Environ Monit Assess (2021) 193: 515
https://doi.org/10.1007/s10661-021-09286-6

Efficacy of rhizobacterial exopolysaccharides in improving plant growth, physiology, and soil properties

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Received: 23 March 2021 / Accepted: 12 July 2021 / Published online: 24 July 2021

Abstract The current study investigated the influence of exopolysaccharides (EPSs) producing plant growth–promoting rhizobacteria (PGPR) on the growth, physiology, and soil properties. The pre-isolated and compatible EPS producing PGPR strains were first screened based on improvement in soil aggregates in an incubation study. The screened strains (*Rhizobium phaseoli* strain Mn-6, *Pseudomonas bathysetes* strain LB_5, and unidentified strain R_2) were then employed in pot study for assessing improvements in maize growth, physiology, and soil properties. Eight treatments including T_1 = control, T_2 = Mn-6, T_3 = R_2, T_4 = LB_5, T_5 = Mn-6 + R_2, T_6 = Mn-6 + LB_5, T_7 = R_2 + LB_5, and T_8 = Mn-6 + R_2 + LB_5 were applied in completely randomized design (CRD) hexa replicated (half for root and half for soil, and yield attributes). The results depicted that among various treatments, the application of PGPR strain Mn-6 increased plant height, root length, root fresh and dry weight, root length density, SPAD value, leaf areas index, photosynthesis rate, transpiration, and stomatal conductance by 24, 79, 72, 90, 49, 35, 23, 21, 75, and 77%, respectively, compared with non-inoculated treatment. Similarly, significant improvement in maize yield and soil physical properties was also observed in response to the application of EPS-producing PGPR. Therefore, it is concluded that the application of EPS producing PGPR is an effective strategy to improve plant growth, physiology, yield, and soil physical properties. Moreover, EPS-producing PGPR should be exploited in field studies for their potential in improving plant growth and soil properties.

Keywords Exopolysaccharides · Plant growth–promoting rhizobacteria · Maize · Soil aggregates

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Introduction

The intensive agricultural practices with excessive inputs of chemicals (pesticides, herbicides, and synthetic fertilizers) and the use of improved crop plants (via targeted breeding and advanced genetic manipulation) enabled unprecedented gains in global food production to feed the increasing population (Fahad et al., 2021). Also, the undue inputs of chemical fertilizers give birth to various environmental problems. The situation demands a bio-revolution based on the biological inputs through the utilization of phytomicrobiome including inoculants (biofertilizers), microbially produced

compounds like EPS, and the manipulation of the phytomicrobiome community structure (Murgese et al., 2020; Timmusk et al., 2017). This will not only ensure agricultural sustainability with reduced environmental impact but also provide food to the growing population on a sustainable basis (Babur et al., 2021a).

Microorganism associations with plant roots play a significant role in plants to withstand various biotic and abiotic stresses (Etesami & Alikhani, 2017; Meena et al., 2017). Likewise, the involvement of microbes in various below ground processes like soil aggregation, cycling of nutrients, organic matter decomposition, and removal of toxins advocates their potential in uplifting agricultural productivity and soil fertility (Babur et al., 2021b; Deka et al., 2019; Shrivastava & Kumar, 2015). They also aid in developing tolerance in plants against various diseases and pathogens (Yang et al., 2009). Moreover, microbes and leguminous plants improve soil fertility and quality through bio-mineralization and synergistic co-evolution relationship (Agler et al., 2016; Paredes & Lebeis, 2016; Rosenberg & Rosenberg, 2016; Babur & Dindarogul, 2020). The co-evolution relationship also increases soil fertility, economic viability, and environmental sustainability by helping plants to respond to the extreme abiotic environment (Compant et al., 2016; Khan et al., 2016).

Plant growth–promoting rhizobacteria, which enhance plant growth through antagonistic and synergistic interactions, are the most explored microbes and plant associations (Bhardwaj et al., 2014; Rout & Callaway, 2012). The PGPR has the ability to convert barren poor-quality land into cultivatable land by influencing various soil characteristics. Therefore, PGPRs have been extensively exploited for the revitalization of soil quality and crop growth for improving agricultural productivity and sustainability (Fasciglione et al., 2015). Furthermore, some strains of PGPR can produce exopolysaccharides which have shown to possess excellent ability to improve soil properties and crop productivity (Costa et al., 2018). The term EPS includes the high molecular weight polymers composed of sugar moieties, a major component (40–95%) of the microbial biofilm (Davey & O'toole, 2000). Bacterial EPSs perform several functions regarding soil aggregate formation, enhancing water and nutrients available in the soil, and enhance the important enzyme activities (phosphomonoesterase, β-glucosidase, protease, arylsulfatase, and urease) in soil (Alami et al., 2000; Deka et al., 2019). The EPS-producing PGPR can increase water and fertilizer availability to the plant by enhancing the volume of soil macro-pores and the rhizosphere soil aggregation (Upadhyay et al., 2011).

The EPSs develop biofilms which not only help in the regulation of nutrients and water flow across the plant roots but also responsible for the regulation of soil aggregation (Khan et al., 2016). The bacterial EPSs form slime polymers (negatively charged) having adhesive potential for joining clay particles. These adhesive forces including hydrogen bonding, cation bridging, anion adsorption, and Van der Wall forces are responsible for the formation of microaggregates (Ashraf et al., 2005), which further adhere to the formation of macroaggregates (Cheng et al., 2020; Nwodo et al., 2012). Therefore, based on the above background and role of EPS producing PGPR in improving soil aggregation and plant growth, the current study was conducted to investigate the influence of EPS producing PGPR on soil aggregation, maize growth, physiology, and production.

Materials and methods

The current study was carried out at the Institute of Soil & Environmental Science (ISES), University of Agriculture, Faisalabad (UAF), Pakistan.

Experimental materials

Pre-isolated, characterized, and compatible microbial strains namely M_2, M_3, M_{11}, M_{19}, M_{22}, Mn-6, LB_5, and R_2 were taken from the Soil Microbiology and Biochemistry Lab, ISES, UAF. The soil used in the study was collected from the farm area of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Physicochemical characteristics of the soil used in the study are given in Table 1 was determined by the method of Ryan et al. (2001).

