



Hungarian University of Agriculture and Life Sciences, MATE

**THE ROLE OF SOIL BACTERIA IN IMPROVING DROUGHT
TOLERANCE AND ADAPTABILITY OF HYBRID MAIZE**

DOI: 10.54598/005300

Summery of Ph.D. Thesis

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Gödöllő

2024

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BACKGROUND AND OBJECTIVES

The appearance of the “Green Revolution” in the latter half of the 20th century sparked a global resurgence in agriculture. By breeding new, high-yielding varieties and hybrids, and by increasing the use of fertilizers, pesticides, and other agricultural chemicals, the “Green Revolution” has significantly contributed to enhancing crop productivity and yields, thereby saving millions of people from starvation. However, the excessive use of synthetic inputs to maximize yields has degraded the biological, physical, and chemical condition of arable land, leading to a downward trend in agricultural productivity worldwide in recent decades. Additionally, the overuse of chemicals has caused the accumulation of minerals and nutrients in the soil, which plants struggle to utilize effectively, ultimately resulting in soil pollution and toxicity.

Phosphorus is an essential nutrient for plants. However, only a small fraction of the total phosphorus content in the soil is directly available to plants through chemical and biological processes, which does not meet the total phosphorus requirement of the plants. In Hungary, phosphorus supply to the soil is mainly carried out through fertilization, organic fertilization, and other methods such as applying propagating materials. During the years 2019–2021, fertilizer application predominated in the distribution of phosphorus, with an average of 93,000 tons sold over the past 10 years, increasing to 113,000 tons on average for the years 2019–2021 according to data from the Hungarian Central Statistical Office (KSH). Only a small percentage of the active ingredient in the applied phosphorus fertilizer can be directly utilized by plants, with the rest being bound in various forms of phosphates in the soil. Consequently, the phosphorus balance in soils tends to shift towards the export side. In Hungary, considering the average of the last 10 years, the phosphorus nutrient balance of soils was –4102 tons. The average amount of nutrients per unit of agricultural land is –0.8 kg/ha (KSH, 2022).

With the growth of population and climate change becoming increasingly relevant, there is a growing need for the development of high-yielding varieties and hybrids that are resilient to biotic and abiotic stresses. Drought and heat are significant environmental stressors that continually affect the growth and development of plants. Maize (*Zea mays* L.) is known for its sensitivity to abiotic stresses, which often results in significant yield losses. In Hungary, the average maize yield reached 8.1 and 8.6 t/ha in 2019 and 2020, respectively. In 2021, yields

averaged 6.0 t/ha, representing a 26–30% yield loss in the maize sector compared to the previous two years (KSH 2022). A number of adaptation and mitigation strategies to drought stress were needed. The manifestation of drought tolerance required a combination of environmental factors and several genes. A complex perspective was warranted to develop it, in which the collective application of biological, genetic, breeding, and agrotechnical knowledge could pay off.

Promoting sustainable agriculture, the search for environmentally friendly technologies to gradually reduce the use of synthetic agricultural chemicals and mitigate their harmful effects has led to the discovery and subsequent use of biofertilizers and other microbial-based formulations. Efficient plant growth-promoting rhizobacteria (PGPR) actively colonize the roots of plants and establish mutually beneficial associations with host plants. The favorable effects of PGPR on plants are well known. They enhance water and nutrient uptake and protect plants against biotic and abiotic stressors. The use of PGPR as biofertilizers offers an environmentally friendly biotechnological alternative to synthetic agricultural inputs in plant cultivation.

Based on all these factors, I set the following objectives at the beginning of my research:

1. Testing the phosphate mobilization capacity of PGPRs *in vitro* by measuring the diameter of clearing zones, phosphate mobilization index, and dissolved phosphate concentration values.
2. Investigation of the effect of PGPR inoculation on promoting early plant growth by monitoring changes in coleoptile and radicle in seedlings of hybrid maize (GKT 3213, GKT 3385, GKT 376, GK Silostar).
3. How does PGPR inoculation affect the phenological parameters (plant height, root wet mass, shoot wet and dry mass, water use efficiency, wet mass/dry mass ratio, dry matter content) of hybrid maize (GKT 3385) under abiotic stress (drought stress) during the 50% female flowering and milk ripening phenophases?
4. Investigation of the adaptive capacity of some maize hybrids developed by Szeged breeding (GKT 3213, GKT 3385, GKT 376, GK Silostar) under the influence of PGPR soil inoculation, by monitoring certain crop-determining traits (grain yield, grain moisture at harvest, grain dry matter content) and

nutritional value (protein content, starch content, and oil content) under field conditions.

MATERIALS AND METHODS

Experimental Plants and PGPRs

During greenhouse experiments, the GKT 3385 F1 (early FAO 390) maize hybrid was used as a model plant. In laboratory and field trials, in addition to GKT 3385, three other genotypes were tested: GKT 3213 F1 (super early FAO 230) maize, GKT 376 F1 (early FAO 380) maize, and GK Silostar F1 (medium maturity FAO 490) silage maize.

In laboratory experiments, the inoculation treatments were conducted using isolates of *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. *Escherichia coli* was used as a control during the assessment of phosphate mobilization ability. In field trials, three inoculation treatments and one untreated control were applied. The first inoculant contained *Bacillus megaterium* and *Pseudomonas fluorescens*, the second *Bacillus pumilus* and *Pseudomonas putida*, while the third included a mixture of *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Saccharomyces cerevisiae* strains (Micro-Logi Tech Ltd., Szolnok, Hungary).

The examination of PGPR activity

To investigate the phosphate-mobilizing ability, modified solid Pikovskaya agar medium was prepared. The medium was sterilized before use. 10 x 50 mL of the medium was dispensed into 9 cm diameter Petri dishes in a laminar flow hood and allowed to solidify. Using a sterile inoculation loop, the bacteria were inoculated onto the plates at four points. Five inoculation treatments were set up with eight replicates per bacterium: BM (*Bacillus megaterium*), BP (*Bacillus pumilus*), PF (*Pseudomonas fluorescens*), PP (*Pseudomonas putida*), and K (*Escherichia coli* control). The plates were then incubated at 30°C for 7 days in a thermostat. After the incubation period, photographic documentation of the plates was captured and analyzed using the Fiji ImageJ image analysis software. The properties examined during the experiment included: CD (colony diameter), HD (diameter of clearance zone), and SI (phosphate-mobilizing index). For the quantitative analysis of tricalcium phosphate, modified liquid Pikovskaya medium was prepared. The medium was sterilized before use. 500 mL of the medium was

dispensed into 500 mL wide-neck Erlenmeyer flasks in a laminar flow hood, with 5 x 100 mL portions. Five treatments were set up for the experiment: four treated with bacteria and one untreated control. The bacteria were inoculated onto the medium using a sterile inoculation loop, and the flasks were sealed with parafilm. The control group was left uninoculated. Five inoculation treatments were established, with four replicates per bacterium: BM (*Bacillus megaterium*), BP (*Bacillus pumilus*), PF (*Pseudomonas fluorescens*), PP (*Pseudomonas putida*), and K (untreated modified liquid Pikovskaya medium control). The cultures were incubated in a shaking incubator at 28°C for 7 days. At the end of the incubation period, the cultures were centrifuged, and the concentration of soluble inorganic phosphate in the supernatant was determined using the molybdenum blue method (Murphy and Riley, 1962) with a spectrophotometer. The property examined during the experiment was PC (soluble inorganic phosphate concentration).

