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Plant growth promoting rhizobacteria and their role in the improvement of growth and yield of sesame

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Abstract

Among the oilseed crops, sesame is recognized as the queen of oilseeds. Phosphorus (P) precipitation and adsorption takes place due to the alkaline and calcareous nature of Pakistani soils which makes P unavailable to the plants and causes a reduction in the sesame yield. Therefore, development of novel and improved technologies should be a major concern to gain maximum yield. In addition to conventional ways of mineral phosphate fertilization, P-solubilization by microbes may increase phosphate availability in arable soils. P-solubilizing bacteria release phosphatase enzyme which converts organic P into inorganic form through hydrolyzation and enhance uptake of P. Some microorganisms having ACC-deaminase activity increase the root growth resulting increased acquisition of P by plant indirectly. A series of studies were carried out to assess the response of bacterial inoculation on sesame growth and yield. Results of initial screening experiment under axenic conditions showed that inoculation resulted in up to 51% improvement in seedling length, 36% increase in seedling fresh weight, 25% enhancement in seedling dry weight over uninoculated control. Bacterial inoculation in pot trial, resulted in 19, 22, 30 and 48% improvement in root length, shoot length, 1000-grain weight and seed yield of sesame compared to uninoculated control, respectively. Regarding biochemical parameters, bacterial inoculation resulted in enhanced seed oil, oil yield, protein yield and seed protein content as compared to uninoculated control. From the results, it can be concluded that multi-trait bacteria could be more efficient PGPR than single trait to promote plant growth.

Keywords: Bacterial inoculation; P-solubilizing rhizobacterium; Phosphatase; ACC-deaminase; Sesame

Introduction

Sesame (*Sesamum indicum* L.) recognized as benniseed or sesamum belongs to *Pedaliaceae* family. Sesame is among the global ancient oilseed crops (Shin *et al.*, 2016). It is a vital source of good quality edible oil (42-54%) (Anilakumar *et al.*, 2010), protein contents (18–25%) (Agricultural Marketing Resources Center, 2018) and carbohydrates (16-18%) (Kiranmayi, 2016). Approximately 100 g of sesame seed contains lipid contents 48 g, ash 6 g and fiber 14 (Pusadkar *et al.*, 2015). Seeds of sesame are rich in mineral nutrients including Ca (calcium), K (potassium), P (phosphorus), Fe (iron), Zn (zinc), B (boron) and Cu (copper) (USDA Nutrient Database, 2015). It contains a high amount of essential fatty acids such as 35 to 54% oleic acid, 39 to 59% linoleic acid, 5% of stearic acid and 10% palmitic acid (Peng *et al.*, 2015). Compared to other oilseed crops, the sesame seed oil is highly stable due to a greater concentration of antioxidants such as sesamol, sesamin, sesaminol, squalene and sesamolinol (Gharby *et al.*, 2017). It has several nutritious and health aspects for humans, as it is used to cure hoarseness, asthma problem, eyes disorders, convulsions and bowel obstruction, etc. (Shi *et al.*, 2018).

Taking in account the current scenario of oilseed crops in Pakistan, total demand for edible oil was 3.255 million tonnes (during the year 2019-20). Locally produced edible oil was 0.507 million tonnes while 2.748 million tonnes of oilseeds/edible oil was imported. The import of edible oil cost US $2.663 billion (Economic Survey of Pakistan, 2019-20).

In Pakistan, area under sesame cultivation is 83 thousand hectares with average yield and annual production of 430 kg per hectare and 35.7 thousand tons, respectively (Economic Survey of Pakistan, 2018-19). Yield and production of sesame is affected by several environmental

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factors such as expensive fertilizers, pathogens, poor crop management, rainfall fluctuation, limited use of certified seeds, and conventional farming operations, etc. Among various factors of sesame production, a good supply of major plant nutrients (N, P, K) is required. Dry weight of plant contains about 0.3 to 0.5% of P. It is essential for formation of albumin, fat and nucleus, cell division and organization, transmission of hereditary material, sugar and starch consumption, photosynthesis, and several other attributes of plant development (Lai, 2002). P is involved in the production of RNA and DNA. Marschner (1990) reported that phosphorus is a constituent of ATP and other hydrophilic phospholipids which in turn, are important part of cell membrane. Symptoms of P deficiency are appearance of reddish color and subsequently decreased photosynthesis and growth retardation, while reduced P supply to plant results in darker green leaves (Malhotra *et al.,* 2018).

Alkaline and calcareous nature of Pakistani soils is main driver of P deficiency for sesame. Owing to adsorption, precipitation and transformation of P, 80-90% soils of arid and semiarid regions suffer from deficiency of plant available P. Out of 0.05% (w/w) P in soil, only 0.1% is available for plant (Zhu *et al.,* 2011). It exists in organic as well as in inorganic forms (Rodrigues *et al.,* 2016). Most of the P is present in organic form such as nucleotides, inositol-phosphate, phospholipids, etc. (Nash *et al.,* 2014). In soils which are calcareous, soil organic phosphorus provides maximum of plant nutrition.

Due to the presence of P in the form of insoluble compounds in majority of the soils that is not available to plants, synthetic or chemical fertilizers are applied to maintain the nutrient levels in soil. However, P containing fertilizers either get precipitated or adsorbed in the soil (Fayiga *et al.,* 2016). Rock phosphate (RP) is the main source of mineral P fertilizers. It is estimated that scarcity of RP reserves (Jasinski 2006) will lead to exhaustion of these reserves within 50-100 years. Furthermore, quality of RP is decreasing in contrary to its price which is increasing day by day (Zhu *et al.,* 2018), indicating that the supply of high-quality P fertilizers will be limited in future (Cordell *et al.,* 2009). The increasing food demands and agriculture products in developing countries has put pressure on culturable land. This situation has resulted in increased prices of fertilizers throughout the world (Chowdhury *et al.,* 2017).