Preparation of media

Yeast mannitol broth (YMB) media following the recipe described by Busse and Bottomley (1989) was prepared using 10 g Mannitol, 0.50 g yeast extract, 0.50 K_2PO_4, 0.20 g $MgSO_4 \cdot 7H_2O$, and 0.1 g NaCl for the preparation of 1 L YMB. The pH of the media was maintained at a value of 6.5 and

Table 1 Physicochemical characteristics of the soil used in the study

Parameter	Symbol	Unit	Value
Sand	–	%	62.5
Silt	–	%	17.5
Clay	–	%	20
Textural class	–		
Bulk density	ℓb	Mg m^{-3}	1.40
Electrical conductivity	EC_e	dS m^{-1}	1.45
pH	–	–	7.9
Saturation percentage	SP	%	35.5
Sodium	Na^+	meq L^{-1}	3.58
Potassium	K^+	meq L^{-1}	0.67
Calcium and magnesium	$Ca^{2+} + Mg^{2+}$	meq L^{-1}	10.15
Carbonates	CO_3^{2-}	meq L^{-1}	0.72
Bicarbonates	HCO_3^-	meq L^{-1}	8.2
Chloride	Cl^-	meq L^{-1}	4.80
Sulfate	SO_4^{2-}	meq L^{-1}	0.78
Organic matter	OM	%	0.34
Total nitrogen	TN	g kg^{-1}	0.45
Available phosphorus	TP	mg kg^{-1}	9.5
Available potassium	TK	mg kg^{-1}	124.6
Field capacity	FC	%	19.6
Permanent wilting point	PWP	%	11.2
Plant available water	PAW	%	8.4

sterilized at 121 °C and 15 psi for 20 min. Furthermore, strains were inoculated in the respective broth and incubated at 28 ± 1 ºC for 48 h. The microbial cells were harvested by centrifuging the inoculated broth with a refrigerated centrifuge at 22 ºC and 9000 g for 15 min. The pellets were resuspended in 0.01 M $MgSO_4$ buffer and diluted to a population density of 10^{-6} to10^{-7} cells per mL ($OD_{560} = 0.45$). The consortium was made by taking equal volumes of resuspended cells in a sterilized container and vortexed 1 min for homogeneous mixing (Dar et al., 2020). Soil application of the consortium was done by 10 ml and 100 ml of broth in incubation and pot study, respectively, using a graduated cylinder.

Incubation study

An incubation study was undertaken for the screening of the effective PGPR strains pre-isolated and characterized for producing EPS. The soil with a field capacity of around 20% was first sterilized at 121 °C for 20 min for three consecutive days (Kohler et al., 2010), then about 200 g of this soil was taken in plastic cups of capacity 250 g. Nine treatments including T_1 = Control, $T_2 = M_2$, $T_3 = M_3$, $T_4 = M_{11}$, $T_5 = M_{19}$, $T_6 = M_{22}$, T_7 = Mn-6, $T_8 = R_2$, and $T_9 = LB_5$ were applied following completely randomized design (CRD) with three replicates. The plastic cups with microbial media were incubated at 28 °C in an incubator for 45 days, and field capacity was maintained by sterilized water through the gravimetric method. After 45 days, the screening of the microbial strains was done in accordance with the increased stabilized aggregates.

Pot study

The efficiency of screened microbial strains in improving maize growth, physiology, yield, and soil properties was evaluated by conducting a pot study. These strains include *Rhizobium phaseoli* strain Mn-6, *Pseudomonas bathysetes* strain LB_5, and unidentified strain R_2. Eight treatments namely T_1 = control, T_2 = Mn-6, $T_3 = R_2$, $T4 = LB_5$, T_5 = Mn-6 + R_2, T_6 = Mn-6 + LB_5,

$T_7 = R_2 + LB_5$, and $T_8 = Mn\text{-}6 + R_2 + LB_5$ were applied in CRD hexa replicated (half for root and half for soil, and yield attributes). About 1 kg of the soil was taken from the air-dried/sieved soil as a composite sample for the analysis of the initial fertility status of the soil before sowing. Chemical fertilizer was applied as per the recommended dose, i.e., 300:150:150 kg ha^{-1} NPK using urea, DAP, and SOP, respectively. Full P and K were applied at sowing while N was applied in three splits. About 100 ml of the culture was applied in each experimental pot after the germination of maize. The data for various growth parameters, i.e., plant height, leaf area index, SPAD value, photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency, were taken at physiological maturity, whereas the data regarding yield and root parameters, i.e., 100-grain weight, root fresh and dry weights, root length density, and cob weight, was recorded at harvesting. Plant and soil samples were taken for the determination of N, P, and K determination along with aggregate stability, soil bulk density, total porosity, soil organic matter concentration, soil field capacity, permanent wilting point, and plant-available water contents.

Analytical methods

Plant agronomical parameters like plant height, root length, root fresh and dry weight, cob, and 100-grain weight were determined using a measuring scale and analytical weight balance. The leaf area index (LAI) of maize was determined following Dwyer and Stewart (1986) formula as described below:

$$\mathrm{LAI} = L \times W \times A \quad (1)$$

where L represents maximum leaf length (cm), W represents maximum leaf width (cm), and "A" represents a correction factor, and for maize, its value is 0.75. The greenness of leaves (SPAD value) was measured by using SPAD-502 plus chlorophyll meter while portable photosynthesis system (CIRAS-3) was used for the determination of transpiration rate, photosynthetic rate, and stomatal conductance, and water use efficiency.

Grain and straw samples were digested using concentrated sulfuric acid and hydrogen peroxide following the method given by Wolf (1982) for the determination of N, P, and K. Nitrogen was measured using the Kjeldahl method (Jackson, 1962). Phosphorus in plant samples was determined by the yellow color method using a spectrophotometer, and a Flame photometer (Janway PFP-7) was employed to determine potassium.

Soil texture was determined by the Bouyoucos hydrometer method (Gee & Bauder, 1986). The water-stable aggregates were fractionated by soil wet sieving with vertically placed set of sieves ranging from 100 to 2000 µm (Six et al., 2006), and fractions were analyzed by soil aggregate analyzer. The method described by Nelson and Sommers (1996) was used for the determination of soil organic matter. Soil bulk density was determined by using a core sampler following the method described by Blake and Hartge (1986). Total porosity was calculated by using the formula described by Brady and Weil (1996) while the field capacity and permanent wilting point were measured by pressure membrane apparatus (Dane & Hopmans, 2002). The plant-available water contents were calculated by subtracting the water contents at the permanent wilting point from the contents at field capacity.

Statistical computation of the data

The recorded data was computed statistically by applying the one-way analysis of variance (ANOVA) technique under the CRD design. Whereas the significant differences between treatments were determined through Tukey's test/honestly significant difference (HSD) test at 5% probability (Montogomery, 2013). Sigma plot software (12.5 version, Sigma, Inc.) was used for plotting and data analyses.

Results

The current study was conducted for the assessment of EPS-producing PGPR in improving maize growth and production as well as soil aggregation stability. The outcomes of the study are presented here.