During the investigation of plant growth-promoting effects, the seeds (GKT 3213 F1, GKT 3385 F1, GKT 376 F1, GK Silostar F1) were surface-sterilized. After surface sterilization, the seeds were germinated on moist filter paper in 9 cm diameter Petri dishes in a thermostat for 48 hours. Diseased and non-germinated seeds were removed, and the remaining healthy germinated seeds were transferred onto filter paper in 9 cm diameter Petri dishes. Throughout the experiment, the bacteria (*Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Pseudomonas putida*) were cultured in liquid Luria–Bertani medium. The nutrient solution was sterilized before use. From 1000 ml of Luria–Bertani medium, we measured 5 x 100 mL portions into 500 mL wide-mouth Erlenmeyer flasks within a laminar flow hood. Bacteria were inoculated into the media using an inoculating loop, and the flasks were sealed with parafilm after inoculation. Following inoculation, the flasks were incubated in a shaking incubator at 28°C and 180 rpm for five days. At the end of the incubation period, the cultures were centrifuged. The supernatant was decanted, and the pellet was diluted with sterile reverse osmosis (RO) water to a volume of 100 mL. The optical density of the bacterial solution was measured using a spectrophotometer. For the experiment, five inoculation treatments were established: four with bacteria and one untreated control. Germinated seeds were inoculated with 5 mL of bacterial suspension using a pipette, and then incubated at 21°C for four days. The control group was not inoculated. The germinated seeds were examined with five inoculation treatments

per hybrid and eight replicates: BM: *Bacillus megaterium*, BP: *Bacillus pumilus*, PF: *Pseudomonas fluorescens*, PP: *Pseudomonas putida*, K: uninoculated seeds (control). After the incubation period, the seedlings were separated from the seeds. The coleoptiles and radicles were prepared separately, and photographic documentation was performed. Subsequently, image analysis was conducted using the Fiji ImageJ software. The properties recorded during the experiment were: CL: coleoptile length, RL: radicle length, SVI: seedling vigor index. Healthy germinated seeds were selected for the experiment, hence the germination percentage (GP) was considered 100%. The seedling vigor index (SVI) was calculated based on the lengths of coleoptiles and radicles.

During the investigation of enhancing tolerance against drought stress factors in container experiments, the seeds (GKT 3213 F1, GKT 3385 F1, GKT 376 F1, GK Silostar F1) were sterilized. The seeds were germinated in 4 kg of sterilized medium, consisting of a 1:1 mixture of sand and Garri ground black peat (organic matter content: > 40% m/m; pH: 7.0 ± 0.5), supplemented with 20 g of Compo Trio per pot (NPK 12:8:16(+3) EC, a 90-day slow-release fertilizer). During the experiment, the bacteria (*Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Pseudomonas putida*) were cultured in liquid Luria Bertani medium. The nutrient solution was sterilized before use. From 1000 ml of Luria–Bertani medium, we measured 5 x 100 mL portions into 500 mL wide-mouth Erlenmeyer flasks within a laminar flow hood. Bacteria were inoculated into the media using an inoculating loop, and the flasks were sealed with parafilm after inoculation. Following inoculation, the flasks were incubated in a shaking incubator at 28°C and 180 rpm for five days. At the end of the incubation period, the cultures were centrifuged. The supernatant was decanted, and the pellet was diluted with sterile reverse osmosis (RO) water to a volume of 100 mL. The optical density of the bacterial solution was measured using a spectrophotometer. Five inoculation treatments were set up in the experiment, including four treated with different bacteria and one untreated control. At the time of seeding, 10 mL of bacterial suspension was inoculated 5 cm deep directly around the seed in the growth medium of the treated plants. During the experiment, the wet weight (WW) of the medium was measured, then dried to a constant weight at 70°C, and the dry weight (DW) was also measured. With knowledge of the wet and dry weights of the medium, the moisture content was calculated as a percentage of the total mass. At

seeding, the moisture content of the medium was adjusted to 70% of its water-holding capacity. Based on these conditions, the following treatments with four repetitions each were applied: BM: *Bacillus megaterium*, BP: *Bacillus pumilus*, PF: *Pseudomonas fluorescens*, PP: *Pseudomonas putida*, K: untreated plants (control), O: optimal irrigation, S: water deprivation. Data collection was conducted twice during the experiment. The first sampling occurred during the R1 growth stage of the plants (50% female flowering), and the second sampling was conducted during the R3 stage (milk ripeness). Recorded properties during the experiment included: PH: plant height, FWR: fresh weight of roots, FWB: fresh weight of shoots, DWB: dry weight of shoots, WUE: water use efficiency, FW/DW: wet weight/dry weight ratio, DMC: dry matter content.

Studying the adaptability of hybrids to soil inoculation under field conditions

The experiment was conducted for three consecutive years (2019, 2020, and 2021) in the maize breeding field of the Cereal Research Nonprofit Ltd. in Kiszombor. The bacteria (*Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Pseudomonas putida*) for inoculation were cultured in liquid Luria–Bertani medium. The medium was sterilized before use. 1000 mL of Luria–Bertani medium was measured into 4 x 100 mL portions in a laminar flow hood and transferred into 500 mL wide-neck Erlenmeyer flasks. The bacteria were inoculated into the media using an inoculating loop, and the flasks were sealed with parafilm. After inoculation, the flasks were incubated at 28°C with agitation at 180 rpm for five days. At the end of the incubation period, the cultures were centrifuged. The supernatant was decanted, and the pellet was diluted with sterilized reverse osmosis (RO) water to a final volume of 500 mL. The optical density of the bacterial solution was measured using a spectrophotometer. The hybrids (GKT 3213 F1, GKT 3385 F1, GKT 376 F1, GK Silostar F1) were tested in a randomized complete block design with three replications in a 4-row small plot experimental system. After winter wheat (*Triticum aestivum*) as a preceding crop, the experimental plots were plowed to a depth of 28–32 cm in November. Following plowing, 250 kg/ha of Genezis NPK-8:21:21 compact fertilizer was applied as autumn basal fertilizer using a fertilizer spreader. In March, 300 kg/ha of Genezis petis oil cake was applied as spring basal fertilizer. Bed preparation was carried out in April using a suspended cultivator. In May, three bacterial inoculants were applied to the soil before sowing. The first inoculant contained *Bacillus megaterium* and *Pseudomonas fluorescens*

(KD1), the second one contained *Bacillus pumilus* and *Pseudomonas putida* (KD2), and the third one contained a mixture of *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Saccharomyces cerevisiae* (MLT) (Micro-Logi Tech Ltd., Szolnok, Hungary). Untreated areas were used as controls (K). For inoculation, a mixture of 1 liter of bacterial suspension and 13 liters of tap water was sprayed onto the soil surface using a self-propelled field sprayer, covering an area of 252 m² per treatment. After application, the inoculant was incorporated into the soil to a depth of 10 cm. Seeds were sown at a depth of 5 cm, with row spacing of 70 cm and plant spacing of 22.3 cm, using a self-propelled plot seeder. Plant density was adjusted to 60,000 plants/ha. Post-emergence weed control was performed using a self-propelled field sprayer. In 2019, a broad-spectrum herbicide was applied at a rate of 0.4 L/ha (Adengo, Bayer Crop Science). In 2020 and 2021, a total-spectrum herbicide was applied at a rate of 400 g/ha (Principal Plus + Successor T, Corteva Agriscience). Cultivation of inter-row spaces was done using a cultivator. Harvesting took place in October with a plot combine harvester. Following the outlined procedures, the experiment was set up with four hybrid maize genotypes, each subjected to four inoculation treatments with three replications: KD1: *Bacillus megaterium* + *Pseudomonas fluorescens*, KD2: *Bacillus pumilus* + *Pseudomonas putida*, MLT: *Rhodopseudomonas palustris* + *Lactobacillus plantarum* + *Lactobacillus casei* + *Saccharomyces cerevisiae*, K: untreated control. Data collection occurred at harvest and during processing. Among the properties determining yield, records were made for GY: grain yield, WC: grain moisture content at harvest, DMC: grain dry matter content. Nutritional properties measured included: PC: grain protein content, SC: grain starch content, OC: grain oil content.

In terms of maize cultivation, the years 2019 and 2020 can be considered intense due to warm and rainy weather conditions. However, 2021 was unfavorable due to high temperatures, prolonged drought, and atmospheric dryness during flowering, all of which negatively impacted the average yield of the crops.

