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ORIGINAL PAPER

Assessment of the influence of soil inoculation on changes in the adaptability of maize hybrids

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Abstract

The aim of this paper is to present the results of the field trial carried out to collect and assess data on the interaction of maize (*Zea mays* L) genotypes and beneficial microorganisms. The small plot field trial consisting of untreated control plots and plots treated with biostimulants was conducted in three consecutive years (2019, 2020 and 2021). Yield is a particularly important trait from the aspect of maize breeding as well as maize production; therefore, the present study focused more closely on how it was influenced by the biostimulant treatments. The level of grain yield, grain moisture content at harvest and grain dry-matter content were observed and recorded as the components of yield. The nutritional value of kernels was also tested, and protein, oil and starch contents were analysed as the most important components of this trait. The results reflected that the treatment with biostimulants constituted from beneficial microorganisms can be listed among the factors influencing the grain yield, in addition to the seasonal effect, the genotype and the nutrient supply of the soil. The treatment with biostimulants, even on its own among the factors, had an impact on the quantity and components of yield, and on the characteristics determining the kernel nutritional value. The interaction between the genotypes and the interacting microorganisms is of specific importance. The most spectacular result was attained with the application of one of the biostimulants leading to elevated grain yield in 75% of the maize genotypes in the study, along with a kernel nutritive value equal to the control group over all of the three years of the trial.

Keywords *Zea mays* L. · PGPR · Biostimulant · Adaptability · Quantity of yield

Introduction

The role of soil microorganisms in crop production

The microbes living in the soil contribute to determining its physical, chemical and biological traits through their vital activities. With their ability to help nutrient efficiency, microorganisms enhance the nutrient acquisition and mobilization and directly stimulate the nutrient uptake. The results of the earlier researches by Gerretsen (1948) and Katznelson and Bose (1959) showed that bacterial inoculation improved phosphorus acquisition by concentrating insoluble phosphates and boosting the mineralization of organic phosphates. Morgenstein and Okon (1987) reported

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that soil inoculation with *Azospirillum brasilense* significantly intensified the nitrogen, phosphorus and potassium uptake for wheat, sorghum and maize plants. The interaction between soil, microorganism and plant is crucial from the aspect of the nutrient supply of the plants. The complexity of the effect of microbes includes that they can either promote, or inhibit or be neutral for root development depending on the crop, the environmental conditions and the type of microorganisms. The degree of the nutrient acquisition and use by plants is highly influenced by the environmental conditions of the growing area. The microbial life in the soil is of fundamental importance in mobilizing the nutrients. Regarding the relationship with the host plant, the beneficial role of soil bacteria is very versatile. PGPR designates beneficial microbes and is an acronym for Plant Growth Promoting *Rhizobacteria*, a group of bacteria that live in the root zone, promote the plant growth directly or indirectly and belong to various taxonomic groups. These bacteria account for 2–5% of bacteria present in the rhizosphere (Kloepper et al. 1980). They enhance the solubility and the uptake of the nutrients and stimulate plant growth by suppressing the harmful effects of pathogens (Vessey 2003). This is why the use of biofertilizers, which contain microbes that promote plant growth, has become important. Due to the changes in livestock farming, the amount of animal manure used as organic fertilizer has diminished, leading to the reduction in useful bacteria in the soil. It was shown that in soil inoculated with complex rhizosphere microflora, primary root growth and hair root formation were promoted compared to the plants grown on sterile soil (Rovira et al. 1983; Fusseder 1984). By the decomposition of organic matter in the soil, the beneficial soil bacteria also contribute to the faster breakdown of stubble residues. PGPR bacteria have been studied for a long time and extensively reviewed by the scientific literature (Katznelson and Bose 1959; Gerretsen 1948; Kloepper et al. 1988; Bowen and Rovira 1991; Sudhakar et al. 2000; Veres et al. 2009; Nagy et al. 2013; Tóth et al. 2015). The Acronym PHPR (Plant Health Promoting *Rhizobacteria*) is also used for bacteria that stimulate plant growth, that is, bacteria that promote healthy plant development (Burr and Ceasar 1984). Plant growth regulating substances secreted by microbes are known collectively as PGR (Plant Growth Regulators). Two groups are distinguished; free-living and symbiotic soil bacteria (Khan 2005). According to estimates, 80% of biologically bound nitrogen is provided by nitrogen bound symbiotically by beneficial bacteria under field conditions (Hamzei 2012). Since a significant number of PGPRs are incapable of colonizing roots, their beneficial effects are indirect (Suslow 1982). PGPRs can exert their activity essentially in three ways (Glick 2001): they synthesize plant-specific compounds (Zahir et al. 2004), protect the plant from diseases (Guo et al. 2004) and participate in nutrient uptake (Cakmakci et al. 2006). The process of plant growth stimulation facilitated by PGPRs has not yet been fully understood. Some possible explanations are that they can solubilize and mineralize elements, especially phosphorus (Richardson 2001); they have an important growth-stimulating effect through symbiotic nitrogen fixation (Kennedy et al. 2004); they increase the amount of secondary metabolites and contribute to long-term tolerance (Egamberdiyeva and Hoflick 2004); the potassium ion plays an important role in enhancing drought tolerance (Alvarez et al. 1996); they improve the resistance to oxidative stress (Stajner et al. 1997; Gururani et al. 2012); they have a positive effect in counterbalancing flooding and salt stress (Saleem et al. 2007); they activate plant defence mechanisms (Dutta et al. 2005); they produce hormones: auxins, cytokinins, gibberellins, abscisic acid and these have a stimulating effect on root cells (Patten and Glick 2002); they are able to produce vitamins such as the synthesize of water-soluble B vitamins (Revilla et al. 2000); they are antagonistic to phytopathogenic bacteria mainly through siderophore secretion, but they can also secrete antibiotics, cyanides and chitinase (Pal et al. 2001; Glick and Pasternak 2003); they synthesize a vital enzyme (1-amylocyclopropane-1-carboxylase deaminase—ACC), which reduces the ethylene level in the root of the developing plants, thereby increasing root length and stimulating plant growth (Penrose and Glick 2003; Glick 2014). A longer and more developed root system enables access to larger soil areas for the crop. The different microbes in commercially available biofertilizers may have various positive effects. They promote plant development through phytohormones, increasing the availability of nutrients to plants by biological nitrogen fixation, mobilizing chemically the poorly soluble nutrients (P, K, NH_4^+, Fe, Mn, Zn), and root differentiation or mycorrhization (Wu et al. 2011). By developing resistance to abiotic and biotic stress factors, as well as by pathogenic antagonism, they improve the health status of the plants (Vessey 2003). Wide-ranging studies have been conducted with various microorganisms to discover whether they have the potential to form the basis of biofertilizers and soil inoculants. The results show that *Bacillus subtilis* can be the basis of microbe-based fertilizers primarily due to secreting antibiotics. *Bacillus megaterium* is one of the most active phosphorus mobilizers (Han and Supanjani 2006) and also has a cytokinin receptor stimulating effect (Ortíz-Castro et al. 2008). It has both a growth-stimulating and an anti-ageing effect, which influences the life of plant positively. It may protect wheat from the deleterious effect of *Mycosphaerella graminicola*, which causes *Septoria* leaf spot (Kildea et al. 2008), and it also produces vitamin B12 (Moorel et al. 2014). In the case of *Bacillus polymyxa*, useful nitrogen-fixing ability (Priest 1993) and phosphate-dissolving ability (Gaur 1990) were described. The bacterium *Azotobacter choroococcum* is known as a free-living nitrogen fixer. Its ability to fix nitrogen depends largely on the soil traits, physical and chemical characteristics, and nutrient content. The *Azospirillum* family is one of the most-studied PGPR groups. Certain members of the family are phosphorus solubilizers, others are free-living nitrogen fixers, and their hormone production is also useful for plants (Steenhoudt and Vanderleyden 2000). *Azotobacter vinelandii* is also a member of the PGPR group owing to its ability to bind nitrogen and mobilize phosphorus (Nostrati et al. 2014). Microbe-based biofertilizers containing the aforementioned species proved to have a broad range of beneficial effects. (Barett and Marsh 2001; Lévai et al. 2008, 2010; Tóth et al. 2015): They stimulate the microbial life in the soil, increase the nutrient efficiency, facilitate nutrient acquisition, humification, nutrient deposition, improve the structure and water balance of the soil, significantly limit the living space of overwintering plant pathogens, fungi and pests; make soil cultivation more economical and environmentally friendly. As a result of the beneficial effects, plants can grow stronger hair roots and achieve higher tolerance to drought.

The most important yield determining factors of maize

The year effect, genotype and nutrient supply have the greatest influence on the yield of maize. Water supply influences fundamentally the success of maize production. In favourable years, fertilization increased the yield by 40–50%, but in extremely dry, drought years, fertilization had no yield increasing effect. Drought during the period of tasselling can reduce the yield up to 40–50% (Claassen and Shaw 1970). Attention must be paid to the appropriate choice of the genetic base in maize breeding (Pepó and Pepó 1993). The biological base has also become extremely important in maize production are increasing in frequency due to climate change. The genetic yielding ability of maize usually ranges from 15 to 18 t/ha. It can be exploited in farm production only under optimal environmental and weather conditions for the given genotype. Among the agrotechnological factors, nitrogen fertilization has primary importance in increasing the yield of maize. The effect of nitrogen fertilization depends to a large extent on the climatic conditions in the growing season. The effect of nitrogen is mostly favourable, but in unfavourable growing seasons it can have adverse effects. It is generally known that N efficiency varies between different maize genotypes. Therefore, the optimization of the nitrogen dose to be applied is a basic precondition for environmentally friendly fertilization.

The efficiency of biostimulants has to be estimated under field conditions, where the survival of the applied microorganisms and their impact on the host plant might be inhibited by competition with the microflora in the soil. The aim of this study was to identify and characterize the effect of the interaction of the beneficial microorganisms exerted on maize hybrids (*Zea mays* L.) grown under field conditions, with specific attention to yield, grain moisture content, dry-matter content, and the contents of protein, oil and starch, respectively.