Microbial screening under incubation study

The effective EPS-producing PGPR strains were screened based on their improvement offered for soil aggregate stability in an incubation study. The findings depicted that the stability of macro-aggregates (with the size of > 2 mm and between 2 and 1 mm) was maximum in PGPR strain Mn-6 which was

29.3% and 20% higher than the values obtained in un-inoculated control (Table 2). The order of > 2 mm aggregate stability was T_7 (Mn-6) > T_9 (LB_5) > T8 (R_2) > T_3 (M_3) > T_5 (M_{19}) > T_6 (M_{22}) > T_4 (M_{11}) > T_2 (M_2) > T_1 (control). Similarly, the stability of 1–2-mm aggregates was 19.19, 18.37, 15.56, 7.41, and 5.19% higher than the control for T_9, T_8, T_5, T_2, and T_6 respectively. The PGPR strain M_3 reduced the stability of aggregates with size from 1 to 2 mm because the aggregate stability value was lower than the control without any inoculation.

Similarly, the stability of 1–0.5 mm micro-aggregates was observed to be lowest (19.3%) in PGPR stain T_3 (M_3) which was about 8.72% lower than control. However, the other treatments T_6 (M_{22}), T_2 (M_2), T_5 (M_{19}), T_9 (LB_5), T4 (M_{11}), and T_7 (Mn-6) have, respectively, around 4.36, 9.48, 12.8, 14.2, 17.5, and 19.9% higher stability values than the control for aggregates of size 1–0.5 mm. Likewise, the stability values for aggregates of size 0.5–0.25 mm in T_4 (M_{11}), T_5 (M_{19}), and T_3 (M_3) were about 12.6, 2.94, and 1.26% higher than the control, respectively, while other strains have lower stability values for 0.5–0.25 mm aggregates compared with the control. While in the case of aggregates of size 0.25–0.10 mm, all treatments have lower stability values than control.

Hence, it was concluded from the aggregate stabilization potential of these strains that the soil application of *Rhizobium phaseoli* strain Mn-6, *Pseudomonas bathysetes* strain LB_5, and unidentified strain R_2 significantly improved the aggregate stabilization capacity of soil under study and were selected for further investigation in pot studies.

Effect of EPS-producing PGPR on plant physiology under pot study

The findings regarding the influence of EPS producing PGPR application on the physiological parameters are presented in Fig. 1a–d. The results depicted that the use of a single microbial strain has an increasing effect on SPAD value (Fig. 1a); however, the maximum SPAD value was observed in the leaves of the plants receiving treatment of Mn-6 with a SPAD value of 51.6 which was around 35% higher than the control. The application of the PGPR consortium has both increasing and decreasing effect on SPAD value, i.e., the use of Mn-6 + LB_5 and R_2 + LB_5 have increased SPAD value as compared to control while application of either Mn-6 + R_2 or Mn-6 + R_2 + LB_5 decreased leaf SPAD value compared with the control. Similarly, the individual application of PGPR strain has increased the photosynthetic rate (Fig. 1b), and about 21, 19, and 12% higher photosynthetic rates were observed in treatments where Mn-6, R_2, and LB_5 have been applied alone, respectively. The consortium application has shown both increasing and decreasing effects on the photosynthetic rate. A similar trend of change was observed in transpiration rate and stomatal conductance (Fig. 1c, d) in response to the application of EPS producing PGPR.

Effect of EPS-producing PGPR on plant growth and yield

The results showed that the application of EPS producing PGPR significantly improved the growth and yield of maize (Fig. 2). The maximum plant

Table 2 Screening of EPS-producing PGPR on the basis of aggregate stability

Treatments	> 2 mm	2–1 mm	1–0.5 mm	0.5–0.25 mm	0.25–0.10 mm
Control	12.3 ± 0.12c	13.5 ± 0.10b	21.1 ± 0.03de	23.8 ± 0.10a–c	29.3 ± 0.09a
M2	13.2 ± 0.15bc	14.5 ± 0.11ab	23.1 ± 0.11b-d	20.5 ± 0.11c	28.7 ± 0.14a
M3	14.3 ± 0.08a–c	13.3 ± 0.09b	19.3 ± 0.14e	24.5 ± 0.14ab	28.6 ± 0.11a
M11	13.6 ± 0.14a–c	13.2 ± 0.14b	24.8 ± 0.09ab	26.8 ± 0.12a	21.6 ± 0.16b
M19	14.2 ± 0.15a–c	15.6 ± 0.27ab	23.8 ± 0.20bc	24.1 ± 0.10a–c	22.3 ± 0.14b
M22	13.8 ± 0.16a–c	14.2 ± 0.24ab	22.0 ± 0.19 cd	22.6 ± 0.16bc	27.4 ± 0.20a
Mn − 6	15.9 ± 0.17a	16.2 ± 0.16a	25.3 ± 0.24ab	22.0 ± 0.18bc	20.6 ± 0.21b
R2	15.3 ± 0.13ab	16.0 ± 0.17a	26.7 ± 0.06a	21.3 ± 0.15bc	20.6 ± 0.18b
LB5	15.7 ± 0.14a	16.1 ± 0.15a	24.1 ± 0.21bc	22.6 ± 0.14bc	21.5 ± 0.10b
LSD ($p < 0.05$)	**2.3823**	**2.4061**	**2.3598**	**3.8466**	**3.6954**

