Promotion of plant growth of maize by plant growth promoting bacteria in different temperatures and soils

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ABSTRACT

Plant-growth-promoting bacteria isolated from the plants grown in different climatic regions of Germany and Uzbekistan were analyzed for plant-growth-promoting effects and nutrient uptake in maize on different soils and under different temperature regimes. The investigations were carried out in pot and field experiments using loamy sand soil from Müncheberg, Germany and Calcisol soil from Tashkent, Uzbekistan. The temperature and soil types were found to influence growth-promoting effects. Inoculation with bacterial strains, *Pseudomonas fluorescens* PsIA12, *Pantoea agglomerans* strain 370320, strain 020315 and strain 050309 isolated from a temperate-climate location (Müncheberg, Germany) was found to significantly increase the root and shoot growth of maize (*Zea mays* L.) grown in loamy sand at 16°C compared to 26°C . Bacterial inoculation also resulted in significantly higher values for plant growth and N, P, and K content of plant components in field experiments. Bacteria isolate *Bacillus amyloliguefaciens* BcA12, isolated from Tashkent in a semi-arid climate, was found to significantly increase the root, shoot growth and nutrient uptake of maize in nutrient-poor Calcisol at 38°C than in nutrient-rich loamy sand at 16°C.

KEY WORDS

Plant growth promoting bacteria, maize, nutrient uptake, soil type, temperature

INTRODUCTION

Beneficial effects of rhizosphere bacteria are most often based on increased plant growth and called plant-growth-promoting rhizobacteria (PGPR) (Kloepper *et al*., 1980). Rhizosphere bacteria *Pseudomonas* spp., *Azospirillium* spp., *Pantoea* spp., *Agrobacterium* spp., increased plant growth and the nutrient uptake of maize, wheat and legumes (Ruppel, 1987; Höflich and Kuhn, 1996; Höflich *et al*., 1994; 1997; Boddey and Döbereiner, 1995; Okon, 1991).

The mechanisms of PGPR are mobilization of nutrients, production of phytohormones, and nonsymbiotic nitrogen fixation (Bothe *et al*., 1992; Sarwar, 1992; Höflich *et al*., 1994). Increased uptake of nutrients such as N, P, and K was suggested as one of the mechanisms by which PGPR increased crop yield (Kapulnik *et al*., 1985).

Many factors could contribute to the inconsistent performance of PGPR, including complex interactions among host, rhizobacteria and the soil environment. Two of the most important factors are soil type and temperature. Therefore, studies on the effect of different temperatures and soils on plant-growth-promoting bacteria efficiency would be very important. The major objective of our research was to study the effect of the plant-growth-promoting bacteria isolated from the different climatic regions on the growth of maize at different temperatures and soils.

Table 1. Soil chemical properties, and soil particle distribution at 0-30 cm soil layer

Site	Type	C_{tot}	$N_{tot.}$	$P_{tot.}$	K	Mg	pH	soil particle size, mm		
		---------mg (100 g)$^{-1}$----------						2 – 0.2 %	0.2 – 0.02 %	< 0.02 %
Müncheberg	Loamy sand	700	60	6.2	7.4	3.7	6.9	7.6	79.8	12.6
Tashkent	Calcisol	200	6	3.0	12.0	6.0	8.5	2.2	54.5	43.3

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MATERIALS AND METHODS

Plant and Soil

The experiments were carried out on loamy sand soil from Müncheberg and a Calcisol soil from Tashkent, Uzbekistan. The soil chemical and physical properties are presented in Table1. The total carbon content, C_{tot}, was identified by elementary analysis while total nitrogen, N_{tot}, content was determined by the Kjeldahl method. The molybdenum blue method determined the total phosphorus content, P_{tot}, in the soil. Potassium, K, was determined using the Flame Photometric Method (Riehm, 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chloride ($CaCl_2$) and extractable magnesium (Schachtschnabel and Heinemann, 1974). Soil pH-value was measured by means of electrometer. Soil particle distribution was determined using sodium phosphate**.** Maize cvs. Felix, Larix (Germany) and cv. Wir-200 (Uzbekistan) were employed as the inoculation experiments. Seeds of these plants were obtained from the Center for Agricultural Landscape and Land Use Research in Müncheberg, Northeastern Germany and from the University of Agriculture, Uzbekista.

