of 22.3 cm. The total experimental area is 1008 m² (252 m² per treatment) (Fig. 1). Our model plant was an early grain maize hybrid, GKT 3385 (FAO 390) bred in Szeged. The tested bacteria species (*Bacillus megaterium* (BM), *Bacillus pumilus* (BP), *Pseudomonas fluorescens* (PF), and *Pseudomonas putida* (PP)) were grown on liquid Luria Bertani (LB) medium. The liquid medium was poured into 100 ml Erlenmeyer flasks and inoculated with the tested bacterial species, then incubated in a shaking thermostat for 5 days at 28 °C at a speed of 180 rpm. Following that, the supernatant was poured off and the pellet was dissolved up to 500 ml in water purified by sterile reverse osmosis (RO). The optical density of the bacterial solution was adjusted to one at 600 nm (OD_{600}) using a spectrophotometer. The physical and chemical properties of the soil of the experimental area are demonstrated in Table 2.

The pre-crop was winter wheat (*Triticum aestivum*). In autumn, after 26–32 cm deep ploughing, in the month of November, 170 kg/ha N, 100 kg/ha P and 100 kg/ha K were applied in the form of 8:21:21 NPK complex fertilizer. In the spring in March, 300 kg/ha CAM with 27% nitrogen content was applied and then the seed bed was prepared by a combinator. The soil inoculation was carried out before sowing with a self-propelled field sprayer, in the ratio of 1000 ml of suspension (10^8 CFU/ml) + 13 l of water/283.5 m², and immediately after application it was mixed into the soil with a rotavator. Sowing was done with a Wintersteiger Plotseed TC self-propelled plot seeder. The number of plants was set at 60,000 plants/ha. Early post-emergence weed control was carried out with Principal Plus + Successor T (Corteva) at a dose of 400 g/ha. The rows were cultivated by a cultivator. The harvest was carried out by a Wintersteiger Quantum Plotech plot combine. Among the traits determining the yield, the yield level, the grain moisture content and the dry matter content at harvest were recorded during harvesting, after harvesting, and during seed conditioning.

Statistical analysis

The collected data were analysed by using Microsoft Excel 2019 XLSTAT software. One-way Analysis of Variance (ANOVA) and three-way Analysis of Variance (ANOVA) were used to analyse the data of the individual factors and those of the interactions between the factors. The least significant difference was calculated by using the Fisher's Least Significant Difference (LSD) testing method. Correlation
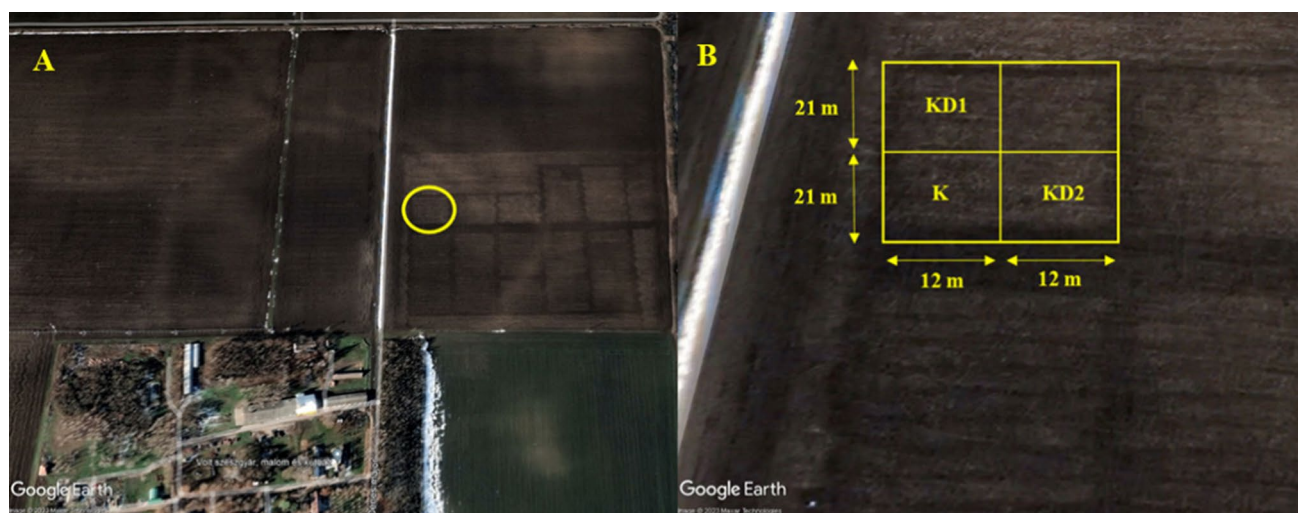
Fig. 1 Satellite image **A** and layout of the 2021 field experiment **B** in Kiszombor (46°11′27.93″N) (Google Earth, 2023) Remarks: K controll; KD1: *Bacillus megaterium* and *Pseudomonas fluorescens*; KD2: *Bacillus pumilus and Pseudomonas putida;* m: meter

Table 2 The physical and chemical properties of the soil of the experimental area

Year	pH (KCl)	KA	Total salt (%)	Na (mg/kg)	CaCO₃ (%)	NO₃-N (mg/kg)	Humus (%)	P₂O₅ (mg/kg)	K₂O	Mg	Zn	Cu	Mn	SO₄
2021	7.14	53	0.08	75	1.9	13.4	2.30	288	335	303	1.3	7.2	148	18.3

and Principal Component Analysis (PCA) were used to analyse the correlations.

Results

Phosphate-solubilizing ability of the tested bacteria

All of the 5 bacterial isolates were tested for phosphate solubilization. Among the isolates, 4 species formed a clear zone around the colonies after 7 days of incubation, proving the realization of the phosphate solubilization (Fig. 2).

Table S1 shows the results of the Analysis of Variance of the recorded data of the observed traits.

The difference was significant for each parameter if the factor bacterium was considered.

The solubilization index (SI) was measured by the formation of clearing zones around the colonies growing on the solid modified Pikovskaya medium. Clearing indicated that these bacteria solubilize insoluble tricalcium phosphate $(Ca_3(PO_4)_2)$. The varying diameters of the clearing zones showed that the bacteria solubilize tricalcium phosphate $(Ca_3(PO_4)_2)$ to different degrees. The results demonstrated that BM was the most efficient in solubilizing inorganic phosphate on solid modified Pikovskaya medium with a solubilization index of 1.805, followed by BP with a solubilization index of 1.752, PF with 1.727 and finally PP with the value of 1.585. In the present study, the maximum release of soluble orthophosphate was recorded for PP (51.636 µg/cm^3) after 7 days of development. It was followed by BP (15.601 µg/cm^3), PF (14.817 µg/cm^3) and finally by BM (14.621 µg/cm^3) after the same incubation period (Table 3).

Table 4 shows the correlations for the parameters determining phosphate solubilization. Negative correlation was found between the diameter of the colonies and the size of the diameter of the clearing zones. The size of the colony diameter had positive correlation with the phosphate solubilization index and the soluble phosphate concentration. The results showed that there was positive correlation between the size of the clearing zone diameter and the solubilization index, and between the solubilization index and the phosphate concentration, respectively. No correlation was found between the size of the diameter of the clearing zone and the soluble phosphate concentration.

Figure 3 displays the result of the Principal Component Analysis, presenting the correlation between the bacteria and the recorded parameters. The Principal Component Analysis was performed based on the data of the parameters determining the phosphate solubilization (CD: colony diameter, HD: clearing zone diameter, SI: phosphate solubilization index, and PC: phosphate concentration). The figure shows that the PP bacterium was the most efficient in facilitating phosphate mobilization. The first principal component represents 71.18% of all correlations and can be interpreted as phosphate-solubilizing potential.

Growth-promoting effect of the bacteria tested on maize seedlings

Four bacterial isolates were tested concerning the plant growth promoting effect (Fig. 4).

