International Journal of Plant & Soil Science

Volume 36, Issue 2, Page 214-227, 2024; Article no.IJPSS.112658
ISSN: 2320-7035

Isolation, Characterization, and Screening of Endospore Forming Endophytic Bacteria from Cowpea [*Vigna unguiculata* (L.) Walp.] for Plant Growth Promotion and Abiotic Stress Mitigation

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2024/v36i24383

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112658

Original Research Article

Received: 22/11/2023
Accepted: 27/01/2024
Published: 01/02/2024

ABSTRACT

We performed isolation and characterization of endospore forming endophytic bacteria (EEB) from endorhizospheric region of healthy bush cowpea var. Bhagyalakshmi. All the EEB isolates were evaluated for their ability to produce plant growth promoting traits such as production of indole acetic acid (IAA), gibberellic acid, ammonia, ACC deaminase, volatile organic compounds and siderophore, and capacity for nitrogen fixation, and solubilization of phosphorus and potassium. Roll towel assay was used for assessing the seedling vigour index of cowpea after seed priming with the

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EEB isolates. Based on growth promotion and seedling vigour index, seven superior bacterial isolates were selected, and antagonistic activity against the fungal pathogens *Fusarium oxysporum, Rhizoctonia solani, Colletotrichum* sp.*, Phytophthora* sp.*, and Pythium* sp.*,* was assayed. The isolates were pooled together to form a consortium and biopriming of cowpea seeds were done. Bioprimed seeds were tested for plant growth promotion and abiotic stress management in cowpea under protray condition. The seedlings of the bioprimed cowpea seeds showed better seedling vigour index and stress tolerance compared to hydro-primed seeds and the unprimed control. EEB isolated from the host plant could be developed as plant growth promoting bioagents.

Keywords: Bacillus spp.; biopriming; seedling vigour; antagonism.

1. INTRODUCTION

Integration of plant growth-promoting bacteria (PGPB) into agricultural practices emerges as a holistic and sustainable strategy for disease management, fostering both plant health and environmental stewardship [1]. This innovative approach harnesses the beneficial interactions between the plants and specific bacteria, enhancing nutrient uptake, improving soil structure, and bolstering plant defense against phytopathogens. By incorporating PGPB, agricultural systems can move beyond traditional biotic and abiotic stress management methods, promoting a more balanced and environmentally friendly approach. This approach not only contributes to the resilience of crops but also aligns with the broader goals of sustainable agriculture, where productivity and ecological well-being go hand in hand.

Among the PGPB, plant growth-promoting endophytes (PGPEs) stand out as microorganisms that colonize the internal tissues of plants without causing harm to the host [2]. This unique characteristic makes PGPEs promising alternative agents as potential biocontrol agents and plant growth promoters. By establishing a symbiotic relationship with the host plant, PGPEs can contribute to enhanced nutrient uptake, improved stress tolerance, and protection against pathogens [3,4].

Seed priming with endophytic microorganisms represents a valuable and eco-friendly approach in ensuring better crop growth and production. Beneficial endophytic microorganisms often produce phytohormones, such as auxins, cytokinins, and gibberellins, which play crucial roles in plant growth and development [5]. Seed priming with such microorganisms can stimulate germination, enhance root and shoot growth, and contribute to overall plant vigour, and can also induce resistance mechanisms in plants, making them more robust and capable of defending against various plant pathogens [6]. This proactive approach helps mitigate the risk of resistance development in plant pathogens, and also reduce the reliance on chemical interventions. Some endophytes contribute to the plant's tolerance to abiotic stresses, such as drought, salinity, and temperature fluctuations [7]. This can be especially beneficial in regions prone to environmental challenges, enhancing crop resilience under adverse conditions. Endophytic microorganisms can also confer tolerance to biotic stresses by activating the plant's defense mechanisms [8]. This helps reduce the impacts of pests and diseases on crop yields. Utilizing endophytic microorganisms for seed priming aligns with eco-friendly agricultural practices.