The average temperature and the amount of precipitation during the growing season of corn from 2019 to 2021

Month	At. (°C) 2019	Rf. (mm) 2019	At. (°C) 2020	Rf. (mm) 2020	At. (°C) 2021	Rf. (mm) 2021
April	13,14	29,5	11,91	5	9,03	25,2
May	14,85	125,6	15,12	26	15,26	55
June	22,81	117,9	20,30	150,4	22,62	21,8
July	22,23	63,2	22,25	79,8	25,33	28,3
August	23,95	17	23,64	46,5	22,45	23,4
September	18,10	62,5	19,44	22,5	17,96	25,8
Mean/Sum	19,18	415,7	18,78	330,2	18,78	179,5
TX30GE (days)	58		42		54	
TX35GE (days)	2		0		16	

At: average temperature (°C), Rf.: precipitation quantity (mm), TX30GE: Number of heat days,
TX35GE: Number of hot days

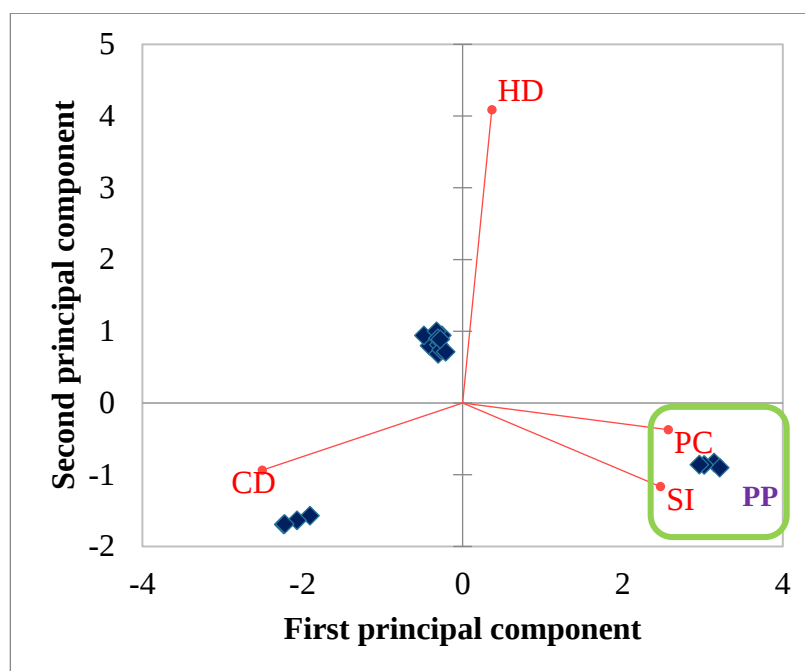
Analysis of the collected data was performed using Microsoft Excel 2019 with XLSTAT software.

RESULTS

The examination of PGPR activity

Investigation of phosphate-mobilizing capacity

Principal component analysis was conducted based on the data of colony diameter (CD), diameter of clearance zone (HD), phosphate mobilization index (SI), and inorganic phosphate concentration (PC). A positive correlation was found between the phosphate mobilization index of the bacteria and the level of inorganic phosphate concentration. Among the strains tested in the experiment, the treatment labeled PP showed the most efficient phosphate mobilization. The first two components explained 71.49% of all correlations, indicating a considerable phosphate mobilization potential.

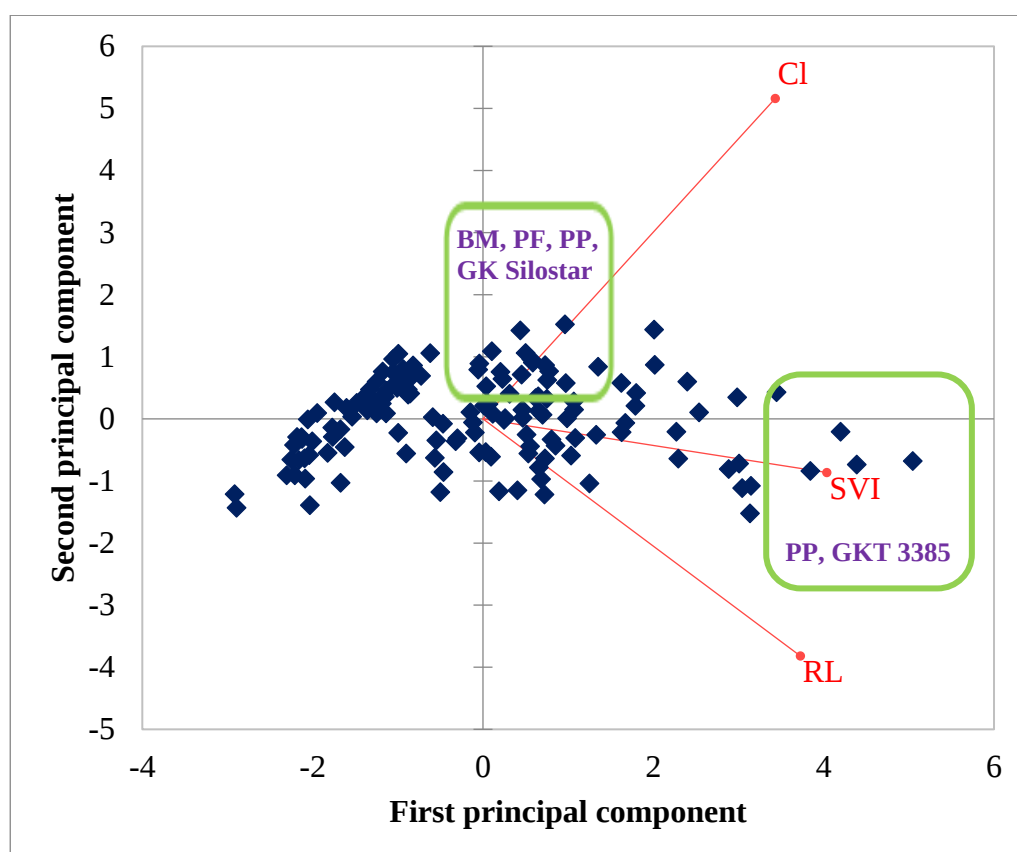


Results of the principal component analysis based on the data of the tested properties

CD: colony diameter, HD: diameter of clearance zone, SI: phosphate mobilization index,
PC: phosphate concentration, PP: *Pseudomonas putida*.

Investigation of plant growth-promoting effects

The principal component analysis was conducted based on the data of coleoptile length (CL), radicle length (RL), and seedling vigor index (SVI). Among the tested strains, the bacterial inoculation marked with PP enhanced the growth of GKT 3385 and GK Silostar hybrids, while the inoculations with BM and PF promoted the growth of GK Silostar seedlings. The first two components explain 85.15% of all correlations, indicating growth-promoting potential.



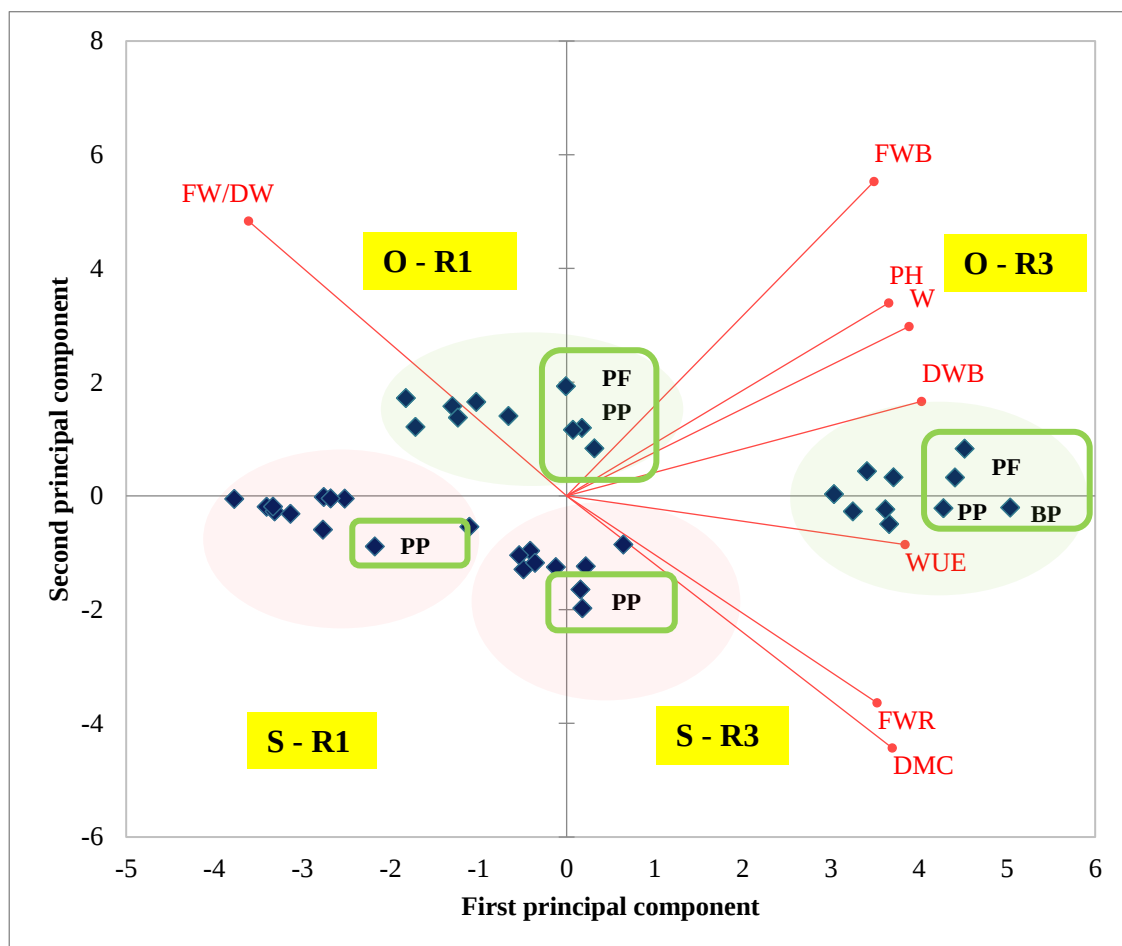
Results of the principal component analysis based on the data of the tested properties

CL: coleoptile length, RL: radicle length, SVI: seedling vigor index, BM: *Bacillus megaterium*, PF: *Pseudomonas fluorescens*, PP: *Pseudomonas putida*.

