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Original Research

Evaluating Multiples Trait of Rhizosphere Bacteria Aimed at Improving the Growth, Crop Output, and Oil Fraction of Sesame

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Abstract

The crop growing of sesame is of important significance due to its nutritional, medicinal, and biological value, which drives the need for improved production. Various environmental factors pose challenges to sesame yield, necessitating the development and promotion of cultivation technologies. One such method involves the application of plant growth-promoting rhizobacteria (PGPR) possessing enzymatic capabilities linked to plant growth promotion. In this study, rhizobacteria with enzymatic activity were isolated and screened, resulting in the identification of five promising strains (F-06, F-18, F-27, F-39, and F-45) from different investigative series. These strains, representing a variety of species, including *Pseudomonas, Psychrobacter, and Bacillus*, were assessed for their effects on field-grown sesame. The data revealed that bacterial inoculation led to notable improvements in various growth parameters of sesame compared to uninoculated controls. Specifically, inoculated plants exhibited increases of up to 26%, 12%, and 37% the plant, 1000-grain weight, and grain yield, respectively. Moreover, biotic yield, straw yield, and yield index were also significantly enhanced by 25%, 15%, and 17%, respectively, over inoculated controls. Additionally, there were substantial increases in oil content (47%) and protein content (62%) in response to inoculation with selected rhizobacteria compared to controls. These findings suggest the positive effect of multitrait microorganisms over PGPR and single-trait microbes when improving plant development and yield in sesame cultivation.

Keywords: sesame, bacterial inoculation, phosphatase, ACC-deaminase

Introduction

Sesame, scientifically known as *Sesamum indicum L.*, is a globally recognized oilseed crop that belongs to the Pedaliaceae family [1]. It is also commonly referred to as benniseed or sesamum. Sesame possesses high nutritional value, providing a variety of essential nutrients to consumers [2]. It has high-grade edible oil, comprising a noteworthy proportion of 42% to 54%. Moreover, sesame seeds harbor substantial quantities of protein (18-25%), carbohydrates (16-18%), and ash [3, 4]. Furthermore, they possess notable concentrations of minerals like calcium, phosphorus, potassium, zinc, iron, boron, and copper, rendering sesame a prized dietary reservoir of these essential elements [5]. This plant kind comprises plentiful important fatty acids as well as substantial

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quantities of oleic, linoleic, stearic, and palmitic acids [6]. Sesame seed oil has notable stability due to its high antioxidant content, including sesamin, sesame, sesamol, sesamolin, and squalene, which surpass the antioxidant levels present in other types of oils [7]. Furthermore, it has potential health benefits against several diseases, such as asthma, eye illnesses, and intestinal blockage [8].

Nutrients such as phosphorus (P) are vital for plant development and metabolic activities, providing essential energy for various biochemical functions [9]. Additionally, it is necessary to produce nucleic acids like DNA and RNA. Previous studies have indicated that approximately 80-90% of soils in arid and semi-arid regions exhibit limited phosphorus availability [10]. Phosphorus availability in soil is commonly restricted, presenting a substantial hurdle for plant absorption [11]. Moreover, both soil chemical characteristics and human agricultural practices can impact the accessibility of phosphorus in soil [11]. Phosphorus-based chemical fertilizers are vulnerable to being adsorbed [12]. Phosphorus in soil is present in both chemical and elemental states, with the majority of total phosphorus being found in organic compounds such as nucleotides, phospholipids, and inositol phosphate [13]. Soil organic phosphorus (SOP) plays a vital role in facilitating P absorption by various crops, especially in alkaline soils [14]. In most mineral soils, SOP may constitute a significant proportion of the overall P content, ranging from 20% to 80% [15]. It serves as a notable source for plant-accessible P through mineral accumulation [16]. Plants cannot directly uptake P in organic form; soil phosphatases facilitate the conversion of SOP into inorganic forms that plant roots can uptake [17, 18]. Phosphatases are classified into acid and alkaline types according to their optimal activity levels, occurring at pH levels of 11 and 6.5, respectively [19].