Materials and methods

Weather conditions during the three years of the trial

Figure 1 and Table 1 show the weather conditions in Kiszombor over the three years of the trial. In 2019, the average temperature during the maize growing season was 19.18 °C. The hottest months were June, July and August. The total precipitation amounted to 415.7 mm, and the

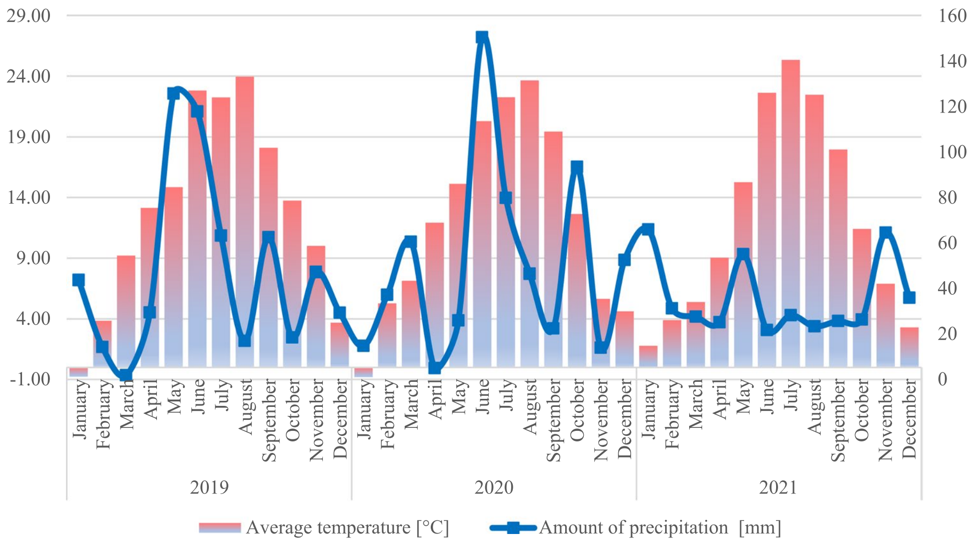

Fig. 1 Average temperature (°C) and amount of precipitation (mm) in 2019, 2020 and 2021, respectively

Table 1 Average temperature (At.) expressed in °C and rainfall (Rf.) expressed in mm during the maize growing seasons in 2019, 2020, and 2021, respectively)

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	19,18	415,7	18,78	330,2	18,78	179,5
TX30GE (days)	58		42		54	
TX35GE (days)	2		0		16	

TX30GE, number of hot days; TX35GE, number of heat days

rainiest months were May, June and July. The number of hot days totalled 58 (TX30GE: the average temperature was permanently above 30 °C), and the number of heat days totalled 2 (TX35GE: the average temperature was permanently above 35 °C). It was an intensive year in terms of maize cultivation with warm and rainy weather. In 2020, the average temperature during the maize growing season was 18.78 °C, which was 0.4 °C lower than in 2019. The hottest months were June, July and August, just like in the year before. The total amount of precipitation was 330.2 mm, which was 85.5 mm less than in 2019. June was the rainiest month. The number of hot days was 42 (16 days less than in 2019), and there were no heat days (2 days less than in 2019). From the aspect of maize cultivation, 2020 can also be regarded as an intensive year, although the cool weather delayed the emergence in the spring. In 2021, the average temperature during the maize growing season was 18.78 °C, similar to 2020. The hottest months were again June, July and August. The total precipitation was 179.5 mm, which was 236.2 mm less than in 2019 and 150.7 mm less than in 2020. May was the rainiest month. The number of hot days was 54 (4 days less than in 2019 and 12 days more than in 2020). The number of heat days was 16 (14 days more than in 2019 and 16 days more than in 2020). From the aspect of maize production, 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 average crop yield.

Plant material, PGPR bacteria, efficient microorganisms and trial conditions

The maize genotypes in the trial were provided by the Maize Breeding Department of the Cereal Research Non-profit Ltd., Szeged. Seeds for the trial were produced annually in the top-cross maize hybrid production program of the CR Ltd. in Kiszombor. The bacterial species were supplied by the entrepreneur Toximent LP., Szolnok and Hungarian University of Agriculture and Life Sciences. The experimental microbiological products of Micro-Logi Tech Ltd. Szolnok were also applied in the trial for research purposes only.

GKT 3213 is a super-early grain maize in the maturity group FAO 230 (registered in the variety list 9 March, 2015). It is a hybrid with superior yield potential in its maturity group, and its performance is above the average under high input conditions. GKT 3385 is an early grain maize in the FAO 390 maturity group (a variety candidate in the state trials). GKT 376 is an early, FAO 380 grain maize (registered in the variety list 12 March, 2014). It is characterized by excellent yield stability over a wide range of growing areas and seasons. It has a strong stalk, good root traits and fast dry-down rate. GK Silostar is a mid-ripening FAO 490 silage maize (registered in the variety list 8 March, 2017). It performs best under intensive nutrient supply and adequate rainfall conditions. *Bacillus megaterium* and *Bacillus pumilus* are rod-shaped, Gram-positive, obligate aerobic and saprophytic soil-borne bacteria. *Pseudomonas fluorescens* and *Pseudomonas putida* are common, Gram-negative and rod-shaped bacteria. *Rhodopseudomonas palustris* (rod-shaped and Gram-negative), *Lactobacillus plantarum* and *Lactobacillus casei* (rod-shaped and Gram-positive), and *Saccharomyces cerevisiae* (single-celled yeast fungus) are efficient microorganisms.

The trial was conducted in 2019, 2020 and 2021, respectively, in the nursery of the Cereal Research Non-profit Ltd. in Kiszombor (46° 11′ 16″ N 20° 23′ 43″ E). Four treatments were applied in the trial; (1) The untreated control (K); (2) the bacterial combination of *Bacillus megaterium*, *Pseudomonas fluorescens* (KD1); (3) *Bacillus pumilus*, *Pseudomonas putida* (KD2) and (4) the experimental microbiological product of Micro-Logi Tech Ltd. involving *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Saccharomyces cerevisiae* (MLT). The trial was carried out in 3 replicates, 4 hybrids (GKT 3213, GKT 3385, GKT 376 and GK Silostar, respectively) in a randomized block design. The bacterial combinations (with the exception of the Micro-Logi Tech Ltd. product, which was received ready-made) were incubated on Luria Bertani (LB) medium (5 g/l yeast extract, 10 g/l NaCl, pH 7.5 ± 0.2). The prepared medium was sterilised before use, at high temperature and pressure for 45 min. The liquid medium was poured into 100 ml Erlenmeyer flasks and inoculated with rennet with the test bacterial species, then incubated in a shaking thermostat for 5 days at 28 °C at 180 rpm, then the supernatant was drained and the pellet dissolved in sterile reverse-osmosis (RO) purified water up to 500 ml. The optical density of the bacterial solution was determined at 600 nm (OD_{600}) by spectrophotometer. The physical and chemical traits of the soil of the experimental area are shown in Table 2.

The pre-crop was winter wheat (*Triticum aestivum L.*). In the autumn, in November, the soil was ploughed 26–32 cm deep and 170 kg/ha N, 100 kg/ha P and 100 kg/ha K were spread in the form of a complex fertilizer with 8:21:21 ratio of NPK. In the spring in March, 300 kg/ha CAN (calcium–ammonium-nitrate) containing 27% N was spread, and then, the seed beds were prepared by a combinator. The soil inoculation was carried out before sowing with a self-propelled field sprayer, in the proportion of 1 l suspension (10^8 CFU/ml) + 13 l distilled water/283.5 m^2 (dosage of 494 l/ha), and it was mixed 7 cm deep into the soil immediately after application by a rotavator. Sowing was done with a Wintersteiger Plotseed TC self-propelled plot seeder. The number of plants was adjusted to 60,000 plants/ha. Early postemergence weed control was accomplished with Adengo (Bayer) at a dose of 0.4 l/ha and Principal Plus + Successor T (Corvetva) at a dose of 400 g/ha. The tillage in the line spacing was done by a cultivator. The plots were harvested by a Wintersteiger Quantum Plotech plot combine.

Observed and recorded data

The levels of grain yield, the grain moisture content at harvest, and the grain dry-matter content were recorded as the most significant components of yield. The protein, oil and starch content, considered as the most significant components of the nutritional value of kernel, were analysed by a Foss Infratech 1241 Analyzer equipped with the software program CO361108. The respective data were recorded either at harvest, after harvest, or during seed conditioning, as required.

Statistical analysis

The collected and recorded data were analysed by using the Microsoft Excel 2019 XLSTAT software. Two-way Analysis of Variance (ANOVA) was used to analyse the interactions among the individual components. The least significant difference was calculated by using Fisher's Least Significant Difference (LSD) procedure. The correlations were analysed by Principal Component Analysis (PCA).

Results

In order to determine the effect of the biostimulant treatment with PGPR bacteria, the traits were observed and recorded that are considered to have the highest significance in influencing the yield and the kernel nutritional value from the point of view of both maize breeding and maize production.

Analysis of the data of the three-year field trial

The data recorded during each of the three experimental years were analysed by two-way ANOVA, and the results are shown in Table 3.

The factors hybrid and year caused significant differences in the case of each trait. A statistically verifiable significant difference was obtained for each trait except grain dry matter and starch content in the case of biostimulant as factor. The hybrid and year interaction showed significant differences for all traits. A significant difference was found for the quantity of grain yield and grain moisture content in the analysis of the hybrid and biostimulant interaction. A statistically verifiable significant difference was found for each of the characteristic traits apart from grain dry matter content in the case of the interaction between year and biostimulant.

