Enterobacter sp. strain Fs-11 adapted to diverse ecological conditions and promoted sunflower achene yield, nutrient uptake, and oil contents

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Abstract

Plant growth-promoting rhizobacteria are under extensive investigation to supplement the chemical fertilizers due to cost-effective and eco-friendly nature. However, their consistency in heterogeneous soil and diverse ecological settings is unclear. The current study presents in vitro and field evaluation of pre-characterized PGPR strain *Enterobacter* sp. Fs-11 (GenBank accession # GQ179978) in terms of its potential to enhance sunflower yield and oil contents under diverse environmental conditions. Under in vitro conditions, strain Fs-11 showed optimal growth at a range of temperature (15 to 40 °C) and pH values (6.5 to 8.5). Extracellular and intracellular localizations of the strain Fs-11 in sunflower root cortical cells through transmission electron microscopy confirmed its epiphytic and endophytic colonization patterns, respectively. In field experiments, conducted at three different agro-climatic locations, inoculation of strain Fs-11 at 50% reduced NP fertilizer resulted in a significant increase in growth, achene yield, nutrient uptake, and oil contents. Inoculation also responded significantly in terms of increase in mono- and polyunsaturated fatty acids (oleic and linoleic acids, respectively) without rising saturated fatty acid (palmitic and stearic acids) contents. We concluded that *Enterobacter* sp. Fs-11 is a potential candidate for biofertilizer formulations to supplement chemical fertilizer requirements of sunflower crop under diverse climatic conditions.

Keywords *Enterobacter* sp. · Field evaluation · Transmission electron microscopy · Fatty acids · Agro-climatic locations

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Introduction

Plant growth-promoting rhizobacteria (PGPR) are able to associate and persist in the dynamic rhizosphere and endosphere environments [1], thereby benefiting plants in terms of nutrient acquisition from soil. Due to its nutritional value, rhizosphere serves as a PGPR hot spot for interactions between plants and microbes [2]. In such nutritionally rich environment, PGPR execute many phytobeneficial functions like atmospheric nitrogen fixation, mineral phosphate solubilization, phytohormone production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, quorum sensing, growth suppression of many plant pathogens, and confer tolerance in plants against biotic and abiotic stresses [3–5]. The genera identified from rhizosphere with known PGPR traits are *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Serratia*, *Acinetobacter*, *Erwinia*, *Flavobacterium*, *Proteus*, *Xanthomonas*, *Agrobacterium*, *Frankia*, etc. [3, 6–8]. In developing and developed countries, there has been a continuous applied research on

PGPR inoculation with taxonomically different crop plants like wheat [9], rice [10], sunflower [11, 12], maize [13], and bean [4] to establish sustainable agricultural practices.

PGPR-based bioinoculants are being commercialized to promote plant growth and control disease-causing microorganisms [14, 15]. Plant-beneficial bacteria belonging to the genera *Azospirillum*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Bacillus*, *Erwinia*, and *Flavobacterium* have been extensively characterized and used as bioinoculants for crop plants with variable degree of success [16, 17]. Such PGPR-based biofertilizers are useful in minimizing the application of environmentally unsafe and economically expensive chemical fertilizers [18, 19]. These bioinoculants can be classified into biofertilizers (mobilizing macro- and micro-nutrients from the environment), phytoestimators (growth hormone producing), and biopesticide (suppressing phytopathogens) [3].

There exists a great need to benefit from such promising, renewable, and eco-friendly bioinoculants to cope with a continuously decreasing fertilizer use efficiency and food security issues in the technologically advanced world [20]. Farmers around the globe, especially in developing countries, are diverting to the bacterial inoculants due to the cheaper rates, eco-friendly nature, and availability throughout the planting seasons [21]. Hence, the global acceptance of biofertilizers may reduce the farmer's dependence on agro-chemicals and make the bioinoculants globally available [22]. Consequently, the synthetic fertilizers are expected to be substituted with bacterial inoculants in the future [23].