Among plant growth-promoting endophytic bacteria, endospore-forming *Bacillus* spp. are recognized for their safety profile and have the potential to produce a diverse array of beneficial substances for agricultural applications. The endospore-forming nature of *Bacillus* provides them with a protective mechanism, allowing them to endure harsh environmental conditions and persist in the soil for extended periods. This resilience enhances their effectiveness as plant growth promoters and biocontrol agents*. Bacillus* species are known for their ability to synthesize various compounds, including enzymes, antibiotics, and plant growth regulators, which can positively influence plant growth and development [9]. Most of them are generally considered non-pathogenic to humans and animals [5]. The present study focused on potency of biopriming with endospore forming endophytic bacteria for growth promotion and stress management in cowpea.

2. MATERIALS AND METHODS

2.1 Sample Collection and Surface Sterilization

Endospores forming endophytic bacteria were isolated from endorhizosphere of cowpea var.

Bhagyalakshmi, collected from Instructional farm, College of Agriculture, Vellayani, Thiruvanatha-puram, Kerala. The root samples, ranging from 5 to 10 cm in size, were washed under running tap water, followed by cutting into smaller pieces. Subsequently, four pre-washes with sterile distilled water were given. To ensure surface sterilization, the plant bits were immersed in a 1% sodium hypochlorite solution for three minutes. Afterward, the samples underwent four additional washes with sterile distilled water under aseptic conditions to remove any remaining sodium hypochlorite. Sterility checks were conducted to evaluate the effectiveness of the disinfection process. For these checks, 0.1 ml of the final wash was plated on Nutrient Agar (NA) medium, and also transferred to 9.9 ml of Nutrient Broth (NB). The samples were then incubated at 28 ± 2°C for 48 hours and observed for bacterial growth, and when no growth occurred it indicated efficiency of the sanitization method.

2.2 Isolation of Endospore Forming Endophytic Bacteria (EEB)

A double enrichment method described by Yashaswini et al. [10] was used to isolate endospore-forming endophytic bacteria. Surface sterilized root bits were made free of excess moisture using sterile tissue paper. The root bits were then placed in sterilized Petri plates and allowed to dry at 35°C for two consecutive days. The dried root bits (approximately 1 g) were macerated in 10 mL phosphate-buffered saline solution (PBS) under aseptic conditions using sterilized pestle and mortar. The macerated root tissue was collected in a sterile glass vial and placed in a hot water bath at 80°C for 10 minutes. The heat-treated samples were serially diluted using sterile distilled water. From the serial dilutions 0.1 ml each was spread plated on nutrient agar (NA) plates. The agar plates were then incubated at 28°C.

2.3 Characterization of EEB Isolates

The bacterial isolates were streak purified on nutrient agar (NA) medium to obtain individual, well-isolated colonies. Colony characters such as elevation, colour, form, margin of colony, colony size (mm), Gram's reaction and cell arrangement were examined, and the results were recorded [11]. Endospore staining was done for conformation of isolated bacterial cultures as endospore forming endophytes.

2.4 Plant Growth Promoting Traits of EEB Isolates

The isolated endospore forming endophytic bacteria were screened for their plant growth promotion traits using standard protocols. Qualitative or quantitative assessment of nitrogen fixation, phosphorous solubilization [12], potassium solubilization [13], IAA production [14], gibberellic acid production [15], ammonia [16], Siderophore production [17] and ACC deaminase activity [18] were done for all the isolates.

2.5 Seedling Vigour Index

The roll towel assay was employed for assessing seedling vigour index [19]. Seeds of cowpea were surface sterilized using 4% sodium hypochlorite for 3 minutes, followed by three washes with sterile distilled water, and blot dried with sterile tissue paper. EEB isolates selected based on plant growth promotion traits were separately cross-streaked heavily on nutrient agar plates and incubated at 28°C for 24 hours. Bacterial suspension was obtained by drenching the culture plates with 10 ml sterile distilled water under aseptic conditions [20]. The surface sterilized seeds were soaked in the bacterial suspension for 3 h and kept overnight for drying [21]. These bacterized seeds were then arranged in rows of ten on moist towel paper. The rolled paper towels were kept at 28°C in a beaker with sterile water at the bottom, and adequate moisture was maintained by daily watering. Three replications were kept for each treatment. After 10 days, the rolled towel papers were retrieved, and observations were recorded. Unprimed seeds were kept as control.