To overcome this problem for promotion of sustainable agriculture, researchers are now exploring the use of microbes for their potential to solubilize the insoluble source of P (inorganic and organic). The free-living bacteria colonized in the plant roots and known for plant growth promotion are called PGPR (plant growth-promoting rhizobacteria) (Mehmood *et al.,* 2018). PGPR influence plant growth through various direct and indirect mechanisms (Mantelin and Touraine, 2004). They promote the growth of plants via siderophores production, solubilization of minerals (Khan and Ahemad, 2012), regulation of nutrient cycling (Walker *et al.*, 2003), fixation of atmospheric nitrogen (Glick, 2012), enhancement of enzymatic activities (Dakora and Phillips, 2002), regulation of ACC deaminase, organic matter decomposition and synthesis of plant hormones (Odoh, 2017).

Availability of P can be improved either by introducing certain fungi or bacteria or by maintaining the native soil microbial communities to improve their potential for P transformation (Richardson, 1994). PGPR having phosphate solubilization potential can be a promising and ecofriendly way to ensure phosphate availability for plants. Microbial enzymatic activities play a central role in biochemical functions in soil including cycling of nutrients, decomposition and formation of organic matter, in catalysis of various important reactions and offering information about soil history (Adetunji *et al.,* 2017).

Soil phosphatase is mainly produced by the bacteria. It assists in breakdown of organic P to release available form of P. Because of this hydrolysis, organic P is transformed to inorganic forms of P which can be uptaken by the plant roots from soil solution (Lemanowicz *et al.,* 2016). The reactions catalyzed by the phosphatase are involved in the hydrolysis (anhydrides as well as esters of H_3PO_4) (Platkowski and Telesinski, 2016).

Phosphatases are divided into alkaline phosphatase (which shows optimal activity at high pH 11) and acid phosphatase (showing maximum activity at low pH 6.5) (Zhu *et al.,* 2018). Both higher plants and microbes can synthesize acid phosphatase, but microbes specifically synthesize alkaline phosphatase (Nannipieri, 2011). Margalef *et al.* (2017) reported that both microbial and plant phosphatases control the orthophosphate ions (HPO^{-}_4 and $H_2PO^{-}_4$) release from soil organic P, but efficiency of microbial phosphatases is greater in release of P.

ACC-deaminase containing soil microbes are responsible for elongation of roots by depressing the ethylene production through breakdown of ACC into NH_3 and α-ketobutyrate in roots (Nadeem *et al.,* 2010). This improve growth of roots causes high P uptake and subsequently plant growth. Thus, available fraction of P increases due to phosphatase-producing bacteria along with its ACC deaminase trait, which eventually increase the yield.

Biochemical characteristics of sesame (seed protein and seed oil) are affected by P together with growth and yield. Our

hypothesis was to test the P-solubilizing PGPR for improving the growth and yield due to enhanced P uptake by the plant. Therefore, present study was designed with an objective to evaluate the role of PGPR having capability to produce different enzymes linked with phosphorus availability for improving growth, yield and oil content of sesame.

Materials and Methods

Isolation, purification, and preservation of rhizobacteria

Various bacteria were isolated from the rhizosphere of sesame grown in semiarid and arid regions of Punjab, Pakistan. The plants were uprooted along with non-rhizospheric soil to prevent root injury and transferred to the laboratory in polythene bags. The plants were gently agitated to remove non-rhizospheric and loosely attached soil. Dilution plate technique was followed to isolate the rhizobacteria. The autoclaved Luria Bertani (LB) agar media was used in this technique. Various bacteria were isolated in the form of pure culture through streaking them 3-4 times. Based on fast growth *in vitro*, 62 bacterial isolates were collected and tested for P-solubilization potential. Nineteen isolates were positive for P-solubilization and were characterized against phosphatase activity assay (alkaline and acidic), coded as AA with numbers, and preserved at -20 $\pm$ 1 ºC (by pouring 400 µL glycerol and 600 µL broth in 1.5 mL Eppendorf) for further characterization. ACC-deaminase activity (qualitative & quantitative estimation) was found only in 10 rhizobacterial isolates. These bacteria were also tested *in vitro* against various growth-promoting attributes such as exopolysaccharide synthesis, hydrogen cyanide production, catalase activity, and oxidase test.

Functional testing of the bacterial isolates

P-solubilization potential

Method of Mehta and Nautiyal (2001) was used for the estimation of P-solubilization potential. Petri plates were prepared with the National Botanical Research Institute's Phosphate (NBRIP) growth agar medium and a well-isolated fresh colony of rhizobacteria was inoculated at 3-5 different places on the plates. The Petri plates were incubated at 28 $\pm$ 1^0C for 48-72 hours and clear halo zone surrounding the colonies was marked as a positive sign of phosphate solubilizing ability of rhizobacteria.

Exopolysaccharide (EPS) synthesis

The method described by Ashraf *et al.* (2004) was followed to determine the EPS synthesis. Three-day old broth culture of strains having 0.5 OD (10^7−10^8 CFU) (determined by using an optical density meter Den-1 Densitometer, McFarland, UK) was inoculated at 4 points on RCV-glucose media containing plates. Inoculated plates were incubated at 28 $\pm$ 1 ºC for four days. Mucoid growth of isolates indicated the EPS production activity.

Determination of hydrogen cyanide (HCN)

For the estimation of HCN production, Abd El-Rahman and Shaheen (2016) method was followed. Bacterial strains were inoculated on Petri plates except the control plates, having the quarter strength King's B media. Filter paper was dipped into the picrate solution and placed inside of the Petri plate lid. Petri plates were sealed using parafilm and incubated at 28 $\pm$ 1 ºC. After 24 hours, the color of the filter paper changed from yellow to brown indicating the production of cyanide by the bacterial strain.

Catalase test

For catalase assay, bacterial culture was taken on a glass slide. One drop of 3% H_2O_2 was added on the culture. Bubbling within 5 to 10 seconds was indication of the catalase activity. The assay was repeated three times (MacFaddin, 1980).