The outcome of microbial inoculants is variable especially in field conditions due to their narrow adaptation level under heterogeneous biotic and abiotic factors [24, 25]. In addition, adaptability of a particular bioinoculant or bacterial strain to diverse agro-climatic conditions is always challenging [26]. Thus, the objective of this research was to evaluate the inoculation effect of a potent and characterized *Enterobacter* sp. strain Fs-11 on various agronomic and physiological attributes of sunflower (*Helianthus annuus* L.) under dissimilar ecological conditions of Faisalabad, Peshawar, and Rawalakot, Pakistan. Sunflower carries ideal combination of saturated and unsaturated fatty acids, and thus, the crop has a great potential to fill the gap between edible oil production and consumption in the country [27]. Thus, the study also includes the field inoculation of sunflower with strain Fs-11 followed by its effect on sunflower oil contents under diverse environmental conditions.

Materials and methods

Background and significance of *Enterobacter* sp. strain Fs-11

Enterobacter sp. Fs-11 was originally isolated from sunflower (*Helianthus annuus* L.) rhizosphere soil (EC 0.76 dS m⁻¹,

pH 8.02, organic matter 0.79%, total N 0.039%, and available P 5.6 mg kg⁻¹), sampled from Faisalabad (30°44'27.10 N, 72°38'18.39 E), Pakistan, during a sampling visit in April 2010. The strain has already been reported as mineral phosphate-solubilizing, auxin-producing, and growth-promoting bacterium [11].

In vitro growth studies

Before inoculating with sunflower seeds in field experiments, strain Fs-11 was investigated in vitro to thrive at a range of temperature and pH values. A 50-mL culture of strain Fs-11 was grown in Luria-Bertani (LB) broth at 150 rpm at a range of temperatures (15, 20, 25, 30, 35, and 40 °C) and a fixed pH value of 7. In the second experiment, the culture was grown at a range of pH values (6.5, 7.0, 7.5, 8.0, and 8.5) and fixed temperature of 30 °C. The culture was harvested after 0.5, 4, 16, and 24 h followed by serial dilution and plating as described by Somasegaran and Hoben [28] to record bacterial cell density.

Ultrastructure studies through transmission electron microscopy

The strain was already reported as efficient root colonizer which was validated through confocal laser scanning microscopic studies and recovering rifampicin-resistant derivatives of strain Fs-11 from roots [11]. To further investigate whether strain Fs-11 is rhizosphere colonizer or an endophyte, colonization pattern was studied through transmission electron microscopy (TEM). For ultrastructure studies, surface sterilization of *H. annuus* (cv. FH-331) seeds was carried out by immersing them in sodium hypochlorite (5%, w/v) for 10 min followed by rinsing five to six times with sterilized water. The seeds were inoculated with strain Fs-11 (1×10^8 CFU mL⁻¹) and germinated on 1.5% (w/v) water agar plates. Root hairs of 10-day old gnotobiotically grown *H. annuus* seedlings were cut into pieces (1–3 cm) and processed for observation under transmission electron microscope (TEM, JEOL 1010, Japan) according to the procedure described by Shahid et al. [12]

Site selection, soil physicochemical analysis, and meteorological data

Three agro-climatically different experimental sites (Fig. 1) of Pakistan, i.e., National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, (31°23'42.13 N, 73°1'37.24 E), Nuclear Institute for Food and Agriculture (NIFA), Peshawar (34°1'13.50 N, 71°28'53.02 E), and The University of Poonch, Rawalakot (UPR), Azad Jammu and Kashmir, Pakistan (33°49'48.04 N, 73°48'17.32 E), were selected to evaluate the effect of strain Fs-11 inoculation at

Fig. 1 Experimental sites NIBGE, NIFA, and UPR located in three different agro-climatic zones of Pakistan showing the meteorological data collected separately for each location during the crop growth period

reduced fertilizer dose [29]. Five soil samples from each experimental site were collected for physicochemical analysis prior to the onset of experiments. Soil physical and chemical properties are presented in Table 1. Meteorological data of the three sites for the entire crop growth period were recorded in collaboration with Pakistan Meteorological Department and are presented Fig. 1. For the measurement of bacterial population density before field experiments at NIBGE, NIFA, and UPR, the soil was randomly collected from ten locations of layout area and was mixed, and 1 g aliquot was used for measurement through plate count method [28]. After field experiments, the same methodology was used to record bacterial population density.