Table S2 shows the results of the Analysis of Variance of the recoded data.
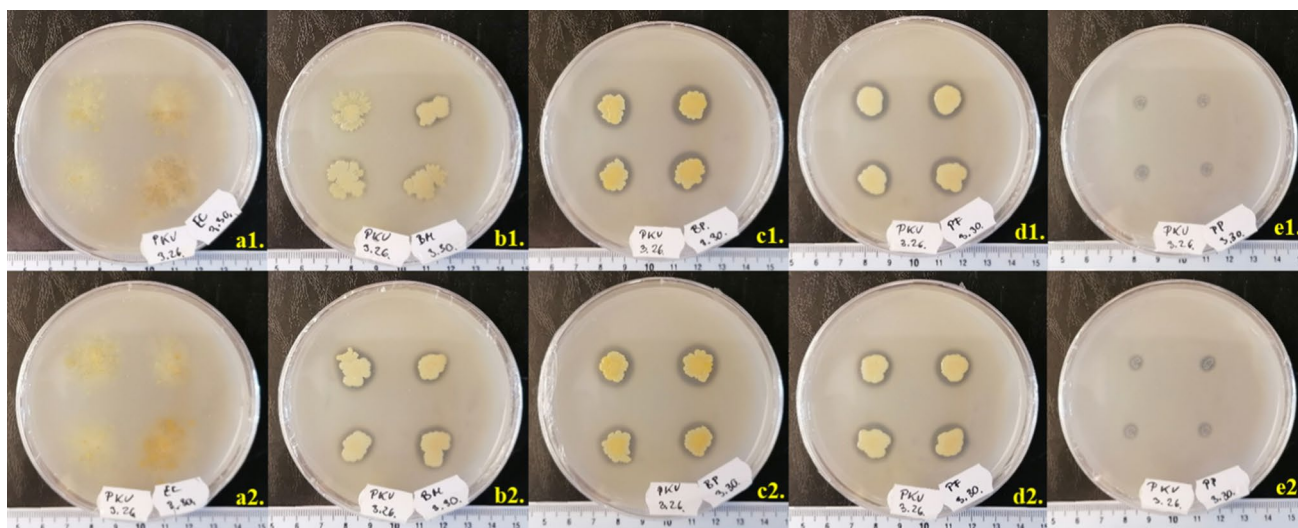
Fig. 2 No clearing zone was formed around the control *Esherichi coli* (a1., a2.) colony. The clearing zones formed around the colony of *Bacillus megaterium* (b1., b2.), *Bacillus pumilus* (c1., c2.), *Pseudomonas fluorescens* (d1., d2.) and *Pseudomonas putida* (e1., e2.) reflect the accomplishment of phosphate solubilization

Table 3 Least Significant Difference (LSD) among the most important parameters (colony diameter, clearing zone diameter, phosphate solubilization index and phosphate concentration) of the phosphate-solubilizing ability of bacteria at 95% confidence interval for the factor bacterium

95% Confidence interval							
LS means							
CD		HD		SI		PC	
Bacterium LSD							
K	25.051 a	K	0.000 c	K	0.000 d	K	0.758 d
BM	13.690 b	BM	16.992 a	BM	1.805 a	BM	14.621 c
BP	12.796 bc	BP	17.033 a	BP	1.752 ab	BP	15.601 b
PF	11.982 c	PF	16.497 a	PF	1.727 bc	PF	14.817 bc
PP	4.669 d	PP	7.960 b	PP	1.585 c	PP	51.636 a
LSD5%	1.665		0.1502		0.039		0.913
p	**< 0.0001**		**< 0.0001**		**< 0.0001**		**< 0.0001**

CD Colony diameter, *HD* Clearing zone diameter in mm, *SI* Phosphate solubilization index, *PC* Phosphate concentration expressed in $\mu g/cm^3$. Small letters show the significance correlations among the obtained values

Table 4 Correlation analysis of the parameters including colony diameter, clearing zone diameter, phosphate solubilization index and phosphate concentration

	CD	HD	SI
HD	**−0.492***		
SI	**0.806*****	**0.903*****	
PC	**0.874*****	0.052 ns	**0.464***

CD Colony diameter, *HD* Clearing zone diameter, *SI* Phosphate solubilization index, *PC* Phosphate concentration. $*P = 0.05\%$, $***P = 0.001\%$, Ns Not significant
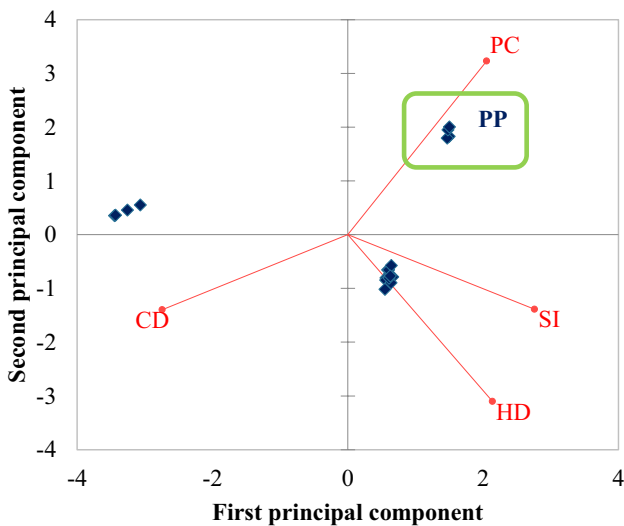
Fig. 3 Principal Component Analysis of the tested bacteria (PP: *Pseudomonas putida*) considering the most important parameters of phosphate solubilization (CD: colony diameter, HD: clearing zone diameter, SI: phosphate solubilization index, and PC: phosphate concentration)

Considering the factor bacterium, the difference was significant for radicle length and germination index.

The germination vigour index was determined by measuring the coleoptile and radicle length of the treated and control seedlings. The various length of the coleoptiles and radicles suggests that the bacteria stimulated the growth of the seedlings to a different extent. In the current study, it was found that the length of the coleoptiles was characterized by a higher value in all bacterial treatments compared to the control group. In the case of the length of the radicles, the maximum length was attained by the PP-treated seedlings with 94.979 mm. The seedlings treated with PF, BP and BM presented a radicle length of 41.067, 40.247 and 38.659 mm, respectively, which were lower than the control with a value of 51.144 mm. Based on the results, the PP treatment proved to be the most effective with a germination vigour index of 15,237.125. This was followed by the untreated control with a germination vigour index of 9792.188, and then by the indices of the other treatments, namely, PF: 9421.088, BP: 8907.813 and BM: 8657.55 (Table 5).

Table 6 shows the correlations for the parameters determining the germination vigour. Positive correlation was found for each of the tested parameters.

Figure 5 shows the results of the Principal Component Analysis, which gives information about the correlation between the bacterial treatments and the parameters recorded about the seedlings. The basis of the Principal Component Analysis were the data of the parameters determining the germination vigour, that is, coleoptile length and radicle length. Based on the figure, it can be concluded that the PP bacterial treatment was the most efficient plant growth stimulant in the case of GKT 3385 maize seedlings in relation to early germination vigour.

Fig. 4 In the photo series No. 1, a1. and a2. show the control treatment in the case of the dissected coleoptiles. The photos b1. and b2. reflect the treatment with *Bacillus megaterium* (BM), c1. and c2. the treatment with *Bacillus pumilus* (BP), d1. and d2. the treatment with *Pseudomonas fluorescens* (PF) and e1., e2. the treatment with *Pseudomonas putida* (PP). In the photo series No. 2, a1. and a2. show the control treatment for the dissected radicles. The photos b1. and b2. show the treatment with *Bacillus megaterium* (BM), c1. and c2. the treatment with *Bacillus pumilus* (BP), d1. and d2. the treatment with *Pseudomonas fluorescens* (PF), and e1.and e2. the treatment with *Pseudomonas putida* (PP), respectively

The first principal component compiles 81.82% of all correlations and can be considered as the indicator for increasing early germination vigour.

Testing the protection of hybrid maize against the factor drought

Four bacterial isolates were tested to determine their role in the protection of hybrid maize against the factor drought stress under optimal and drought-stressed conditions (Fig. 6).

Table S3 demonstrates the evaluation of the data of the traits recorded at 50% female flowering and at milk stage of development by Analysis of Variance.

The various factors and interactions showed a significant difference in relation to the recorded phenological parameters in almost all cases. However, in the case of plant height, the water capacity*phenophase and bacteria*water capacity*phenophase interactions did not show significant difference. No significant differences were found in the bacteria*phenophase and bacteria*water capacity*phenophase interactions for the above-ground biomass fresh mass. When examining the water use efficiency, the factor bacterium, bacteria*water capacity and bacteria*phenophase interactions did not result in significant differences. Considering the bacteria*phenophase interaction, no significant difference was found for the ratio of fresh weight/dry weight.