The majority of bacteria and higher plant species are known to synthesize acidic phosphatases, whereas alkaline phosphatases are predominantly produced by a few microorganisms [20]. Plant and microbial phosphatases play an important role in effectively releasing orthophosphate ions (HPO_4 and H_2PO_4) from SOP [21]. Soil bacteria play a crucial role in facilitating the release of P from organic compounds within the soil, thereby contributing to the overall mineralization process of total soil P [22, 23]. Chemical or synthetic fertilizers such as phosphatic fertilizers (e.g., rock phosphate) are commonly used to restore soil P content, which leads to serious environmental concerns [24, 25]. Rock phosphate stocks are predicted to be depleted in the next 50 to 100 years due to their high consumption [26]. Additionally, there are financial constraints, such as the fact that the cost of rock phosphate is globally increasing and its standard is deteriorating over time [27]. To address these challenges, microbial inoculants are currently under global investigation, making it possible to convert P into accessible forms, thereby fostering the adoption of sustainable agricultural methods [28]. The bacteria present in plant roots are well recognized for their beneficial effects on plant growth and are commonly identified as plant growth-promoting rhizobacteria (PGPR) [29]. PGPR encourages and boosts the growth of plants through the fixation of nitrogen that is present in the atmosphere, enzyme production, mineral dissolution, production of siderophores, cycling of nutrients, plant hormone production, and ACC-deaminase (1-aminocyclopropane-1-carboxylic acid) regulation [30]. PGPR improves the growth and development of plants through enhanced enzymatic activities [31, 32]. Additionally, bacteria-producing ACC-deaminase can enhance seed root elongation and germination by reducing the levels of ethylene in plant. It is well-reported that bacterial strains with both ACC-deaminase and phosphate solubilizing capabilities have a substantial effect on root length [33, 34]. The objectives of our study were to investigate the microbial capability of P-solubilization and mineralization along with ACC-deaminase activity under sesame cultivation. In this study, we elucidated the potential of PGPR to enhance enzymatic activity, soil properties, crop output, and the oil percentage of sesame.

Materials and Methodology

Isolation and Identification of Bacterial Strains

Rhizosphere soil samples underwent bacterial strain isolation utilizing Luria Bertani (LB) agar medium. The dilution plate technique was utilized to isolate rhizobacteria under sterile conditions. To obtain each distinct rhizobacterial strain, purification was repeated two or three times via streaking onto fresh plates [35].

The assessment of rhizobacterial phosphatase activity in different conditions (acidic/alkaline) was performed utilizing the techniques provided by [36], while the measurement of whole and the effects of inorganic phosphates in strains of bacteria was carried out using [37]. Organic phosphorus was calculated by deducting the effects of inorganic phosphates from the total soil phosphorus. The bacterial strain's ACC-deaminase activity was evaluated *in vitro* using the method reported by Shaharoona et al. [38]. The activity of ACC-deaminase was quantified using the methods of [39, 40].

Seed Inoculation

Rhizobacterial strains were cultured in 250 mL conical flasks using an LB broth medium comprising yeast extract (5g/L), sodium hydroxide (10g/L), and tryptone (10g/L). The orbital shaking incubator was used to shake the flasks at a speed of 100 revolutions per minute over a period of three days at 28 ± 1°C. Before seed inoculation, the optical density (OD) at 535 nm was determined using an OD meter (Biolog-21907, USA) and carefully adjusted by the serial dilution technique to ensure a uniform bacterial population in the LB broth [41]. The sesame seeds were rinsed with double-deionized water and subsequently inoculated using a slurry, technique. Sterilized peat was combined with a 10% solution of sugar to make a uniform slurry and then the inoculum was combined with peat (1:1 volume/weight). The seeds in the uninoculated control group were only rinsed with a 10% sterilized sugar solution. The inoculated seeds were used for sowing under shade following a 6–8-hour air drying period. After harvesting, the sesame seeds were cleaned, dried, and used for further analysis. According to AOAC (1990), the Soxhlet apparatus was used to quantify the oil content of the seeds, and the Kjeldhal technique was used to evaluate the amount of protein.

Statistical Analysis

The data was analyzed statistically using Statistix 8.1.

Results and Discussion

Identification

In Table 1, the bacterial strains were categorized for various biochemical characteristics. The Pseudomonas fluorescence isolate F-6 and the *Psychrobacter* sp. isolate F-27 showed a P-solubilization index (PSI) of 7.25 and 2.94, respectively. The quantitative analysis of the phosphate-solubilization of bacterial isolates highlights that it not only showed solubilization in solid media but also had solubilization ability in liquid media when supplemented with tricalcium phosphate (TCA).

At 515.29 and 725.63 µg mL^{-1}, the isolates' levels of phosphate solubilization from inorganic P sources contrasted. The isolates of bacteria had varying degrees of acidic and alkaline phosphatase enzyme activity in liquid culture in the presence of β-glycerophosphate as a substrate.