Preparation of inoculum and seed surface sterilization

Inoculum of *Enterobacter* sp. Fs-11 was prepared by growing the strain up to 1×10^8 CFU mL⁻¹. The culture was centrifuged at 8000g and washed twice with 0.85% (w/v) saline. The cells were re-suspended in equal volume of saline and diluted to maintain the cell density of 1×10^8 CFU mL⁻¹. Inoculation was completed by dipping the seeds in inoculum for 30 min. Hybrid sunflower (cv. FH-331) seeds were procured from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The seeds were surface-sterilized by 5% (w/v) sodium hypochlorite as described by Shahid et al. [11]

Experimental design, crop husbandry, and treatments

Field experiments were conducted in a randomized complete block design (RCBD) with three replications. A tractor-mounted cultivator was employed to plow the seed-beds two to three times followed by planking. Plot size and seed rate were kept as 3×2 m² and 8 kg ha⁻¹, respectively. Recommended doses of NP fertilizers ($N = 50$ kg ha⁻¹ and $P = 90$ kg ha⁻¹) were applied in the form of urea and diammonium phosphate (DAP), respectively. All DAP was applied at the time of sowing, while half urea was applied at sowing and the remaining half at first irrigation. Manual sowing by the dibbling method was carried out with three seeds per hill, and between-row and between-plant distances of 75 and 25 cm, respectively, were maintained. All other standard agronomic and plant protection measures were followed for whole set of experiments. The following three non-inoculated treatments were compared with their respective inoculated ones:

- T₀ non-inoculated control without fertilizer application
- T₁ non-inoculated control with full N and half P fertilizers
- T₂ non-inoculated control with full NP fertilizer
- T₃ inoculated with strain Fs-11 without fertilizer application
- T₄ inoculated with strain Fs-11 with full N and half P fertilizers
- T₅ inoculated with strain Fs-11 with full NP fertilizer

Table 1 Physicochemical properties of soil samples collected from experimental locations of NIBGE, NIFA, and UPR

Parameters	Locations		
	NIBGE	NIFA	UPR
EC (dS m ⁻¹)	0.75	0.81	0.82
Soil pH	8.3	8.2	7.3
Soil texture	Sandy loam	Clay loam	Loam
Bulk density (mg m ⁻³)	1.37	1.59	1.51
Organic matter (%)	0.68	1.12	1.78
Organic C (g kg ⁻¹)	3.8	4.4	4.5
Total N (g kg ⁻¹)	0.55	0.44	0.76
C:N ratio	6.9	10	5.9
Available P (mg kg ⁻¹)	6.54	4.74	6.1
Available K (mg kg ⁻¹)	203	164	224
Bacterial cell density before experiment (log CFU g ⁻¹ soil)	6.1	5.9	6.9
Bacterial cell density before experiment (log CFU g ⁻¹ soil)	6.8	6.6	7.5

Measurement of growth parameters

Crop was sun-dried for 5 days after harvesting. The threshing was done by physical beating. Height and head diameters were measured with the help of scale by randomly selecting 20 plants from each treatment. Average value of 10 randomly selected plants from each treatment was measured to calculate number of achenes per head and 1000-achene weight. Achene yield was measured per plot basis and then converted to kg ha⁻¹.

Measurement of physiological parameters

Achene phosphorus (P) contents were measured by the vanadium phosphomolybdate yellow color method [30], using UV-visible spectrophotometer (JENWAY6305, UK), while nitrogen (N) contents were determined by micro-Kjeldahl method as described by Sparks et al. [31]. Furthermore, achene oil and fatty acid contents were recorded through gas chromatography as described by Erickson [32].

Statistical analysis

Data were analyzed by one- and two-way analysis of variance [33] and presented as the mean of three replications ($n=3$) using Statistix ver. 8.1 software (Tallahassee, Florida). Values that differ significantly ($P \leq 0.05$) according to Fisher's least significant difference (LSD) method are shown with different low-case letters. To determine the effect of locations on various parameters, principal component analysis (PCA) was conducted using SPSS 17 software (SPSS Inc., USA).