Enhancement of drought stress tolerance factor investigation

During the experiment, adequately watered plants absorbed an average of 421 mL of water, while the water-stressed plants received only 206 mL. The difference between the two treatments averaged 215 mL, representing a significant, nearly 50% reduction in water availability compared to plants growing under optimal conditions. At the tasseling (VT) and the stage of lactic ripeness (R3), both treatments showed increased water demand in the plants. The principal component analysis was conducted based on the data of water requirement (W), plant height (PH), fresh weight of roots (FWR), fresh weight of shoots (FWB), dry weight of shoots (DWB), water use efficiency (WUE), ratio of fresh weight/dry weight (FW/DW), and dry matter content (DMC). Among the strains investigated in the experiment, the bacteria labeled as PP in the graph reduced the wet weight/dry weight ratio of the GKT 3385 hybrid during water stress at the 50% tasseling stage compared to control plants and increased the dry matter content value during the

milk stage. Under optimal watering conditions, treatments labeled with PF and PP resulted in taller plants at the 50% tasseling stage compared to control plants. During the milk stage, the treatment labeled with PP, PF, and BP stimulated root development compared to the control. The first two components explain 81.30% of all correlations.

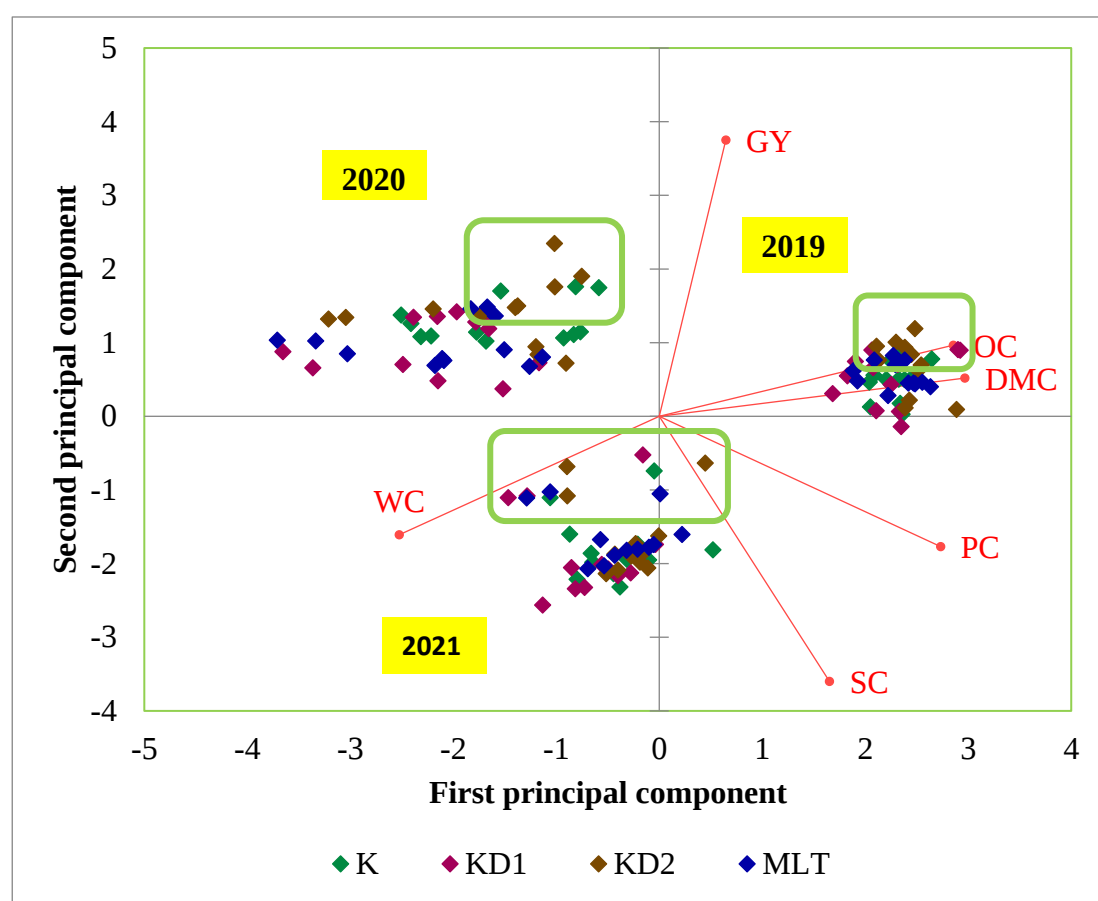


Results of the principal component analysis based on the data of the tested properties

W: water requirement, PH: plant height, RFW: root fresh weight, BFW: shoot fresh weight, BDW: shoot dry weight, WUE: water use efficiency, FW/DW: ratio of fresh weight/dry weight, DMC: dry matter content, R1: 50% silking, R3: lactic ripening, O: optimal water supply, S: water deprivation, BP: *Bacillus pumilus*, PF: *Pseudomonas fluorescens*, PP: *Pseudomonas putida*.

Examination of hybrids adaptability under field conditions due to soil inoculation

The principal component analysis was conducted based on the data of grain yield (GY), grain moisture content at harvest (WC), grain dry matter content (DMC), grain protein content (PC), grain oil content (OC), and grain starch content (SC). Among the different hybrid varieties treated with various inoculants, in 2019, 2020, and 2021, the GKT 3385 and GK Silostar hybrids exhibited higher average yields due to the effect of KD2 and MLT inoculation compared to treatments with K and KD1, as well as compared to the GKT 3213 and GKT 376 hybrids. The first two components explain 83.17% of all correlations, indicating a potential for higher yields.



Results of the principal component analysis based on the data of the tested properties

K: Control, KD1: *Bacillus megaterium* + *Pseudomonas fluorescens*, KD2: *Bacillus pumilus* + *Pseudomonas Putida*, MLT: *Rhodopseudomonas palustris* + *Lactobacillus plantarum* + *Lactobacillus casei* + *Saccharomyces cerevisiae*, GY: grain yield (t/ha), WC: grain moisture content at harvest (%), DMC: grain dry matter content (%), PC: grain protein content (%), OC: grain oil content (%), SC: grain starch content (%).

Novel scientific findings

1. The various PGPR (*Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Pseudomonas putida*) inoculations elicited hybrid-specific differences in the coleoptile and radicle length, as well as the seedling vigor index of hybrids across different maturity groups and within the same maturity group.
2. The inoculation with *Pseudomonas putida* significantly improved the germination capacity of GKT 3385 (early FAO 390) grain hybrid maize and GK Silostar (medium FAO 490) silage hybrid, stimulating seedling growth.
3. Inoculation with *Bacillus megaterium* and *Pseudomonas fluorescens* significantly enhanced the germination capacity of GK Silostar (medium FAO 490) silage hybrid and promoted seedling growth.
4. We presented novel data on the effects of various PGPR (*Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Pseudomonas putida*) inoculations on the physiological properties of maize plants under optimal water supply and prolonged water deprivation during 50% silking and lactic ripeness stages.
5. During drought stress, the *Pseudomonas putida* treatment moderately increased the fresh root weight of GKT 3385 (early FAO 390) maize hybrid at the 50% silking and lactic ripeness stages. At the 50% silking stage, it improved the ratio of fresh weight/dry weight, and significantly increased the dry matter content in both examined phenophases.
6. Based on our 3-year experimental results, the combined inoculation of *Bacillus pumilus* and *Pseudomonas putida* significantly increased the grain yield in a hybrid-specific manner under field conditions, regardless of the crop year.
7. Based on our 3-year experimental results, the combined inoculation of *Bacillus pumilus* and *Pseudomonas putida* significantly increased the grain yield of GKT 3385 (early FAO 390) dent corn hybrid and GK Silostar (mid-season FAO 490) silage corn hybrid under field conditions, regardless of the crop year.