The highest acid phosphatase activity (43.65 µg PNP g^{-1} h^{-1}) for isolate F-18 (Pseudomonas sp.) was found. The lowest recorded acid phosphatase activity (20.54 µg PNP g^{-1} h^{-1}) was observed with F-39 (Bacillus sp.) inoculation. The alkaline phosphatase activity of these isolates ranges from 16.35 to 29.59 µg PNP g^{-1} h^{-1}. The qualitative ACC-deaminase activity was measured using optical density, also referred to as bacterial growth on ACC as a substrate. With an OD value of 0.66, Bacillus aquimaris (F-45) had the lowest bacterial isolate, while Psychrobacter sp. (F-27) had the highest. The isolates displayed ACC-deaminase activity at varying levels (325-337 nmol α-ketobutyrate g-1 biomass h-1).

Table 1. Bacterial characterization.

Bacterial Isolates	Phosphate Solubilizing Index (PSI)	Available P (µg mL^{-1})	Phosphatase activities (µg PNP g^{-1} h^{-1})		ACC-deaminase assay Qualitative (OD value)	ACC-deaminase activity (nmol α-ketobutyrate g^{-1} biomass h^{-1}
			Acid phosphatase	Alkaline phosphatase		
F-12	7.25 ± 0.19	626.34 ± 0.22	34.92 ± 1.4	23.47 ± 0.56	0.65 ± 0.03	325 ± 0.01
F-11	4.91 ± 0.93	663.01 ± 0.84	43.65 ± 1.3	29.59 ± 1.17	0.72 ± 0.03	350 ± 0.05
F-13	2.94 ± 0.30	515.29 ± 0.21	29.31 ± 1.73	16..35 ± 0.86	0.75 ± 0.02	355 ± 0.01
F-32	4.36 ± 0.83	619.41 ± 0.22	20.54 ± 0.74	15.61 ± 1.1	0.71 ± 0.01	336 ± 0.09
F-12	4.32 ± 0.50	725.63 ± 0.12	24.87 ± 1.6	21.13 ± 0.9	0.66 ± 0.02	337 ± 0.01

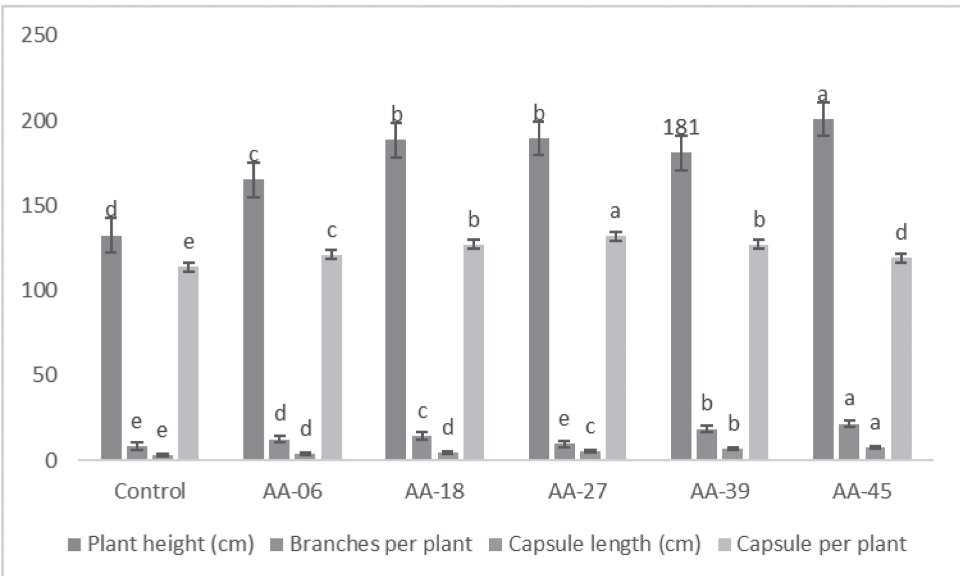

Fig. 1. Effects of seed inoculation on sesame plant height, branches per plant, capsule length, and capsules per plant (n=3).