Results

In vitro growth studies

Growth curves constructed after in vitro experimentation resulted in optimal cell density of *Enterobacter* sp. Fs-11 at the tested temperature and pH values (Fig. 2). After 24 h of incubation, cell density at 30 °C (10.21 ± 0.39 CFU mL⁻¹) and pH 7.5 (10.34 ± 0.41 CFU mL⁻¹) was found maximum. The minimum cell density of strain Fs-11 after same incubation period was determined at 15 °C (7.97 ± 0.38 CFU mL⁻¹) and pH 6.5 (8.47 ± 0.34 CFU mL⁻¹). On an average, the growth of strain Fs-11 at variable temperature and pH settings was found optimum which confirmed its potential to evaluate the strain as inoculum under diverse ecological conditions.

Ultrastructure studies through TEM

Cells of *Enterobacter* sp. Fs-11 were localized in rhizoplane of sunflower (Fig. 3). Some bacterial cells were found embedded in an extracellular matrix of root hair cells (Fig. 3b). Strain Fs-11 was also clearly spotted inside the root cortical cells (Fig. 3c,d). Thus, *Enterobacter* sp. Fs-11 was found to be a rhizocompetent strain with substantial ability to colonize sunflower rhizosphere and to demonstrate both ectophytic and endophytic behaviors.

Soil physicochemical analysis

The soil textures of the three experimental locations NIBGE, NIFA, and UPR were sandy loam, clay loam, and loam with 8.3, 8.2, and 7.3 pH values, respectively. Similarly, organic matter percentage was higher in UPR (1.78) than NIBGE and NIFA (0.68 and 1.12, respectively). The total N was calculated as 0.55, 0.44, and 0.76 g kg⁻¹. The available P (6.54, 4.74, and

Fig. 2 Time-lapse in vitro growth of *Enterobacter* sp. Fs-11 at different temperature (a) and pH (b) levels measured through plate count method. Error bars represent standard deviation ($n = 3$)

6.1 mg kg⁻¹) and K (203, 164, and 224 mg kg⁻¹) were measured at NIBGE, NIFA, and UPR, respectively (Table 1). Thus, the soil conditions were quite heterogeneous for field experiments. After the harvesting of field experiments, the bacterial population density was recorded higher (6.8, 6.6, and 7.5 log CFUg⁻¹ of soil) at NIBGE, NIFA, and UPR, respectively, as compared with the population density calculated before the start of experiments.

Yield response of sunflower to inoculation

Inoculation of strain Fs-11 along with half dose of recommended NP-fertilizer (T₄) significantly (Fisher's LSD; $P \leq 0.05$) boosted sunflower plant height (ca. 149, 112, and 140 cm) and number of achenes per head (ca. 917, 709, and 282) at NIBGE, NIFA, and UPR, respectively, along with head diameter (ca. 40 cm) at UPR (Table 2) as compared to non-inoculated plants with 50% reduced P fertilizer (T₁). The inoculation response of sunflower in terms of growth parameters was even statistically at par with non-inoculated plants with full dose of NP-fertilizer (T₂). A non-significant effect of T₄ was recorded at all locations in terms of 1000-achene weight (ca 69, 57, and 69 g, respectively) as compared to T₂. Subsequently, achene yield of T₄ (2138 and 3182 kg ha⁻¹) of locations NIFA and UPR, respectively, was significantly higher as compared to T₁ and statistically with T₂. Moreover, treatment T₅ (inoculation with full dose of

Fig. 3 Localization of *Enterobacter* sp. Fs-11 with gnotobiotically grown sunflower rhizoplane (a), extracellular matrix of root hair cells (b) inside root cortical cells (c, d), and rhizosphere (d) through transmission electron microscopy. B bacteria, CW cell wall, RC root cell, EM Extracellular matrix, RS Rhizosphere

Table 2 Effect of seed inoculation of *Enterobacter* sp. Fs-11 on sunflower growth and yield