Oxidase activity

For oxidase activity, 1% Kovacs oxidase reagent as proposed by Kovacs, 1956 (tetra-methyl-*p*-phenylenediamine dihydrochloride; TMPD) was used. The filter paper was dipped in TMPD and air-dried. Fresh bacterial culture was rubbed on the filter paper. The appearance of dark purple color on filter paper indicated the oxidase activity (Steel, 1961).

Phosphatase activity assay

Acidic/alkaline phosphatase activity was determined by following Eivazi and Tabatabai (1977). Phosphatase enzyme catalyzes the conversion of *p*-Nitrophenyl phosphate (*p*NPP) to *p*-Nitrophenol through hydrolysis. *P*-Nitrophenol can absorb wavelength of 405 nm, thus absorbance at this wavelength indicated the phosphatase enzyme activity.

ACC metabolism assay (Qualitative)

Method of Shaharoona *et al.* (2006) was followed to perform the *In vitro* qualitative test for ACC metabolism. The media containing 3mM ACC and 0.1M ammonium sulfate as a source of nitrogen was used to grow PGPR isolates. The control contained 0.1M magnesium sulfate. Bacterial growth on nitrogen sources was assessed. OD meter (DEN-1, McFarland Densitometer, Grant Instruments Ltd Shepreth, Cambridgeshire, England) was used to measure the OD at 535 nm after 0, 24, 48, 72, and 96 hours

of incubation. The strains that showed increase in their OD were considered positive for ACC metabolism.

ACC-deaminase activity (Quantitative)

Quantitative testing of ACC-deaminase was performed by determining the amount of α-ketobutyrate (µmol) produced using standard curve. This enzyme cleaves the ACC to α-ketobutyrate. The range of the standard curve for α-ketobutyrate was 0.1-1.0 at 540 nm (Penrose and Glick, 2003).

Growth room trial

Inoculum preparation

The culture of isolates was prepared in conical flask containing 250 mL LB growth media. The isolates were inoculated in LB media and incubated in orbital shaking incubator (Firstek Scientific, Tokyo, Japan) for 48-72 hours at 28 ± 1ºC and 120 rpm. The culture was centrifuged for 10 min at 4000 × g at 4°C and cells were harvested. An inoculum having OD 0.5 McFarland units (i.e., $>10^8$ cells mL^{-1}) was formed with the help of densitometer (Den-1 Densitometer, McFarland, UK) in sterilized LB broth medium. The control was uninoculated and its OD was subtracted from the OD of inoculum

Seed surface sterilization

Seeds were dipped in 95% ethanol for few seconds and then rinsed 3-4 times using sterilized distilled water. After this, seeds were immersed in a 5% $NaClO_4$ solution for 8-10 minutes and then washed with sterilized distill water (Abd-Alla *et al.,* 2012)

Seed inoculation

Surface sterilized seeds were inoculated separately for each bacterial isolate by dipping for 10 min in their respective culture of 0.5 OD (i.e., $\gg 10^8$ cells mL^{-1}). In the case of control, surface-sterilized seeds were dipped in uninoculated sterilized broth.

Screening of rhizobacteria for plant growth-promoting activity under gnotobiotic conditions

Ten promising strains were selected on the basis of *in vitro* characterization. The selected strains were further evaluated for their role in plant growth promotion under axenic conditions by using sesame (*Sesamum indicum* L.) as the test plant. For this purpose, a jar trial was carried out in the growth room of Soil Microbiology & Biochemistry Lab (SBML), Institute of Soil and Environmental Science (ISES), University of Agriculture, Faisalabad (UAF). Plastic glasses were filled with growth media (sand) and covered with an aluminum file. These glasses were autoclaved for 3 times to remove all the contamination. The inoculum was prepared as described above. Four surface-sterilized seeds (as described earlier) were inoculated by dipping in their respective culture (as explained previously) and for the control, surface-sterilized seeds were immersed in autoclaved broth solution. Inoculated seeds were transplanted to autoclaved glass jars according to the method described by Hussain *et al.* (2002). Then, sterilized full strength Hoagland solution (Hoagland and Arnon, 1950) was added to jars after every three days for providing water and nutrients to seedlings. The jars were arranged according to completely randomized design. Each treatment was replicated thrice. Initially, jars were placed in dark throughout the pre-germination phase. After germination, the temperature of the growth room was maintained at 8 $\pm$ 1ºC adjusted to 8 hours dark and 16 hours light period under continuous light white fluorescent light (300 µmol m^{-2} S^{-1} photosynthetic photon flux density). After 21 days of sowing, data was collected for comparison with the least significant difference test (Montgomery, 2001). The rhizobacterial isolates demonstrating substantial improvement in growth were considered as effective plant growth promoters and further used in the pot experiment.

Pot experiment

For physiochemical characteristics of the soil, standard procedures were followed (U.S. Salinity Laboratory Staff, 1954; Page *et al.,* 1965). The methodology described by Rehman *et al.* (2015) was followed for the determination of soil properties. In short, particle size of soil was measured by using hydrometer method. pH of the soil was observed with pH meter (Lovibond, model Sensodirect 100). EC of the extract (EC_e) was recorded using conductivity meter (Jenway model-4070). For determination of saturation percentage (SP%), mass of oven-dried soil was subtracted form wet soil and resulted value was divided by the mass of oven-dried soil. The obtained value was then multiplied with 100 to get SP%. Soil organic matter was determined following Walkley-Black method (Walkley and Black., 1934; FAO, 1974)

Table 1: Characterization of rhizobacterial isolates

Bacterial isolates	Phosphate solubilization	Exopolysaccharide	HCN production	Catalase	oxidase
AA-06	+	+	+	+	+
AA-09	+	+	-	-	+
AA-15	+	-	+	-	+
AA-18	+	+	+	+	+
AA-21	+	-	+	-	-
AA-27	+	+	-	+	+
AA-32	+	-	+	+	-
AA-39	+	+	-	+	+
AA-45	+	+	-	+	+
AA-49	+	+	+	-	-