Table 5 The Least Significant Difference (LSD) of the germination vigour-determining parameters (coleoptile length, radicle length and germination vigour index) of the seedlings in the treatment with bacteria, calculated at 95% confidence interval and projected on the factor bacterium

95% Confidence interval					
LS means					
CL		RL		SVI	
Bacterium LSD					
K	46.778 b	K	51.144 b	K	9792.188 b
BM	47.917 b	BM	38.659 c	BM	8657.550 b
BP	48.832 b	BP	40.247 c	BP	8907.813 b
PF	53.144 ab	PF	41.067 c	PF	9421.088 b
PP	57.393 a	PP	94.979 a	PP	15,237.125 a
LSD5%	7.808		8.754		1419.431
p	0.052		< 0.0001		< 0.0001

CL Coleoptile length, *RL* Radicle length in mm, *SVI* Seedling germination vigour index. Small letters show the significance correlations between the obtained values

Table 6 Correlation between the following traits, coleoptile length, radicle length, and germination vigour index

	CL	RL
RL	**0.490****	
SVI	**0.698*****	**0966*****

CL Coleoptile length, *RL* Radicle length, *SVI* Seedling germination vigour index. **P = 0.01%, ***P = 0.001%
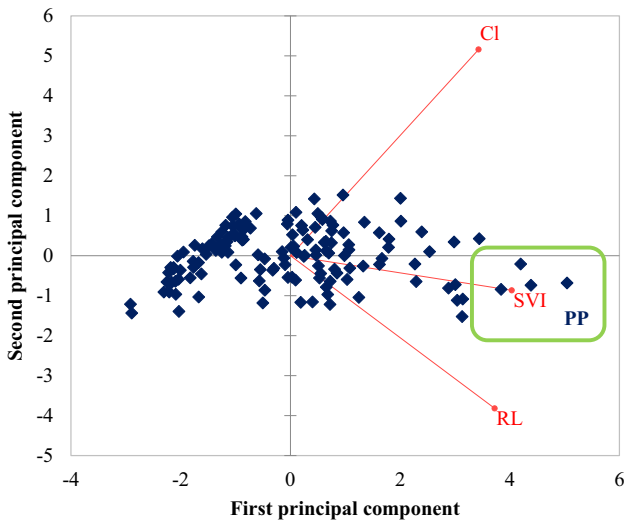
Fig. 5 Principal Component Analysis based on the data of the bacterial treatments (PP: *Pseudomonas putida*) and the parameters determining the germination vigour of the seedlings (CL: coleoptile length, RL: radicle length and SVI: germination vigour index)
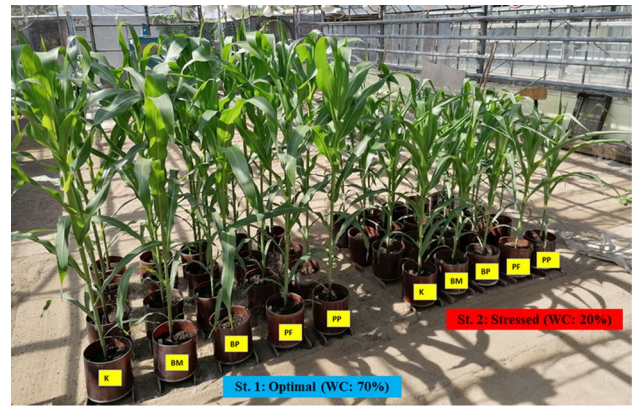
Fig. 6 Subtrial 1 (St. 1): In this part of the trial, the water supply was optimal, meaning that water was added up to 70% of the water capacity of the soil (WC: 70%). Subtrial 2 (St. 2) constituted the drought-stressed treatment, meaning that water was added up to 20% of the water capacity of the soil (WC: 20%). The treatments were as follows: K: controll, BM: *Bacillus megaterium*, BP: *Bacillus pumilus*, PF: *Pseudomonas fluorescens*, and PP: *Pseudomonas putida*

The water demand of plants

In the present study, it was experienced that the impact of the bacterial treatments was diverse on the recorded traits of the plants, and varied over the individual factors and the interactions. The data recorded at 50% female flowering and milk stage of development reflected that the plants of the maize hybrid GKT 3385, which were grown in the greenhouse at two water regimes, needed less water when treated with bacteria than the control plants. The plants in the BM treatment used the least amount of water, which was 6080.240 ml, while the water use of the control plants was 7552.125 ml. Under irrigated conditions the plants absorbed 8059.600 ml of water, while under stressed conditions it was much less at 5132.000 ml, reflecting considerable water deprivation. The plants absorbed 7540.000 ml of water up to the stage of milk stage of development, while they used 5651.000 ml up to 50% female flowering. Under optimal water supply, the plants treated with BP needed 7278.750 ml of water compared to the control plants, which used 8923.750 ml water. Under stressed condition, the plants under BM treatment were irrigated with 4603.000 ml of water and the control plants with 5580.500 ml. The above-mentioned results of water use could be reinforced from the aspect of the interaction of water capacity and phenophase as well. Both at 50% female flowering (BM: 5176.750 ml, BP: 5226.750 ml, and control: 6199.500 ml) and at milk stage of development (BM: 6983.750 ml, BP: 6971.000 ml, and control: 8304.750 ml), the BM and BP treatments resulted in lower water demand of the plants. Considering all three factors, it can be concluded that the plants treated with bacteria required less water than the control in both

phenophases and irrigation regimes. With optimal water supply, in both phenophases, the plants treated with BP absorbed the least amount of water compared to the control. It was 8352.500 ml at 50% female flowering (control: 10,332.500 ml), and 6205.000 ml at milk stage of development (control: 7515.000 ml). Under stressed condition, the least amount of water was applied to the BM-treated plants at 50% female flowering (3966.000 ml, control: 4884.000 ml) and milk stage of development (5240.000 ml, control: 6277.000 ml) (Fig. 7, Table S4).

The plant height of plants

Considering the factor bacterium, no difference was found between the height of the treated and control plants. The lowest value was measured for the plants treated with BP with 180.55 cm, while the control plants where as tall as 195.00 cm. Significant difference could be observed in the height of the plants, when the irrigated and water-stressed conditions were compared. With optimal water supply, the average height of the plants was 229.92 cm, while under water deprivation this value was only 148.13 cm. The impact of the drought was that the plant height reduced. The height of the plants reached 216.69 cm at the milk stage of development, and 161.35 cm at the 50% female flowering stage. Under optimal water supply, the plants treated with PP (248.96 cm) and PF (238.85 cm) grew taller than the control, which was 225.28 cm. Under water deprivation, the PP-treated plants (215.33 cm) were taller than the control (149.46 cm). At 50% female flowering, the height of the plants treated with bacteria was lower than

the control. However, the height of the plants treated with PF (230.75 cm) and PP (225.84 cm) was above the control (207.85 cm) at milk stage of development. In the case of optimal water supply, taller plants were found in both phenophases than in the drought-stressed environment. Taking into account the interaction between all three factors, it was found that the plants treated with PF and PP in irrigated conditions both at 50% female flowering (PF: 207.00 cm, PP: 242.00 cm, and control: 195.50 cm) and at milk stage of development (PF: 270.70 cm, PP: 255.93 cm, and control: 255.07 cm) achieved higher plant height than the control. Under non-irrigated conditions, at the time of 50% female flowering and milk stage of development, no significant difference could be found in the height of the treated and control plants (Fig. 8, Table S4).