2.6 Assessment of *in vitro* Biocontrol Potential by EEB Isolates

Dual culture plate technique [22] was employed to assess the antagonistic activity of EEB isolates against various fungal and bacterial pathogens, with inhibition zones measured at specific time points depending on the pathogens being tested. Fungal pathogens, *Fusarium oxysporum, Rhizoctonia solani, Colletotrichum* sp.*, Phytophthora* sp.*, and Pythium* sp. obtained from the Department of Agricultural Microbiology, College of Agriculture, were used for the assay. To initiate the assay, the fungal pathogens were cultured on PDA plates for 3-5 days, and mycelial discs were cut out from the advancing growing region of the fungal colony using a sterile 5 mm corkborer. These discs were then placed at the

centre of PDA plates prepared for the dual culture plate assay. On both sides of the mycelial disc, bacterial streaks of 1.5 cm were made at a distance of 3.5 cm from the center. The plates were incubated at a temperature of 28±2°C. After 48 hours, the inhibition zone was measured (in mm) for the test plates containing *Rhizoctonia solani* and *Pythium* sp. For *Fusarium oxysporum, Colletotrichum* sp., and *Phytophthora* sp., the inhibition zone was measured (in mm) after 96 hours of incubation.

2.7 Biochemical Characterization of Bacterial Isolates

The biochemical characterization of EEB isolates involved conducting various biochemical tests and carbohydrate utilization pattern using commercially available HiMedia© kits (KB013 HiIMViC Biochemical Test Kit). By analyzing the color changes and reactions of the isolates in these tests, the biochemical properties and carbohydrate utilization patterns of the endospore-forming endophytic bacterial isolates were determined**.**

2.8 Molecular Identification of the Bacterial Isolates

The molecular identification of the selected endospore forming endophytic bacterial isolates was done by 16S rRNA sequence analysis using universal primers. The sequences of the 16S rRNA were compared by BLAST search from the database of NCBI GenBank. The sequences were then subjected to alignment using the Multiple Alignment software tool Clustal W. To further elucidate the evolutionary relationships among the selected gene sequences, a phylogenetic tree and a distance matrix were constructed. This process was facilitated using MEGA11, a comprehensive software package designed for Molecular Evolutionary Genetics Analysis [23].

2.9 Evaluation of effects of biopriming with EEB isolates

2.9.1 Plant growth promotion

Bacterial isolates were heavily cross streaked in NA plates. Fully grown plates were drenched with 10 mL of sterile water and the growth was scraped out and transferred to separate glass vials. OD value was adjusted to 0.8 and the approximate bacterial population in the suspension was standardized as 10^8 cfu/mL. Consortium was prepared by mixing equal amount of all the bacterial isolates. Surface sterilized seeds were kept in the bacterial suspension for 3 h and then kept for overnight drying [19]. Hydropriming (HP) was performed by immersing surface-sterilized seeds in sterile distilled water for 3 h [24]. Untreated seeds without any priming served as control. The primed seeds were screened by paper towel method for seedling vigour under *in vitro* conditions [25].

Growth promotion of bioprimed cowpea seeds were also done by a protray growth assessment. Seed priming was done as described above. The pro-trays were filled with sterilized vermiculite and coir pith (1:1) as planting medium, which had been autoclaved for three consecutive days at 121°C for one hour each. A single bio-primed seed of cowpea was planted in each cell, and the seedlings were watered twice in a day.

2.9.2 Salinity stress tolerances

The bioprimed seed were screened for their ability to tolerate osmotic stress induced by the application of NaCl [26]. Different osmotic concentrations were made by adding 50, 100, 150 and 200 mM of NaCl to the *in vitro* paper towel assay system. The highest tolerant limit of NaCl obtained from roll towel assay was selected for salinity stress tolerance under protray conditions. A single bioprimed seed of cowpea was planted in each cell, and appropriate concentration of NaCl were poured daily.

2.9.3 Moisture stress tolerances

The ability of bioprimed seeds to tolerate moisture stress was screened by roll towel method. Moisture stress was induced by different concentrations of PEG in the range of 10, 20 and 30%. In protray, moisture stress was induced by the complete withdrawal of watering 20 days after seedling emergence until the plant dry up.

3. RESULTS

3.1 Isolation and characterization of EEB from cowpea

Colony characters such as elevation, colour, shape, margin of colony and texture were noted and the results are listed in Table 1. Totally thirty five isolates were obtained by double enrichment method and majority the isolates had irregular shape undulated margin and raised elevation, and they showed various textures like mucoid, brittle and butyrous. Colonies of all the isolates

showed either white, off white or creamy white colour. Pure cultures of endospore forming endophytic bacteria were checked for the production of endospores. In all the isolates the spores were produced centrally.