Treatments	Plant height (cm)	Head diameter (cm)	Number of achenes head ⁻¹	1000-achene weight (g)	Achene yield (kg ha ⁻¹)
NIBGE, Faisalabad					
T ₀	114.67 C	30.33 C	550.71 B	58.02 C	2321.1 C
T ₁	123.11 C	33.17 BC	604.67 B	66.63 ABC	2665.3 AB
T ₂	139.23 AB	38.33 AB	704 AB	72.57 A	2902.0 A
T ₃	126 BC	34 ABC	628.34 B	62.55 BC	2449.0 BC
T ₄	149.32 A	37.67 AB	916.67 A	67.60 AB	2804.1 A
T ₅	142.33 A	39.33 A	901.12 A	69.25 AB	2854.5 A
LSD ($P \leq 0.05$)	15.55	5.33	240.79	9.24	238.07
NIFA, Peshawar					
T ₀	87.18 C	23.67 C	402.76 B	40.61 C	1493.3 C
T ₁	94.14 C	30.67 BC	482.33 B	51.60 B	1806.2 B
T ₂	120.66 AB	35.69 AB	787.44 A	63.99 A	2218.7 A
T ₃	94.00 C	26.33 C	434.70 B	50.61 B	1656.3 BC
T ₄	111.67 B	34.89 AB	708.67 A	57.09 AB	2138.0 A
T ₅	129.48 A	39.25 A	811.00 A	62.25 A	2154.3 A
LSD ($P \leq 0.05$)	9	7.08	108.78	7.94	292.96
UPR, Rawalakot					
T ₀	108.37 C	25.73 C	490.11 C	51.09 D	2549.7 B
T ₁	124.19 B	33.93 B	696.46 B	61.70 BC	2738.2 B
T ₂	152.39 A	42.17 A	986.67 A	72.83 A	3140.6 A
T ₃	119.01 BC	28.68 BC	570 BC	57.83 CD	2739.4 B
T ₄	139.67 A	40.26 A	882.33 A	68.73 AB	3182.3 A
T ₅	143.35 A	39.87 A	901 A	73.91 A	3254.3 A
LSD ($P \leq 0.05$)	15.03	5.53	177.09	10.15	312.37

Data analyzed by one-way analysis of variance (Steel et al. 1997) are presented as the mean of three replications ($n = 3$). Values that differ significantly ($P \leq 0.05$) according to Fisher's least significant difference (LSD) method are presented with different letters

T₀ = non-inoculated control without fertilizer application

T₁ = non-inoculated control with full N and half P fertilizers

T₂ = non-inoculated control with full NP fertilizer

T₃ = inoculated with strain Fs-11 without fertilizer application

T₄ = inoculated with strain Fs-11 with full N and half P fertilizers

T₅ = inoculated with strain Fs-11 with full NP fertilizer

recommend fertilizer) did not enhance sunflower growth and yield significantly as compared to T₄, suggesting that strain Fs-11 showed plant growth promoting potential under reduced NP fertilizer application.

Effect of inoculation on sunflower nutrient uptake

Inoculation affected non-significantly in terms of achene and leaf N contents at all three locations. The N contents of achenes (37.33, 35.39, and 38.98 g kg⁻¹) and leaves (36.83, 34.81, and 35.15 g kg⁻¹) of T₄ at NIBGE, NIFA, and UPR, respectively, were found statistically at par with T₁ and even T₂ (Table 3). On the other hand, inoculation of *Enterobacter* sp. Fs-11 at 50% reduced P fertilizer (T₄) significantly promoted P uptake of achenes (14.94 and 15.37 g kg⁻¹) at

NIBGE and UPR, respectively, and leaves (12.65, 14.20, and 16.32 g kg⁻¹) at NIBGE, NIFA, and UPR, respectively, as compared its corresponding non-inoculated treatment (T₁). Inoculation effect with 50% reduced P fertilizer (T₄) on the P contents of sunflower achenes and leaves was measured statistically at par with non-inoculated plants with full dose of NP fertilizer (T₂).

Effect of inoculation of sunflower achene oil and fatty acid contents

The inoculation with strain Fs-11 at half dose of P fertilizer (T₄) enhanced achene oil contents significantly at NIBGE (41.05%) and NIFA (42.72%) as compared to non-inoculated treatment (T₁). At UPR, the oil contents of T₄ (43.24%) were found

Table 3 Effect of seed inoculation of *Enterobacter* sp. Fs-11 on sunflower nitrogen and phosphorus uptake