The fresh weight of the roots of plants

When taking the factor bacterium, the PF treatment was found to increase the fresh weight of the roots to the highest extent with a value of 166.250 g compared to the control result of 151.500 g. Under drought-stressed conditions, the roots of the plants had lower fresh weight (132.300 g) than the plants with optimal water supply (171.850 g). The severe water deprivation also affected the root development of the plants. The sampling in the two different phenophases proved that the roots of the plants at milk stage of development had higher fresh weight (204.150 g) than at the stage of 50% female flowering (100.000 g). Under irrigated conditions, the plants treated with PF produced the highest root fresh weight with a result of 198.000 g, while the related
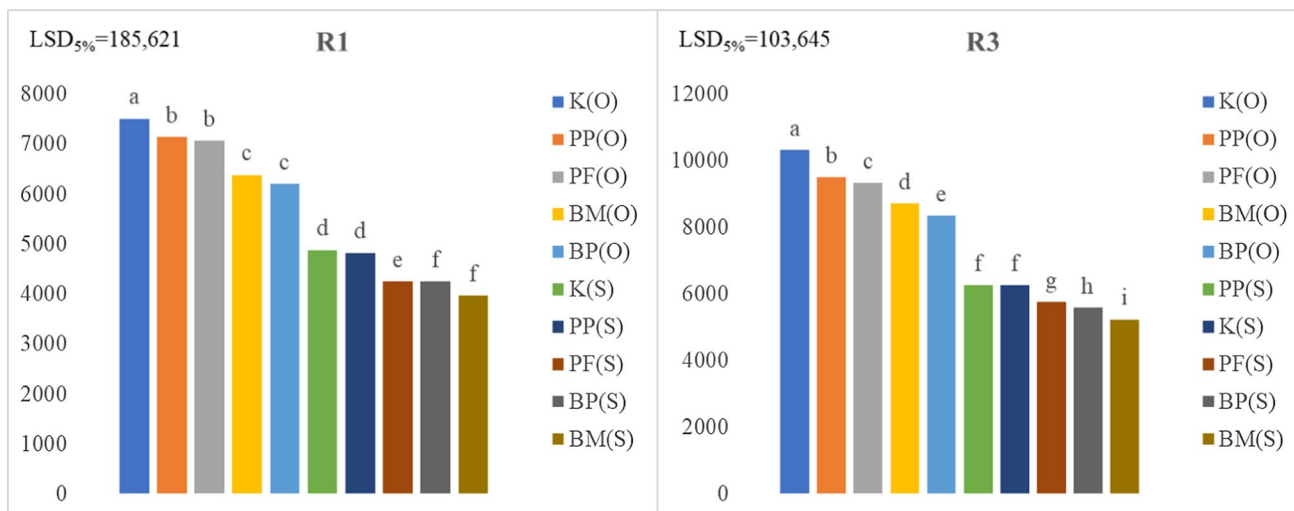
Fig. 7 Evolution of the water demand of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in ml. Small letters indicate the significance relationships between the obtained values
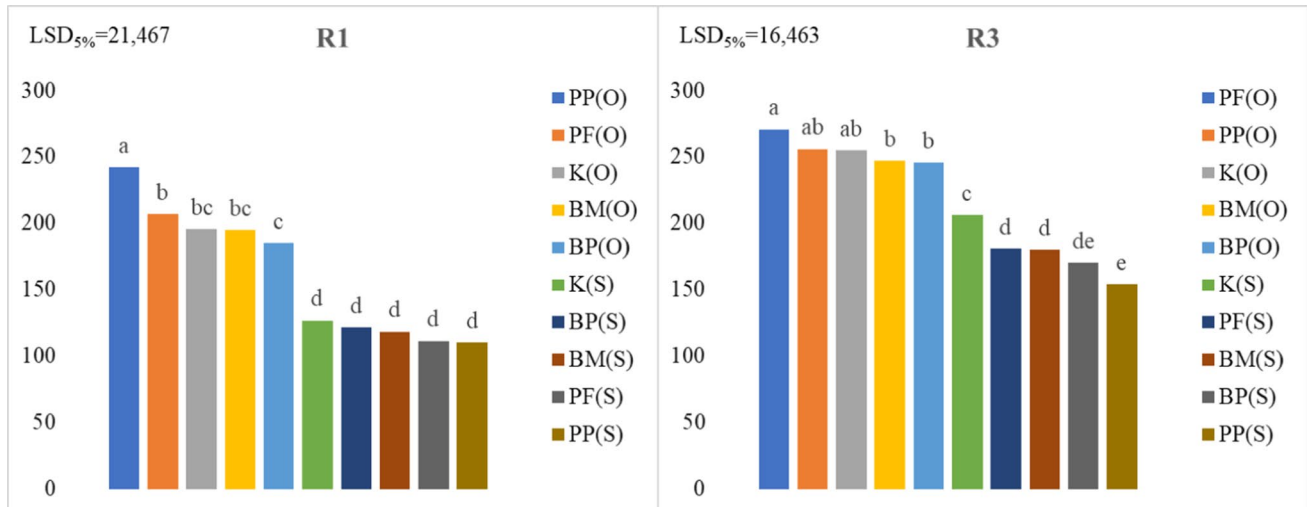
Fig. 8 Evolution of plant height of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two pheno- phases (R1: 50% female flowering; R3: milk stage of development) expressed in cm. Small letters indicate the significance relationships between the obtained values

value of the control was only 164.000 g. In the case of water deprivation, the PP treatment was the most efficient, because the roots of the plants had 148.500 g fresh weight, compared to the control, which had only 139.000 g. At 50% female flowering stage, the root fresh weight of the plants treated with bacteria presented a lower value for all bacteria than that of the control group. At the stage of milk stage of development, 224.500, 219.250, and 219.000 g of root fresh weight could be recorded in the PF, PP and BP treatments, compared to the result of the control, which was 188.500 g. In both irrigation regimes, higher root fresh weight was measured at the milk stage of development. With optimal water supply, at the milk stage of development, higher root fresh weight was measured in the PF (279.000 g), PP (270.000 g) and BP (246.500 g) treatments compared to the control (203.000 g). Under water deprivation, no higher value could be found for this trait than that of the control at the milk stage of development. The same prevailed also for the 50% female flowering stage. The root fresh weight results of the treated plants did not exceed those of the control plants neither under optimal water supply nor under water-stressed condition (Fig. 9, Table S4).

The above-ground biomass fresh weight of plants

Regarding the factor bacterium and the interactions, the bio-mass fresh weight did not become higher than the control as a result of the treatments. Under water deprivation, the fresh weight of the stressed plants was lower compared to that of the plants under optimal water supply, both at the 50%

female flowering stage and the milk stage of development (Fig. 10, Table S4).

The dry mass of the above-ground biomass of plants

The results were in full compliance with those found for the above-ground fresh plant biomass for each factor and interaction (Fig. 11, Table S4).

The water use efficiency of plants

In the case of water use efficiency, no difference was found between the bacterial treatments and the control group. In case of the optimal water supply, the water use of the plants was more efficient than in the drought-stressed treatment. The water use of the plants at the milk stage of development was more efficient (0.007 g/ml) than at 50% female flowering (0.005 g/ml). The same could be observed in the case of the interaction of both factors (Fig. 12, Table S4).