Microorganisms

Rahnella aguatilis 6, *Pseudomonas fluorescens* PsIA12, *Pantoea agglomerans* strain 050309, strain 370320, strain 370308, strain 020315 and *Bacillus amyloliguefacines* BcA12 were used as the test microorganisms. The bacterial strains were isolated from the following plants: *R. aguatilis* 6, *P. fluorescens* PsIA12 from the rhizosphere of wheat, *P. agglomerans* strain 050309, strain 370320, strain 370308, and strain 020315 from the phyllosphere of triticale grown in loamy sand (Müncheberg) and *B. amyloliguefaciens BcA12* from soil of the root zone of wheat grown in Calcisol soil (Tashkent). For isolation of bacteria from the rhizosphere, 10 g of washed roots and bacteria from the phyllosphere and 10 g of leaves were macerated and shaken with 10 ml sterile water. For isolation of bacteria from the soil of the root zone, 10 g of soil from the root surface were shaken with 10 ml sterile water. The resulting suspensions were spread over the surface of a glycerol-peptone agar. After an incubation time of seven days at 28 °C, the bacterial strains were isolated from the plates and identified.

Identification of strains

The identification of strains relied on standard biochemical and physiological tests according to the classification of Bergey (1984) and using the Biological System (Behrendt, 1997). Gram stain, morphology, spore formation, motility, nitrate reduction, and gas production from glucose were determined according to methods by Gerhardt (1981). The auxin production was tested using Salkowsky's reagent (Sarwar *et al*., 1992). The strains were tested for properties such as nitrogenase activity (Ruppel, 1987), and antagonistic activity. *Fusarium culmorum,* were used as indicator strains for antagonistic bacteria. Bacteria isolates were tested on growth-plates on Hirte agar (Hirte, 1961). A small block of peptone dextrose agar with fungus was placed on the test plate. Bacteria isolates were streaked on the test plates perpendicular to the fungus. Plates were incubated at 28^{o}C until the fungi had grown over the control plates without bacteria. Antifungal activity was recorded as the width of the zone of growth inhibition between the fungus and the organism tested. Salt tolerance was determined in Hirte agar medium containing NaCl at 5-7%.

Plant growth and inoculation in pots

The study of the effect of isolated strains on plant growth and nutrient uptake was carried out in pot experiments using a nutrient-rich loamy sand soil originating from a moderate climate (Germany) and a nutrient-poor calcareous, Calcisol, soil from a semi-arid climate (Uzbekistan). Plants were grown in pots for four weeks under open natural conditions with a temperature of 36 °C to 38 °C during the day and 20 °C to 24 °C at night in summer (Uzbekistan). Also, the study of the effect of bacteria on plant growth was tested in plastic containers (5 cm diameter and 18 cm deep) with 350 g of soil placed in a temperature regulated growth chamber at a light intensity of 20 kLux for 16 h. at a temperature of 16 °C during the day, 12 °C at night, and 24kLux with 26 °C day and 16 °C night (Germany). The soil was moistened with water and maintained at 60% of its moisture holding capacity (MHC). The inoculation treatments were set-up in a randomized design with eight replicates. The day before sowing, pots (10 cm diameter and 13 cm deep) were filled with 500 g soil. Three seeds of maize were sown per pot. After the emergence of the seeds, plants were thinned to two per pot. The bacteria were grown in a glycerol-peptone-medium. Tubes were secured on a rotary shaker (120 rpm; 23 °C) and agitated for three days. Seedlings of these plants were inoculated with 1 ml of the bacterial suspension that resulted, with an inoculum density of ca. 10^6 cfu/ml. Control seeds received 1 ml glycerol-peptone-medium. Four weeks after germination, shoots and roots were separated and dried overnight at 105 °C before determining the root and shoot dry weight. The criteria for growth promotion were studied as root and shoot dry matter in a 6-leaf-stage and N, P, and K content of plants.

Field experiment

In Germany, another experimental field site on Salmtieflehm-Fahlerde (Arbeitsgruppe Boden, 1994) was established in a randomized block design with six replications (plot size: 15 m2, harvest was performed at the 12-leave stadium). Preceding crops were yellow lupin (*Lupinus luteus* L.) with under-sown cocksfoot (*Dactylis glomerata* L.). Farmyard manure (300 dt ha-1 fresh weight) was mixed into the soil by a milling machine before the sowing of corn. The seeds of plants were inoculated with the bacterial preparation (10^8 cfu / g preparation) (Höflich, 1987). The criteria for growth promotion were studied as root and shoot dry matter and the N, P, K and Mg content of plants.

Statistical analysis

The data were analyzed with a two-way ANOVA and Student-Newman-Keuls test for testing the significant differences ($\alpha = 0.05$) of main effects.

RESULTS

Growth promotion of maize by bacterial inoculants at different temperatures and soils.

Bacterial inoculation affected the early plant growth and the nutrient content of maize grown at different soils and temperatures.

Inoculation experiments at different temperatures showed that plant growth promoting bacteria

P. fluorescens Ps1A12, *P. agglomerans* strain 050309, strain 370320, and strain 020315 isolated from moderate climate were more effective at 16 °C than at 26 °C. The strain significantly increased shoot and root dry matter from 21 to 27% at 16 °C (Fig. 1).