Table 2: Characterization of rhizobacterial isolates

Bacterial isolates	Phosphatase activities (µg PNP g^{-1} h^{-1})		ACC-deaminase activities	
	Acidic phosphatase activity	Alkaline phosphatase activity	Qualitative ACC-deaminase assay	Quantitative ACC-deaminase activity (nmol α-ketobutyrate g^{-1} biomass h^{-1})
AA-06	35.2 ± 1.35	24.1 ± 0.61	+	33.9 ± 0.01
AA-09	12.7 ± 0.91	11.3 ± 1.35	+	314.6 ± 0.06
AA-15	19.6 ± 1.48	15.6 ± 0.90	+	294.8 ± 0.04
AA-18	42.4 ± 1.26	28.1 ± 1.18	+	349.5 ± 0.05
AA-21	12.9 ± 1.19	13.2 ± 1.39	+	318.7 ± 0.08
AA-27	28.6 ± 1.67	13.5 ± 0.78	+	354.6 ± 0.01
AA-32	15.8 ± 0.85	11.9 ± 1.29	+	277.5 ± 0.07
AA-39	22.3 ± 0.66	16.4 ± 1.07	+	335.7 ± 0.09
AA-45	25.1 ± 1.59	20.6 ± 0.86	+	334.8 ± 0.01
AA-49	19.2 ± 0.47	13.8 ± 0.76	+	260.9 ± 0.01

Values for qualitative test of ACC-deaminase are confusing It may be +ve or -ve

From screening trial, five best strains of rhizobacteria (AA-06, AA-18, AA-27, AA-39 and AA-45) were further assessed in a pot experiment for their potential to promote sesame growth, biochemical characteristics and yield. Each pot (diameter of 23 cm, height of 30 cm) was filled with 12 kg soil collected form experimental field of ISES, UAF. The experiment was conducted in wire house at ambient temperature and light. The inoculum was prepared as detailed in screening trial above. Sesame seeds were surface sterilized (as explained earlier) and coated by using slurry method (mixing respective bacterial culture with a 15% (w/v) table sugar solution and autoclaved peat: clay (3:1)) whereas the seeds for control treatment were prepared without bacterial inoculation. Inoculated seeds were air dried for 6-8 hours under shade. Recommended dose of NPK (50, 60, 45 kg ha^{-1}, calculated according to the weight of soil) was applied in the form of urea, diammonium phosphate (DAP), and sulfate of potash (SOP) fertilizers. Pots were irrigated with canal water. Experiment was conducted following completely randomized design with three replications of each treatment. Plants were harvested at maturity (after 120 days of sowing), and various yield and growth parameters were recorded.

Biochemical parameters

Seed oil (%)

Collected seed samples of sesame were cleaned properly to remove foreign materials if any. These seeds were placed in an oven at 130ºC temperature to 12% moisture content. Seeds were crushed by using Thomas Willey Mill (Model ED-5) into powder form. N-hexane (5 mL) was added to 12 g of crushed sample, folded in filter paper, and inserted in Soxhlet apparatus. Then, 150 mL of N-hexane in a 500 mL round bottom flask was heated for 6 hours at 600°C using heating mantle. The vapors of N-hexane were collected in reflux condenser and cooled with the help of water flow in Soxhlet arrangement. In a portion of the Soxhlet containing flooded sample, the cooled solvent was condensed back to help extraction of oil from the

sample. Oil was removed from the extracted sample and remaining oil was extracted through hot-presses by hydraulic press. The weight of the sample was determined and seed oil yield (%) was calculated as below.

Seed oil yield (%) = Sample weigt before extraction – Sample weigt after extraction / Weight of the sample before extraction × 100

Seed protein (%)

Kjeldahl method was followed to determine the nitrogen (AOAC, 1990). The formula described by James (1995) was used to calculate total protein.

Total protein (%) = N (%) × 6.25.

Bacterial Identification

Identification of rhizobacterial isolates (AA-06, AA-18, AA-27, AA-39 and AA-45) was performed by Macrogen, Korea using 16S rRNA sequence of isolates. For this purpose, freshly grown colonies were picked and stabbed in LB-agar slants and sent to Macrogen Korea. The results of the sequences were compared with NCBI library using BLAST tool. These isolates were identified as *Pseudomonas fluorescens* (AA-06), *Pseudomonas* sp. (AA-18), *Psychrobacter* sp. (AA-27), *Bacillus* sp. (AA-39) and *Bacillus aquimaris* (AA-45).

Statistical analysis

The effect of all treatments on growth, biochemical and yield parameters was assessed using Linear models in Statistix 8.1. Treatments were compared using analysis of variance (ANOVA). Treatment means were compared using Least significant difference (LSD) test at 5% probability level (Montgomery, 2001).

Results

Characterization

Characteristics such as P-solubilization , exopolysaccharide, hydrogen cyanide, catalase, and oxidase production were observed for all ten rhizobacteria (Table 1) that were used in the growth room experiment.

Rhizobacterial isolates 1 and 4 (AA-06 and AA-18, respectively) were the only isolates positive for all functional testing, however phosphate solubilization activity was found in all bacterial isolates. Results for exopolysaccharide production showed that tested rhizobacteria had ability to synthesize exopolysaccharide although, AA-15, AA-21 and AA-32 were non-producers. Hydrogen cyanide test was negative for isolates AA-09, AA-27, AA-39 followed by AA-45. However, catalase activity was recorded for six bacterial isolates. Furthermore, oxidase production was found in all rhizobacteria except, AA-21, AA-32 and AA-49.