The fresh weight/dry weight ratio of plants

In the case of the bacterial treatments, no difference was found between the results of the treated and control plants. Due to water deprivation, the fresh weight/dry weight ratio of the plants (6.873) was below the value with optimal water supply (6.406). Regarding the fresh/dry weight ratio of the plants, more favourable value was found at milk stage of development (5.573) than at 50% female flowering stage (7.705). Under irrigated conditions, the fresh/dry weight ratio of the plants treated with bacteria presented a lower
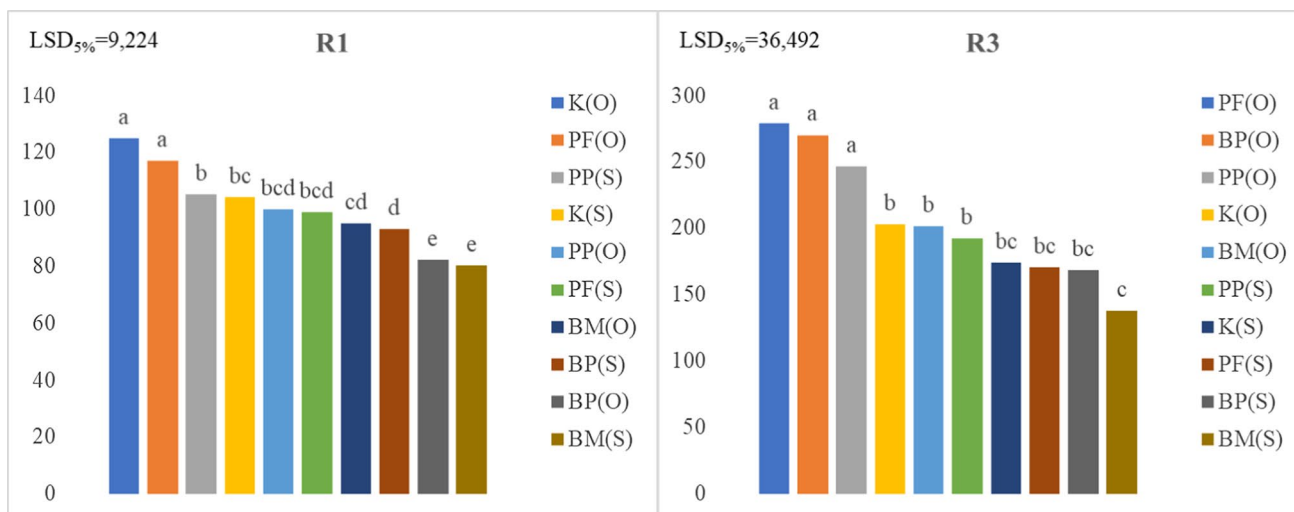
Fig. 9 Evolution the fresh weight of the roots of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by t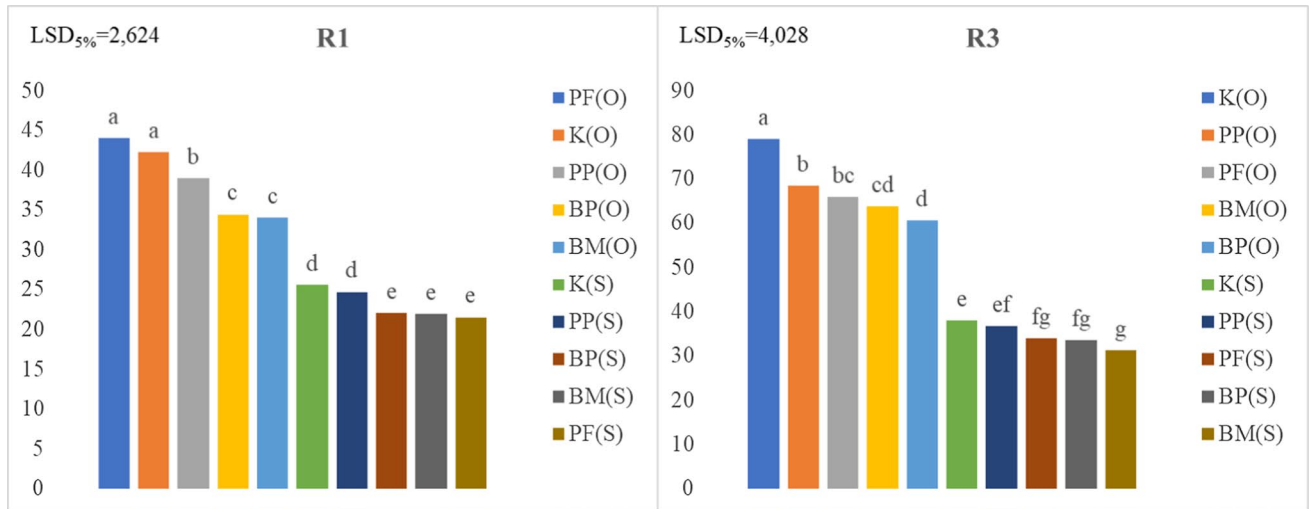wo types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in g. Small letters indicate the significance relationships between the obtained values
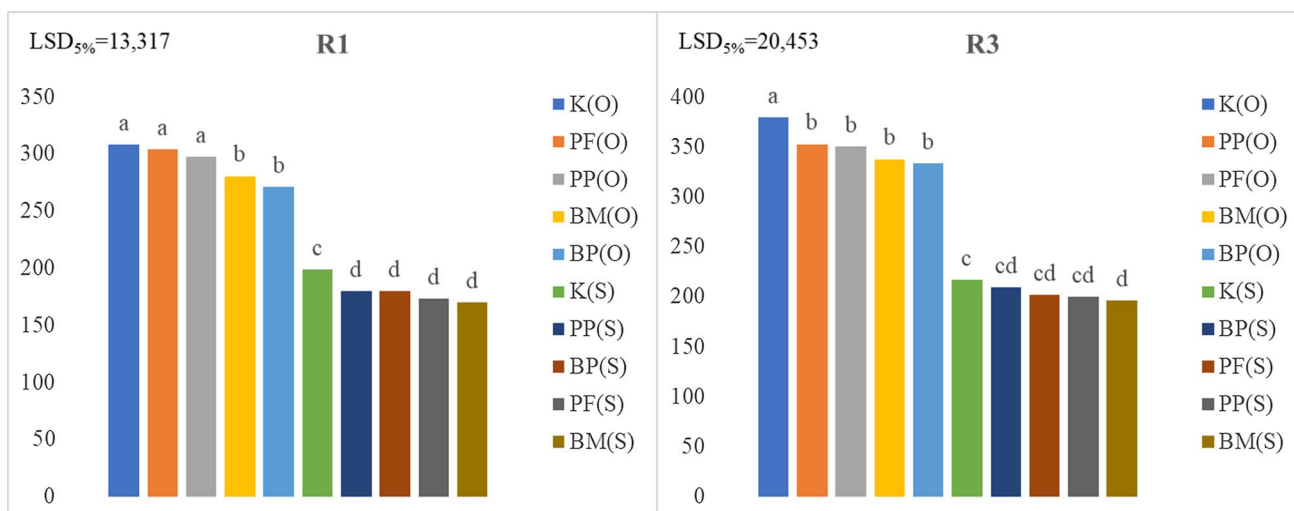
Fig. 10 Evolution of the biomass fresh weight of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in g. Small letters indicate the significance relationships between the obtained values

value compared to the control group. In the case of drought stress, a more favourable fresh weight/dry weight ratio was realized in the PP treatment (6.383) than at the control (6.748). The PP treatment resulted in the most outstanding fresh/dry weight ratio (7.312) compared to the control (7.780), when the interaction between all factors was taken into account under stressed conditions at 50% female flowering stage. In the case of optimal water supply, the fresh weight/dry weight ratio (6.905) was more advantageous in

the case of the plants in the PF treatment, compared to the control value of 7.306 at 50% female flowering (Fig. 13, Table S4).

The dry matter content of plants

Regarding the factor bacterium, no higher dry matter content was found at the treated plants than in the control group. Under irrigated conditions, higher plant dry matter

Fig. 11 Evolution of the dry mass of the above-ground biomass of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in g. Small letters indicate the significance relationships between the obtained values
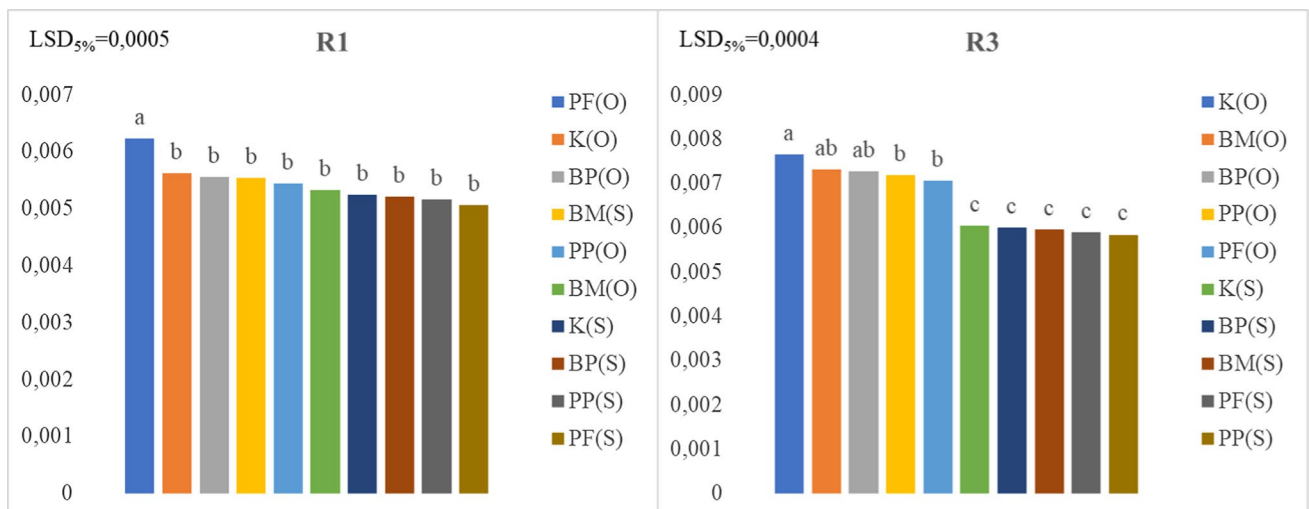
Fig. 12 Evolution of the water use efficiency of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in g/ml. Small letters indicate the significance relationships between the obtained values

content was attained (16.230%) than under non-irrigated conditions (14.875%). The dry matter content of the plants at the milk stage of development (18.081%) was higher than at 50% female flowering (13.024%). In the case of water deprivation, the plants treated with PP exhibited the highest dry matter content (16.021%), compared to the control (15.175%). This value is almost the same as the result of the PP treatment under optimal water supply conditions (16.258%). Both under water stress and under optimal water supply, the dry matter content of the plants was higher during the milk stage of development. Considering the interaction between all the factors, the highest dry matter content of the plants (18.344%) compared to the control (17.344%) was measured in the case of the PP treatment during the milk stage of development under stressed conditions (Fig. 14, Table S4).