Grain yield is one of the most important aims in maize breeding and has the highest importance in agricultural practice. Therefore, particular importance has been given to the analysis of the effect of biostimulants on grain yield in the present study. On one hand, the grain yield was analysed from the aspect of hybrids as influenced by treatments,

Table 2 The physical and chemical traits of the experimental site

Year	pH (KCl)	KA	Salt content (total) (%)	Na (mg/kg)	$CaCO_3$ (%)	NO_3-N (mg/kg)	Humous (%)	P_2O_5 (mg/kg)	K_2O	Mg	Zn	Cu	Mn	SO_4
2019	7.12	54	0.08	53	2.6	15.3	2.37	316	455	511	1.3	8.6	184	12.5
2020	7.16	50	0.07	66	4.9	11.2	2.54	634	437	347	1.3	6.0	99	17.0
2021	7.14	53	0.08	75	1.9	13.4	2.30	288	335	303	1.3	7.2	148	18.3

Table 3 Results of the two-way Analysis of Variance (ANOVA) involving the three-year data of the yield determining traits (yield, grain moisture content and dry matter content) and the kernel nutritional value determining traits (protein, oil and starch content) recorded about the maize hybrids

	DF	MS	F	*p*
Grain yield				
Hybrid	3	50.627	225.670	0.000
Year	2	214.691	956.995	0.000
Biostimulant	3	3.321	14.802	0.000
Hybrid*Year	6	25.428	113.348	0.000
Hybrid*Biostimulant	9	1.361	6.068	0.000
Year*Biostimulant	6	0.877	3.910	0.001
Grain moisture content				
Hybrid	3	67.309	414.643	0.000
Year	2	405.981	2500.973	0.000
Biostimulant	3	1.755	10.808	0.000
Hybrid*Year	6	16.906	104.146	0.000
Hybrid*Biostimulant	9	0.655	4.037	0.000
Year*Biostimulant	6	1.302	8.023	0.000
Grain dry matter content				
Hybrid	3	2.771	6.770	0.000
Year	2	115.840	283.040	0.000
Biostimulant	3	0.239	0.584	0.627
Hybrid*Year	6	1.446	3.533	0.003
Hybrid*Biostimulant	9	0.184	0.449	0.905
Year*Biostimulant	6	0.122	0.299	0.936
Protein content				
Hybrid	3	2.076	27.972	0.000
Year	2	120.480	1623.578	0.000
Biostimulant	3	0.722	9.728	0.000
Hybrid*Year	6	1.914	25.787	0.000
Hybrid*Biostimulant	9	0.074	0.998	0.446
Year*Biostimulant	6	0.752	10.138	0.000
Oil content				
Hybrid	3	0.301	12.460	0.000
Year	2	6.857	283.624	0.000
Biostimulant	3	0.124	5.130	0.002
Hybrid*Year	6	0.204	8.458	0.000
Hybrid*Biostimulant	9	0.017	0.714	0.695
Year*Biostimulant	6	0.068	2.815	0.014
Starch content				
Hybrid	3	1.052	6.476	0.000
Year	2	383.075	2358.035	0.000
Biostimulant	3	0.261	1.606	0.192
Hybrid*Year	6	0.864	5.317	0.000
Hybrid*Biostimulant	9	0.114	0.700	0.707
Year*Biostimulant	6	0.486	2.995	0.009

DF, degrees of freedom; MS, mean squares; F, F ratio; Sig, *P* values

involving the comparison between the treated hybrids compared to the untreated control (A); on the other hand, yield was compared over the experimental years, by taking the year effect as an independent variable (B) (Fig. 2). In case of the hybrid and biostimulant interaction, the KD2 treatment induced an increase in grain yield for all the genotypes, except for GKT 3213. It generated an additional grain yield of 1.034 t/ha for GKT 3385, 0.438 t/ha for GKT 376, and 0.845 t/ha for GK Silostar. Considering the seasonal effect and biostimulant interaction, the KD2 treatment led to a grain yield increase of 0.198 t/ha in 2019, 0.556 t/ha in 2020, and 0.839 t/ha in 2021, respectively.

Regarding the hybrid and biostimulant interaction, the KD2 treatment showed a reduction in the grain moisture content at harvest for all genotypes, except for GKT 3213. The decrease was 0.538% for GKT 3385, 0.314% for GKT 376 and 0.482% for GK Silostar (Fig. 3A). Regarding the year effect and biostimulant interaction, the KD2 treatment led to lower grain moisture content in intensive year (2019 and in 2020), with a decrease of 0.617% and 0.621%, respectively, and higher grain moisture content in unfavourable year (2021), with 0.226% increase (Fig. 3B). The treatments did not influence the grain dry-matter content of any of the treated hybrids compared to the controls (Fig. 3C, D).

In the case of the hybrid and biostimulant interaction, the KD2 treatment was identified as inducing higher protein content for the genotype GKT 3213 compared to the control (K), the KD1 and MLT treatments, and the other hybrids (Fig. 4A). For the interaction of year and biostimulant, in 2019 the hybrids treated with KD1 produced the highest protein content (Fig. 4B). Regarding the oil content, the highest value was measured in the hybrid and biostimulant interaction in the control (K) treatment for GKT 3385 (Fig. 4C). Regarding the interaction of year effect and biostimulant, in 2019 the hybrids treated with KD1 had the highest oil content (Fig. 4D). In the case of the hybrid and biostimulant interaction, the MLT treatment led to the highest starch content for the GKT 3213 genotype (Fig. 4E). Regarding the interaction between the year effect and the biostimulant, in 2021 the plants treated with KD1 exhibited the best results (Fig. 4F).

Based on Table 4, it could be shown, that taking into account the biostimulant factor, the plants treated with KD2 produced significantly higher average grain yield than the control (K), and the hybrids treated with KD1 and MLT, respectively. The hybrids treated with MLT gave significantly higher grain yield compared to the plants treated with KD1. No significant difference was found between the average crop grain yield of the control (K) and MLT-treated hybrids, and the control (K) and KD1-treated hybrids. Regarding the hybrid and biostimulant interaction, in the case of GK Silostar and GKT 3385, the KD2 treatment resulted in significantly higher grain yield than the control

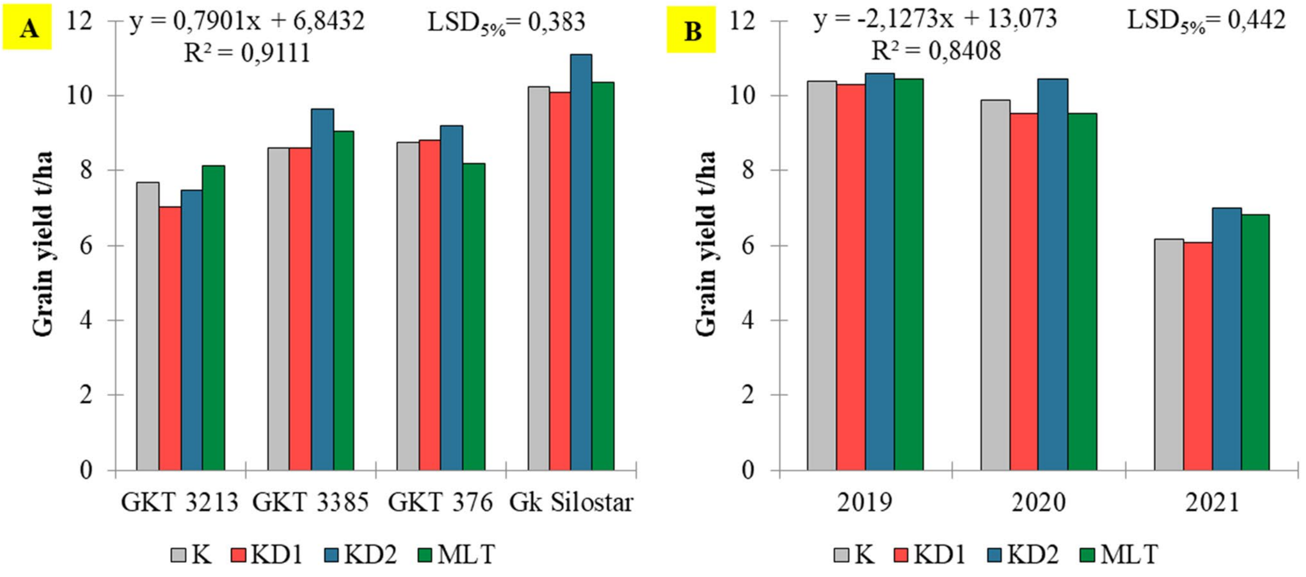

Fig. 2 The effect of the treatments with biostimulants on the average crop grain yield (t/ha) over maize hybrids (**A**); and over year effects (**B**). The applied treatments were (K): control; (KD1): *Bacillus megaterium*, *Pseudomonas fluorescens*; (KD2): *Bacillus pumilus*, *Pseudomonas putida;* (MLT): *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*

(K) and the other two bacterial treatments. The only exception was GKT 376, for which no significant difference was found in the KD2 treatment.

As far as the interaction of year and biostimulant was concerned, the results showed that in 2019, 2020, as well as in 2021, the hybrids treated with KD2 had significantly higher average yield compared to the controls (K) and the other treatments. The analysis of the effect of the biostimulant showed that the grain moisture content of the hybrids treated with KD2 was significantly lower at harvest than that of the plants in the control (K), KD1 and MLT treatments. No significant difference could be found between the grain moisture content of the hybrids in the control (K), KD1 and MLT treatments, respectively. The effect of the hybrid and biostimulant interaction was that the hybrid GK Silostar treated with KD2 showed significantly lower grain moisture content than in the control (K), MLT and KD1 treatments. The KD2 treatment resulted in significantly lower grain moisture content in GKT 376 and GKT 3385 than the control (K) and the other two treatments. Regarding the year and biostimulant interaction, in 2019 and 2020, the hybrids treated with KD2 had significantly lower grain moisture content than the control (K) and the plants treated with KD1 or MLT. However, in 2021, the KD2 treatment resulted in significantly higher grain moisture content compared to the results observed in the previous two years. No significant difference could be found for the grain dry matter content of the hybrids on the influence of the biostimulant factor, the hybrid and biostimulant interaction, and the year and biostimulant interaction, respectively.