8. The combined inoculation of *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Saccharomyces cerevisiae* significantly increased the grain yield of the GK Silostar (mid-season FAO 490) silage corn hybrid under field conditions during a drought year.

CONCLUSION AND RECOMMENDATIONS

The phosphate mobilization capacity of PGPRs

For plants, phosphorus is the second most limiting nutrient after nitrogen. Numerous microorganisms (bacteria, fungi, algae) possess phosphate mobilization capabilities. To apply the accumulated phosphorus in the soil, phosphate-mobilizing bacteria can function as biofertilizers and play a crucial role in increasing the accessibility of phosphorus that is otherwise difficult for plants to obtain (Alori *et al.*, 2017). In our study, we investigated the phosphate mobilization ability of *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, and *Pseudomonas putida* bacteria, using tricalcium phosphate as an inorganic phosphorus source.

We observed varying sizes of clear zones around the tested PGPR colonies after 7 days of incubation post-inoculation. Similar conclusions were drawn by Gaur (1990), Nguyen *et al.* (1992), Vazquez *et al.* (2000), and Khan *et al.* (2022), indicating that only phosphate-mobilizing bacteria are capable of forming clear zones in media containing tricalcium phosphate. The formation of clear zones is a result of the activity of organic acids produced by the bacteria or phosphatase enzymes (Paul and Shina, 2013). Song *et al.* (2008) attributed the phosphate mobilization efficiency of *Burkholderia cepacia* to high levels of organic acid production, specifically gluconic acid. The varying diameters of the clear zones indicate that bacteria mobilize tricalcium phosphate to different extents, a finding supported by the results of Mei *et al.* (2021). The diameters of the zones ranged from 7 to 18 mm, and the phosphate mobilization indexes of the bacteria ranged from 14 to 52. Similar results were reported by Kumar *et al.* (2010), Tariq *et al.* (2022), and Amri *et al.* (2023).

Based on the results of the measured phosphate mobilization index values during the experiment, among the tested PGPRs, *Pseudomonas putida* exhibited the highest efficiency in phosphate mobilization. This was supported by the values of dissolved phosphate concentration measured after the 7th day, ranging from 14 to

52 $\mu\text{g}/\text{cm}^3$. The *Pseudomonas putida* inoculation resulted in the highest phosphorus concentration, reaching 51.636 $\mu\text{g}/\text{cm}^3$. Similar effects were observed by Pandey *et al.* (2006) on liquid Pikovskaya medium at 28°C after 7 days of incubation. Our findings suggested that the bacteria tested in the experiment were capable of dissolving tricalcium phosphate, with *Pseudomonas putida* being the most effective phosphate mobilizer based on both the phosphate mobilization index and dissolved phosphorus concentration values.

The plant growth-promoting effects of PGPRs

The assessment of the plant growth-promoting potential of bacteria requires a comprehensive approach, which includes, among other things, the examination of seed germination. Enhancing the germination capacity and initial growth of maize genotypes is an important breeding guideline, with one of the main selection criteria being the seedling vigor index. The plant growth-promoting effects of PGPR strains have been supported by numerous studies (Lugtenberg *et al.*, 2002; El-Hawary *et al.*, 2002; Wu *et al.*, 2005; Fahad *et al.*, 2015; Saleem *et al.*, 2021). Previous investigations have demonstrated that *Bacillus spp.* and *Pseudomonas spp.* can be used as plant growth-promoting bacteria, benefiting from their rapid proliferation and strong environmental compatibility (Gravel *et al.*, 2007; Rehman *et al.*, 2019).

In our experiment, we investigated the potential plant growth-promoting effects of *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, and *Pseudomonas putida* bacterial isolates on the maize seedlings of GKT 3213, GKT 376, GKT 3385, and GK Silostar, monitoring the growth of coleoptiles and radicles, as well as the seedling vigor index. Based on our results, we found that the maize genotype played a key role. The highest values for coleoptile and radicle growth, as well as for the seedling vigor index, were observed in the case of the GKT 3385 hybrid. GKT 3385 is an early-maturing maize variety, whose germination outcomes yielded higher values compared to the early-maturing GKT 376, the super-early GKT 3213, and the mid-early GK Silostar.

PGPRs had a moderately positive effect on coleoptile length, and *Bacillus pumilus* inoculation had a lower effect compared to the control treatment. In terms of radicle length, we observed a significant plant growth stimulating effect for *Pseudomonas putida* inoculation and the treatment moderately increased the seedling vigor index of plants. Nezarat and Gholami (2009), Noumavo *et al.* (2013) also reported an improvement in the germination of maize seeds after inoculation

with *Pseudomonas putida*. In an investigation of genotype-bacteria interaction, our results showed that different bacterial inoculations had different effects on coleoptile and radicle length and seedling vigor index of hybrids with different maturity groups and within the same maturity group after 4 days of incubation. The bacterial inoculations did not increase the coleoptile and radicle length of the super early hybrid GKT 3213, but rather resulted in a lower value, thus not improving the germination seedling vigor of the hybrid, and the same effect was observed in the early hybrid GKT 376. When examining the coleoptile length of the early-maturing GKT 3385 hybrid, significant growth-promoting effects were observed with the inoculation treatments of *Pseudomonas fluorescens* and *Pseudomonas putida*. Furthermore, *Pseudomonas putida* increased the length of the radicles, improving the hybrid's seedling vigor index. Investigations on the mid-maturing GK Silostar maize revealed that inoculations with *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, and *Pseudomonas putida* significantly stimulated coleoptile growth, with *Pseudomonas putida* treatment resulting in growth-promoting effects on radicles as well. Based on the aforementioned results, it can be concluded that treatments with *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Pseudomonas putida* increased the seedling vigor index of the plants, indicating that the tested bacteria possess plant growth-promoting properties that enhance maize germination, a crucial aspect in seedling emergence. Presumably, these results are attributed to the activity of hydrolytic enzymes, which provide intensive substrate assimilation (such as starch α -amylases), facilitating early germination. Increased hormone biosynthesis may trigger the activity of these hydrolytic enzymes. Additionally, hormone dependence seems to enhance the seedling vigor index due to the synthesis of indole-3-acetic acid (Meliani *et al.*, 2017). Similar results were obtained in maize plants following inoculation with *Bacillus megaterium* by Kifle and Laing (2016), with *Pseudomonas fluorescens* and *Pseudomonas putida* treatments by Agbodjato *et al.* (2016), and after *Pseudomonas putida* inoculation by Georgieva *et al.* (2023).

The hybrid specificity of the bacteria can be explained by the significant influence of the plant on the composition of the rhizosphere microbiome (Zhang *et al.*, 2017; Massalha *et al.*, 2017; Turner *et al.*, 2013). Even within a plant species, simple genotype differences can have significant effects (Winston *et al.*, 2014). According to Smith *et al.* (2017), part of this regulation is the result of interorganism

signaling molecules. Starting from seed germination, through root growth, to root senescence, signaling molecules are released into the surrounding soil. These molecules support microbial growth and activity in the rhizosphere. The selection of signaling molecules (timing, changes in quantity, and/or quality) is a mechanism by which plants can manipulate the quantity and composition of their root-associated microbiota (Bakker *et al.*, 2012). It is presumed that the exudates mainly consist of sugars, amino acids, and organic acids, which are present in high concentrations in the plant cytoplasm. They also contain complex secondary metabolites in smaller amounts, such as flavonoids, terpenes, and phenolic compounds, which specifically attract microbes (Musilova *et al.*, 2016). It has also been suggested that the exudation of jasmonic acid and salicylic acid signaling molecules into the rhizosphere may participate in the interaction between roots and microbes (Doornbos *et al.*, 2011). The release of root exudates is a genetically regulated process, so different plant genotypes can establish distinct rhizobacterial communities. This results in extraordinary variability among plant species, within the same species among different plant types, at different developmental stages, under different conditions, and in different biotic interactions (Gransee and Wittenmayer, 2000; Micallef *et al.*, 2009; Badri *et al.*, 2013; Kristin and Miranda, 2013).