Table 7 demonstrates the correlations between the tested parameters. The results reflect positive correlation for almost
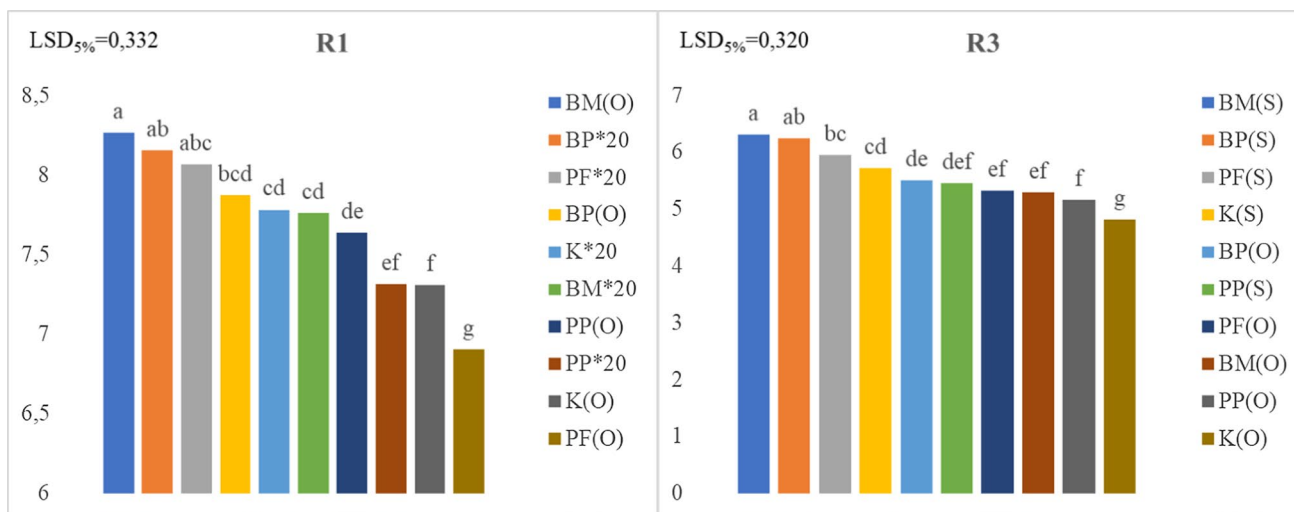
Fig. 13 Evolution of the fresh weight/dry weight ratio of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development). Small letters indicate the significance relationships between the obtained values
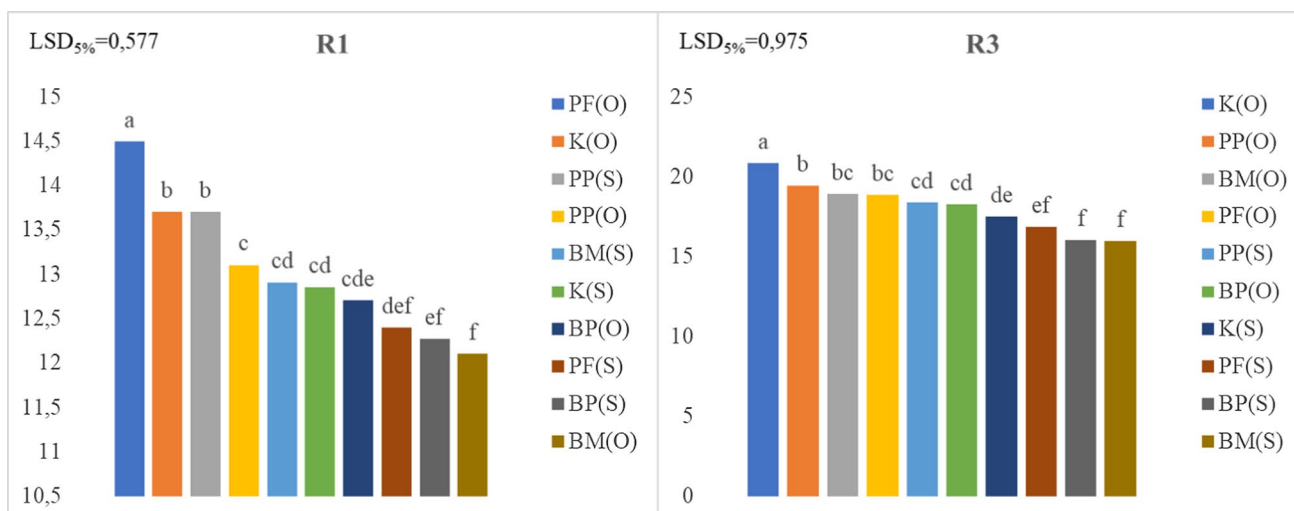
Fig. 14 Evolution of the dry matter content of plants due to bacterial treatments (K: control; BM: *Bacillus megatherium*; BP: *Bacillus pumilus*; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida*) is influenced by two types of water norms (O: optimal; S: stressed), in two phenophases (R1: 50% female flowering; R3: milk stage of development) expressed in %. Small letters indicate the significance relationships between the obtained values

all the parameters. Only the fresh/dry weight ratio showed negative correlation.

In Fig. 15, the results of the Principal Component Analysis are displayed. It reflects the correlation between the recorded plant traits as influenced by the bacterial treatments under two irrigation regimes (WC 70% and WC 20%) and at two phenophases (R1: 50% female flowering and R3: milk stage of development). The Principal Component Analysis was performed with the data of the phenological parameters

of the plants (water, plant height, root fresh weight, above-ground biomass fresh weight, above-ground biomass dry weight, water use efficiency, ratio of fresh weight to dry weight, and dry matter content). The figure shows, that the response of the plants varied across the bacterial treatments influenced by the irrigation conditions and the stages of phenophase. Under stressed conditions, it was the PP treatment that had a significant effect on the physiological condition of the plants, by reducing the fresh/dry weight ratio

Table 7 Correlation analysis of the following parameters, water, plant height, root fresh weight, above-ground biomass fresh weight, above-ground biomass dry weight, water use efficiency, fresh weight/dry weight ratio and dry matter content

	W	PH	RFW	BFW	BDW	WUE	FW/DW
PH	0.904***						
RFW	0.718***	0.662***					
BFW	0.947***	0.885***	0.548***				
BDW	0.971***	0.872***	0.761***	0.915***			
WUE	0.804***	0.762***	0.759***	0.756***	0.913***		
FW/DW	− 0.700***	− 0.643***	− 0.859***	− 0.497***	− 0.778***	− 0.845***	
DMC	0.737***	0.655***	0.863***	0.534***	0.816***	0.862***	− 0.992***

W Water, *PH* Plant height, *RFW* Root fresh weight, *BFW* Above-ground biomass fresh weight, *BDW* Above-ground biomass dry weight, *WUE* Water use efficiency, *FW/DW* Fresh weight/dry weight ratio, *DMC* Dry matter content, ***$P = 0.001$%

Fig. 15 Principal component analysis of the applied bacterial treatments (BP: *Bacillus pumilus*, PF: *Pseudomonas fluorescens*, and PP: *Pseudomonas putida*) based on the phenological parameters of the plants (W: water, PH: plant height, RFW: root fresh weight, BFW: above-ground biomass fresh weight, BDW: above-ground biomass dry weight, WUE: water use efficiency, FW/DW: ratio of fresh weight to dry weight, and DMC: dry matter content) under unirrigated (WC 20%) and irrigated (WC 70%) conditions, at 50% female flowering (R1) and milk stage of development (R3), respectively
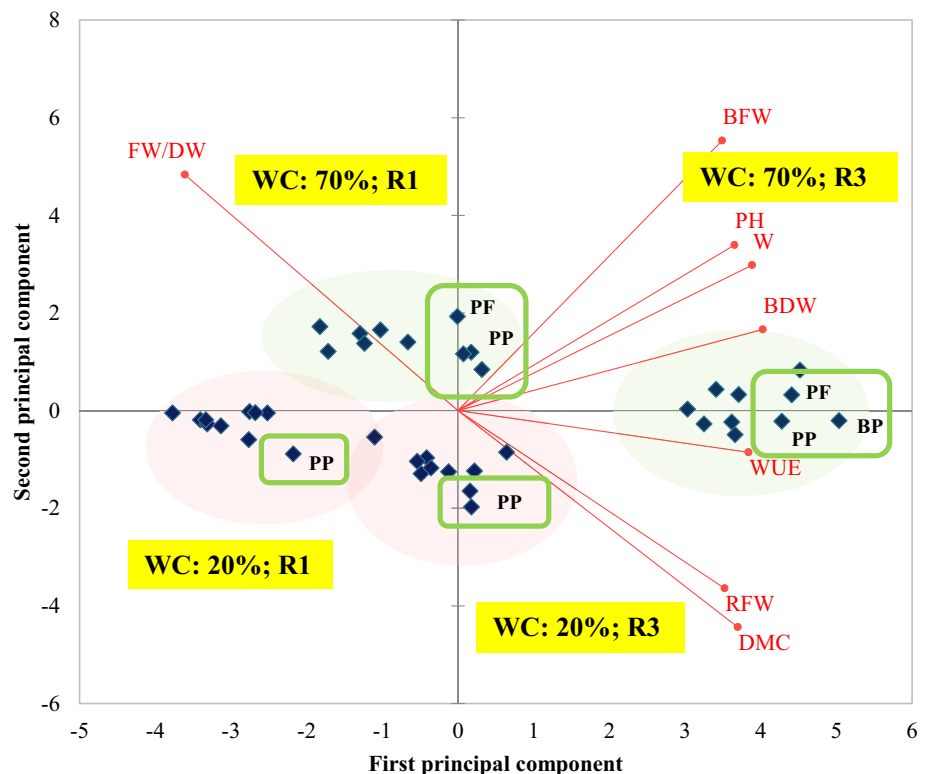
compared to the control at 50% female flowering stage, and by increasing the dry matter content at the milk stage of development. With optimal water supply, the PF and PP treatments enhanced the plant height at 50% female flowering compared to the control, which implied growth-stimulating effect. During the milk stage of development, the PP, PF and BP treatments stimulated the root development of the plants compared to the control. The first principal component represents 81.30% of all correlations.