Table 5 reflects the influence of the biostimulant treatment on the protein content. The plants treated with KD2 had significantly higher average protein yield than the hybrids treated with KD1 or MLT, but no significant difference could be observed, if compared to the control (K). There was no significant difference between the hybrids treated with KD1 and MLT, but significantly less protein content was recorded in both cases than for the control (K). In the hybrid and biostimulant interaction, GKT 3213 treated with KD2 had significantly higher protein content compared to the other hybrids and treatments. For the year and biostimulant interaction, significantly higher protein content was found in the case of the plants treated with KD1 in 2019 in comparison with the other years and treatments, but no significant difference could be found compared to the control (K) in 2019. Considering all the treatments in 2020 and 2021, the protein yield proved to be significantly lower than the value of the control (K) in 2019. Regarding the effect of the biostimulant factor on oil content, no significant difference was found between the KD2 treatment and the control (K); however, in the KD1 and MLT treatments the oil content was significantly lower than in the control (K). In the case of the hybrid GKT 3385, the oil content of the plants treated with KD1 or KD2 did not differ significantly from the control (K) on the effect of the hybrid and biostimulant interaction. The oil content was found significantly lower in the case of the other hybrids and treatments. Regarding the interaction of year and biostimulant, significantly higher oil content was found in the plants treated with KD1 in 2019 compared to the other years and treatments, but no significant difference

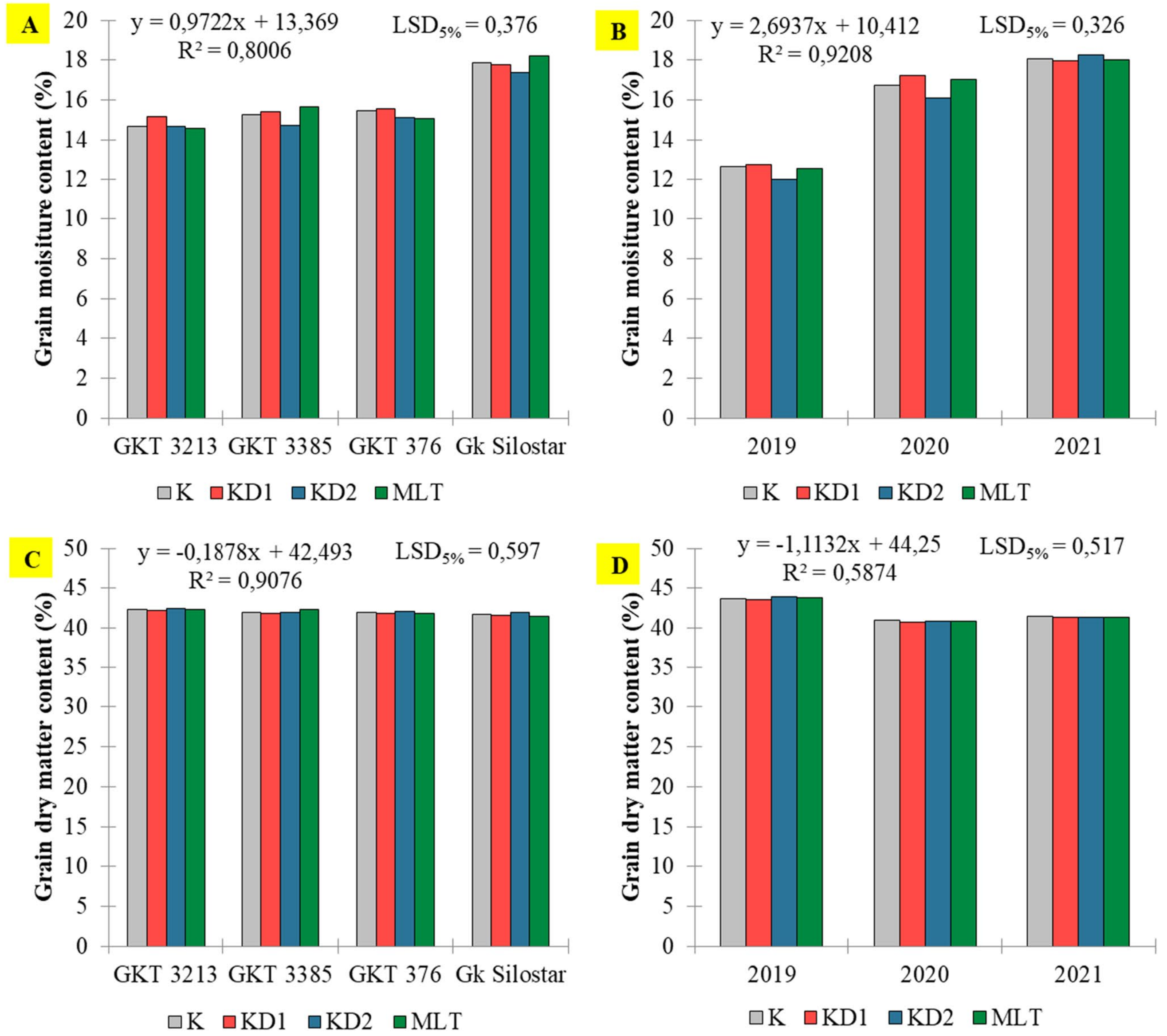

Fig. 3 The effect of the treatments with biostimulants on the grain moisture content (%) influenced by the hybrid (**A**) and year effect (**B**) interaction, and on the average of grain dry-matter content (%) influenced by the hybrid (**C**) and year effect (**D**). The applied treatments were (K): control; (KD1): *Bacillus megaterium*, *Pseudomonas fluorescens*; (KD2): *Bacillus pumilus*, *Pseudomonas putida;* (MLT): *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*

could be observed compared to the control (K) in 2019, and the KD2 and MLT treatments, respectively. In 2020 and 2021, the protein content proved to be significantly lower in all the treatments compared to the 2019 values. As regards the effect of the biostimulant factor on starch, no significant difference was found between the treatments. No significant difference was found either in the case of the hybrid and biostimulant interaction. For the year and biostimulant interaction, significantly higher starch content was found in the plants treated with KD1 in 2021 compared to the other years and treatments, but this did not differ significantly from the control (K) and the MLT treatment in 2021.

Table 6 shows the correlation of the yield-determining parameters and the nutritional value determining parameters. A negative correlation was found between grain yield and grain moisture/starch content. The grain yield and grain dry-matter/oil content showed a positive correlation. A negative correlation could be identified between grain moisture content and grain dry matter/protein/oil content. The grain dry matter content showed a positive correlation with protein/oil/starch content. A positive correlation was found between protein and oil/starch content, and between oil and starch content, respectively.

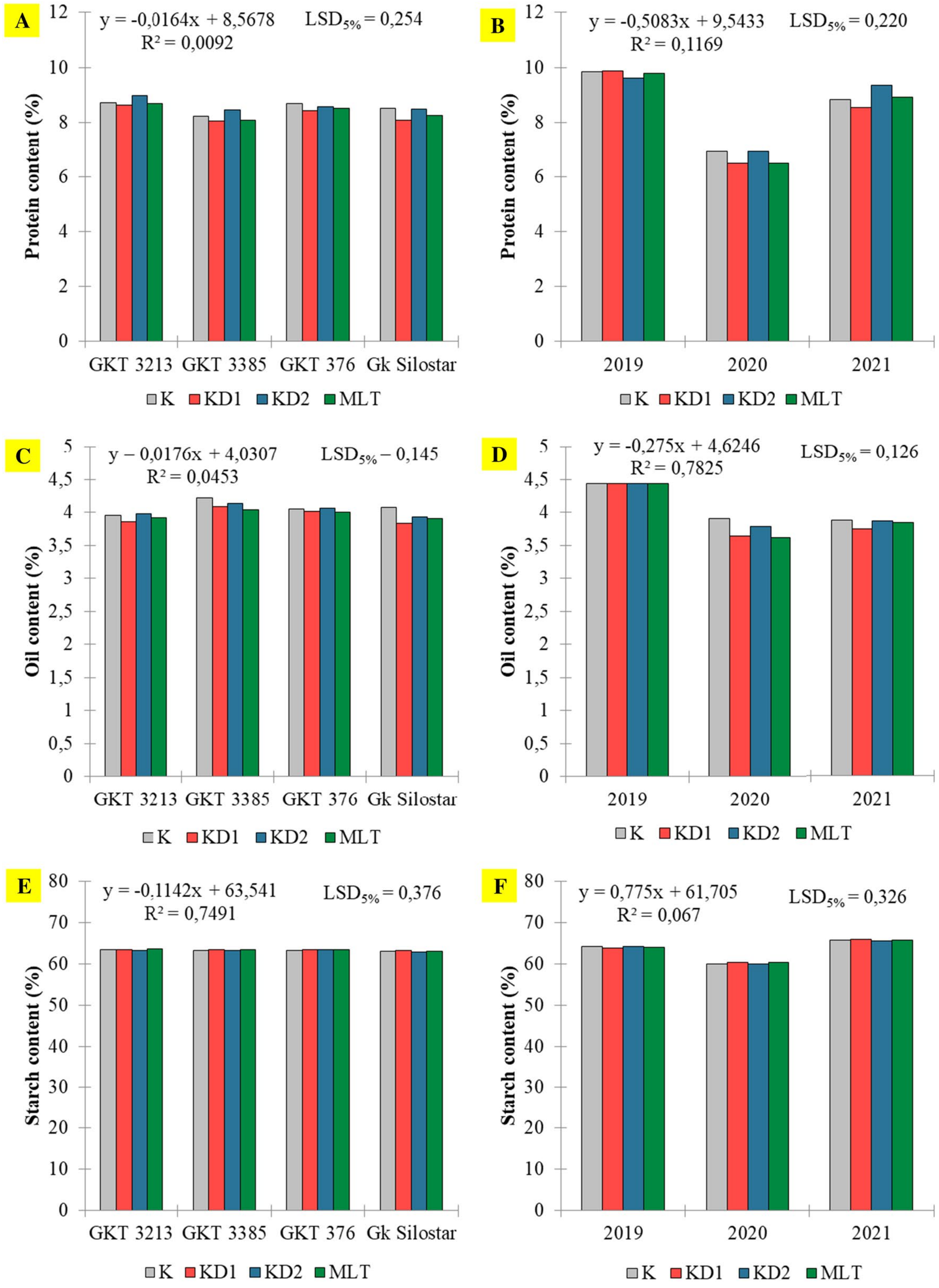

Fig. 4 The effect of the treatments with bacteria on the average of protein content (%) influenced by the hybrid (**A**) and year effect (**B**) interaction, on the average of oil content (%) influenced by the hybrid (**C**) and year effect (**D**) and on the average of the starch content (%) influenced by the hybrid (**E**) and year effect (**F**) interaction. The applied treatments were (K): control; (KD1): *Bacillus megaterium*, *Pseudomonas fluorescens*; (KD2): *Bacillus pumilus*, *Pseudomonas putida*; (MLT): *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*