Growth Factors

The results from Fig. 1 of the study showed that introducing all the chosen bacterial strains to sesame plants grown in the field significantly increased their height. Among them, plants treated with Bacillus aquimaris (F-45) grew the most. Following inoculation with F-27 (*Psychrobacter* sp.), plant height increased by 11% compared to the untreated control group. While the inoculation had a significant impact, statistical analysis didn't distinguish it from the control. Furthermore, the induction of enzymatically active bacteria had a noticeable effect on the number of branches per plant. The *Pseudomonas fluorescence* strain F-06 proved to be the most effective, increasing the number of branches per plant by 45% compared to the control. In comparison to the untreated control, inoculating specific bacteria led to a significant increase in capsule length. *Psychrobacter* sp. (F-27) showed the most substantial improvement, with capsule height increasing by up to 57% compared to the control, while Bacillus sp. (F-39) showed the least improvement, with an increase of up to 19%. Moreover, all rhizobacterial strains were successful in boosting the number of capsules per plant compared to the control, as illustrated in Fig. 1. When comparing the effects of F-18 (*Pseudomonas* sp.) inoculation to the control treatment, there was a remarkable 48% increase in the number of capsules per plant. After inoculating with F-18 (Pseudomonas sp.), the total number of capsules per plant saw a 48% increase compared to the control treatment. Fig. 2 presents data on the impact of inoculation on the number of seeds per capsule. In response to inoculation with F-27 (Psychrobacter sp.), there was an increase in seeds per capsule of up to 47% compared to the control. Following closely was F-06 (Pseudomonas fluorescence), which resulted in a 38% increase in seed number per capsule compared to the uninoculated control. On the other hand, F-39 (Bacillus sp.) was the least effective, causing only a 12% increase in seed number per capsule compared to the control. Additionally, the data from Fig. 2 indicated that the maximum 1000-grain weight was achieved with inoculation by F-27 (Psychrobacter sp.), which was 12% higher than the control. Although the inoculation effect with the selected bacterial strains was statistically similar, it was significantly different from the control.

Yield Factors

Grain yield was most significantly impacted by Pseudomonas fluorescence strain F-06, which increased it by 37% more than the control. When compared to the control, the increase in grain production resulting from the inoculation of enzymatically active rhizobacteria ranged from 8% to 37%. Pseudomonas fluorescence, or F-06, was the inoculant that produced the biggest increase in biological yield, up to 29% over the control.

Table 2. Effects seed inoculation growth attributes of sesame plants (n=3).

Treatment	Seed per capsule	1000 grain weight (g)	Grain yield (kg/hac)	Biological yield (Lg/hac)
Control	67.54 d	2.98 d	1188.45 f	2730.12 c
F-12	93.25 ab	3.17 c	1620.45 a	3398.45 a
F-11	84.56 bd	3.11 bc	1530.24 b	3240.47 a
F-13	99.45 a	3.27 a	1420.31 c	3058.49 b
F-32	74.54 cd	3.08 c	1264.21 e	2841.25 c
F-12	84.65 bc	2.47 ab	1344.28 d	3017.58 ab

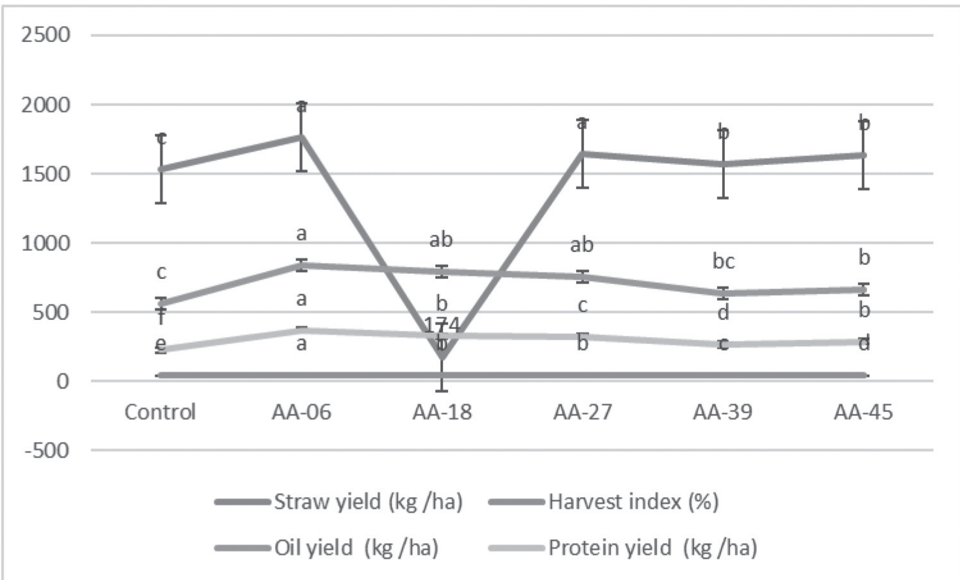

Fig. 2. Effects of a field trial's inoculation with a particular PGPR that exhibits enzymatic activity on sesame harvest index, oil yield, protein yield, and straw yield (average of three replicates).