Field trial

As it is shown in Table 8, the average temperature was 18.78 °C in the experimental area during the maize growing season in 2021. The hottest months were June, July and August. The total precipitation was 179.5 mm during the maize growing season, but its distribution was not uniform, with the most rainfall in May. The number of hot days was 54, and those of heat days was 16. From the aspect of maize production, the year 2021 was rather unfavourable. The hot weather, the lack of precipitation, and the atmospheric drought during flowering exposed the plants to extreme drought stress, which was also reflected in the yield averages.

The Analysis of Variance of the recorded plant traits is demonstrated in Table S5.

The factor bacterium caused significant difference for each of the yield components.

Table 8 Average temperature (At.) (°C) during the maize growing season in Kiszombor in 2021

Month	At. (°C) 2021	Rf. (mm) 2021
April	9,03	25,2
May	15,26	55
June	22,62	21,8
July	25,33	28,3
August	22,45	23,4
September	17,96	25,8
Mean/Sum	18,78	179,5
TX30GE (days)	**54**	
TX35GE (days)	**16**	

Remarks: TX30GE: No. of hot days; TX35GE: No. of heat days

TX30GE: No. of hot days; TX35GE: No. of heat days

Table 9 Least Significant Difference (LSD) at 95% confidence interval between the yield-determining traits of the maize plants (grain yield, grain moisture content and dry matter content) treated with bacteria, with bacterium as the determining factor

95% Confidence interval

LS means

Y		W		DMC	
Bacterium LSD					
K	5.468 b	K	17.472 a	K	41.150 a
KD1	5.339 b	KD1	17.667 a	KD1	41.167 a
KD2	6.278 a	KD2	17.700 a	KD2	41.150 a
LSD5%	0.498		0.486		0.348
p	**0.007**		0.505		0.991

Y Grain yield (t/ha), *W* Grain moisture content (%), *DMC* Dry matter content (%). Small letters indicate the significance relationships between the obtained values

The highest grain yield (6.278 t/ha) was produced by the plants treated with KD2. It was followed by the control with 5.468 t/ha and KD1 with 5.339 t/ha, respectively. In the case of grain moisture content and dry matter content, no difference could be found between the treatments (Table 9).

Table 10 shows the correlation for the yield-determining plant traits. No correlation was found between any of the parameters involved in the analysis.

Principal Component Analysis of the correlation between the bacterial treatment and the yield-determining traits. The analysis was performed with the data of the yield-determining properties, including grain yield, grain moisture content and dry matter content. The figure confirms the conclusion that the KD2 bacterial treatment was the most efficient yield increasing stimulant in the case of the maize hybrid GKT 3385. The first principal component comprises 57.72% of

Table 10 Correlation analysis of the yield-determining traits, involving grain yield, grain moisture content and dry matter content

	Y	W
W	0.213 ns	
DMC	−0.095 ns	−0.666 ns

Y Grain yield, *W* Grain moisture content, *DMC* Dry matter content, Ns Not significant
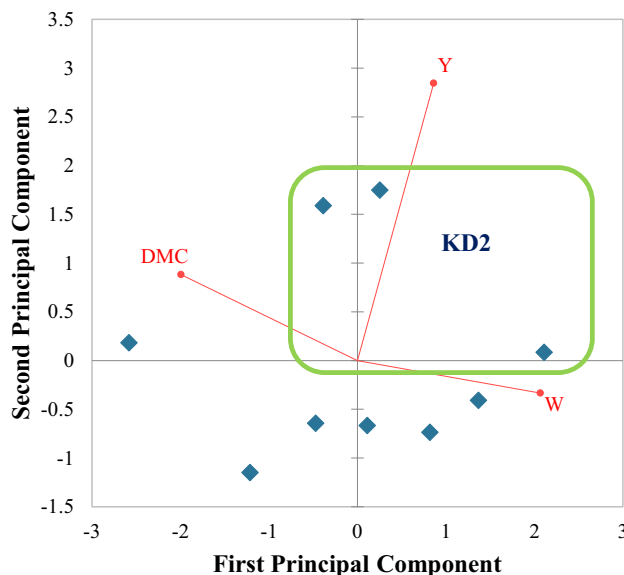
Fig. 16 Principal component analysis of the influence of the bacterial treatments (KD2: *Bacillus pumilus* and *Pseudomonas putida)* on the yield-determining plant traits. (Y: grain yield, W: grain moisture content, and DMC: dry matter content)

all correlations and can be interpreted as yield potential (Fig. 16).

Discussion

Phosphate-solubilizing ability of bacteria

In the current study, all five bacterial isolates were tested for phosphate solubilization. Four bacteria formed a clearing zone around the colonies after 7 days of incubation, indicating the completion of phosphate solubilization. The result of the present investigation was in compliance with those of Gaur 1990 and Khan et al. 2005, who also reported that only phosphate-solubilizing bacteria are able to create clearing zones in an environment containing tricalcium phosphate $(Ca_3(PO_4)_2)$. No significant difference could be observed between the phosphate solubilization index of *Bacillus megaterium* (1.805), *Bacillus pumilus* (1.752) and *Pseudomonas fluorescens* (1.727). In the case of *Pseudomonas*

putida, a lower value was found, with a solubilization index of 1.585. In the present study, the largest amount of soluble orthophosphate was produced by *Pseudomonas putida* (51.636 µg/cm^3), demonstrated by the quantitative analysis of the orthophosphate generated in the solution. Pandey et al. 2006 reported about a similar result on liquid Pikovskaya medium at 28 °C after 7 days of incubation. This is significantly more than the amount of phosphate solubilized by *Bacillus pumilus* (15.601 µg/cm3), *Pseudomonas fluorescens* (14.817 µg/cm3) and *Bacillus megaterium* (14.621 µg/cm3). *Pseudomonas putida* proved to be the most efficient phosphate-solubilizing bacterium, mobilizing 70% more insoluble phosphate than *Bacillus megaterium*, *Bacillus pumilus* and *Pseudomonas fluorescens* on modified liquid Pikovskaya medium after 7 days of incubation. In the current experiment, all of the 4 tested bacterial species proved to have phosphate-solubilizing ability, with *Pseudomona putida* to have the highest efficiency. This bacterium was identified to have the capability to solubilize the majority of the insoluble phosphate forms, and release soluble P as orthophosphate, an available form of phosphorus for maize plants, and thus supplying the plants with additional nutrients.

Growth-promoting effect of bacteria on maize seedlings

The plant growth-stimulating effect of PGPR strains has been unambiguously proved for various crops (El-Hawary et al. 2002; Wu et al. 2005). Bacterial inoculants are capable to increase plant growth and germination rates, enhance the plant emergence and the responses to external stressors, and protect plants from diseases (Lugtenberg et al. 2002). In previous studies it was established that *Bacillus spp*. and *Pseudomonas spp*. are apt to be used as plant growth-stimulating bacteria, and their positive characteristics manifest themselves in rapid reproduction, simple nutrition and wide environmental compatibility (Gravel et al. 2007, Rehman et al. 2019). In the present experiment, all four bacterial isolates were tested for their plant growth-stimulating effect on maize seedlings, with focus on early germination vigour. No significant difference was found in the coleoptile length of the treated plants compared to the control, so it became obvious, that the bacteria did not stimulate the elongation of the coleoptiles after 6 days of incubation. The treatment with *Pseudomonas putida* significantly increased the length of the radicles (by 85%), up to 94.979 mm, compared to the control group with 51.144 mm, so its root-growth stimulating effect had been verified on maize seedlings. Significant difference was also found for the treatment with *Pseudomonas putida* from the aspect of germination vigour index, because this bacterium strain was spotted to be very efficient in facilitating this trait compared to the result of the control. The

germination vigour of the seedlings in the treatment with *Pseudomonas putida* was 55% higher, represented by the value of 15,237.125, in comparison with the control, with the value of 9792.188. In the scientific literature, Nezarat et Gholami, 2009 and Noumavo et al. 2013, also reinforced the efficiency of *Pseudomonas putida* in stimulating the germination vigour of maize seedlings on the basis of their findings. As regards the plant growth stimulating effect, the results of the present study confirmed that *Pseudomonas putida* beneficially influenced the growth of radicles in the early stage of germination, and thus, it fortified the early vigour of maize, which again, influenced the emergence beneficially.