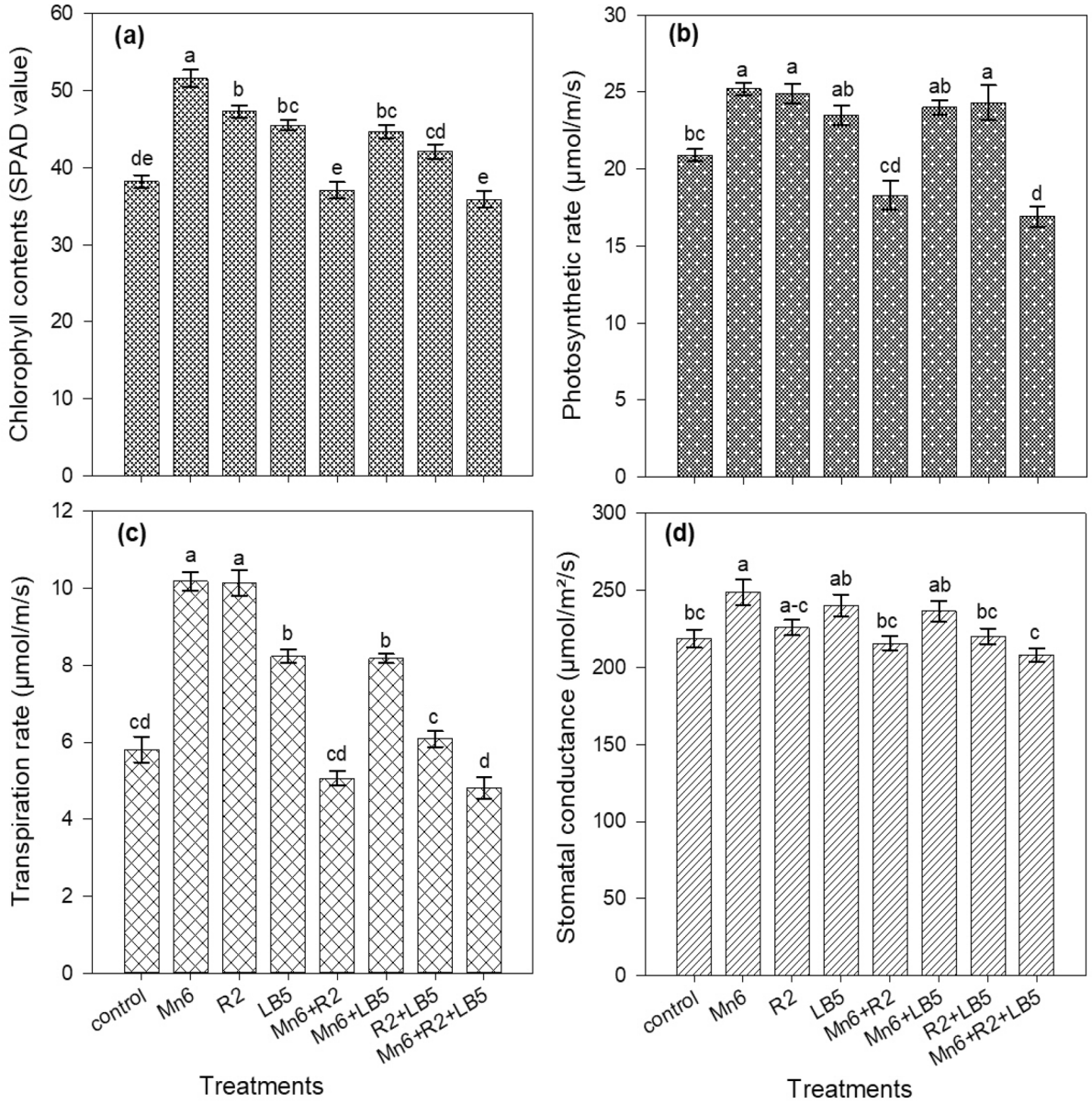

Fig. 1 Effect of EPS-producing PGPRs on physiological parameters: **a** SPAD value, **b** photosynthetic rate, **c** transpiration rate, **d** stomatal conductance ($p < 0.05$)

height (147.4 cm) and root length (38.3 cm) were recorded in treatment where only PGPR strain Mn-6 was applied (Fig. 2a). The observed plant height and root length were, respectively, 24.4% and 91% higher than the control treatment. Moreover, the plant height was 21% higher than the control with the application of Mn-6 + LB_5 and R_2 separately. On the other hand, the root length was about 76% and 42% higher compared with the control in the treatments LB_5 and Mn-6 + LB_5, respectively. The use of Mn-6 + R_2 and Mn-6 + R_2 + LB_5 negatively affected both plant height and root length.

Similarly, the highest root fresh and dry weight (79.4 g and 34.6 g, respectively) were observed

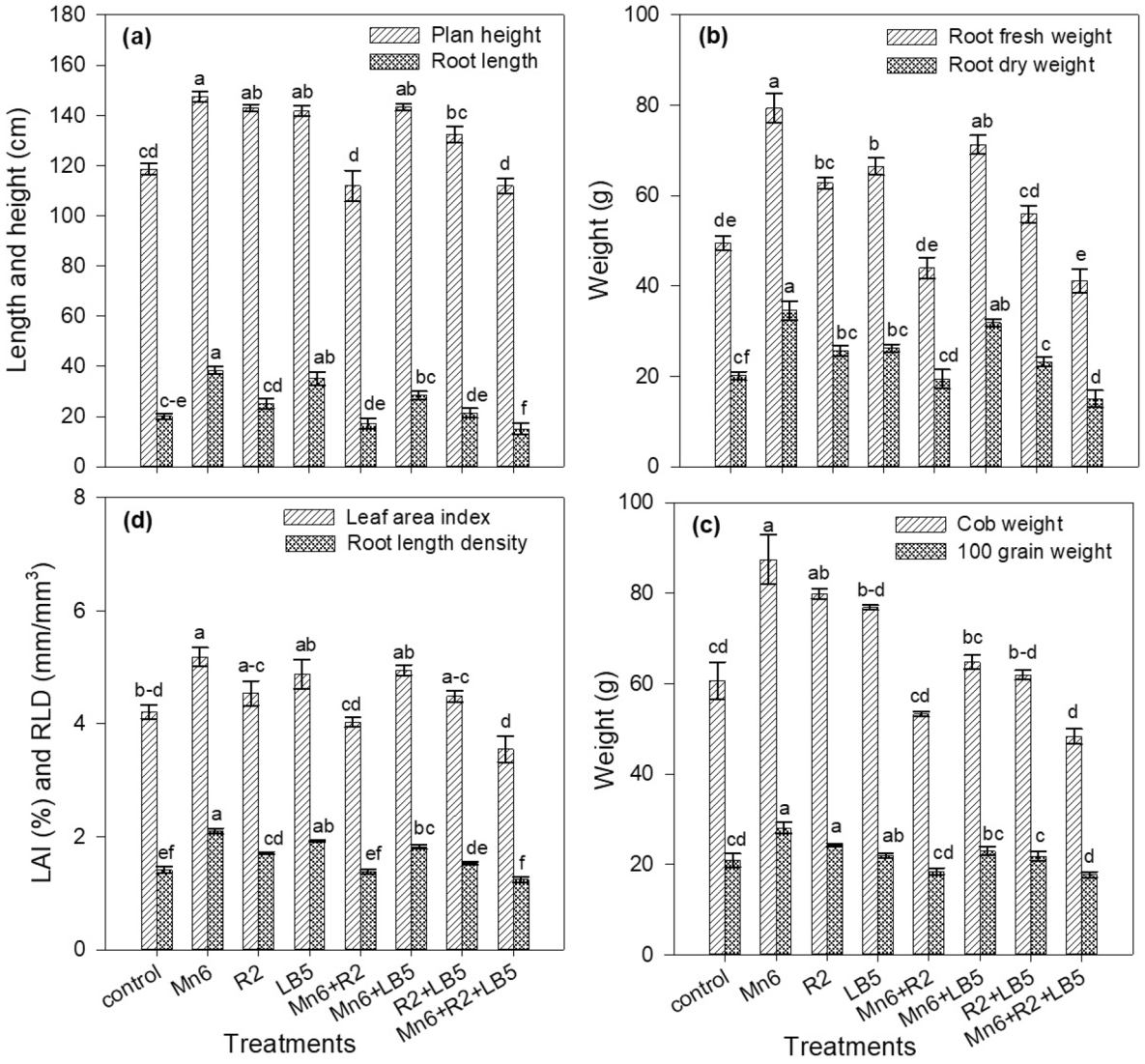

Fig. 2 Effect of EPS producing PGPR's on agronomical and yield parameters: **a** plant height and root length, **b** root fresh and dry weight, **c** Leaf area index and root length density, **d** cob weight, 100 grain weight ($p < 0.05$)