Phosphatase activities assay

Results of *in vitro* testing of phosphatase activities depicted that all isolates showed both alkaline and acid phosphatase activities β-glycerophosphate (a substrate) containing liquid culture (Table 2). Bacterial isolate AA-18 showed maximum acid phosphatase activity (41.65 µg PNP g^{-1} h^{-1}) while isolate AA-09 inoculation exhibited minimum acid phosphatase activity (12.65 µg PNP g^{-1} h^{-1}). The activity of alkaline phosphatase showed by these isolates ranged from 11.32 to 27.59 µg PNP g^{-1} h^{-1} with the minimum of 11.32 µg PNP g^{-1} h^{-1} shown by isolate AA-09.

ACC metabolism assay

Qualitative assessment of ACC deaminase activity was performed and all the isolates were observed as positive for ACC metabolism (Table 2).

ACC-deaminase activity

Isolates showed different degree of efficiency regarding quantitative ACC deaminase (Table 2). Low ACC deaminase activity (ranging from 261 to 278 nmol α-ketobutyrate g^{-1} biomass h^{-1}) was observed in isolates AA-15, AA-32, and AA-49. The remaining seven isolates exhibited highert activity ranging from 355 to 315 nmol α-ketobutyrate g^{-1} biomass h^{-1}.

Screening on the basis of plant growth promoting activity

Some of the isolates had positive effect on sesame seedlings while the others did not.

Isolates had different response regarding length of shoot (Figure 1A). Isolate AA-18 caused maximum improvement and increased the shoot length up to 35% as compared to uninoculated control followed by AA-06, AA-27 and AA-39 that caused 32, 28 and 30 % more shoot length, respectively, than uninoculated control. Three bacterial isolates (AA-21, AA-32 and AA-45) also improved the shoot length which ranged from 12 to 16 %, compared with uninoculated control. Similarly, other isolates (AA-09 and AA-49) caused 10 and 11 % more shoot length respectively, compared to control treatment. The isolates AA-15 was least efficient to improve shoot length.

In sesame seedlings, rhizobacterial inoculation caused significant increase in the root length when compared with uninoculated control (Figure 1B). Results showed that

maximum improvement of 84 % in root length was observed due to inoculation with bacterial isolate AA-06 followed by AA-18, AA-27 and AA-39 that increased the root length ranging from 75 to 81% compared to uninoculated control. Similarly, the other four isolates (AA-21, AA-32, AA-45 and AA-49) also increased the root length effectively than uninoculated control. Two isolates (AA-09 and AA-15) caused minimum increase of 12% in root length over uninoculated control.

Inoculation with rhizobacteria caused significant improvement in the fresh weight of sesame seedlings as compared with un-inoculated control; however, isolates were not significantly different (Figure 1C). Data showed that isolate AA-27 gave maximum results and increased the fresh weight of seedlings up to 36% as compared to uninoculated control. Bacterial isolates AA-21, AA-32 and AA-39 were statistically at par with each other and caused up to 26, 29 and 31% more seedling fresh weight, respectively, than control treatment. Rhizobacteria AA-09