Table 4 The values of the 95% confidence interval for the Least Significant Difference (LSD) between the yield components of the hybrids (grain yield, grain moisture content and grain dry-matter content), taking into consideration each independent variable (hybrid, year and biostimulant) and their modes of interaction (hybrid*year, hybrid*biostimulant and year*biostimulant)

95% confidence interval					
LS means					
Grain yield		Grain moisture content		Grain dry matter content	
Hybrid LSD					
GK Silostar	10.463a	GK Silostar	17.796a	GKT 3213	42.342a
GKT 3385	8.958b	GKT 376	15.294b	GKT 3385	42.043ab
GKT 376	8.730c	GKT 3385	15.247b	GKT 376	41.931bc
GKT 3213	7.577d	GKT 3213	14.767c	Gk Silostar	41.672c
p	**< 0.0001**		**< 0.0001**		**0.000**
Year LSD					
2019	10.442a	2021	18.067a	2019	43.759a
2020	9.840b	2020	16.756b	2021	41.404b
2021	6.515c	2019	12.504c	2020	40.827c
p	**< 0.0001**		**< 0.0001**		**< 0.0001**
Biostimulant					
KD2	9.349a	KD1	15.974a	KD2	42.086a
MLT	8.928b	MLT	15.867a	K	42.023a
K	8.818bc	K	15.800a	MLT	41.987a
KD1	8.633c	KD2	15.463b	KD1	41.891a
p	**< 0.0001**		**< 0.0001**		0.627
*Hybrid*Year LSD*					
GKT 376*2019	11.168a	GK Silostar*2021	21.244a	GKT 3385*2019	43.896a
GKT 3385*2020	10.736b	GK Silostar*2020	19.559b	GKT 3213*2019	43.762a
GK Silostar*2019	10.596b	GKT 3385*2021	17.610c	GK Silostar*2019	43.704a
GK Silostar*2021	10.422b	GKT 376*2021	16.823d	GKT 376*2019	43.675a
GKT 3385*2019	10.380b	GKT 3213*2021	16.592de	GKT 3213*2021	41.704b
GK Silostar*2020	10.369b	GKT 376*2020	16.493e	GKT 376*2021	41.629bc
GKT 376*2020	9.890c	GKT 3385*2020	15.689f	GKT 3213*2020	41.558bcd
GKT 3213*2019	9.622c	GKT 3213*2020	15.284g	GKT 3385*2021	41.167cd
GKT 3213*2020	8.364d	GK Silostar*2019	12.583h	GK Silostar*2021	41.118cd
GKT 3385*2021	5.758e	GKT 376*2019	12.567h	GKT 3385*2020	41.067d
GKT 376*2021	5.132f	GKT 3385*2019	12.442h	GKT 376*2020	40.488e
GKT 3213*2021	4.746g	GKT 3213*2019	12.425h	GK Silostar*2020	40.194e
p	**< 0.0001**		**< 0.0001**		**0.003**
*Hybrid*Biostimulant LSD*					
GK Silostar*KD2	11.100a	GK Silostar*MLT	18.213a	GKT 3213*KD2	42.400a
GK Silostar*MLT	10.380b	GK Silostar*K	17.846ab	GKT 3213*K	42.372a
GK Silostar*K	10.255b	GK Silostar*KD1	17.759b	GKT 3213*MLT	42.344ab
GK Silostar*KD1	10.115b	GK Silostar*KD2	17.364c	GKT 3385*MLT	42.306ab
GKT 3385*KD2	9.623c	GKT 3385*MLT	15.635d	GKT 3213*KD1	42.250ab
GKT 376*KD2	9.191cd	GKT 376*KD1	15.557de	GKT 376*KD2	42.028abc
GKT 3385*MLT	9.028de	GKT 376*K	15.439def	GKT 3385*KD2	42.017abc
GKT 376*KD1	8.806de	GKT 3385*KD1	15.402defg	GKT 3385*K	42.006abc
GKT 376*K	8.753de	GKT 3385*K	15.244efg	GKT 376*K	41.950abc
GKT 3385*KD1	8.591ef	GKT 3213*KD1	15.180fg	GK Silostar*KD2	41.900abc
GKT 3385*K	8.589ef	GKT 376*KD2	15.124fg	GKT 376*KD1	41.883abc
GKT 376*MLT	8.170fg	GKT 376*MLT	15.058gh	GKT 376*MLT	41.861abc
GKT 3213*MLT	8.133g	GKT 3385*KD2	14.706hi	GKT 3385*KD1	41.844abc
GKT 3213*K	7.676h	GKT 3213*K	14.670i	GK Silostar*K	41.765bc
GKT 3213*KD2	7.483h	GKT 3213*KD2	14.657i	GK Silostar*KD1	41.587c
GKT 3213*KD1	7.018i	GKT 3213*MLT	14.560i	GK Silostar*MLT	41.436c

Table 4 (continued)

95% confidence interval					
LS means					
Grain yield		Grain moisture content		Grain dry matter content	
p	**< 0.0001**		**0.000**		0.905
*Year*Biostimulant LSD*					
2019*KD2	10.609a	2021*KD2	18.264a	2019*KD2	43.983a
2019*MLT	10.448a	2021*K	18.038a	2019*MLT	43.767a
2020*KD2	10.443a	2021*MLT	18.026a	2019*K	43.675a
2019*K	10.411a	2021*KD1	17.942a	2019*KD1	43.613a
2019*KD1	10.298a	2020*KD1	17.207b	2021*K	41.449b
2020*K	9.887b	2020*MLT	17.015bc	2021*MLT	41.398bc
2020*KD1	9.516b	2020*K	16.712c	2021*KD2	41.392bc
2020*MLT	9.512b	2020*KD2	16.092d	2021*KD1	41.379bc
2021*KD2	6.996c	2019*KD1	12.775e	2020*K	40.946bcd
2021*MLT	6.823c	2019*K	12.650e	2020*KD2	40.883cd
2021*K	6.157d	2019*MLT	12.558e	2020*MLT	40.796d
2021*KD1	6.084d	2019*KD2	12.033f	2020*KD1	40.682d
p	**0.001**		**< 0.0001**		0.936

The lowercase letters represent the status of significance among the values obtained from the analysis

p value, the significant differences were highlighted

The results of the principal component analysis are presented in Fig. 5, demonstrating the correlation between the treatments and the traits. The analysis was based on the three-year data of the yield-related components (grain yield, grain moisture content and grain dry matter content), and the components of the kernel nutritional value (protein, oil and starch content). It is obvious from the figure that 75% of the hybrids responded to the KD2 treatment with increased grain yield compared to the control groups in each of the three experimental years. The principal component explains 83.17% of the correlations in total and can be defined as yield potential.

Discussion

The effect of years on the observed traits

According to Sárvári and Pepó (2014), the resilience of the agro-ecosystem in its response to unfavourable weather conditions is essentially determined by the intensity level of maize cultivation. It can be shown that the extremes in the weather caused by climate change have a great influence on the maize yield (Brown and Rosenberg 1999). In this study, the results of the three-year field trial supported the conclusion that the year had the greatest effect on the level of maize grain yield. The first two experimental years, 2019 and 2020, happened to be intensive years for maize production, in which the average grain yield of the examined maize hybrids was nearly 10 t/ha. However, the third experimental year, 2021, was an extremely unfavourable year for maize. The lack of precipitation, the high average temperature and the atmospheric drought during tasselling caused 38% average grain yield reduction in the tested hybrids, which was 6.5 t/ha in 2021. Among the determinant traits for grain yield, the highest value was found for the average of the grain moisture content at harvest in 2021 (18.1%), with a lower value of 16.8% in 2020, and with the lowest value of 12.5% in 2019. As regards the grain dry matter content of the hybrids, its value was the highest in 2019 (43.8%), with a lower value of 41.4% in 2021 and with the lowest value of 40.8% in 2020. The year factor also affected the traits determining the kernel nutritional value of hybrids. For protein and oil content, the highest values were found in 2019 (9.8% and 4.4%, respectively), with lower values of 8.9% and 3.8%, respectively, in 2021, and with the lowest values of 6.7% and 3.7%, respectively, in 2020. In the average of the hybrids, the starch content was the highest in 2021 (65.7%), with a lower value of 64.0% in 2019, with the lowest value of 60.2% in 2020. Results of long-term experiments proved that the equilibrium of different crop models and consequently, its productivity were greatly influenced by environmental, primarily weather factors (Sárvári and Pepó 2014).