3.2 Assessment of Plant Growth Promotion Properties

The plant growth promotion properties like nitrogen fixation, phosphorous solubilization, IAA production, gibberellic acid production, ammonia, Siderophore production and ACC deaminase activity are listed in Table 2. Isolates *viz*. EEB-C9, EEB-C16, EEB-C19, EEB-C22, EEB-C26, EEB-C29 and EEB-C33 exhibited all such PGP traits, all others such as EEB-C10, EEB-C11, EEB-C20, EEB-C21, EEB-C30 and EEB-C31 produced only IAA and ammonia.

3.3 Seedling Vigour Index

The isolates showed significant difference in germination percentage. The isolate EEB-C9 showed maximum germination percentage (79.995%) followed by EEB-C16 and EEB-C31. With regard to seedling vigour index (SVI), maximum value was recorded with the seedlings treated with the isolate EEB-C9 (2847.157) followed by EEB-C31 (2299.836). The minimum SVI was recorded in the isolate EEB-C33 (1033.186) (Table 3).

3.4 Biocontrol Potential of EEB Isolates against Phytopathogens

The results of the interaction between EEB and the pathogens in the dual culture plate assay are presented in Fig. 1. The detection of an inhibition zone in the dual-culture plate assay was interpreted as evidence of antagonism exhibited by the endospore-forming endophytic bacterial isolates against fungal pathogens. Maximum zone of inhibition was measured in the isolate EEB-C33 against *F. oxysporum* (7mm), *R.solani* (7mm), *Colletotrichum* sp. (8.3mm), and *Pythium* sp. (2.5mm) (Table 4).

3.5 Biochemical and Molecular Characterization of Selected EEB Isolates

Results of the biochemical reactions of the selected bacterial isolates after assessment of the plant growth promotion traits and biocontrol potential are given in Table 5. The phylogenetic relatedness of the selected bacterial isolates after the 16S rRNA sequence analysis is given in Fig. 2.

3.6 Evaluation of EEB Bioprimed Cowpea Seeds for Plant Growth Promotion

The bacterial consortium exhibited a significant influence on the germination % and seedling vigour index in cowpea seeds, surpassing the control group. In protray assessment 100% germination was recorded in cowpea seeds treated with consortium and Seedling vigour index (2752) was also higher in treatment with the consortium. The least values were recorded in hydropriming and control (Table 6).

Table 1. Colony characteristics of endospore forming endophytic bacteria isolated from cowpea var. Bhagyalakshmy

Isolates	Shape	Margin	Elevation	Colour	Texture
EEB-C9	Irregular	Filamentous	Raised	White	Mucoid
EEB-C10	Irregular	Undulate	Umbonate	Creamy white	Butyrous
EEB-C11	Irregular	Undulate	Umbonate	Creamy white	Butyrous
EEB-C16	Punctiform	Smooth	Flat	Cream	Butyrous
EEB-C19	Circular	Wavey	Flat	Cream	Butyrous
EEB-C20	Irregular	Undulate	Raised	White	Brittle
EEB-C21	Irregular	Undulate	Raised	Off white	Brittle
EEB-C22	Irregular	Smooth	Raised	White	Brittle
EEB-C26	Irregular	Lobated	Raised	Cream	Mucoid
EEB-C29	Irregular	Wavey	Raised	Cream	Mucoid
EEB-C30	Punctiform	Smooth	Umbonate	Creamy white	Mucoid
EEB-C31	Irregular	Smooth	Umbonate	Creamy white	Butyrous
EEB-C32	Irregular	Undulate	Umbonate	Creamy white	Butyrous
EEB-C33	Irregular	Lobated	Raised	White	Mucoid

Table. 2 Plant growth promotion traits of endospore forming endophytic bacterial isolates of cowpea