in the treatment containing PGPR strain Mn-6 alone (Fig. 2b). The observed root fresh and dry weights were 60.5% and 59.5% higher than control, respectively. The application of Mn-6 + R_2 and Mn-6 + R_2 + LB_5 consortia adversely influenced both root fresh and dry weight. A similar trend of change was observed for Leaf area index and root length density because of the application of EPS-producing PGPR as seen for root fresh and dry weight (Fig. 2c). Moreover, the yield of the crop significantly improved with the use of EPS producing PGPR (Fig. 2d). The maximum cob (87.4 g) and 100-grain weight (28 g) was recorded in the treatment inoculated with Mn-6 followed by the treatment containing R_2 strain. The minimum cob and 100-grain weight was observed in treatments containing consortia of microbial strains (Mn-6 + R_2 and Mn-6 + R_2 + LB_5).

Effect of EPS-producing PGPR on nutrient contents

The results regarding the macronutrient contents in corn grain and straw are given in Fig. 3. The maximum contents of nitrogen in grain and straw (1.52 and 0.84%, respectively) were observed in plants treated with EPS-producing strain Mn-6 followed by the treatment containing microbial strain LB_5. Regarding the application of strain in combinations, two combinations, i.e., Mn-6 + LB_5 and R_2 + LB_5 depicted a positive response toward the nitrogen contents in grain and straw. Under these treatments, respectively, 42.3 and 22.7% higher contents of nitrogen than control were found in grain while the nitrogen contents were 54 and 18% more than the control in the straw samples of the same treatments. Moreover, the statistical analysis depicted that the treatment effect was significant in the nutrient contents in grain and straw. A similar sequence of improvement in response to the application of EPS-producing PGPR was recorded for phosphorus and potassium in grain and straw of maize.

EPS-producing PGPR in relation to water use efficiency and soil water contents

The findings of the influence of EPS production by rhizobacteria on water contents at field capacity (FC) depicted that minimum water contents at field capacity of 19.5% were observed in the pots receiving consortium of all strains, i.e., T_8 (Mn-6 + B_2 + LB_5) which were 1.2% lower than the control treatment having 19.76% water contents at FC (Fig. 4a). The maximum water at field capacity (about 21%) was held by the pots treated with Mn-6 single strain which were around 6% higher than the water contents in the control treatment. Similarly, the results regarding soil water contents at permanent wilting point (PWP) showed that the lower water contents were observed in the treatments where a single microbial strain or a combination of two strains was applied (Fig. 4b). However, the minimum water contents at permanent welting point (10.1%) were observed in pots where Mn-6 microbial strain was incubated. Furthermore, around 11 and 10% higher water contents at PWP were observed in the pots receiving treatment of T5 (Mn-6 + R_2) and T8 (Mn-6 + R_2 + LB_5), respectively.

The amount of water freely available in the soil for the plant's growth and developments is termed plant-available water (PAW). The maximum value (10.9%) for PAW was found in the pots receiving the single strain of EPS-producing PGPR, i.e., Mn-6 (Fig. 4c). The contents of water available for plant uptake were about 18.6 and 15.2% lower in the pots containing the application of Mn-6 + R_2 + LB_5 and Mn-6 + R_2,

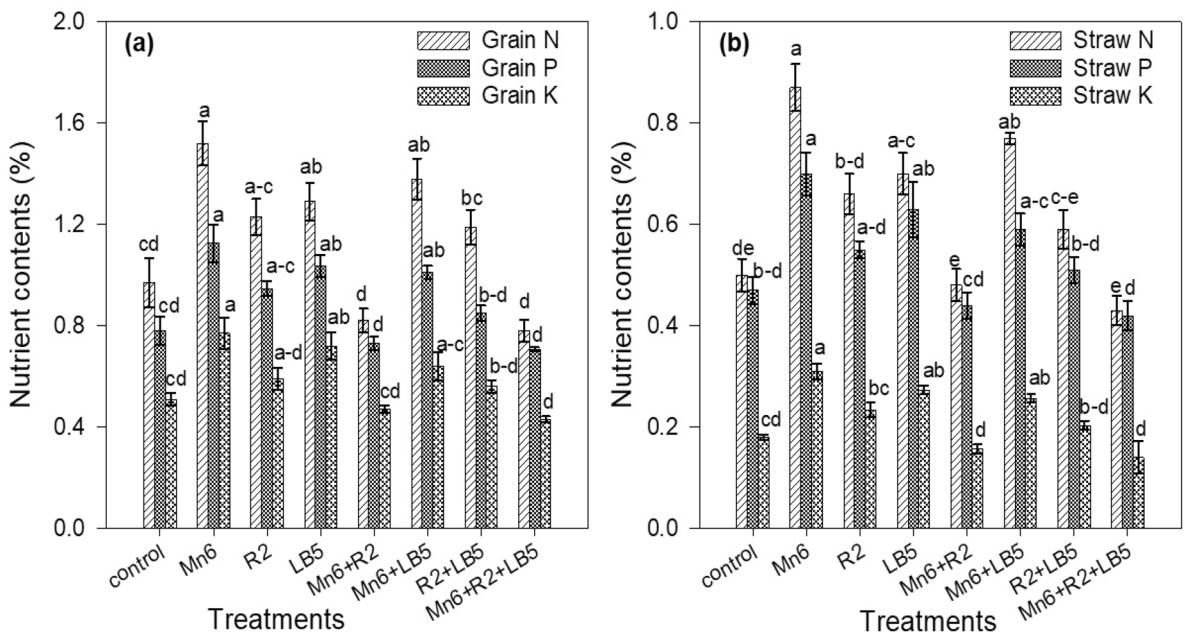

Fig. 3 Effect of EPS producing PGPR's on macro-nutrients contents: **a** grain N, P, K and **b** straw N, P, K ($p < 0.05$)