On the other hand, F-39 (Bacillus sp.) only increased its biological yield by 5% over the untreated reference. Similar to this, inoculating with particular bacterial strains increased the yield of sesame straw. Pseudomonas fluorescence, or F-06, produced the highest output, up 16% over the control. The next most effective strain, Pseudomonas sp. F-18 was found to improve the yield of straw by up to 27% compared to the uninoculated control. Conversely, a slight rise in the harvest index was observed upon inoculation with Bacillus sp. F-39 (in comparison to other strains); that being said, it was still significantly better compared to the control treatment (Table 2).

Discussion

The rhizosphere is the part of the soil where plants and soil MO interact with each other. Microorganisms had optimistic effects on plant development and yield production through both direct and indirect mechanisms [42]. Our investigation demonstrated the efficiency of plant growth-promoting rhizobacteria (PGPR) retaining traits such as phosphate solubilization, phosphatase production, and ACC-deaminase activity in enhancing the growth, yield, and oil content of sesame plants under field conditions. Similarly, a previous study found that inoculation with PGPR increased grain yield by suppressing diseases [43]. The results from these laboratory tests indicate that both the solubilization of inorganic phosphorus and phosphatase activity (mineralization) can coexist within the same bacteria [43]. In laboratory experiments, most of the bacterial isolates displayed both acidic and alkaline phosphatase activities to varying extents. Research conducted on a strain of *Rhizobium leguminosarum* showed that when grown in liquid media supplemented with four different organic phosphorus compounds, the strain produced both types of phosphatases. These findings indicate the strain's ability to utilize various organic phosphorus sources. Regarding ACC-deaminase activity, our data indicated that the tested isolates possessed varying levels of effectiveness in this regard [42]. Similarly, Nagaraju et al. [43] noted that certain phosphate-solubilizing bacteria also produce ACC-deaminase, which serves as an enzyme promoting plant growth. In our field trial, all the bacterial strains showed an increase in the growth, yield parameters, and oil content of sesame compared to the control treatments. The enhanced performance of the selected strains in improving various growth aspects, yield parameters, and oil content could be attributed to increased microbial activities triggered by their inoculation. These activities might have led to the dissolution of fixed forms of soil phosphorus (inorganic P) or the mineralization of organic matter, releasing organic P, as suggested by Richardson et al. [44]. The combination of phosphate-solubilizing microorganisms with phosphatic fertilizer and organic manure significantly boosted the phosphorus content of seeds, tillers per square meter, grain yield, and overall biological yield of wheat.

The plant growth promotion observed in our study with the chosen bacterial strains was linked to their production of ACC-deaminase in addition to phosphate solubilization. Moreover, the increased activity of rhizosphere phosphatase following inoculation with these bacterial strains suggested that they secreted phosphatase enzymes to break down soil phosphorus from organic compounds. In a study conducted by Ahmad et al. [14], the effectiveness of Rhizobium and Pseudomonas strains in enhancing the physiology, ionic balance, and quality of mung beans grown in salt-affected fields, Pseudomonas fluorescence biotype G (N3), not only exhibited ACC-deaminase activity but also showed positive results for phosphate solubilization.

The field trial results further demonstrated a significant improvement in the oil and protein content of sesame plants following microbial inoculation. This could be attributed to the microbial production of phosphatase enzymes, which facilitated the mobilization of organic and inorganic phosphorus compounds through enhanced microbial solubilization and mineralization, as suggested by Richardson et al. [44]. These findings align with those of Chandra [45], who observed higher oil content in inoculated plants compared to uninoculated controls. Similarly, it was reported increased plant biomass, oil, and protein content in a pot trial due to inoculation with phosphatase-producing isolates exhibiting ACC-deaminase activity, which indirectly enhanced phosphorus acquisition by promoting root growth [46].

The research suggests that using plant growth-promoting rhizobacteria (PGPR) with abilities like phosphate solubilization, phosphatase production, and ACC deaminase activity could significantly enhance the growth, yield, and oil content of sesame crops. The experiments showed that phosphate-solubilizing bacteria contribute to growth enhancement by releasing organic acids (which lower pH) and/or producing phosphatases and ACC deaminase simultaneously. This highlights the importance of choosing bacteria with diverse traits during screening and selection processes, offering an appealing approach to improving crop performance.

Conclusion

Rhizobacteria, found in the root zone of plants, play a crucial role in supporting plant growth by breaking down both organic and inorganic phosphates and displaying ACC-deaminase activity. These beneficial bacteria help release phosphorus from different sources, including organic and inorganic compounds, by producing enzymes like phosphatases and organic acids. This study suggests that harnessing these microorganisms could be a reliable and effective strategy for improving crop growth and productivity in real-world farming situations.

Conflict of Interest

The authors declare no conflict of interest.

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