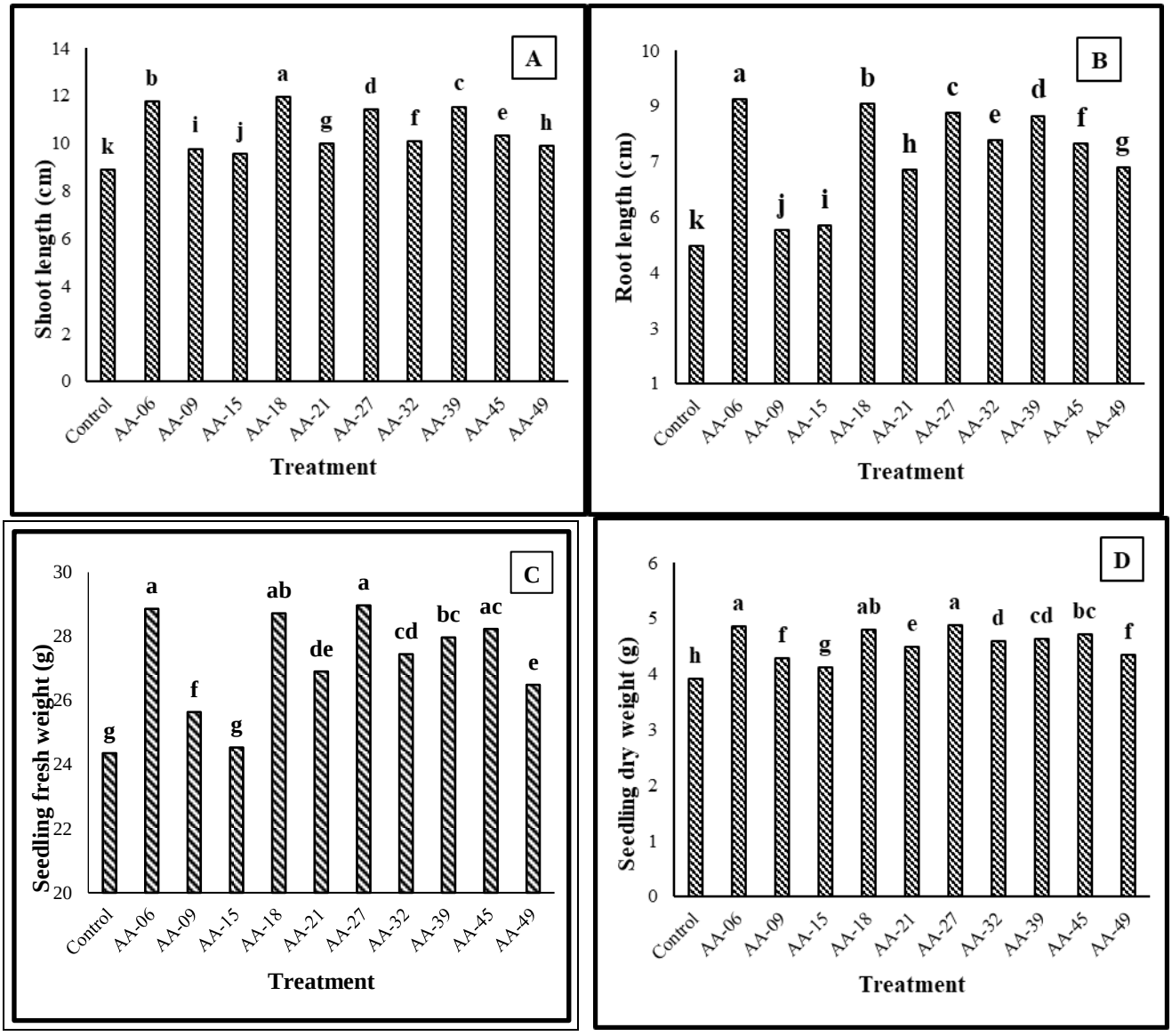

Figure 1: Effect of PGPR enzymatic activity on shoot and root length, fresh and dry weight of sesame seedling under growth room experiment (Average of three replicates)

and AA-49 increased the fresh weight up to 24% compared to uninoculated control. Isolate AA-15 gave minimum results in increasing seedling fresh weight.

Dry weight of sesame seedlings was significantly improved due to bacterial inoculation over un-inoculated control; however, some isolates could not show the significant behavior. Maximum improvement in dry weight of seedling was achieved with the rhizobacteria AA-27 that caused up to 25 % more dry weight of seedling as compared to control treatment. Enhancement in dry weight of seedling was also observed in case of inoculation with isolates AA-

Table 3: Effect of PGPR on growth parameters of sesame crop under pot conditions (Average of three replication), means sharing the same letter (s) are statistically at par at 5% probability level

Treatments		Shoot parameters		
		Length (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
Un-inoculation	**Control**	148.17 ± 2.1 d	163.33 ± 3.5 e	98.79 ± 4.7 d
	AA-06	165.91 ± 1.7 b	237.00 ± 3.8 a	125.48 ± 1.2 ab
	AA-18	170.43 ± 1.7 b	240.33 ± 1.8 a	130.76 ± 2.3 a
Inoculation	**AA-27**	176.25 ± 2.0 a	217.67 ± 3.8 b	118.83 ± 4.0 bc
	AA-39	181.10 ± 1.7 a	203.33 ± 2.3 c	115.73 ± 5.5 bc
	AA-45	159.88 ± 2.0 c	188.00 ± 3.2 d	108.82 ± 3.4 cd
LSD ($\rho \leq 0.05$)		5.79	9.75	11.69
Treatments		**Root parameters**		
		Length (cm)	**Fresh weight (g plant^{-1})**	**Dry weight (g plant^{-1})**
Un-inoculation	**Control**	13.60 ± 0.02 f	30.34 ± 1.44 d	18.49 ± 0.02 f
	AA-06	15.27 ± 0.02 c	36.72 ± 0.02 ab	21.17 ± 0.05 b
	AA-18	15.79 ± 0.03 b	35.86 ± 0.03 b	20.53 ± 0.02 c
Inoculation	**AA-27**	14.96 ± 0.02 d	38.36 ± 0.03 a	21.84 ± 0.03 a
	AA-39	14.42 ± 0.04 e	32.28 ± 0.02 c	19.80 ± 0.03 e
	AA-45	16.20 ± 0.02 a	33.38 ± 0.02 c	20.37 ± 0.02 d
LSD ($\rho \leq 0.05$		0.08	1.81	0.09

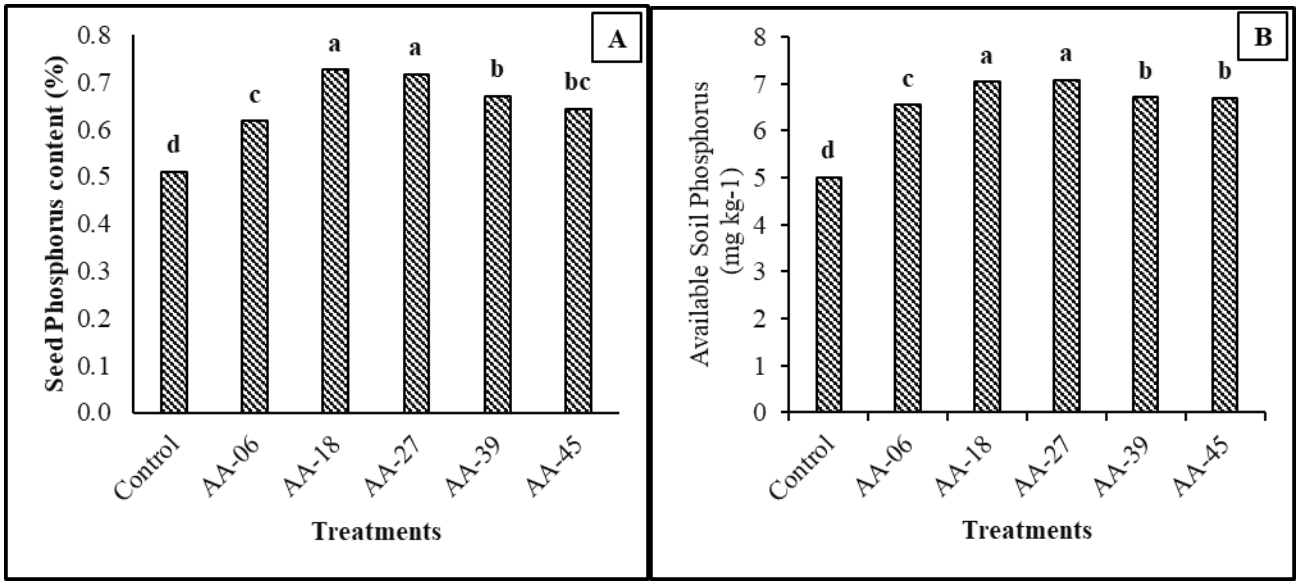

Figure 2: Effect of PGPR on phosphorus content in soil (A) and seed of sesame (B) seedling under pot conditions (Average of three replicates), Bars sharing the same letter (s) are statistically at par at 5% probability level

21 and AA-32 that caused 14 and 27% increase in dry weight of seedling over uninoculated control. Results indicated that two bacterial isolates (AA-39 and AA-45) were statistically similar to each other but significant with up to 20% more seedling dry weight over control. Minimum increase in seedling dry weight was recorded with isolate AA-15 (Figure 1D).