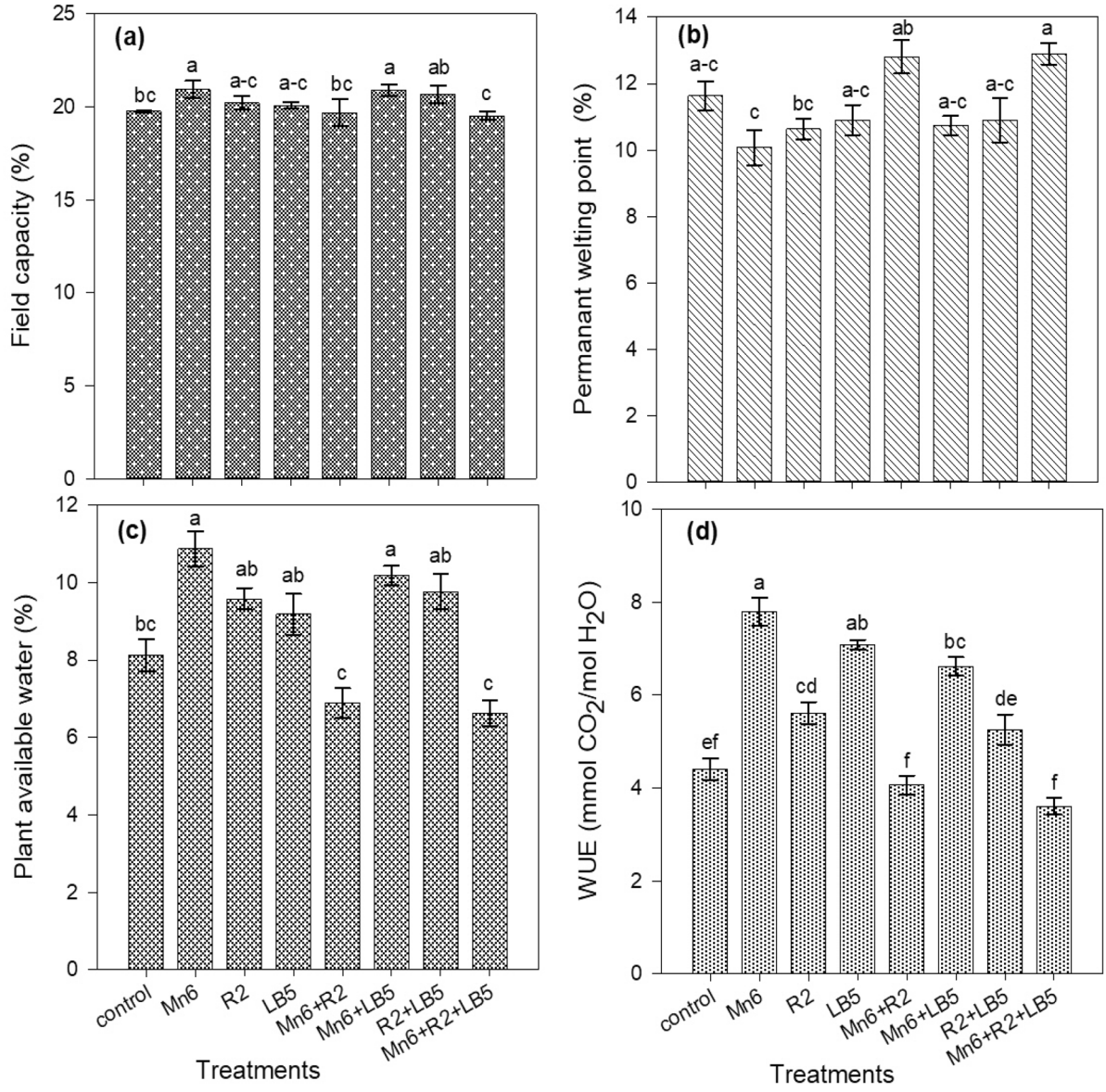

Fig. 4 Effect of EPS producing PGPR's on **a** filed capacity, **b** permanent welting point, **c** plant available water, **d** water use efficiency (WUE) ($p < 0.05$)

respectively. The results regarding water use efficiency (WUE) of maize depicted that about 77, 61, and 27.4% higher water use efficiency were observed in the plants grown on soils incubated with microbial strains Mn-6, LB_5, and R_2, respectively, compared with the control, while the WUE was about 8 and 18% lower than the control in the plants grown in the pots incubated with Mn-6 + R_2 and Mn-6 + R_2 + LB_5, respectively.

Effects of EPS-producing PGPR on soil properties

The influence of EPS production by PGPR on the stability of the aggregates was also determined by aggregate stability analyzer through wet sieving, and the results are presented in Table 3. It was observed that the stability of the macroaggregates of size > 2 mm and from 2 to 1 mm was adversely affected in response to the application of PGPR consortia, i.e.,

Mn-6 + R_2 and Mn-6 + R_2 + LB_5. However, the minimum stability of macroaggregates of formerly mentioned sizes was obtained in the soil incubated with Mn-6 + R_2 + LB_5 which was 3.8 and 9.2% lower than the control for the aggregate size of > 2 mm and from 2 to 1 mm, respectively. All other treatments receiving the either single or combined application of EPS-producing PGPR strains depicted a positive response toward the stability of macroaggregates. However, the maximum value for macro size aggregates (21.9 and 20.3% for aggregate size of > 2 mm and 2 to 1 mm, respectively) was observed in the soil incubated with the microbial strain of Mn-6. Moreover, the stability of the macroaggregates with the application of Mn-6 was about 13.6 and 5% higher than the control for the aggregate size of > 2 mm and 2–1 mm, respectively.

Similarly, the stability of micro-aggregates of size 1–0.5 mm was lowest in the soil receiving Mn-6 strain which was around 1.63% lower than the control. On the other hand, the stability of micro-aggregates (1–0.5 mm) in the treatments including LB_5, Mn-6 + R_2 + LB_5, R_2, LB_5 + R_2, Mn-6 + LB_5, and Mn-6 + R_2 was respectively 0.12, 1.05, 3.87, 5.34, 12.0, and 13.7% higher stability than control. Likewise, in the case of the stability of the aggregates of size from 0.5 to 0.25 mm, the maximum value (22.6%) was obtained with the use of Mn-6 + R_2 + LB_5 which was about 13.6% greater than the control stability. After this treatment, the application of R_2 and Mn-6 + R_2 improves the stability of the aggregates while all other treatments give off the values of stability lower than the control. Moreover, the stability of micro-aggregates of size from 0.25 to 0.10 mm was increased with the use of microbial strain LB_5 only whereas the application of all other treatments resulted in lowering the stability of the aggregates of the previously mentioned size compared with un-inoculated control.

