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Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4

Sandeep Kumar [a], Piyush Pandey [b], D.K. Maheshwari [a,*]

[a] Department of Botany and Microbiology, Gurukul Kangri University, Haridwar (U.A.) 249404, India
[b] S.B.S.P.G. Institute of Biomedical Sciences and Research, Balawala, Dehradun (U.A.) 248161, India

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ABSTRACT

Pseudomonas aeruginosa LES4, an isolate of tomato rhizosphere was found to be positive for several plant growth-promoting attributes like production of indole acetic acid, HCN and siderophore, solubilization of inorganic phosphate along with urease, chitinase and β-1-3-glucanase activity. In addition, it showed strong antagonistic effect against *Macrophomina phaseolina* and *Fusarium oxysporum*. *P. aeruginosa* LES4 caused halo cell formation and other morphological deformities in mycelia of *M. phaseolina* and *F. oxysporum*. Root colonization was studied with *Tn5* induced streptomycin resistant transconjugants of spontaneous tetracycline-resistant LES4 (designated $LES4^{tetra+strep+}$) after different durations. The strain was significantly rhizospheric competent, as 17.4% increase in its population was recorded in sesame rhizosphere. Seed bacterization with LES4 resulted in significant increase in vegetative growth parameters and yield of sesame over non-bacterized seeds. However, application of LES4 with half dose of fertilizers resulted in growth equivalent to full dose treatment, without compromising with the growth and yield of sesame. Moreover, the oil yield increased by 33.3%, while protein yield increased by 47.5% with treatment of half dose of fertilizer along with LES 4 bacterized seeds, as compared to full dose of fertilizers.

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1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop, next to soybean and groundnut but has low yield potential [35]. India rank first in the world in area (about 2.47 m ha annually, 40% of the world) and production (0.74 m tones, 27% of the world). In intensive cropping system, supplementing soil nutrients by use of chemical fertilizer is considered inevitable for obtaining optimum yield of crops, however, their utilization efficiency remains low, due to loss by volatization, denitrification, leaching and conversion into unavailable forms. Continuous use of chemical fertilizers subverts the soil ecology, disrupt environment, degrade soil fertility and consequently shows harmful effects on human health [1] and contaminates ground water [15]. Presence of chemicals in sesame had been major impediment in the promotion of sesame export. Export consignments of sesame are sometimes unsuitable in the international market due to the presence of pesticide residue, resulting in loss of revenues [6].

Role of plant growth-promoting rhizobacteria (PGPR) in plant growth promotion and biological control of soil borne pathogens has been intensively investigated [17,19]. Integration of PGPR with traditional inorganic fertilizers in the field may prove to be effective means to increase the solubility of insoluble phosphorous ions and other minerals to plants with simultaneous reduction in diseases incidence. They take systemic and simultaneous account of environmental aspects, quality of the produce and profitability of agriculture [22]. Alternatively, substitution of chemicals with bacterial fertilizers and biopesticides, especially blending of chemical fertilizers with chemical adaptive PGPR is a promising approach to obtain sustainable fertility of the soil and plant growth [39]. There are several PGPR currently commercialized whose growth-promoting activity in crop plants have been demonstrated in several ways including production of iron-sequestering siderophores and antimicrobial compounds that hinder colonization of hosts by phytopathogens [40], induction of host systemic disease resistance, solubilization of precipitated mineral nutrients and for production of plant growth hormones, thereby enhancing the plants ability to take up nutrients from soil and increasing yield [8].

* Corresponding author. Tel.: +91 1334 246 767 (O), 265 469 (R), +91 983 730 8897 (M); fax: +91 1334 246 767 (O).
E-mail addresses: piyushgkp@rediffmail.com (P. Pandey), maheshwaridk@gmail.com (D.K. Maheshwari).

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Hence, the present work was aimed to blend chemical fertilizers with effective PGPR to obtain the optimum benefits. This strategy was designed to allow reduction in the dose of fertilizers, along with an approach to increase productivity of sesame without associated ecological harm.

2. Materials and methods

2.1. Microorganisms

A number of bacterial strains were isolated using standard microbiological technique from the rhizosphere of tomato (*Lycopersicon esculantum* L.) grown in nutrient deficient soil in wasteland. Healthy and young tomato seedlings were gently uprooted at Dehradun (Alt. 640 m, Lat. 30°30′40″N, Long. 77°52′12″E, Rainfall 216 cm) in India. The roots were cut into 2 cm long segments and vortexed in 25 ml sterilized distilled water for few minutes. Suitable dilution was plated by serial dilution plate technique on nutrient agar medium (NAM). The bacterial colonies were isolated and maintained on NAM slants at 4 °C. The isolates were characterized for direct and indirect plant growth-promoting (PGP) activities including solubilization of inorganic phosphate, IAA production, and HCN production along with antagonism against two fungal pathogens. Seven isolates were selected and identified on the basis of morphological, physiological and a biochemical characteristic according to Bergey's manual of determinative bacteriology [14], and compared against *Pseudomonas aeruginosa* MTCC-1934, *Pseudomonas putida* MTCC-102 and *Pseudomonas fluorescens* MTCC-103 as standard strains. All isolates were maintained on tryptic soy agar medium (TSM) at 4 °C for further use.