Pot experiment

Results of jar trial showed that bacterial isolates AA-06, AA-18, AA-27, AA-39 and AA-45 gave more significant results than other tested isolates in terms of plant growth promotion. Results of pot trial are summarized as under.

Growth and yield parameters

Application of PGPR strains significantly improved the sesame shoot length in pot conditions (Table 3). Maximum plant height was noted when plants were inoculated with AA-39 (*Bacillus aquimaris)*. Inoculation with AA-45 (*Bacillus* spp.) resulted in 8% more shoot length than uninoculated control. The results of shoot length upon inoculation were statistically at par with each other but significantly different from uninoculated control. The results suggested that PGPR inoculation significantly improved the shoot fresh weight (Table 3). Maximum shoot fresh weight (47%) was recorded upon inoculation with AA-18 (*Pseudomonas* spp.) compared to uninoculated control; however, AA-45 (*Bacillus* sp.) caused minimum increase (15%) as compared to uninoculated control.

Likewise, inoculation resulted in significant increase of dry weight of shoot as compared to uninoculated plants (Table 3). Strain AA-18 (*Pseudomonas* spp.) and AA-45 (*Bacillus* spp.) caused 43 and 19% improvement in shoot dry weight over uninoculated control. Inoculation also caused prominent improvement in root length (Figure 2). Strain AA-45 (*Bacillus* spp.) gave maximum results and increased the root length by 19% compared to uninoculated control. Minimum increase of 6% over uninoculated control was recorded upon inoculation with AA-39 (*Bacillus* spp.).

As shown in Table 3, inoculation with all strains caused increase in root fresh weight than uninoculated control. The strain AA-39 (*Bacillus* sp.) gave minimum increase (6%) in the root fresh weight over uninoculated control. While AA-27 (*Psychrobacter* spp.) produced 26% higher root fresh weight than uninoculated control. Data on effects of inoculation on dry weight of sesame root showed (Table 3) that root dry weight was improved by 18 and 14% when plants were inoculated with AA-27 (*Psychrobacter* sp.) and AA-06 (*Pseudomonas fluorescens*), respectively when compared with uninoculated control. The strain AA-39 (*Bacillus* sp.) gave least significant results causing only 7% improvement in root dry weight compared with uninoculated control.

Table 4: Effect of PGPR on yield parameters of sesame crop under pot conditions (Average of three replication), means sharing the same letter (s) are statistically at par at 5% probability level

Treatment		Yield Parameter		
		1000 grain weight (g)	**Seed yield (g plant^{-1})**	**Seed oil (%)**
Un-inoculation	**Control**	2.32 ± 0.02 e	7.14 ± 0.08 f	46.51 ± 0.03 f
	AA-06	2.90 ± 0.03 a	10.32 ± 0.02 b	50.16 ± 0.02 a
	AA-18	2.67 ± 0.04 c	10.54 ± 0.02 a	49.78 ± 0.02 b
Inoculation	**AA-27**	2.81 ± 0.02 b	9.49 ± 0.03 c	49.42 ± 0.02 c
	AA-39	2.76 ± 0.03 b	9.11 ± 0.03 d	47.54 ± 0.02 e
	AA-45	2.58 ± 0.02 d	8.15 ± 0.02 e	48.58 ± 0.04 d
LSD ($\rho \leq 0.05$)		0.08	0.10	0.07
		Oil yield (g plant^{-1})	**Seed protein (%)**	**Protein yield (g plant^{-1})**
Un-inoculation	**Control**	3.32 ± 0.03 f	18.88 ± 0.02 f	1.35 ± 0.02 f
	AA-06	5.17 ± 0.01 b	21.28 ± 0.02 b	2.20 ± 0.01 b
	AA-18	5.24 ± 0.01 a	22.40 ± 0.02 a	2.36 ± 0.01 a
Inoculation	**AA-27**	4.69 ± 0.01 c	20.25 ± 0.05 c	1.92 ± 0.01 c
	AA-39	4.33 ± 0.01 d	19.33 ± 0.02 e	1.76 ± 0.01 d
	AA-45	3.96 ± 0.01 e	19.62 ± 0.02 d	1.60 ± 0.01 e
LSD ($\rho \leq 0.05$)		0.05	0.08	0.02

LSD shows least significant difference among means.

Data regarding 1000 grain weight shown in Table 4 indicated that AA-06 (*Pseudomonas fluorescens*) produced highest (25% over uninoculated control) 1000 grain weight. Minimum increase up to 11% in 1000 grain weight was observed in case of AA-45 (*Bacillus* spp.) inoculation. Similarly, inoculation with rhizobacterial strains significantly improved the sesame yield with the maximum increase of 48% over uninoculated control due to AA-18 (*Pseudomonas* spp.) (Table 4).

Biochemical parameters

It was observed that inoculated plants produced more oil than the control plants (Table 4). However, AA-06 (*Pseudomonas fluorescens*) strain was more effective than other strains. Only 2% increase in oil production was recorded in case of AA-39 (*Bacillus* spp.) while comparing with uninoculated control. All strains posed significant positive effects on oil yield, but AA-18 (*Pseudomonas* spp.) gave maximum results and caused 58% more oil yield than uninoculated control (Table 4).