The influence of genotype on the observed traits

The second factor that affected the yield was the genotype. The three-year averages showed that GK Silostar delivered

Table 5 The values of the 95% confidence interval for the Least Significant Difference (LSD) between the components determining the kernel nutritional value of the hybrids (protein, oil and starch content), taking into consideration each independent variable (hybrid, year and biostimulant) and their modes of interaction (hybrid*year, hybrid*biostimulant and year*biostimulant)

95% confidence interval					
LS means					
Protein content		Oil content		Starch content	
Hybrid LSD					
GKT 3213	8.748a	GKT 3385	4.121a	GKT 3213	63.443a
GKT 376	8.550b	GKT 376	4.032b	GKT 376	63.393a
GK Silostar	8.331c	GK Silostar	3.935c	GKT 3385	63.313a
GKT 3385	8.204c	GKT 3213	3.927c	GK Silostar	63.058b
p	**< 0.0001**		**< 0.0001**		**0.000**
Year LSD					
2019	9.772a	2019	4.436a	2021	65.685a
2021	8.904b	2021	3.838b	2019	64.039b
2020	6.699c	2020	3.737c	2020	60.181c
p	**< 0.0001**		**< 0.0001**		**< 0.0001**
Biostimulant LSd					
KD2	8.618a	K	4.075a	MLT	63.375a
K	8.527a	KD2	4.027ab	KD1	63.372a
MLT	8.391b	MLT	3.968bc	K	63.255a
KD1	8.299b	KD1	3.945c	KD2	63.205a
p	**< 0.0001**		**0.002**		0.192
*Hybrid*Year LSD*					
GKT 3213*2019	10.356a	GKT 3385*2019	4.511a	GK Silostar*2021	65.750a
GKT 376*2019	10.300a	GKT 376*2019	4.467ab	GKT 3385*2021	65.733a
GK Silostar*2019	9.344b	GK Silostar*2019	4.411ab	GKT 3213*2021	65.658a
GKT 3385*2019	9.089c	GKT 3213*2019	4.356b	GKT 376*2021	65.600a
GK Silostar*2021	9.000c	GKT 3385*2021	3.950c	GKT 3385*2019	64.100b
GKT 376*2021	8.950cd	GK Silostar*2021	3.917cd	GKT 3213*2019	64.100b
GKT 3213*2021	8.917cd	GKT 3385*2020	3.901cd	GKT 376*2019	64.089b
GKT 3385*2021	8.750d	GKT 376*2021	3.858cde	GK Silostar*2019	63.867b
GKT 3213*2020	6.972e	GKT 3213*2020	3.800de	GKT 3213*2020	60.572c
GKT 3385*2020	6.774ef	GKT 376*2020	3.770e	GKT 376*2020	60.489c
GK Silostar*2020	6.649f	GKT 3213*2021	3.625f	GKT 3385*2020	60.106d
GKT 376*2020	6.401g	GK Silostar*2020	3.477g	GK Silostar*2020	59.559e
p	**< 0.0001**		**< 0.0001**		**< 0.0001**
*Hybrid*Biostimulant LSD*					
GKT 3213*KD2	8.963a	GKT 3385*K	4.216a	GKT 3213*MLT	63.567a
GKT 3213*K	8.706b	GKT 3385*KD2	4.141ab	GKT 376*KD2	63.511ab
GKT 3213*MLT	8.700b	GKT 3385*KD1	4.083abc	GKT 3213*KD1	63.486ab
GKT 376*K	8.676bc	GK Silostar*K	4.073abcd	GKT 3385*KD1	63.477abc
GKT 3213*KD1	8.623bc	GKT 376*KD2	4.059bcde	GKT 3213*K	63.475abc
GKT 376*KD2	8.574bc	GKT 376*K	4.051bcde	GKT 376*MLT	63.438abcd
GKT 376*MLT	8.516bcd	GKT 3385*MLT	4.043bcdef	GKT 3385*MLT	63.389abcd
GK Silostar*K	8.501bcd	GKT 376*KD1	4.010bcdef	GKT 376*KD1	63.343abcd
GK Silostar*KD2	8.473bcde	GKT 376*MLT	4.008bcdef	GKT 376*K	63.279abcd
GKT 3385*KD2	8.461bcde	GKT 3213*KD2	3.979cdefg	GKT 3213*KD2	63.245abcde
GKT 376*KD1	8.435cde	GKT 3213*K	3.959cdefgh	GKT 3385*K	63.200abcde
GK Silostar*MLT	8.265def	GK Silostar*KD2	3.930defgh	GKT 3385*KD2	63.187bcde
GKT 3385*K	8.224ef	GKT 3213*MLT	3.916efgh	GK Silostar*KD1	63.183bcde
GK Silostar*KD1	8.086f	GK Silostar*MLT	3.904fgh	GK Silostar*MLT	63.105cde
GKT 3385*MLT	8.082f	GKT 3213*KD1	3.854gh	GK Silostar*K	63.068de
GKT 3385*KD1	8.051f	GK Silostar*KD1	3.833h	GK Silostar*KD2	62.878e

Table 5 (continued)

95% confidence interval					
LS means					
Protein content		Oil content		Starch content	
p	0.446		0.695		0.707
*Year*Biostimulant LSD*					
2019*KD1	9.875a	2019*KD1	4.442a	2021*KD1	65.875a
2019*K	9.842a	2019*MLT	4.436a	2021*K	65.700ab
2019*MLT	9.772ab	2019*KD2	4.433a	2021*MLT	65.692ab
2019*KD2	9.600b	2019*K	4.433a	2021*KD2	65.475b
2021*KD2	9.342c	2020*K	3.907b	2019*K	64.150c
2021*MLT	8.908d	2021*K	3.883bc	2019*KD2	64.108c
2021*K	8.825d	2021*KD2	3.867bcd	2019*MLT	64.039c
2021*KD1	8.542e	2021*MLT	3.850bcd	2019*KD1	63.858c
2020*K	6.913f	2020*KD2	3.781cd	2020*MLT	60.394d
2020*KD2	6.912f	2021*KD1	3.750de	2020*KD1	60.383d
2020*MLT	6.491g	2020*KD1	3.643ef	2020*KD2	60.033e
2020*KD1	6.480g	2020*MLT	3.617f	2020*K	59.916e
p	**< 0.0001**		**0.014**		**0.009**

The lowercase letters represent the status of significance among the values obtained from the analysis

p value, the significant differences were highlighted

the highest grain yield (10.5 t/ha). This was followed by GKT 3385 with 9 t/ha and GKT 376 with an average grain yield of 8.7 t/ha. The average grain yield of GKT 3213 was the lowest (7.6 t/ha). The other grain yield component, the grain moisture content at harvest was the highest for GK Silostar, with the average value of 17.8%, followed by GKT 376 and GKT 3385 with 15.3% and 15.2%, respectively, and finally, by GKT 3213 with the average value of 12.5%. The average value of grain dry matter content was the highest for the hybrid GKT 3213 (42.3%), followed by GKT 3385 and GKT 376 with 42.0% each, and finally, by GK Silostar with the lowest value of 41.7%. One of the traits that determine the kernel nutritional value of hybrids is protein content. The highest value for protein content was found at hybrid GKT 3213 (8.7%), followed by GKT 3385 with 8.6% and GK Silostar and GKT 3385 with 8.3% and 8.2%, respectively. In the average of the hybrids, GKT 3385 had the highest oil content with the value of 4.1%, followed by GKT 376, GK Silostar and GKT 3213 with 4.0%, each. As regards of starch content, the rank was 63.4% for GKT 3213 and GKT 376, 63.3% for GKT 3385, and 63.1% for GK Silostar. The results found for the traits determining the grain yield and the kernel nutritional value, respectively, also supported the conclusion that the hybrids were at different stages of maturity.

Table 6 Correlation analysis of the yield determining and the nutritional value determining traits

	Y	GM	DM	P	O
GM	− 0.243*				
DM	0.252*	− 0.783***			
P	− 0.062 ns	− 0.433***	0.701***		
O	0.367***	− 0.717***	0.739***	0.648***	
S	− 0.466***	− 0.029 ns	0.364***	0.753***	0.301***

Y, grain yield; GM, grain moisture; DM, grain dry-matter; P, protein; O, oil; S, starch

$^{***}P = 0.1\%$; $^{**}P = 1\%$; $^{*}P = 5\%$; ns, not significant

The effect of the biostimulant treatments on the observed traits

The third factor that affected the yield was the biostimulant treatment. Inoculation of such PGPR showed a positive effect on yield in maize (Ferreira et al. 2013; Noumavo et al. 2013; Abo-kora 2016). Based on the three-year averages, the highest grain yield was measured in the KD2 treatment amounting to 9.3 t/ha, followed by the MLT treatment with 8.9 t/ha, the control (K) with 8.8 t/ha and finally, the KD1 treatment with 8.6 t/ha grain yield. The KD2 treatment resulted in 5.7% and the MLT treatment in 1.1% yield increase compared to the control (K). A yield loss of 2.3% was observed in the KD1 treatment. Zafar-ul-Hye et al. (2014) and Iqbal et al. (2016) reported increased growth and grain yield in maize inoculated with PGPR. According to Ullah and Bano (2015), the yield was higher in *Pseudomonas putida* and *Bacillus pumilus* inoculated plants. Regarding the yield-determining traits, the grain moisture