Macrophomina phaseolina and *Fusarium oxysporum* were procured from culture collection laboratory, Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, India. Pure culture of fungal colonies was maintained on potato dextrose agar (PDA) slants at 4 °C for further use.

2.2. Screening for plant growth-promoting attributes

Phosphate solubilization [38], IAA production [12], HCN production [23], siderophore production [32], and chitinase and β-1-3-glucanase activities [7] were determined as per standard protocols. Antagonistic activity of isolates against phytopathogens was determined according to Skidmore and Dickinson [36].

2.3. In vitro antagonism

Antagonistic properties of bacterial strains were tested against two fungal pathogens *M. phaseolina* and *F. oxysporum* causing charcoal rot and wilt diseases on sesame using a dual culture technique as described previously [19]. Agar blocks (5 days old, 5 mm dia.) containing 5 days old mycelia were placed in four corners of a Petri plate, and inoculated with loopful culture (24 h old) of bacterial strain, spotted 2 cm apart from the fungus. These plates were incubated at 28 °C. Plates inoculated with only fungal agar blocks served as control. Growth inhibition was calculated by measuring the distance between the edge of bacterial and fungal colonies, and percent inhibition was calculated by following formula:

$$\text{Growthinhibition} = [(C - T)/C]100$$

where C = radial growth of fungus in control, T = radial growth of fungus in dual culture. Fungal hypha surrounding zone of inhibition, and from control plates were observed under the microscope by standard procedure.

2.4. Seed bacterization

The certified seeds of sesame (*S. indicum* L. cv. ST-1) were procured from Center for Biotechnology, Jamia Hamdard University, Hamdard Nagar, New Delhi, India. Seed bacterization was done by the method of Weller and Cook [41]. Seeds were surface sterilized with 95% alcohol for 30 s, followed by 0.1% (w/v) $HgCl_2$ for 1–2 min and then washed with sterile distilled water for 5–6 times. These germinated seeds were dried under sterile air stream. 24 h old culture of LES4 was centrifuged at 7000 rpm for 15 min at 4 °C. The pellets were retained and re-suspended in sterile distilled water to obtain a population density of 10^8 cfu ml^{-1} and the cell suspension was mixed with 1% carboxymethyl cellulose (CMC) solution in ratio of 1:0.5. Slurry thus obtained, was coated on the surface of germinated seeds. The seeds coated with 1% CMC slurry without LES4 served as control. The bacterized seeds were dipped in known volume of sterile water and cfu were counted on TSM for standardizing the inoculum. The population of LES4 was recorded by dilution plate technique as 10^8 cfu seedling^{-1}.

2.5. Root colonization

Root colonization of *P. aeruginosa* LES4 was studied by quantitative analysis of population dynamics in the rhizosphere of sesame using antibiotic resistant marker strain. Tetracycline-resistant strain of LES4 was isolated on TSM, containing 100 mg l^{-1} of tetracycline (*P. aeruginosa* LES4^{tetra+}). LES4^{tetra+} was engineered for streptomycin resistance (100 mg l^{-1}) (designated LES4$^{tetra+strep+}$) with *Tn5* delivery suicide vector pGS9 [33] in donor *Escherichia coli* strain WA803 (pGS9) as described [18]. Sesame plants emerged with bacterized seeds was sampled after 30, 60, 90 and 120 DAS, and bacterial population on the roots were measured. Root adhering soil particles were carefully removed and root was cut into 1 cm long segments, which was vortexed in known volume of sterile water to release root-associated bacteria. Suitable dilutions of the suspension were plated on TSM containing tetracycline and streptomycin (100 mg l^{-1} each) to enumerate the bacterial population.

2.6. Field trial

Field trials of sesame were carried in sandy loam soil (80.3% sand, 6.5% silt, 7.7 clay, total organic C 0.0923%, pH 6.8 having 35% water holding capacity). The recommended dose of chemical fertilizer for sesame crop was 120 kg ha^{-1} nitrogen, in three split doses of urea, and 30 kg ha^{-1} phosphate in the form of Diammonium phosphate (DAP) and 30 kg ha^{-1} potassium in the form of Murate of potash (MoP) in single doses. The combination of NPK was $N_{40+40+40}P_{30}K_{30}$. Seeds bacterized with *P. aeruginosa* LES4 and non-bacterized seeds were sown on randomized block design (RBD) in 7 sets of treatments with three replicates of each treatment, (i) seeds coated with *P. aeruginosa* LES4, (ii) *P. aeruginosa* LES4 + half dose of chemical fertilizers, (iii) half dose of chemical fertilizers, (iv) full dose of chemical fertilizers, (v) seeds coated with CMC slurry only without any fertilizer and bacteria (control). The crop was irrigated three times (including one pre-sowing irrigation) at different critical stages, i.e. at flowering/capsule formation and seed filling. Seed germination rate (%) was noted on 15 days after sowing (DAS). Vegetative growth parameters including biomass accumulation, root and shoot lengths, leaf area were recorded at 30, 60, 90 and yield attributes were recorded after 120 DAS on harvesting the crop. The experiment was conducted for two consecutive years and data are presented as mean. The data were analyzed statistically by using analysis of variance (ANOVA) to find out significance at 1% and 5% levels [9].

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