Enhancement of drought stress tolerance

One of the most important tasks in domestication is the selection of genotypes that tolerate drought stress well. The response of plants to drought stress is a complex process, dependent not only on the intensity and duration of the stress but also on the developmental stage of the plant (Çakir 2004, Singh *et al.* 2008, Farooq *et al.* 2009, Liu *et al.* 2010). Corn, as a C₄ plant, has high water and nutrient requirements for rapid growth. Drought stress affects almost every developmental stage of corn. However, developmental stages such as germination and flowering are crucial periods in crop production, and during these stages, plants are more susceptible to drought stress (Delachave and Pinho 2003, Ashagre *et al.* 2014, Khayatnezhad *et al.* 2010, Aslam *et al.* 2015).

PGPR can play an important role in alleviating abiotic stress on host plants and can contribute to mitigating stress effects (Ahluwalia *et al.*, 2021). The stress-reducing mechanisms followed by the application of PGPR are highly complex. The intricate network of signal transduction occurring during plant-microbe

interactions regulates these mechanisms and stress alleviation (Smith *et al.*, 2017). PGPR produce antioxidants, improve root and shoot morphology, form biofilms to enhance plant water supply, and produce secondary metabolites to strengthen plant adaptability (Ahluwalia *et al.*, 2021; Ruzzi *et al.*, 2015; Kumar *et al.*, 2019; Li *et al.*, 2020). Numerous studies have been conducted on microbial-mediated drought tolerance during the germination stage (Grover *et al.*, 2013; Sandhya *et al.*, 2009), and previous findings have shown that inoculating corn with *Pseudomonas putida* FBKV2 enhances plant growth and increases resistance to drought.

In our current study, we inoculated the GKT 3385 hybrid with *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas fluorescens*, and *Pseudomonas putida* under optimal irrigation and drought-stressed conditions, then evaluated the phenological parameters of corn at 50% silking and ripening stages. Similar to the findings of Sandhya *et al.* (2010), drought stress affected the development of corn. Our findings showed that drought stress significantly inhibited the growth of corn, resulting in a decrease in plant height, root fresh weight, bulb fresh weight, and dry matter content compared to plants receiving optimal irrigation. This effect was also observed by Pandey *et al.* (2000), Suralta *et al.* (2010), Hugh and Richard (2003), Pervez *et al.* (2004), and Çakir (2004), demonstrating that with increased drought stress, plants gradually reduced plant height and dry matter content. In our current study, at 50% silking under optimal irrigation, the treatment with *Pseudomonas putida* significantly increased plant height, which was not observed during the ripening stage. At 50% silking, during drought stress, the *Pseudomonas putida* inoculation moderately increased root fresh weight, which was also observed during the ripening stage. Under optimal irrigation during the ripening stage, treatments with *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Bacillus pumilus* resulted in significantly higher root fresh weight in the plants. The water use efficiency of corn, measured as the transpiration efficiency, was significantly increased by *Pseudomonas fluorescens* inoculation at 50% silking under optimal irrigation. In the case of *Bacillus megaterium* treatment, we observed moderate growth during water deprivation. The ratio of fresh weight to dry weight of the plants was significantly improved by *Pseudomonas fluorescens* under optimal irrigation and by *Pseudomonas putida* inoculation during drought stress at 50% silking. *Pseudomonas fluorescens* treatment significantly increased the dry matter content of the plants under optimal irrigation at 50% silking, and the same effect was

observed with *Pseudomonas putida* treatment under drought stress conditions, which was moderately evident during the ripening stage. Our findings are supported by the studies of Ahmad *et al.* (2019) and Mubeen *et al.* (2021), who observed similar effects with *Pseudomonas fluorescens* and *Pseudomonas putida* treatments on corn plant height, bulb fresh and dry weight, root fresh and dry weight, and dry matter content under both optimal and drought stress conditions. Based on our results, we can conclude that *Pseudomonas putida* treatment increased the fresh and dry weight as well as the dry matter content of the GKT 3385 hybrid under drought stress conditions.

The adaptation of hybrids under field conditions in response to soil inoculation

Increasing crop yield while preserving quality is one of the primary goals of agricultural and domestication research (Kayad *et al.*, 2021). The genetic yield potential varies greatly among hybrids, influenced by the interaction between genotype and environment, making the relationship between yield and quality complex and variable. The yield of maize is determined by the number of kernels per row, the number of kernels per ear, and the weight of the kernels, all of which are significantly influenced by various meteorological factors (Yang *et al.*, 2022). In our study, based on the results of traits determining crop quantity and nutritional value, it can be concluded that the examined hybrids differ from each other in terms of maturity and adaptability.

Environmental changes such as climate, soil conditions, water availability, sunlight, and biotic stress can have significant impacts on plant development and productivity. A given genotype may perform well in favorable conditions but show weaker performance in unfavorable environments. Therefore, the adaptability of plants to their environment is a critical factor that must be considered in domesticating and variety selection. There is a complex interaction between genotype and environment, where synergistic or antagonistic effects between genotype and environment may strengthen or reduce plant performance (Olivera *et al.*, 2018). According to Sárvári and Pepo (2014), the resilience of agroecosystems and their response to adverse weather conditions are fundamentally determined by the intensity of maize cultivation. It is evident that extreme weather conditions caused by climate change have a significant impact on maize yield (Brown and Rosenberg, 1999). The results of the three-year field experiments indicated that the yield of maize was the most influenced by the growing season. The years 2019 and

2020 can be considered as intensive years for maize cultivation, resulting in a yield average of nearly 10 t/ha for the studied hybrids. Conversely, 2021 was an extremely unfavorable year, with a 38% decrease in yield average for the examined hybrids (6.5 t/ha) due to precipitation shortage, high average temperatures, and atmospheric drought during tasseling. The number of grains partly depends on the timing of silking and pollination. The processes of silking and pollination are highly sensitive to environmental changes, and if this period is characterized by water scarcity or unfavorable climatic conditions (e.g., excessive rainfall, inadequate humidity, low temperatures, nutrient deficiency), grain setting decreases, resulting in grain shortage. The grain weight reflects the external developmental and internal physiological status of maize, the proper functioning of which is essential for achieving high yields (Wu et al., 2022). Results from long-term experiments have shown that the balance of different yield components and consequently productivity is greatly influenced by environmental factors, primarily weather conditions (Sárvári *et al.*, 2014).

Several studies have reported that PGPR inoculation can increase yield to varying extents, improve soil nutrient availability, and enhance nutrient uptake and utilization in the soil (Cassán *et al.*, 2009; Díaz-Zorita & Fernández-Canigia, 2009; Singh *et al.*, 2011). Inoculation with different strains of *Azospirillum brasilense* isolated from the rhizosphere of maize increased maize grain yield by 5–11% (Salvo *et al.*, 2018; Skonieski *et al.*, 2019). Aquino *et al.* (2019) utilized 40 PGPR isolates to assess their impact on the growth of maize and sorghum. Among the isolates were *Bacillus pumilus* and *Bacillus megaterium*, which primarily promoted increases in plant height, bulb dry weight, and chlorophyll content. Based on the results of Katsenios *et al.* (2022), *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus pseudomycoides* increased the yield of sweet corn kernels. PGPR soil inoculation has shown a positive effect on maize yield (Ferreira *et al.*, 2013; Noumavo *et al.*, 2013; Abo-kora, 2016). In our study, considering the three-year average yields, the highest kernel yield was observed in the KD2 inoculation treatment (9.3 t/ha), followed by the MLT treatment with 8.9 t/ha, then K with 8.8 t/ha, and finally the KD1 treatment with 8.6 t/ha. The KD2 inoculation resulted in a 5.7% increase in yield, while the MLT treatment led to a 1.1% increase compared to the non-inoculated K plants. We observed a 2.3% yield reduction in the KD1 treatment. Zafarulhye *et al.* (2014) and Iqbal *et al.* (2016) reported an increase in maize kernel

yield with PGPR-treated plants. According to Ullah and Bano (2019), the yield was higher in plants treated with *Pseudomonas putida* and *Bacillus pumilus*. Singh *et al.* (2023) stated that *Azospirillum lipoferum* and *Pseudomonas putida* have particularly favorable effects on maize development, growth, and yield potential enhancement.

According to the results of our three-year field experiment, we observed that the combined strain mixture of *Bacillus pumilus* + *Pseudomonas putida*, when applied to the soil, enabled plants to achieve additional yields despite drought conditions, which is highly significant from agricultural, domesticating, and economic perspectives. To gain a more precise understanding of the correlations, it is essential to uncover the genetic background expressing phenological traits, the results of which further deepen and facilitate the selection processes. The effectiveness of microbes serving as potential biostimulants needs to be evaluated within the framework of multi-location performance trials under field conditions and across a wide genotype portfolio. This approach will ensure that the obtained results contribute to the development of truly sustainable agriculture and efficient and environmentally friendly precision farming techniques.