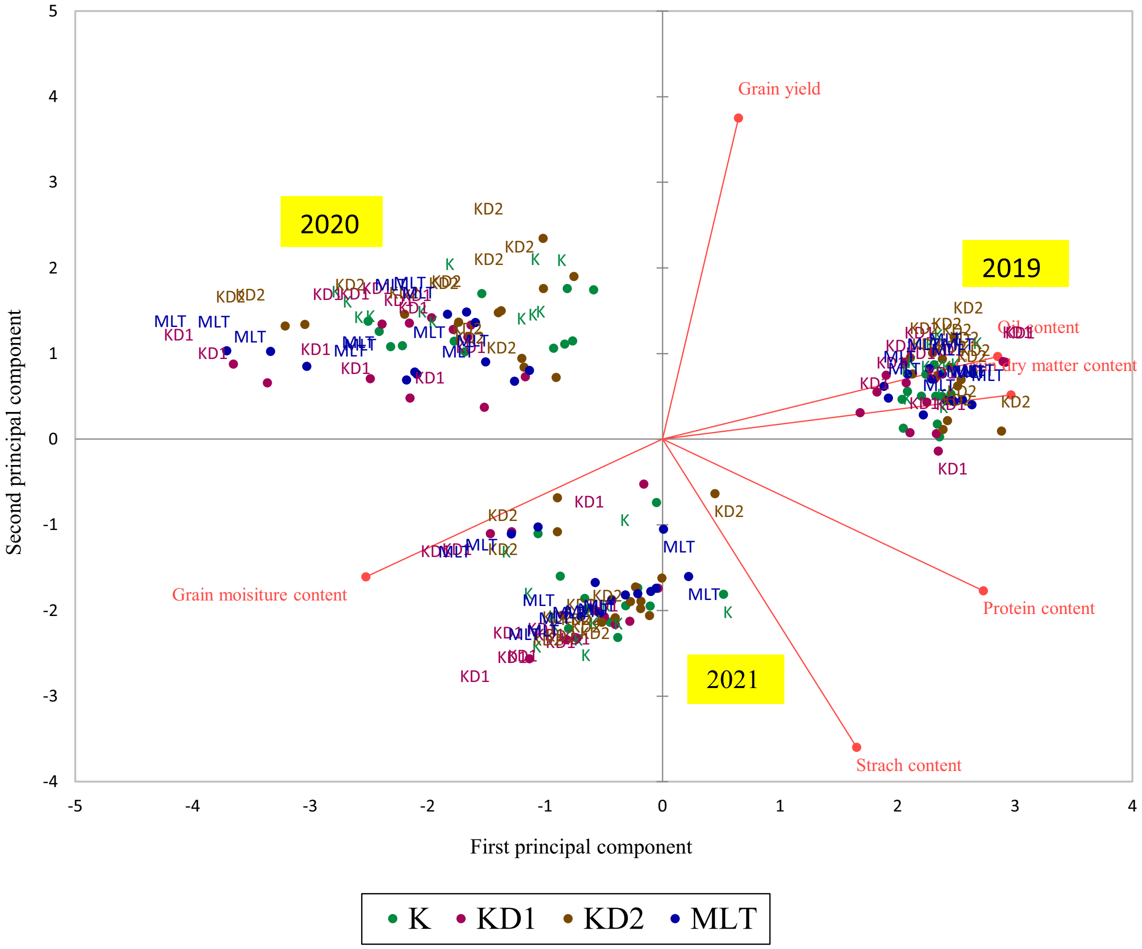

Fig. 5 The results of the principal component analysis exploring the correlation between the applied treatments, including (K): control; (KD1): *Bacillus megaterium*, *Pseudomonas fluorescens;* (KD2): *Bacillus pumilus*, *Pseudomonas putida;* (MLT): *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae;* and the 3-year data on grain yield, grain moisture content, grain dry matter content, and protein, oil and starch content, respectively

content at harvest was higher in the KD1 and MLT treatments (16.0% and 15.9%, respectively), than in the case of the control (K). The lowest grain moisture content at harvest was measured in the KD2 treatment with the value of 15.5%. This is 1.9% below the control (K). No significant difference could be observed due to the treatments compared to the control (K) in the three-year averages of the grain dry matter content. The grain dry matter content with its value of 42.0% proved to be constant throughout the trial. Among the traits that determine the nutritional value of the hybrids, the KD2 treatment increased the protein content to 8.6% compared to the 8.5% of the control (K). The data of the MLT and KD1 treatments with the values of 8.4% and 8.3%, respectively, were below the control (K). The average values of the oil content reflected that the treatments did not cause any difference in this trait compared to the control (K). The hybrids in the trial showed 4.0% oil content. The average values of starch content showed that there was no difference among the treatments compared to the control (K) and values around 63.0% were recorded for each treatment. The results demonstrated that the KD2 and MLT biostimulant treatments gave a yield increase compared to the control (K). Moreover, in the case of the KD2 treatment, even the grain moisture content at harvest was lower, and there was no deterioration in the traits determining the kernel nutritional value of the hybrids.

The effect of the interaction of biostimulant treatments and maize genotypes on the studied traits

The quantity of the yield was also influenced by the interaction of the biostimulant treatment and the maize hybrids. Over the three-year average, the hybrid GK Silostar produced the highest grain yield in the KD2 treatment with a value of 11.1 t/ha, followed by the MLT treatment with

10.4 t/ha. This corresponds to a grain yield increase of 7.8% in KD2 and 0.9% in MLT treatments, respectively, compared to the control (K) with the value of 10.3 t/ha. In the KD1 treatment, a grain yield loss of 1.9% was shown. Among the yield-determining traits, the MLT treatment resulted in a value of 18.2% for the average of the grain moisture content at harvest, which was higher than the value of the control (K) (17.8%). The results of the KD1 treatment did not differ from the data of control (K). On the other hand, the value was 17.4% in the KD2 treatment, which proved to be 2.2% lower compared to the value of the control (K). The average values of grain dry-matter content demonstrated, that in 41.8% of the treatments, the results did not differ from the control (K). Regarding the traits determining the kernel nutritional value, in the case of GK Silostar, the treatments with biostimulants did not cause any difference in the protein content compared to the control (K) with the value of 8.5%. The same conclusion could be drawn based on the averages of oil and starch contents with the values of 4.1% and 63.1%, respectively. Based on the data of its traits and the three-year average values, GK Silostar, which is a mid-ripening silage maize hybrid (FAO 490), proved to deliver 0.8 t/ha higher grain yield as a result of the KD2 (*Bacillus pumilus*, *Pseudomonas putida*) treatment, at 2% lower grain moisture content, and with the grain dry-matter, protein, oil and starch content equal to the control (K) value. The three-year averages showed that the highest grain yield of the GKT 3385 hybrid was found in the KD2 treatment at 9.6 t/ha, followed by the MLT treatment at 9.0 t/ha, corresponding to a yield increase of 11.6% in KD2, and 4.6% in the MLT treatment, compared to the control (K) with a value of 8.6 t/ha. No difference was observed in the KD1 treatment compared to the control (K) (8.6 t/ha). Among the yield determining traits, the grain moisture content at harvest was higher in the MLT treatment with a value of 15.6% than the control (K) (15.2%). In the case of the KD1 treatment, the same value was 15.4%. In case of KD2 treatment, the value was 14.7%, which was 3.3% lower compared to the control (K). The averages of the grain dry-matter content showed that in 42.0% of the applied treatments the results were not different from those in the control (K). Considering the protein content among the traits determining the nutritional value of GKT 3385, the KD2 treatment resulted in 8.5% protein content, compared to the control (K) with the value of 8.2%. The KD1 and MLT treatments did not lead to any difference compared to the control (K). Based on the averages of the oil and starch content, no difference was found on the influence of the biostimulant treatments compared to the control (K), shown by the data of 4.2% and 63.2%, respectively. The analysis of the recorded traits and their three-year average demonstrated that the KD2 (*Bacillus pumilus*, *Pseudomonas putida*) treatment in the case of GKT 3385 increased the grain yield by 3.3%, that is, 1 t/ha, accompanied by lower grain moisture content and the same grain dry-matter, protein, oil and starch content as the control (K). Taking the three-year averages, the highest grain yield of the GKT 376 hybrid was measured in the KD2 treatment with a value of 9.2 t/ha, equivalent to a grain yield increase of 4.5% compared to the control (K) (8.8 t/ha). No difference could be observed in the KD1 treatment compared to the control (K) (8.8 t/ha). In the case of the MLT treatment, however, 6.8% yield loss occurred. The value for the average grain moisture content at harvest among the yield determining traits was in the KD1 treatment higher (15.6%) than at the control (K) (15.4%). The same value in the KD2 and MLT treatment was 15.1%, which was 1.9% lower compared to the control (K). As regards the averages of the grain dry-matter content, in 42.0% of the treatments the results did not differ from the control (K). Among the traits that determined the nutritional value of GKT 376, no difference was found in the case of the KD2 and MLT treatments in the protein content compared to the control (K) with the value of 8.6%. In the KD1 treatment, however, 0.2% less protein content was measured in comparison with the control (K). Based on the averages of the oil and starch contents, no difference was recorded in the biostimulant treatments compared to the control (K), with the values of 4.1% and 63.3%, respectively. The data and their three-year average showed that GKT 376 could deliver a higher grain yield of 0.4 t/ha as a result of the KD2 (*Bacillus pumilus*, *Pseudomonas putida*) treatment, together with 9% lower grain moisture content and the same grain dry-matter, protein, oil and starch content as the control (K). In the three-year averages, the highest grain yield of the GKT 3213 hybrid was measured in the MLT treatment at 8.1 t/ha. This corresponded to a yield increase of 5.2% compared to the control (K) (7.7 t/ha). On the other hand, a yield loss was observed for this hybrid in the KD2 treatment of 2.6% and in the KD1 treatment 9.1%, respectively, compared to the control (K). For the grain moisture content at harvest among the yield-determining traits, the value was higher (15.2%) in the KD1 treatment than for the control (K) with a value of 14.7%. In the case of the MLT treatment, the measured value was 14.6%, which is 0.7% lower compared to the control (K). In the KD2 treatment, this value was the same as for the control (K). Concerning the grain dry-matter content, the analysis revealed no difference in the results in 42.4% of the treatments compared to the control (K). Regarding the protein content among the traits determining the kernel nutritional value, in the case of GKT 3213 no difference was observed in the MLT and KD1 treatments compared to the control (K) (8.7%). In the KD2 treatment, the protein content was 0.3% higher compared to value of the control (K). Based on the averages of the oil and starch contents, no difference was observed due to the biostimulant treatments compared to the control (K), the values being 4.0% and 63.5%, respectively. Based on the data of the tested traits and their three-year average, the MLT (*Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces cerevisiae*) treatment was found to induce a 0.4 t/ha higher grain yield in the case of GKT 3213 to the untreated control (K), in addition to 0.7% lower grain moisture content and with the same grain dry-matter, protein, oil and starch content as the control (K).