Isolates	IAA (µg mL^{-1})	Ammonia (µmol mL^{-1})	GA (µgmL^{-1})	Total Nitrogen (µg mL^{-1})	P. solubilization (mg L^{-1})	Siderophore (%)	ACC deaminase (µmol mL^{-1})
EEB-C 9	30.98	12.42	2.10	0.031	-	74.421	21.478
EEB-C 10	27.44	10.80	-	-	-	-	-
EEB-C 11	18.74	-	-	-	-	-	-
EEB-C 16	43.23	ND	-	0.022	-	77.144	25.149
EEB-C 19	21.94	12.05	-	0.036	-	77.92	25.343
EEB-C 20	19.86	4.84	-	-	-	-	-
EEB-C 21	19.12	6.01	-	-	-	-	-
EEB-C 22	37.35	14.66	-	0.017	-	73.334	27.172
EEB-C 26	47.54	8.95	70.89	0.021	-	11.182	32.567
EEB-C 29	30.43	3.80	-	0.022	-	74.409	27.373
EEB-C 30	58.37	ND	-	-	-	-	-
EEB-C 31	15.13	4.72	13.39	-	-	-	-
EEB-C 32	12.39	12.54	-	-	-	-	-
EEB-C 33	16.47	18.93	42.64	0.025	2.1623	25.794	25.731

Table 3. Seedling vigour index of bioprimed cowpea seeds in roll towel assay

Isolates*	Germination (%) *	Shoot length (cm) / plant *	Root length (cm) / plant *	Seedling vigour index*
EEB-C 9	79.99 ± 9.42 ^{a}	15.65 ± 0.16^{a}	20.02 ± 1.34^{ab}	2847.15 ± 215.82^{a}
EEB-C 10	49.99 ± 4.71 ^{de}	11.07 ± 0.10^{gh}	13.91 ± 1.64^{de}	1338.19 ± 101.26^{gh}
EEB-C 11	53.33 ± 0.00 ^{cd}	14.71 ± 0.57^{abc}	20.59 ± 1.28^{ab}	1883.21 ± 37.71^{cdef}
EEB-C 16	73.33 ± 0.00 ^{ab}	13.68 ± 0.00^{bcde}	14.82 ± 0.31^{cd}	2090.40 ± 22.86^{bc}
EEB-C 19	53.33 ± 0.00 ^{cd}	12.90 ± 1.10^{cdefg}	20.28 ± 3.84^{ab}	1769.88 ± 263.97^{def}
EEB-C 20	43.33 ± 4.70 ^{e}	12.22 ± 0.19^{efg}	17.85 ± 2.32^{bc}	1391.55 ± 181.33^{gh}
EEB-C 21	60.00 ± 0.00 ^{c}	15.61 ± 1.57^{a}	18.44 ± 0.15^{bc}	2043.33 ± 103.70^{bcd}
EEB-C 22	53.33 ± 0.00 ^{cd}	12.46 ± 0.13^{defg}	18.53 ± 2.69^{bc}	1653.23 ± 150.84^{efg}
EEB-C 26	49.99 ± 4.71 ^{de}	9.82 ± 2.25^{h}	15.51 ± 3.20^{cd}	1264.53 ± 71.71^{hi}
EEB-C 29	53.33 ± 0.00 ^{cd}	14.21 ± 0.04^{abcd}	22.31 ± 0.17^{a}	1948.21 ± 7.07^{cde}
EEB-C 30	46.66 ± 0.00 ^{de}	13.00 ± 0.80^{bcdef}	18.00 ± 0.00^{bc}	1446.46 ± 37.70^{fg}
EEB-C 31	69.99 ± 4.71 ^{b}	14.7 6± 1.00^{ab}	18.08 ± 0.59^{bc}	2299.83 ± 183.92^{b}
EEB-C 32	43.33 ± 4.70 ^{e}	13.06 ± 0.21^{bcdef}	17.96 ± 0.05^{bc}	1344.89 ± 153.06^{gh}
EEB-C 33	46.66 ± 0.00 ^{de}	13.89 ± 0.55^{abcde}	10.25 ± 0.35^{e}	1033.18 ± 174.39^{i}
control	46.66 ± 0.00 ^{de}	11.67 ± 0.15^{fg}	9.32 ± 0.31^{e}	979.44 ± 102.67^{j}
SE(m)	2.582	0.612	1.243	104.915
CV (%)	6.652	6.528	9.88	8.58

**Mean of 3 replications having 10 plants. Values with same letters in a column are not significantly different. LSD (p<0.05)*

Table 4. Antagonism of endospore forming endophytic bacterial isolates in dual culture plate assay