The highest porosity among the treatments with a single microbial strain was observed in the soil incubated with Mn-6 which was 4% more than the control (Fig. 5a). The application of combinations of microbial strains Mn-6 + LB_5 and R_2 + LB_5 improves porosity while Mn-6 + R_2 led to decrease porosity compared with the control. The opposite trend of change in particle density than porosity in response to the application of EPS producing microbial strains was observed (Fig. 5b). The maximum particle density was observed in the treatment where the combination Mn-6 + R_2 + LB_5 was applied while the minimum was recorded in the soil receiving Mn-6 treatment. Likewise, the soil organic matter was improved with the application of a single strain with the maximum value (0.61%) for the soil incubated with Mn-6 which was around 72% higher than the control. The application of microbial strains in combination (Mn-6 + LB_5 and R_2 + LB_5) was found to have a negative effect on soil organic matter. All treatments were statistically significant in influencing soil organic matter.

Discussion

Farm mechanization in the twentieth century has posed deterioration of physical properties of soil, i.e., soil structure, soil aggregation, and bulk density. A good soil structure is important for improving aeration, nutrient retention, carbon sequestration, controlling water and wind erosion, and flourishing biodiversity which ultimately is responsible for improved

Table 3 Effect of EPS producing PGPR on aggregate stability of soil in pot studies

Treatments	> 2 mm	2–1 mm	1–0.5 mm	0.5–0.25 mm	0.25–0.10 mm
Control	19.25 ± 0.12 h–n	19.34 ± 0.1 g–m	19.37 ± 0.03 g–l	19.9 ± 0.1d–i	22.13 ± 0.09bc
Mn-6	21.87 ± 0.15bc	20.31 ± 0.17d–f	19.05 ± 0.11i–n	18.44 ± 0.11n–o	20.33 ± 0.14d-f
R2	19.62 ± 0.13e–k	19.77 ± 0.17e–i	20.12 ± 0.06d–g	20.15 ± 0.15d–g	20.35 ± 0.18d-f
LB5	19.94 ± 0.08d–h	19.69 ± 0.11e–j	19.39 ± 0.15 g–l	17.76 ± 0.14op	23.22 ± 0.11a
Mn-6 + R2	18.9 ± 0.14j–n	18.49 ± 0.1 m–o	22.02 ± 0.09bc	20.26 ± 0.12d-f	20.32 ± 0.16d–f
Mn-6 + LB5	20.46 ± 0.15de	19.95 ± 0.14d–h	21.69 ± 0.2c	17.79 ± 0.1op	20.11 ± 0.14d–h
R2 + LB5	20.16 ± 0.16d–g	20.02 ± 0.27d–h	20.46 ± 0.19de	18.73 ± 0.16 k-n	20.63 ± 0.21d
Mn-6 + R2 + LB5	18.53 ± 0.17 l–o	17.57 ± 0.25p	19.57 ± 0.24f–k	22.61 ± 0.17ab	21.73 ± 0.2c
HSD ($p \leq 0.05$)	**0.8612**				

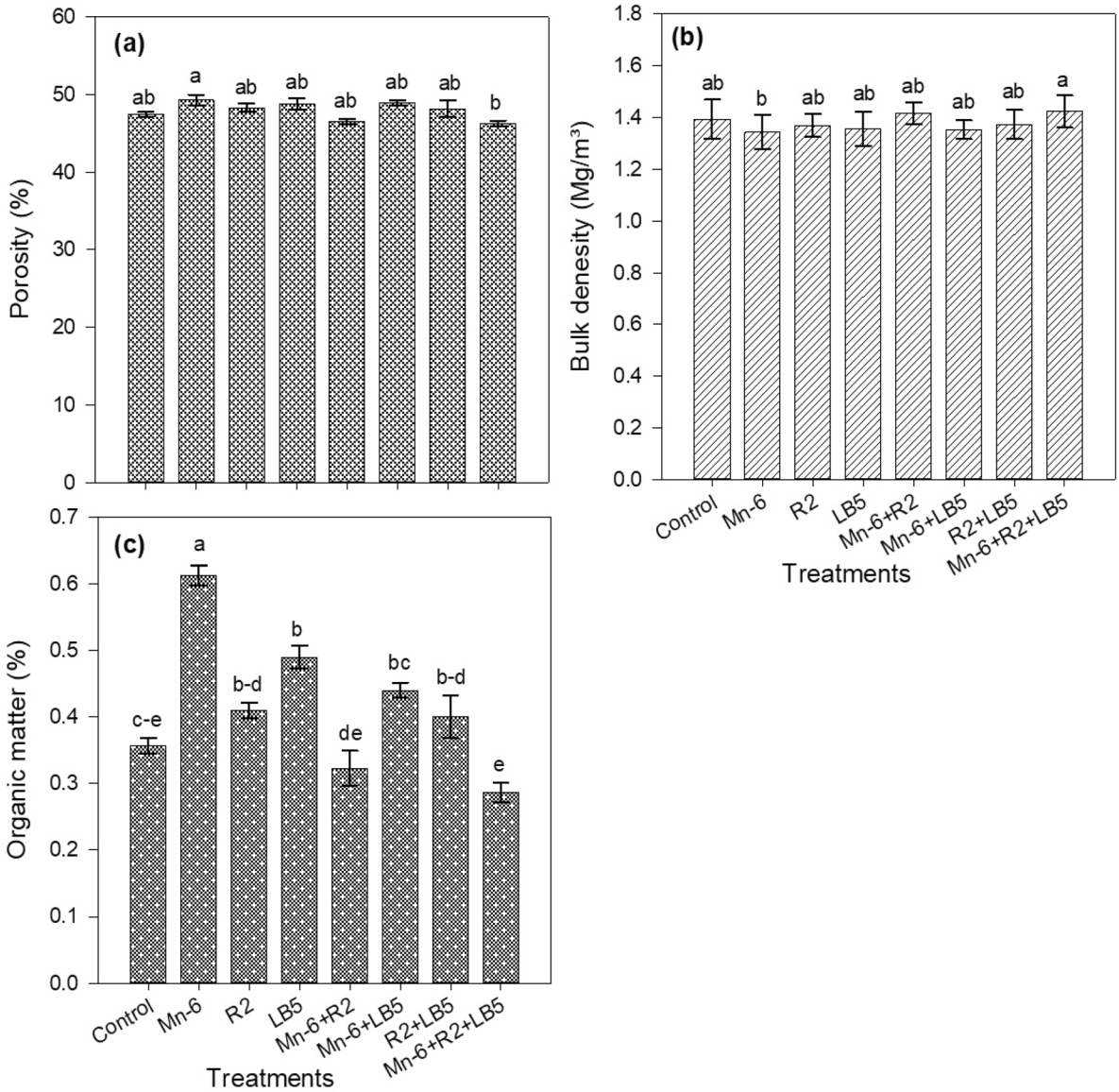

Fig. 5 Effect of EPS-producing PGPRs on soil physicochemical parameters: **a** porosity, **b** bulk density, **c** organic matter ($p < 0.05$)