The results of the present study have revealed that the biostimulants constituted from beneficial microorganisms and applied in maize production, affect the crop yielding ability along with the year effect, genotype and nutrient supply. The efficiency of biostimulant treatment as a single factor has also been shown, as far as the components and quantity of yield, and the traits determining the nutritional value were concerned. The efficacy of biostimulant treatment is predominantly influenced by the interaction of the maize genotypes and the interacting microorganisms.

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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

Alvarez MI, Sueldo RJ, Barassi CA (1996) Effect of Azospirillum on coleoptile growth in wheat seedlings under water stress. Cereal Res Commun 24:101–107

Barett G, Marsh S (2001) Challenges for contemporary extension: the case of Biofertiliser in Vietnam. Retrieved from The Regional Insitute Online Publishing website. http://www.regional.org.au/au/apen/2001/non-refereed/BarrettG.htm

Bowen GD, Rovira AD (1991) The rhizosphere, the hidden half of the hidden half. In: Plant roots: the Hidden half, pp 641–669

Brown RA, Rosenberg NJ (1999) Climate change impacts on the potential productivity of corn and winter wheat in their primary United States growing regions. Clim Change 41:73–107

Burr TJ, Ceasar A (1984) Beneficial plant bacteria. Crit Rev Plant Sci 2:1–20

Cakmakci R, Bönmez F, Aydın A, Sahin F (2006) Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38:137–143

Claassen MM, Shaw RH (1970) Water deficit effects on corn: II. Grain components. Agron J 62:652–655

Dutta S, Signgh PR, Jindal JK (2005) Effect of antagonist bacteria plant defence activators on management of bacterial leaf spot of mungbean. Indian Phytopathol 58:269–275

Egamberdiyeva D, Hoflick G (2004) Effect of plant-growth promoting bacteria growth and nutrient uptake of cotton and pea is a semiarid-region of Uzbekistan. J Arid Environ 56:293–301

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

Fusseder A (1984) Der Einfluss von Bodenart, Durchlüftung des Bodens, Ernährung and Rizosphärenflora auf die Morphologie des seminalen Wurzelsystems von Mais. Z Pflanzenernähr Bodenk 147:553–565

Gaur AC (1990) Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, p 176

Gerretsen FC (1948) The influence of microorganisms on the phosphate intake by the plant. Plant Soil 1:51–81

Glick BR (2001) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21(3):83–393

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39

Glick BR, Pasternak JJ (2003) Plant growth promoting bacteria. In: Glick BR, Pasternak JJ (eds) Molecular biotechnology principles and applications of recombinant DNA, 3rd edn. ASM Press, Washington, pp 436–454

Guo JH, Qi HY, Guo YH, Ge HL, Gong LY, Zhang LX (2004) Biocontrol of tomato wilt by plant growth promoting rhizobacteria. Biol Control 29:66–67

Gururani MA, Upadhyaya HP, Baskar V, Venkatesh J, Nookaraju A, Park SW (2012) Plant growth promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-Scaverning enzymes and improved photosynthetic performance. J Plant Growth Reg 32:245–258

Hamzei J (2012) Seed N concentration, nitrogen use efficiency and growth of barley under combinations of bio and chemical N fertilizer application. Int Res J Appl Basic Sci 3(1):70–76

Han HS, Supanjani LKD (2006) Effect of co- inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52:130–136

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 Agric Biol 18(1):37–45

Katznelson H, Bose B (1959) Metabolic activity and phosphate dissolving capability of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Can J Microbiol 5:79–85

Kennedy IR, Choudhury AIMA, Kecskes ML (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Boil Biochem 36(8):1229–1244

Khan AG (2005) Role of soil microbes in the rhizosphere of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Kildea S, Ransbotyn V, Khan MR, Fagan B, Leonard G, Mullins E, Doohan FM (2008) Bacillus megaterium shows potential for the biocontrol of *Septoria tritici* blotch of wheat. Biol Control 47:37–45

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 268:885–886

Kloepper JW, Lisfhitz R, Schroth MN (1988) *Pseudomonas* inoculants to benefit plant production. Anim Plant Sci 1:60–64

Lévai L, Veres SZ, Gajdos É, Bakonyi N (2008) The possible role of bacteria containing bio-fertilizers in sustainable agriculture. J Soil Sci Plant Nutr Spec Issue 8(3):188–189

Lévai L, Veres SZ, Tóth B, Bákonyi N, Gajdos É, Faragó E, Marozsán M, (2010) Necessity to use living bacteria in plant nutrition. In: Maric S, Loncarié Z (eds) Proceedings of 45th Croatian and 5th international symposium on agriculture, Opatija, 2010. February 14-09, pp 818–822

Moorel SJ, Biedendieck R, Deery E, Warren MJ (2014) Towards a cell factory for vitamin B12 production in *Bacillus megaterium*: bypassing of the cobalamin riboswitch control elements. New Biotechnol 31(6):553–561

Morgenstein E, Okon Y (1987) Promotion of plant growth and NO_3^- and Rb+ uptake in *Sorghum bicolor* × *Sorghum sudanense* inoculated with *Azospirillum brasilense* Cd. Arid Soil Res Rehabil 1:211–217

Nagy L, Nagy LG, Tóth B, Makleit P, Veres Sz (2013) Effect of biofertilizers on physiological parameters of sunflower (Helianthus annuus L.). In: Tóth E (ed) The farm of the future. 55th Georgikon Days international scientific conference. Keszthely, p. 74

Nostrati R, Owlia P, Saderi H, Rasooli I, Malboobi MA (2014) Phosphate solubilization characteristics of efficient nitrogen fixing soil Azotobacter strains. Iran J Microbiol 6(4):285–295

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. Am J Plant Sci 4(05):101

Ortíz-Castro R, Valencia-Cantero E, López-Bucio J (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signalling. Plant Signal Behav 3(4):263–265

Pal KK, Tilak KVBR, Saxena AK, Dey R, Singh CS (2001) Suppression of maize root diseases caused by *Machrophomina phaseolina*, *Fusarium miniliforme* and *Fusarium gramineraum* by plant growth promoting rhizobacteria. Microbiol Res 156:209–223

Patten CL, Glick BR (2002) Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. Can J Microbiol 48:635–642

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Plant Soil 118:10–15

Pepó P, Pepó P (1993) Biological background of sustainable (*Zea mays* L.) production. Landsc Urban Plan 27:179–184

Priest F (1993) Systematics and ecology of Bacillus. In: Sonenshein AL, Hoch J, Losick R (eds) *Bacillus subtilis* and other gram-positive bacteria biochemistry, physiology and molecular genetics. American Society for Microbiology Press, Washington, pp 3–16

Revilla P, Malvar RA, Abuin MC, Ordás B, Soengas P, Ordás A (2000) Genetic background effect on germination of su1 maize and viability of the su1 allele. Maydica 45:109–111

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. Aust J Plant Physiol 28:897–906

Rovira AD, Bowen GD, Foster RC (1983) The significance of rhizosphere microflora and mycorrhizas in plant nutrition. In: Launchli A, Bielski RL (eds) Encyclopaedia of Plant Physiology, New series, vol 15A. Springer, Berlin, pp 61–89

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminas in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Sárvári M, Pepó P (2014) Effect of production factors on maize yieldand yield stability. Cereal Res Commun 42(4):710–720

Stajner D, Kevreaan S, Gasaic O, Mimica-Dudic N, Zongli H (1997) Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. Biol Plant 39:441–445

Steenhoudt O, Vanderleyden J (2000) Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24(4):487–506

Sudhakar P, Chattopadhyay GN, Gangwar SK, Ghosh JK (2000) Effect of foliar application of Azotobacter, Azospirillum and Beijerickia on leaf yield and quality of mulberry (*Morus alba*). J Agric Sci 134:227–234

Suslow TV (1982) Role of root-colonizing bacteria in plant growth. In: Mount MS, Lacy GH (eds) Phytopatonic procariotes, vol. 1. Academic Press, New York, pp. 187–223

Tóth B, Kovács B, Lévai L, Veres SZ (2015) The big question: use of agriculture or not? In: Plant KK, Sinha S, Bajpai S, Govil JN (eds) Fertilizer technology, vol 2. Studium Press LLC, Biofertilizers, pp 132–147

Ullah S, Bano A (2015) Isolation of PGPRs from rhizospheric soil of halophytes and its impact on maize (*Zea mays* L.) under induced soil salinity. Can J Microbiol 11:1–7

Veres SZ, Lévai L, Marozsán M, Gajdos É (2009) The effect of plant growth promoting bacteria containing fertilizer to the photosynthetic activity of maize seedlings. In: Giametta G, Zimbalatti G (Eds) Proceedings of XXXIII CIOSTA CIGIR V conference and IUFRO (Unit 3.06.00)

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wu QS, Li GH, Zou YN (2011) Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* (L.) Batsch) seedlings. J Anim Plant Sci 21(4):746–750

Zafar-ul-Hye M, Farooq HM, Zahir ZA, Hussain M, Hussain A (2014) Application of ACC-deaminase containing rhizobacteria with fertilizer improves maize production under drought and salinity stress. Int J Agric Biol 16:591–596

Zahir AZ, Arshad M, Frankenberger WT Jr (2004) Plant growth promoting rhizobacteria: application and perspectives in agriculture. Adv Agron 81:97–168