Protection of hybrid maize against the drought stress factor

The response of plants to water stress can be multifaceted, because it depends not only on the intensity and duration of the stress, but also on the development stage of the plants when the stress occurs (Çakir 2004; Singh et al. 2008; Farooq et al. 2009; Liu et al. 2010). As a C4 crop, maize plants need substantial water and nutrient supply to support their rapid plant growth. According to Lobell et al. 2011, maize plants are sensitive even to minor water fluctuation. Drought stress affects almost all development stages of maize plants. However, germination and flowering are the most important stages of plant development and plants are more sensitive to drought stress in those stages (Delachiave et Pinho 2003; Ashagre et al. 2014; Khayatnezhad et al. 2010; Aslam et al. 2015). A number of studies focused on the microbial-mediated drought tolerance at seedling stage (Grover et al. 2013; Sandhya et al. 2009), and they came to the conclusion that inoculation of maize with *Pseudomonas putida* FBKV2 strain improved the plant growth and stimulated the drought resistance. In the current study, the maize hybrid GKT 3385 was treated with all four bacteria under irrigated (WC 70%) and non-irrigated (WC 20%) conditions. The phenological parameters of the plants were monitored and recorded at 50% female flowering and at milk stage of development. In accordance with the results of Sandhya et al. 2010, the drought stress drastically affected maize growth, which was reflected by stunted plants and leaf wilting in the present trial as well. It could be observed that both under irrigated and non-irrigated conditions, the plants treated with bacteria absorbed significantly less water than the control at 50% female flowering and at milk stage of development. With optimal water supply, the treatment with *Pseudomonas putida* increased the plant height significantly, by 25% to 242.000 cm compared to the control with 195.500 cm. Under the condition of water deprivation, the bacteria did not increase significantly the plant height compared to the control in either of the studied phenophases.

With optimal water supply, at the stage of milk stage of development, the treatment with *Pseudomonas fluorescens* (279.000 g), *Bacillus pumilus* (270.000 g) and *Pseudomonas putida* (246.500 g) resulted in a significantly higher fresh weight of the roots compared to the control (203.000 g). The fresh root mass increase was 37% under the treatment with *Pseudomonas fluorescens*, 33% under the *Bacillus pumilus* treatment and 21% in the case of the treatment with *Pseudomonas putida*. Under non-irrigated conditions, no significant difference could be observed between the root fresh weight of the bacteria-treated plants and that of the control group during the milk stage of development. There was no significant difference between the root fresh weight of the bacteria-treated and the control plants in either of the investigated phenological stages when the conditions with optimal water supply and drought stress were compared. Regarding the fresh and dry weight of the above-ground biomass, no significant difference was found in favour of the bacteria-treated plants when comparing the two irrigation regimes or the two investigated phenophases. The recorded data reflected that the results of the bacteria-treated plants were very close to those of the control group. The water use efficiency is one of the most important parameters, as it shows how many grams of biomass are produced by the plants using 1 ml of water. In the present experiment, it was found that the water use efficiency of plants is significantly lower under non-irrigated conditions. The comparison of the investigated phenophases demonstrated, that the water use efficiency of the plants at milk stage of development was significantly higher (by 40%, 0.007 g/ml) than at 50% female flowering (0.005 g/ml). There was not significant difference in the water use efficiency of bacteria-treated plants in comparison with the control at 50% female flowering and in the milk stage of development when the plants under optimal water supply and under water stress were compared. The fresh/dry weight ratio of plants was significantly more favourable under optimal water supply (6.406) than under stressed condition (6.873), constituting 7% decline in the rate for the stressed plants. At the milk stage of development of the plants, the ratio was 5.573 compared to the 50% female flowering stage, with the value of 7.705, establishing 38% difference between the two phenophases. The treatment with *Pseudomonas putida* under drought stress condition at 50% female flowering significantly (by 6%) improved the fresh/dry weight ratio of plants (7.312) compared to the control (7.780). The *Pseudomonas fluorescens* treatment enhanced the fresh/dry weight ratio of plants by 6% with a value of 6.905 at 50% female flowering. Due to drought stress, the dry matter content of the plants with the value of 14.875% showed significant decrease (9%), compared to the irrigated plants (16.230%). The recorded data reflected significantly (39%) higher dry matter content (18.801%) at milk stage of development than at 50% female flowering

(13.024%). Under the condition of water deprivation, the plants in the *Pseudomonas putida* treatment produced a significantly higher dry matter content with the value of 18.344%, which was 5% higher than the control (17.497%) at the milk stage of development. With optimal water supply at 50% female flowering, the dry matter content of the plants treated with *Pseudomonas fluorescens* was significantly (6%) higher (14.490%) than the control (13.700%). Under the condition of drought stress, the plants treated with *Pseudomonas putida* delivered a dry matter content of 13.697%, which was significantly more advantageous (by 7%) than that of the control plants (12.853%). The results of the present experiments showed that water stress significantly affected the growth process of the plants during the development stages of maize, which caused severe reduction in plant height, root fresh mass, above-ground biomass accumulation and dry matter content compared to the plants with adequate water supply. These results are in compliance with the results reported in previous studies, stating that the plant height and the shoot dry matter reduced as the drought stress became more and more severe (Pandey et al. 2000; Suralta et al. 2010; Hugh et Richard 2003; Pervez et al. 2004; Çakir 2004). A number of scientific publications focused on the microbial-mediated drought tolerance at the seedling stage (Grover et al. 2013), and found that inoculation of maize with *Pseudomonas putida* was beneficial for the plant development and improved the drought tolerance (Sandhya et al. 2009). Based on the phenological parameters recorded in our drought stress provocation experiment, the bacteria were found to influence the investigated traits in diverse ways during the two phenophases under optimal water supply and in the drought-stressed environment. At optimal water supply, in the stage of 50% female flowering, the height of plants treated with *Pseudomonas fluorescens* and *Pseudomonas putida* exceeded that of the control and other bacteria-treated plants. During the milk stage of development, *Bacillus pumilus*, *Pseudomonas fluorescens* and *Pseudomonas putida* stimulated the root growth of the plants. Due to water deprivation, at 50% female flowering, *Pseudomonas putida* reduced the fresh/dry weight ratio of the plants compared to the control. The same influence could be experienced during the milk stage of development, and in addition, the dry matter content of the plants increased as well. The water demand of the bacteria-treated plants proved to be lower than that of the control group.

Results of the field trial

In the current trial, the plants treated with KD2 (*Bacillus pumilus* and *Pseudomonas putida*) presented significantly higher results (by 15%) in the aspect of grain yield (6.278 t/ha) compared to the control (5.468 t/ha). No significant difference could be found with respect to grain yield and dry

matter content. The plants treated with the bacterial combination KD2 were capable to produce plus yield compared to the control group in 2021, which was an extremely drought-stressed year. The results of the present study and those of Safari (2022) are in agreement and allow the conclusion that in order to improve the yield potential and the drought tolerance of plants, the treatment with PGPR bacteria, in particular with *Pseudomonas putida,* is recommended. The data recorded about the yield-determining traits in the field trial reflected that the plants had the potential to produce plus yield compared to the control even in a dry growing season owing to the soil inoculation with KD2. This result opens new perspectives in economics, plant breeding and crop production. In order to understand the deeper interrelations, it would be important to study the genetic background of the phenological traits, carry out stress gene expression tests and compare the results. The efficiency of potential biostimulators should be reinforced in field trials, where the survival of the applied microorganisms and their influence on the host plants may be limited by the competition with the microflora in the soil.

Declarations

Conflicts of interest There are no conflicts of interest between authors. The authors have no relevant financial or non-financial interests to disclose.

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