B. amyloliguefaciens BcA12 isolated from a semi arid climate was more effective for maize in nutrient poor Calcisol soil at 38 °C than in nutrient-rich loamy sand at 16 °C (Fig.2). The strain significantly ($P = 0.05$) increased the root and shoot dry matter of maize in Calcisol soil at 38 °C from 16 to 37 % as compared to the control. This increase in biomass translated into significantly higher total N, P, and K contents. Bacterial inoculants had no significant effects on the percentage N and P of shoot material in loamy sand.

Increases in plant growth and nutrient uptake were recorded for treated plants (12 leaves stage) in field experiments (Fig.3). Strain *Rahnella aguatilis* 6 gave the best performance and resulted in a 27% increase in plant growth over the control. The various bacterial inoculants differentially influenced the N, P, K, and Mg contents of plant components. K content was increased in all treatments significantly. Only strain *Rahnella aguatilis* 6 resulted in the significant increase of N uptake.

DISCUSSION

This work demonstrated that independent of the origin, selected growth stimulating bacteria isolates (Rhizosphere, Phyllopshere, and Soil of the root zone) are able to increase the growth and nutrient uptake of maize in loamy sand and Calcisol soil at different temperatures and soils.

Increased nutrient uptake by plants inoculated with effective bacteria was attributed to the production of plant growth regulators by the bacteria at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil (Höflich *et al*., 1996). The positive effects of bacterial strains in these experiments indicated that bacterial production of plant-growth-promoting substances might be responsible for the observed effects. Several workers demonstrated the production of phytohormones by rhizosphere bacteria (Haahtela, 1990; Zimmer *et al*., 1995; Turjanista *et al*., 1995). In the present work, Auxin was detected in all bacterial suspensions.

The importance of physiological plant promotion characteristics may vary with

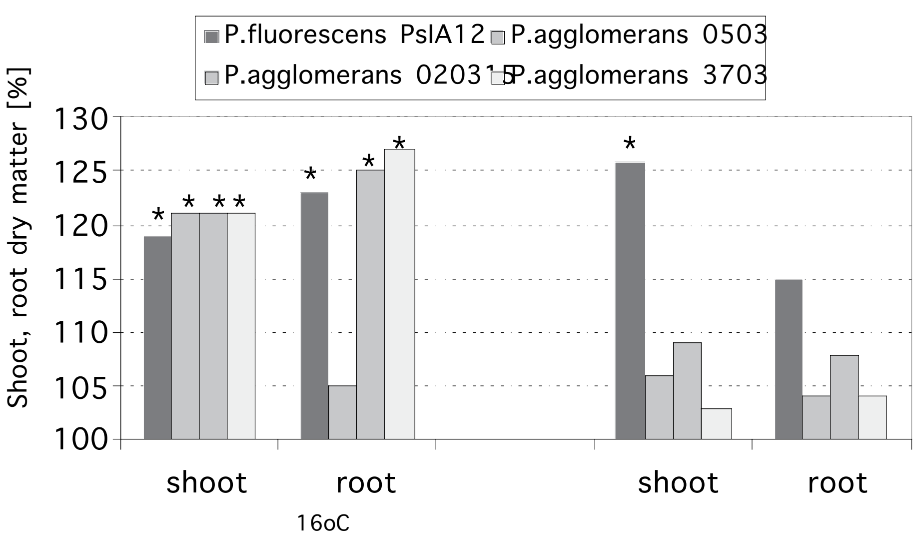

Fig. 1. The Influence of *Pseudomonas fluorescens* Ps1A12, *Pantoea agglomerans* 37/03/20, *P. agglomerans* 03/05/09 and *P. agglomerans* 02/03/15 on plant growth of maize at different temperatures (pot experiment, control=100)

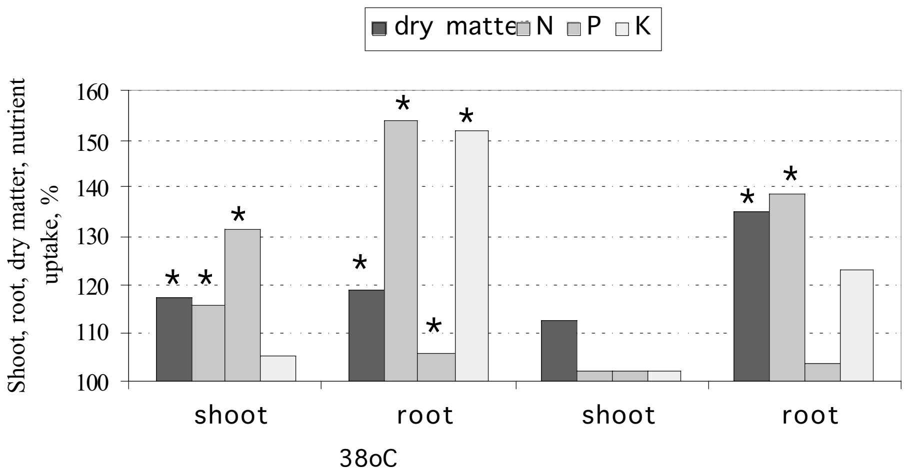

Fig. 2. Inoculation effect of *Bacillus amyloliguefaciens BcA12* on dry matter and nutrient uptake of maize at different soils and temperatures (pot experiment, control=100).