REFERENCES

- Abo-kora HA, (2016) Endophytic colonization of maize (*Zea mays* v.) root plants by PGPRs under salinity stress. *Nat. Sci.*, 14(7): 34-51
- Agbodjato NA, Noumavo PA, Adjanohoun A, Agbessi L, Baba-Moussa L, (2016) Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (*Zea mays* L.). *Biotechnology research international*.
- Ahluwalia O, Singh PC, Bhatia R, (2021) A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resources, Environment and Sustainability*, 5, 100032.
- Alori ET, Glick BR, Babalola OO, (2017) Microbial Phosphorus Solubilization and Its Potential for Use in Sustainable Agriculture. *Front. Microbiol.* 8:971. doi: 10.3389/fmicb.2017.00971
- Amri M, Rjeibi MR, Gatrouni M, Mateus DMR, Asses N, Pinho HJO, Abbes C. Isolation, Identification, and Characterization of Phosphate-Solubilizing Bacteria from Tunisian Soils. *Microorganisms*. 2023; 11(3):783. <https://doi.org/10.3390/microorganisms11030783>

- Ashagre H, Ibrahim A, Hamza, Urgecha F, Worku N, (2014) Influence of boron on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *African Journal of Plant Science*. 8(2) pp. 133-139
- Aslam M, Maqbool MA, Cengiz R, (2015) *Drought Stress in Maize*. Springer US. pp. 19-36
- Aquino JPA, (2019) Plant growth-promoting endophytic bacteria on maize and sorghum1. *Pesqui. Agropecu. Trop*. 49
- Badri DV, and Vivanco JM, (2009) Regulation and function of root exudates. *Plant Cell Environ*. 32, 666–681. doi: 10.1111/j.1365-3040.2008.01926.x
- Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM, (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil* 360, 1–13. doi: 10.1146/annurev.arplant.57.032905.105159
- Brown RA, Rosenberg NJ, (1999) Climate change impacts on the potential productivity of corn and winter wheat in their primary United States growing regions. *Climate Change* 41:73–107
- Çakir R, (2004) Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crop Res*. 89:1–16
- Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V, (2009) Corrigendum to “*Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.) [Eur. J. Soil Biol. 45 (2009) 28–35]”. *Eur J Soil Biol* 45: 28–35.
- Delachiave M, Pinho S, (2003) Germination of *Senna occidentalis* seed at different osmotic potential levels. *Architectural Technologies*. 46:1663-166
- Díaz-Zorita M, Fernández-Canigia MV, (2009) Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. *Eur J Soil Biol* 45: 3–11.
- Doornbos RF, Geraats BP, Kuramae EE, Van Loon LC, Bakker PA, (2011) Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. *Mol. Plant Microbe Interact*. 24, 395–407. doi: 10.1094/MPMI-05-10-0115
- El-Hawary MI, El-Hawary Fatma I, El-Ghamry AM, El-Naggar E, (2002) Effect of application of biofertilizer on the yield and NPK uptake of some wheat genotypes as affected by the biological properties of soil. *Pak. J. Biol. Sci.*, 5: 1181-1185
- Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Huang J, (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, 22, 4907-4921.
- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Souchere V, Alberola C (eds) *Sustainable agriculture*. Springer, pp 153–188

- Georgieva G, Nedeva T, Badalova M, Deleva V, Savov V (2023) Study of the plant growth-promoting capacity of *Pseudomonas putida* 1046 in a model plant system. In: Chankova S, Danova K, Beltcheva M, Radeva G, Petrova V, Vassilev K (Eds) Actual problems of Ecology. BioRisk 20: 115-128. <https://doi.org/10.3897/biorisk.20.97581>
- Ferreira A, Pires RP, Rabelo R, Oliveira J, Brito C, (2013) Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under green house and field conditions. Appl. Soil Ecol., 72: 103-108
- Granssee A, Wittenmayer L, (2000) Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J. Plant Nutr. Soil Sci. 163, 381–385. doi: 10.1002/1522-2624(200008)163:4<381:AID-JPLN381>3.0.CO;2-7
- Gravel V, Antoun H, Tweddell RJ, (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of Indole Acetic Acid (IAA). Soil Biol. Biochem., 39: 1968-1977
- Grover A, Mittal D, Negi M, Lavania D, (2013) Generating high temperature tolerant transgenic plants: Achievements and challenges. Plant Sci. 205-206:38-47
- Hugh JE, Richard FD (2003) Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. Agron J 95:688–696
- Iqbal MA, Khalid M, Zahir ZA, Ahmad R, (2016) Auxin producing plant growth promoting rhizobacteria improve growth, physiology and yield of maize under saline field conditions. Int. J. Agri. Biol., 18(1): 37-45
- Katsenios N, Andreou V, Sparangis P, (2022) Assessment of plant growth promoting bacteria strains on growth, yield and quality of sweet corn. Sci Rep 12, 11598 <https://doi.org/10.1038/s41598-022-16044-2>
- Kayad A, Sozzi M, Gatto S, Whelan B, Sartori L, Marinello F, (2021) Ten years of corn yield dynamics at field scale under digital agriculture solutions: A case study from North Italy. Computers and Electronics in Agriculture, 185, 106126.
- Khan H, Akbar WA, Shah Z, Rahim HU, Taj A, Alatalo JM, (2022) Coupling phosphate-solubilizing bacteria (PSB) with inorganic phosphorus fertilizer improves mungbean (*Vigna radiata*) phosphorus acquisition, nitrogen fixation, and yield in alkaline-calcareous soil. Heliyon, 8(3).
- Khayatnezhad M, Zaeifizadeh M, Gholamin R, (2010): Investigation and Selection Index for Drought Stress. Australian Journal of Basic and Applied Sciences, 4(10): 4815-4822
- Kifle MH and Laing MD (2016) Effects of Selected Diazotrophs on Maize Growth. Front. Plant Sci. 7:1429. doi: 10.3389/fpls.2016.01429
- Kristin A, Miranda H, (2013) The root microbiota—a fingerprint in the soil? Plant Soil 370, 671–686. doi: 10.1007/s11104-013-1647-7

- Kumar A, Bhargava P, Rai, LC (2010) Isolation and molecular characterization of phosphate solubilizing *Enterobacter* and *Exiguobacterium* species from paddy fields of Eastern Uttar Pradesh, India. *African Journal of Microbiology Research*, 4(9), 820-829.
- Kumar A, Patel JS, Meena VS, Srivastava R, (2019) Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. *Biocatalysis and agricultural biotechnology*, 20, 101271.
- Li H, Guo Q, Jing Y, Liu Z, Zheng Z, Sun Y, Xue Q, Lai H (2020) Application of *Streptomyces pactum* Act12 enhances drought resistance in wheat. *J Plant Growth Regul* 39:122–132. <https://doi.org/10.1007/s00344-019-09968-z>
- Liu Y, Li S, Chen F, Yang S, Chen X, (2010) Soil water dynamics and water use efficiency in spring maize (*Zea mays* L.) fields subjected to different water management practices on the Loess Plateau, China. *Agric Water Manage.* 97:769–775
- Lugtenberg BJJ, Chin-A-Woeng TFC, Bloemberg GV, (2002) Microbe-plant interactions: Principles and mechanisms. *Antonie van Leeuwenhoek*, 81: 373-383
- Massalha H, Korenblum E, Tholl D, Aharoni A, (2017) Small molecules below-ground: the role of specialized metabolites in the rhizosphere. *Plant J.* 90, 788–807. doi: 10.1111/tpj.13543
- Mei C, Chretien RL, Amaradasa BS, He Y, Turner A, Lowman S. Characterization of Phosphate Solubilizing Bacterial Endophytes and Plant Growth Promotion In Vitro and in Greenhouse. *Microorganisms*. 2021; 9(9):1935. <https://doi.org/10.3390/microorganisms9091935>
- Meliani A, Bensoltane A, Benidire L, Oufdou K, (2017) Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. *Research & Reviews: Journal of Botanical Sciences*, 6(2), 16-24.
- Micallef SA, Channer S, Shiaris MP, Colon-Carmona A, (2009) Plant age and genotype impact the progression of bacterial community succession in the *Arabidopsis* rhizosphere. *Plant Signal. Behav.* 4, 777–780. doi: 10.1093/jxb/erp053
- Musilova L, Ridl J, Polivkova M, Macek T, Uhlik O, (2016) Effects of secondary plant metabolites on microbial populations: changes in community structure and metabolic activity in contaminated environments. *Int. J. Mol. Sci.* 17:1205. doi: 10.3390/ijms17081205
- Nezarat S, Gholami A, (2009) Screening Plant Growth Promoting Rhizobacteria for Improving Seed Germination, Seedling Growth and Yield of Maize. *Pakistan Journal of Biological Sciences*, 12, 26-32
- Nguyen C, Yan W, Le Tacon F, Lapeyrie F, (1992). Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) P.D. Orton. *Plant Soil* 143: 193-199
- Noumavo PA, Kochoni E, Didagbé YO, Adjanohoun A, Allagbé M, Sikirou R, Gachomo EW, Kotchoni SO, Baba-Moussa L, (2013) Effect of Different Plant Growth Promoting Rhizobacteria on