It was noteworthy that inoculation significantly improved the seed protein. Maximum effects were obtained with AA-18 (*Pseudomonas* spp.) which caused 26% increase in seed protein as compared to respective uninoculated control. Minimum improvement of 9% compared to uninoculated control, in seed protein was recorded in case of strain AA-39 (*Bacillus* spp.). Moreover, 75% more protein yield than uninoculated control was obtained upon inoculation with AA-18 (*Pseudomonas* spp.) strain (Table 4).

Similarly, bacterial inoculation with P solubilizing strains significantly enhanced the P content in soil and grain of sesame (Figure 2A and B). It was observed that AA-18 strain resulted in 42.48% increase in P content in grain of sesame as compared to uninoculated control that was statistically similar to isolate AA-27 that caused 40.52% increase in P content of grain of sesame. Maximum available soil phosphorus (41.32% as compared to uninoculated control) was recorded where AA-27 strain was applied that was statistically similar to AA-18 isolate with 40.52% higher available P in the soil.

Discussion

The rhizosphere supports various soil microbe-plant interactions. The bacteria occupying rhizosphere have been well-studied for their beneficial role in plant growth promotion via different direct or indirect mechanisms (Vejan *et al.*, 2016). The current study demonstrated the PGPR efficiency in enhancing growth, yield and oil content of sesame through their potential to mineralize and solubilize P along with ACC-deaminase trait under natural wirehouse conditions.

In vitro tests of our study revealed that P-solubilizers exhibiting phosphatase activity had solubilized/mineralized the inorganic and organic forms of P. These results are in line with Susila *et al.* (2016) who stated that bacteria can carry out solubilization and mineralization of inorganic P. Correspondingly, Alori *et al.* (2017) found that inorganic P-solubilizing rhizospheric bacteria also produced the phosphatase enzyme under *in vitro* environments. Thus, more synthesis of bacterial phosphatase may result in higher P solubilization.

In vitro tests showed that most of the bacteria exhibited both alkaline and acidic phosphatase activities. While studying the effects of rhizobacterial inoculation on status of phosphatase activity, numerous researchers have been reporting that bacterial strains grown on organic P compounds have potential to synthesize both alkaline and acid phosphatase in liquid medium containing organic P substrate (Behera *et al.*, 2017; Sang *et al.*, 2018). In current study, strains showed both qualitative and quantitative ACC-deaminase activity and these results are supported by Shameer *et al.* (2018) who observed that phosphate solubilizing rhizobacteria had plant growth-promoting ACC deaminase activity.

Our filed study demonstrated that all strains significantly improved the growth, biochemical parameters and yield of the sesame compared to uninoculated control. This might be attributed to higher microbial actions which caused solubilization of insoluble and inorganic P or mineralization of organic matter to release organically bound P in the soil (Richardson and Simpson, 2011; Anand *et al.*, 2016; Bechtaoui *et al.*, 2020).

Generally, P supply is associated with enhanced root density and proliferation causing higher nutrient and water supply to roots and consequently increased plant growth, yield as well as dry matter (Kang *et al.*, 2014). According to Nkaa *et al.* (2014), inoculation with inorganic P solubilizing rhizobacteria along with capability to mineralize the organic P compounds increased the leaves number plant^{-1}, grain numbers pod^{-1}, and seed yield ha^{-1} in cowpea (*Vigna unguiculata* L.). The inoculation of rapeseed with bacteria exhibiting phosphate solubilization trait resulted in increased plant growth and yield and minimized the need of chemical fertilizers (Valetti *et al.* 2018; Tahir *et al.* 2018).

In our study, enhanced plant growth might be due to ACC-deaminase trait of rhizobacterial strains that can assist the plant in P acquisition through promoting root growth. Nevertheless, improved root growth can help the plant in P-

uptake. Mukhtar *et al.* (2020) reported that 54% P-solubilizing strains exhibited ACC-deaminase activity.

The results of our pot study showed that inoculation with bacterial strains enhanced the protein and oil production in sesame. This can be attributed to synthesis of phosphatase enzyme by microbes which helped in insoluble organic and inorganic P compounds (Omer and Abd-Elnaby, 2017). Availability of P contribute to formation of and translocation of carbohydrates, growth of roots, and resistance against pathogens leading to increased seed yield, seeds capsule^{-1}, capsules number plant^{-1}, oil and protein content in sesame (El-Fawy and El-Said, 2018).

Similar results were recorded by the Kutlu *et al.* (2019) who reported that microbial inoculation in plants caused high oil yield than uninoculated control. Moreover, Okon and Labandera-Gonzales (1994) found that upon inoculation with phosphates and ACC-deaminase-producing bacteria in a pot experiment, plants produced more biomass, protein and oil which is result of increased root growth and ultimately P acquisition. The study suggested the multi-trait (such as ACC-deaminase, phosphatase enzyme synthesis, P solubilization ability) exhibiting PGPR can be more effective in enhancing growth, oil and yield in sesame.

Higher phosphorus contents in grain of sesame and soil were observed in inoculated treatment as compared to uninoculated control. Many researchers have reported increased seed P content by phosphate solubilizing microorganisms (Kucey, 1987; Mehana and Wahid, 2002; Zaidi et al., 2004), our study revealed the same effect. The higher P content in plant and soil might be because of the ability of P solubilizing PGPR that enhanced the available P in soil and thus improved the uptake of P by the sesame plant.

Likewise, Minaxi *et al.* (2012) demonstrated the efficiency of PGPR possessing multiple growth promoting traits. In this study, multi-trait bacteria were found to be more effective in improving sesame growth and yield related parameters in pot experiment which might be attributed to multiple plant growth promoting traits of PGPR.

Conclusion

The PGPR with capability to solubilize the inorganic and organic P compounds along with ACC-deaminase can significantly improve the development, growth and yield of sesame by producing phosphatase to release P from insoluble P compounds (either inorganic or organic). The study revealed that bacteria with multi-traits can be a promising and effective tool for enhancing growth and yield of crop under pot conditions.

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