Treatments	Achene N contents (g kg ⁻¹)	Leaf N contents (g kg ⁻¹)	Achene P contents (g kg ⁻¹)	Leaf P contents (g kg ⁻¹)
NIBGE, Faisalabad				
T ₀	30.54 C	27.47 C	11.39 B	7.66 B
T ₁	35.33 AB	34.68 AB	11.13 B	8.26 B
T ₂	37.39 A	35.72 A	14.71 A	11.65 A
T ₃	32.92 BC	30.36 BC	11.66 B	7.37 B
T ₄	37.33 A	36.83 A	14.94 A	12.65 A
T ₅	37.69 A	35.27 A	15.39 A	12.98 A
LSD ($P \leq 0.05$)	3.85	4.44	1.32	1.69
NIFA, Peshawar				
T ₀	26.83 C	23.62 B	10.03 C	7.66 D
T ₁	31.38 BC	29.57 AB	10.61 BC	8.26 CD
T ₂	39.47 A	32.63 A	14.46 A	11.41 BC
T ₃	31.12 BC	29.18 AB	11.33 ABC	8.37 CD
T ₄	35.39 AB	34.81 A	11.99 ABC	14.20 AB
T ₅	35.66 AB	34.36 A	13.72 AB	14.98 A
LSD ($P \leq 0.05$)	5.78	6.07	3.59	3.56
UPR, Rawalakot				
T ₀	32.38 C	28.86 B	9.69 B	9.03 C
T ₁	36.09 BC	36.33 A	11.80 B	10.78 BC
T ₂	40.37 A	35.31 A	15.88 A	13.57 AB
T ₃	34.77 C	29.08 B	12.17 B	10.64 BC
T ₄	38.98 AB	35.15 A	15.37 A	16.32 A
T ₅	38.34 AB	36.27 A	15.26 A	16.65 A
LSD ($P \leq 0.05$)	3.72	5.68	2.89	3.19

Data analyzed by one-way analysis of variance (Steel et al. 1997) are presented as the mean of three replications ($n = 3$). Values that differ significantly ($P \leq 0.05$) according to Fisher's least significant difference (LSD) method are presented with different letters

T₀ = non-inoculated control without fertilizer application

T₁ = non-inoculated control with full N and half P fertilizers

T₂ = non-inoculated control with full NP fertilizer

T₃ = inoculated with strain Fs-11 without fertilizer application

T₄ = inoculated with strain Fs-11 with full N and half P fertilizers

T₅ = inoculated with strain Fs-11 with full NP fertilizer

statistically similar to that of T₁ (41.27%). Similarly, mono- and polyunsaturated fatty acids (oleic and linoleic acids, respectively) were also affected non-significantly after inoculation at 50% reduced P fertilizer (T₄) as compared to non-inoculated plants with half P fertilizer dose (T₁) except the location UPR where oleic acid was measured significantly high. In case of saturated fatty acids (palmitic acid and stearic acid), a similar non-significant effect was recorded after inoculation at all locations except NIBGE where palmitic acid significantly reduced in case of inoculated treatment T₄ as compared to T₁. On the other hand, a comparative decreasing trend was calculated in case of palmitic acid in inoculated treatments as compared to non-inoculated ones (Table 4).

In principal component analysis, all the variables responded to inoculation of strain Fs-11 in similar pattern at

NIBGE and UPR, although these locations have diverse ecological conditions. Only oleic acid C18:1 and linoleic acid C18:2 were influenced positively by NIFA, while all other yield and physiological parameters were favored by the climatic conditions of NIBGE and UPR (Fig. 4).

Discussion

Potential PGPR strains have been isolated, characterized, and commercialized as bioinoculants in various parts of the world, especially in developing countries. The consistency of these bacterial bioinoculants in terms of performance has always been a question mark mainly due their adaptability in diverse climatic conditions, heterogeneous nature of soil, and

Table 4 Effect of seed inoculation of *Enterobacter* sp. Fs-11 on sunflower oil and fatty acid contents