- Maize Seed Germination and Seedling Development. *American Journal of Plant Sciences*.4: 1013-1021
- Pandey RK, Maranville JW, Chetima MM (2000) Deficit irrigation and nitrogen effects on maize in a Sahelian environment II. Shoot growth, nitrogen uptake and water extraction. *Agric Water Manage* 46:15–27
- Paul D, Sinha SN, (2013) Phosphate solubilization potential and phosphatase activity of some bacterial strains isolated from thermal power plant effluent exposed water of river Ganga. *CIBTech J Microbiol*, 2(3), 1-7.
- Pervez HZ, Srinivasan G, Cordova HS, Sanchez C (2004) Gains from improvement for mid-season drought tolerance in tropical maize (*Zea mays* L.). *Field Crop Res* 89:135–152
- Rehman, A., Ullah, A., Nadeem, F., Farooq, M. (2019). Sustainable Nutrient Management. In: Farooq, M., Pisante, M. (eds) *Innovations in Sustainable Agriculture*. Springer, Cham.
- Ruzzi M, Aroca R, (2015) Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae*, 196, 124-134.
- Saleem S, Iqbal A, Ahmed F, Ahmad M, (2021) Phytobeneficial and salt stress mitigating efficacy of IAA producing salt tolerant strains in *Gossypium hirsutum*. *Saudi journal of biological sciences*, 28(9), 5317-5324.
- Salvo LPD, Cellucci GC, Carlino ME, Salamone IEG, (2018) Plant growth-promoting rhizobacteria inoculation and nitrogen fertilization increase maize (*Zea mays* L.) grain yield and modified rhizosphere microbial communities. *Appl Soil Ecol* 126: 113–120.
- Sandhya V, Ali SKZ, Grover M, Reddy G, Venkateswarlu B, (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biology and Fertility of Soils*. 46: 17–26
- Sandhya, V, Ali, SK. Z., Grover, M., Reddy, G. – Venkateswarlu, B. (2010): Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regulation* volume 62: 21–30
- Sárvári M, Pepó P, (2014) Effect of Production Factors on Maize Yield and Yield Stability. *Cereal Research Communications* 42(4): 710–720
- Singh S, Singh G, Singh P, Singh N, (2008) Effect of water stress at different stages of grain development on the characteristics of starch and protein of different wheat varieties. *Food Chem*. 108:130–139
- Singh JS, Pandey VC, Singh DP, (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ* 140: 339–353.
- Singh H, Singh Z, Kashyap R, Yadav SR, (2023) Lateral root branching: evolutionary innovations and mechanistic divergence in land plants. *New Phytologist*, 238(4), 1379-1385.

- Skonieski FR, Viégas J, Martin TN, Mingotti CCA, Naetzold S, Tonin TJ, (2019) Effect of nitrogen topdressing fertilization and inoculation of seeds with *Azospirillum brasilense* on corn yield and agronomic characteristics. *Agronomy* 9: 812.
- Smith DL, Gravel V, Yergeau E, (2017) Editorial: signaling in the phytomicrobiome. *Front. Plant Sci.* 8:611. doi: 10.3389/fpls.2017.00611
- Song OR, Lee SJ, Lee YS, Lee SC, Kim KK, Choi YL, (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. *Brazilian Journal of Microbiology*, 39, 151-156.
- Suralta RR, Inukai Y, Yamauchi A, (2010) Dry matter production in relation to root plastic development, oxygen transport, and water uptake under transient soil moisture stresses. *Plant Soil* 332: 87–104
- Tariq MR, Shaheen F, Mustafa S, ALI S, Fatima A, Shafiq M, Safdar W, Sheas MN, Hameed A, Nasir MA. 2022. Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. *PeerJ* 10:e13782 <https://doi.org/10.7717/peerj.13782>
- Turner TR, James EK, Poole PS, (2013) The plant microbiome. *Genome Biol.* 14:209. doi: 10.1186/gb-2013-14-6-209
- Ullah A, Akbar A, Luo Q, Khan AH, Manghwar H, Shaban M, Yang X, (2019) Microbiome diversity in cotton rhizosphere under normal and drought conditions. *Microb. Ecol.* 77, 429–439
- Vazquez P, Holguin G, Puente ME, (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol Fert Soils* 30(5–6):460–468
- Winston ME, Hampton-Marcell J, Zarraonaindia I, Owens SM, Moreau CS, Gilbert JA, (2014) Understanding cultivar-specificity and soil determinants of the cannabis microbiome. *PLoS One* 9:e99641. doi: 10.1371/journal.pone.0099641
- Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH, (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma*, 125: 155-166
- WU YW, Bo ZHAO, LI XL, LIU QL, FENG DJ, LAN TQ, YUAN JC, (2022) Nitrogen application affects maize grain filling by regulating grain water relations. *Journal of Integrative Agriculture*, 21(4), 977-994.
- Yang B, Wu S, Yan Z, (2022) Effects of Climate Change on Corn Yields: Spatiotemporal Evidence from Geographically and Temporally Weighted Regression Model. *ISPRS International Journal of Geo-Information*, 11(8), 433.
- Zhang R, Vivanco JM, and Shen Q, (2017) The unseen rhizosphere root-soil-microbe interactions for crop production. *Curr. Opin. Microbiol.* 37, 8–14. doi: 10.1016/j.mib.2017.03.008

LIST OF PUBLICATIONS

Peer-reviewed scientific publications in foreign languages related to the thesis

Kálmán CD, Kálmán L, Szél S, Mórocz Salamon K, Nagy Z, Kiss E, Posta K, (2023) Assessment of the influence of soil inoculation on changes in the adaptability of maize hybrids. Cereal Research Communications, 1-17.

Kálmán CD, Nagy Z, Berényi A, Kiss E, Posta K, (2023) Investigating PGPR bacteria for their competence to protect hybrid maize from the factor drought stress. Cereal Research Communications, 1-22.

Peer-reviewed scientific publications in Hungarian related to the thesis

Kálmán CD, Kálmán L, Latos Cs, Pauk J, Bóna L, Nagy Z, (2021) Kukoricahibridek fiatalkori szárazságstresszre és talajtípusra adott reakciói üvegházban. Növénytermelés, 71. 2.

Popular science publication in Hungarian related to the thesis

Kálmán CD, Nagy Z, (2021) A kukorica vízhasznosítása, aszálytűrése. Agrofórum, 92. 26 p.

Conference proceedings

Kálmán CD, Kálmán L, Móroczné S K, Nagy É, Nagy Z, Szél S (2018) Kukorica agrotechnikai kísérletek eredményei az elmúlt három évben. XXIV. Növénynevelési Tudományos Nap, Összefoglalók.

Kálmán CD, Szentgyörgyi A, Kiss E, (2018) Examination of the phosphate solubilizing ability of PGPR bacteria. Journal of Food Processing & Technology, Volume 9. 73 p., 55. p

Kálmán CD, Szentgyörgyi A, Kiss E, (2019) PGPR baktériumok foszfát-szolubilizáló képességének vizsgálata. XXV. Növénynevelési Tudományos Nap, Összefoglalók.

Nagy Z, **Kálmán CD**, Lantos Cs, Palágyi A, Palágyi A, Pauk J, Purgel Sz, Purgis T, Szakál M, Bóna L, (2019) Egyszerű digitális képanalízis alkalmazása kukorica növények szárazságtűrésének vizsgálatában. XXV. Növénynevelési Tudományos Nap, Összefoglalók.