crop growth (Briar et al., 2011; Kong et al., 2011; Peng et al., 2015; Shahzad, 2020). The microbial biopolymers (exopolysaccharides) are important in stabilizing the soil structure through flocculating the primary particles into aggregates (Chen et al., 2015), conserving the soil carbon pool from degradation and reducing soil dispersion (Kong et al., 2011; Shahzad, 2020). This study narrated the effect of microbially produced EPSs on soil aggregation and maize growth, physiology, and yield under pot conditions. The results depicted that the EPS-producing rhizobacterial strains, i.e., Mn-6, LB5, and R2, had a significant effect on soil aggregate stabilization and maize growth improvement when applied alone and in combination, i.e., Mn-6 + LB5. The other combinations negatively affect the soil as well as plant attributes.

The stabilization of the soil aggregates through the wet method depicted 4–28% and 1.2–14% more stable aggregates under EPS-producing PGPR applications in incubation and pot studies, respectively,

which justified the stability of the aggregates against water erosion (Babur et al., 2021a). The results obtained from the pot study were lower as reported by Moncada et al. (2015). The reason for the stabilization of the aggregates might be due to EPS production by PGPR in the plant rhizosphere, more root proliferation, and their gummy exudations (Cheng et al., 2020; Deka et al., 2019; Hallett et al., 2009). Multiple species of bacteria had a significant impact on soil aggregate stabilization through production of high molecular weight extrapolymeric substances as described by Crawford et al. (2012), Lehmann and Rillig (2015), and Costa et al. (2018). The soil porosity, bulk density, and soil water contents, i.e., plant available water, field capacity, and permanent wilting point were also influenced by microbial EPS secretions, and the results were in line with Daynes et al. (2013) and Shahzad (2020). The soil aggregate stabilization by EPS production through the studied rhizobacteria might be the possible reason for improving bulk density, water contents, and porosity (Benard et al., 2019; Cheng et al., 2020; Khan & Bano, 2019; Wang et al., 2009).

The significant increment in the nutrient contents of maize might be due to the ability of some PGPRs to improve nitrogen contents in plants as rhizospheric free living nitrogen fixer or by urease activity and make the non-available soil nitrogen to its available forms. As nitrogen is an integral part of the chlorophyll molecule, the increase in available nitrogen might be used by the plants in the synthesis of chlorophyll which ultimately resulted in the improvement of SPAD value (SPAD value) in the leaves (Curá et al., 2017). Higher SPAD value is directly proportional to high photosynthetic rate due to more light capturing of the chlorophyll molecule, and the higher photosynthetic rate required more entry of carbon dioxide into the plant leaves through stomata (stomatal conductance) and a high rate of water transpires through stomata (Naveed et al., 2014). The findings of the current study are in accordance with the results reported by Naveed et al. (2014) and Curá et al. (2017).

The improvement in plant growth and yield because of EPS producing PGPR application was might be due to higher nutrient uptake of root system, microbial secreted phytohormones, phosphate solubilization activity, antibiotics (protection from pathogens), and ACC-deaminase activity (limit ethylene biosynthesis) and other growth-promoting mechanisms. The higher nutrient uptake from a well-developed root system is responsible for higher biomass production and higher grain production in maize under pot experimentation (Turan et al., 2006). Moreover, the increase in root proliferation in response to the application of PGPR led the root to prone more soil area for nutrient uptake from the soil, thereby resulting in uplift of plant growth and yield (Canbolat et al., 2006). The findings reported by Yazdani et al. (2009) are in line with the outcomes of the current study.

The bacteria residing in the rhizosphere actively participate in all biogeochemical cycles and determine the bioavailability of nutrients for plant and microbial community uptake (Osorio Vega, 2007). Moreover, certain bacteria can fix the atmospheric nitrogen, and they also convert soil-fixed nitrogen into a plant-available form of nitrogen, i.e., NH_4^+, NO_3^-, thereby improving nitrogen uptake (Cakmakci et al., 2001). The improvement in P and K contents by the application of EPS producing PGPR was due to their ability to produce phosphatase enzyme, and some organic acids that modify the pH of rhizospheric soil and increase phosphorus solubility and uptake (Aslantas et al., 2007). Similarly, the release of certain organic acids might be responsible for the improvement in K contents by releasing K from silicate clay minerals along with P-solubilization (Han et al., 2004).

The improvement in the soil aggregation may be due to exopolysaccharides which is a gummy material secreted by the PGPR strains and can bind soil particles (Lehmann & Rillig, 2015). The differences among the treatments may be due to the differences in the ability of PGPR stains in secreting exopolysaccharides. The production of exopolysaccharides by the applied PGPR and buildup of organic matter in plant rhizosphere soil might be responsible for improving plant-available soil water contents in soil (Wang et al., 2009). Moreover, higher physiological activities due to the application of EPS-producing PGPR might be responsible for higher biomass production leading toward the high water use efficiencies in maize (Vivas et al., 2003). Similar findings were reported by Crawford et al. (2012) and Lehmann and Rillig (2015).

The negative effects of the PGPR combinations on physical properties and plant growth might be due to the extracellular secretions consisting of antagonistic phytotoxins which might be the major reason for less

EPS production and less organic matter accumulation in the soil and caused soil structural and other physical properties of deterioration (Dexter & Czyz, 2007). The negative effects of combined application of strains on soil structure equally affect the growth, physiology, and yield of maize (Shahzad, 2020). The improvement in soil physical properties in the current study was a little lower than the values reported by Moncada et al. (2015) while the values observed for improvement in soil physical properties by Daynes et al. (2013) were lower than the current study.

Conclusions

The potential of EPS-producing PGPR in improving maize growth, physiology, yield, and soil properties was evaluated in this study. The results indicated that soil application of EPS-producing PGPR strains improves not only the growth and physiology of the maize but also increased yield. Furthermore, the application of EPS producing PGPR also improves soil physical properties like porosity, bulk density, and soil aggregate stability. Therefore, it is concluded that the application of EPS producing PGPR is beneficial in enhancing not only the plant growth and development but also improving soil properties.

Acknowledgements The authors acknowledged Mr. Haroon Shahzad and the farm staff for helping in the conduction of the research.

Declarations

Competing interests The authors declare no competing interests.

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