Also in our previous experiments, the same strain was effective at 38 °C rather than 16 °C for winter wheat and cotton (Egamberdiyeva and Höflich, 2001). From our results we suggest that plant growth promoting bacteria are effective in such conditions, which supposedly are adapted to the particular climatic conditions of an area. Waldon (1989) also demonstrated that rhizobial bacteria, isolated from nodules of the desert woody legumes *Prosopis glandulosa,* grew better at 36 °C than at 26 °C. They are physiologically distinct, suggesting adaptation to their respective environmental conditions.

soil and weather parameters (Höflich *et al*., 1994). This is partly affected by different growth stimulation effects after inoculation. In our study, a statistical enhancement in maize growth promotion by bacterial strains isolated from moderate climates was observed at a moderate temperature of 16 °C, rather than 26 °C. According to Höflich *et al*., (1994, 1996, 1997) *Pseudomonas* sp., *Rhizobium* sp. and *Agrobacterium* sp. isolated from the temperate climate promoted the growth of young plants and increased the yields of Gramineae, Legume, and Maize in temperate climates under field conditions. Also in our field experiments, bacterial strains *Rahnella aguatilis* 6, *P. agglomerans* 050309, and *P. agglomerans* 370308 increased the growth and nutrient uptake of maize.

Our bacteria *B. amyloliguefaciens* BcA12, isolated from a semi arid climate, significantly increased the plant growth and nutrient uptake of maize at 38°C compared to 16 °C.

Our bacteria *B. amyloliguefaciens* BcA12 from a semi-arid climate was more effective for maize in nutrient-poor Calcisol soil than in nutrient-rich loamy sand. Defreitas (1992 a,b) also demonstrated that in low fertility, Asquith soil, pseudomonas bacteria strains significantly enhanced early plant growth. Also, Paula *et al*. (1992) suggested that the magnitude of the plant response to any microbial inoculation is greatly affected by the nutrient content of soil. Bacterization only marginally increased yields when tested under ideal climatic situations. The greatest benefits occurred when crops encountered stressful conditions for prolonged periods (Lazarovits, 1997). Non-treated plants by comparison performed poorly under such conditions.

In summary, the final results of plant growth promotion in our experiments showed that plant-growth-promoting bacteria can play an essential role in helping the plant establish and grow. Effective bacteria-plant partners must be selected through vegetation trials with consideration to the specific ecological conditions (crop, soil, temperature). However, the extent of stimulation of plants by effective bacteria from Uzbekistan and their persistence in plant-growth-promotion activity under actual field conditions remains unclear. The experiments concerning stimulation of maize by effective strains from Uzbekistan must be followed by investigations under field conditions.

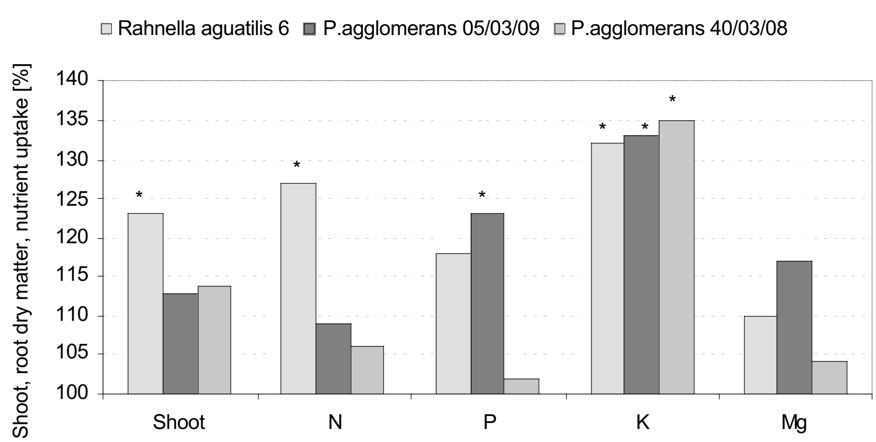

Figure 3. Inoculation effect of *Rahnella aguatilis* 6, *Pantoea agglmerans* 05/03/09 and *P. agglomerans* 37/03/08 on shoot dry matter and nutrient uptake of maize in field experiments with loamy sand (control=100).

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