Treatments	Oil contents (%)	Oleic acid C18:1 (%)	Linoleic acid C18:2 (%)	Palmitic acid C16:0 (%)	Stearic acid C18:0 (%)
NIBGE, Faisalabad					
T ₀	36.65 C	27.74 C	35.84 C	6.03 A	1.20 C
T ₁	38.84 B	27.99 BC	33.17 C	5.63 AB	1.37 BC
T ₂	40.50 A	36.95 A	43.87 A	5.21 BC	1.83 A
T ₃	38.87 B	31.98 ABC	40.01 B	5.28 BC	1.60 AB
T ₄	41.05 A	33.71 ABC	43.44 AB	4.78 C	1.53 AB
T ₅	40.74 A	34.17 AB	42.45 AB	4.89 C	1.38 BC
LSD ($P \leq 0.05$)	1.42	6.34	3.58	0.68	0.32
NIFA, Peshawar					
T ₀	37.34 D	31.09 C	36.20 B	6.35 A	1.23 B
T ₁	39.47 BCD	33.49 BC	40.37 AB	5.36 BC	1.41 AB
T ₂	40.28 ABC	40.78 A	43.99 A	4.76 CD	1.77 A
T ₃	38.84 CD	32.05 BC	36.04 B	5.83 AB	1.15 B
T ₄	42.72 A	35.57 B	42.12 A	5.41 BC	1.31 AB
T ₅	41.96 AB	39.59 A	42.21 A	4.22 D	1.46 AB
LSD ($P \leq 0.05$)	2.59	3.96	5.49	0.93	0.48
UPR, Rawalakot					
T ₀	38.23 C	29.57 C	34.59 C	6.83 A	1.19 B
T ₁	41.27 BC	30.17 C	36.94 BC	5.77 B	1.40 AB
T ₂	44.79 A	33.67 ABC	42.36 A	5.40 B	1.69 A
T ₃	42.24 AB	33.16 BC	35.29 C	5.72 B	1.40 AB
T ₄	43.24 AB	37.49 AB	41.30 AB	5.43 B	1.41 AB
T ₅	43.34 AB	38.09 A	42.13 A	4.99 B	1.42 AB
LSD ($P \leq 0.05$)	3.37	4.82	4.78	0.89	0.44

Data analyzed by one-way analysis of variance (Steel et al. 1997) are presented as the mean of three replications ($n = 3$). Values that differ significantly ($P \leq 0.05$) according to Fisher's least significant difference (LSD) method are presented with different letters

T₀ = non-inoculated control without fertilizer application

T₁ = non-inoculated control with full N and half P fertilizers

T₂ = non-inoculated control with full NP fertilizer

T₃ = inoculated with strain Fs-11 without fertilizer application

T₄ = inoculated with strain Fs-11 with full N and half P fertilizers

T₅ = inoculated with strain Fs-11 with full NP fertilizer

competition of PGPR strains with indigenous microbiota. Thus, success of biofertilizer lies in its adaptation to local environment and its ability to compete with various biotic and abiotic factors. This study reports here environmental adaptability and plant growth-promotion potential of *Enterobacter* sp. Fs-11, which was isolated and characterized reported earlier [11], as mineral phosphate-solubilizing strain with considerable ability to synthesize indole-3-acetic acid in vitro. The strain also survived in rhizosphere of sunflower and enhanced its growth significantly in a pot experiment [11]. Looking at the PGPR potential of strain Fs-11, it was planned to evaluate the strain under diverse field conditions. Before field experiments, the strain was characterized for its adaptability to a range of temperature and pH values in vitro. The purpose of in vitro growth studies was to confirm the ability of the strain Fs-11 to thrive

under variable temperature and pH values. The optimal growth of strain Fs-11 ($8\text{--}10\text{ CFU mL}^{-1}$) at a range of temperature and pH values after 24 h of incubation made it a prospect for inoculation under variable agro-climatic conditions (Fig. 2). In vitro growth studies made it feasible to select agro-ecologically different zones for its field inoculation experiments. Consistent to our study, Pandey et al. [34] isolated some bacterial strains from Uttarakhand, India, with wide range of temperature and pH tolerance ability. In vitro studies also confirmed its epiphytic and endophytic nature through TEM. Localization of the strain Fs-11 in sunflower rhizosphere and endosphere after its inoculation under gnotobiotic conditions reaffirmed the hypothesis that the bacterium is a potential root colonizer (Fig. 3). Despite the fact that colonization studies were conducted in the absence of any indigenous bacterial competition, its colonization in

Fig. 4 Comparative effects of different locations on agronomic and physiological parameters of sunflower as determined by principal component analysis (PCA). Input data were first analyzed by two-way analysis of variance and are presented as the mean of three replications ($n = 3$)

heterogeneous soil is already reported [11]. Localization of various plant growth-promoting rhizobacteria (PGPR) in rhizosphere, rhizoplane, and endosphere of plant roots and nodules through TEM has already been reported by various researchers [12, 35–38].

Selection of sites for field experiments is made after studying the agro-ecological locations of Pakistan. Pakistan is divided into ten agro-climatic zones based on climate, water availability, land use, and geography [39]. After inoculation of strain Fs-11, a significant (Fisher's LSD; $P \leq 0.05$) increase in growth and yield parameters as compared to non-inoculated control plants (T_1) at NIBGE, NIFA, and UPR may be attributed to its rhizosphere adaptation under variable temperatures and soil types, mineral phosphate solubilization, and IAA synthesis (Table 2). Auxin synthesis and mineral phosphate solubilization potential of PGPR have already been reported to enhance plant growth and nutrient acquisition from the soil [5, 40–42]. Andreote et al. [43] and Shankar et al. [44] reported the increased root colonization and plant growth promotion after PGPR inoculation. Plant growth-promoting rhizobacteria are also reported to enhance wheat, lentil, and cotton growth under variable temperature and soil conditions [45, 46]. Similarly, significant increase of achene P contents at 50% reduced P fertilizer dose (T_4) in comparison with non-inoculated plants at NIBGE, NIFA, and UPR was due to export of P from soil to sunflower achenes and leaves after inoculation (Table 3). The strain Fs-11 has already been reported as a potential candidate for inorganic phosphate solubilization and organic acid synthesis, which might be one of the reasons for better P utilization from soil in current study [11, 47]. On the other hand, achene and leaf N contents were not affected significantly after inoculation. This non-significant effect might be due to the inability of strain Fs-11 to fix atmospheric nitrogen. Thus, significant growth and

physiological modulations in sunflower plant after strain Fs-11 inoculation made the strain competitive and potent in diverse environmental conditions.

Being an oilseed crop, sunflower is globally grown for its seed oil yields. After inoculation at NIBGE and NIFA, a significant increase in achene oil contents as compared to non-inoculated plants with 50% reduced P and no NP fertilizer doses (T_0 and T_1) might be due to better vigor of inoculated plants and better acquisition of soil nutrients. These results are contrary to the findings of Akbari et al. [48] who reported that PGPR inoculation does not affect the achene oil contents. On the other hand, Ekin [49] reported that inoculation with phosphate-solubilizing bacteria M-13 resulted in enhanced oil contents in sunflower. Increase in oil contents of inoculated plants might be attributed to the better P nutrient acquisition from soil as P is directly responsible for oil yield increase in plants. Unsaturated fatty acids (linoleic acid and oleic acid) were remained statistically at par in terms of inoculated and non-inoculated treatments at all experimental sites. This might be due to unknown ecological and physiological factors responsible to accumulate these oils in sunflower seeds. Palmitic acid C16:0 was comparatively less-accumulated in inoculated plants which was a healthy sign for humans and animals. The risk of cardiovascular diseases increases with the increased consumption of palmitic acid according to the World Health Organization. At the same time, non-inoculated treatment with full fertilizer (T_2) was also found statistically at par with Fs-11 inoculated treatment (T_4) in key parameters like achene yield and oil contents at NIBGE, NIFA, and UPR. This suggested that inoculation of strain Fs-11 at 50% reduced P fertilizer dose competed with application of full dose of recommended fertilizer. Comparatively, better effect of inoculation at UPR and NIBGE than at NIFA may be due to the better soil and climatic conditions throughout the crop growth period.

Conclusion

The current study proved that *Enterobacter* sp. Fs-11 is a rhizocompetent strain that can boost nutrient acquisition under diverse soil conditions after adhering with plant roots. The strain is adaptable to variable temperature and soil pH conditions and thus a potent candidate to enhance sunflower yield and oil contents. The strain Fs-11 can cut off P fertilizer requirements to half without compromising achene yield and oil contents. Hence, it is a potential candidate for biofertilizer of sunflower crop for sustainable yield in a cost-effective manner.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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