Isolates	Inhibition zone (cm)*				
	F. oxysporum	***Pythium* sp.**	***R. solani***	***Colletotrichum* sp.**	***Phytophthora* sp.**
EEB-C9	5±0^{c}	3±0^{c}	6±0^{d}	5±0^{f}	3±0^{c}
EEB-C16	-	-	-	4±0^{g}	7±0^{b}
EEB-C19	-	-	1±0^{h}	6±0^{d}	4±0.07^{c}
EEB-C22	-	-	2±0^{g}	6.2±0.07^{d}	5±0^{ab}
EEB-C26	4±0^{d}	0.5±0^{b}	3.5±0.071^{f}	7±0^{c}	5.5±0.07^{ab}
EEB-C29	2±0^{e}	0.2±0^{e}	2±0^{g}	8.33±0^{b}	1±0^{e}
EEB-C33	7±0^{ab}	2.5±0.071^{cd}	7±0^{ab}	8.33±0^{b}	1.1±0.07^{d}
Control	-	-	-	-	-
SE(m)	0.026	0.013	0.018	0.022	0.026
CV (%)	11.178	5.423	5.958	3.216	3.381

**Mean of 3 replications. Values with same letters in a column are not significantly different. LSD (P<0.05)*

3.7 Evaluation of Bioprimed Seeds for Salinity Stress Tolerances

Bioprimed cowpea seeds tolerated up to 100mM NaCl concentration in both roll towel and protray assessment and the result are depicted in Table 7. EEB-C treated cowpea seeds showed higher germination percentage and seedling vigour index compared to hydropriming and control seeds under salinity stress.

3.8 Evaluation of Bioprimed Seeds for Moisture stress Tolerances

Cowpea seeds were able to withstand moisture stress induced by the addition of 10% PEG. In protray assessment moisture stress was induced by the complete withdrawal of watering 20 days after seedling emergence until the plant dry. On the 40th days after planting, seeds treated with EEB-C showed the maximum number of live

plants (23 plants) with the survival percentage of 92%, comparing to control (Table 8).

4. DISCUSSION

The present study focused on potency of biopriming of cowpea seed with endospore forming endophytic bacteria for growth promotion and stress tolerance. The common EEB isolates from most of the crops are *Bacillus* species, which are recognized for their safety profile, and with the potential to produce a diverse array of beneficial substances for agricultural applications [27]. Additionally, *Bacillus* species are reported to exhibit high potential as fungal antagonists, making them valuable contributors to plant health management [9]. The prevalence of *Bacillus* species is attributed to several factors, notably the production of endospores, which enables their persistence under adverse environmental conditions. Therefore, an effort has been undertaken for the isolation of endospore forming endophytic bacteria from endorhizospheric region of cowpea, a major tropical vegetable crop by double enrichment method on NA medium. From cowpea, thirty five EEB isolates were obtained and all of them were found to be belonging to the genus *Bacillus.* Using the double enrichment method, which facilitated the formation of endospores by the EEB during the drying process, more viable count of endospore producing bacteria were obtained compared to the conventional one-time physical enrichment method. Similar results on the isolation of EEB from amaranthus has been reported by Yashaswini et al. [10]; 28].

Table 5. Biochemical characterization of selected EEB isolates of cowpea

Biochemical characters	Isolates						
	EEB-C9	EEB-C16	EEB-C19	EEB-C22	EEB-C26	EEB-C29	EEB-C33
Indole	-	-	-	-	-	-	-
Methly red	-	+	+	-	-	-	-
Malonate	-	+	-	-	-	-	-
Voges proskauer's	+	-	-	-	-	+	+
Citrate utilization	+	+	-	-	-	-	-
ONPG	-	-	-	-	+	-	-
Nitrate reduction	+	-	+	-	+	+	+
Catalase	-	-	-	-	-	-	-
Arginine	-	+	+	+	+	+	+
Sucrose utilization	+	-	-	-	+	+	+
Mannitol utilization	+	-	-	-	+	+	+
Glucose utilization	+	-	+	+	+	+	+
Arabinose utilization	-	-	-	-	-	-	-
Trehalose utilization	+	-	+	+	-	-	-

Table 6. Germination and seedling vigour index of cowpea seeds bioprimed with EEB consortium in roll towel and protray assessment

Treatments	Roll towel assay		Protray assay	
	Germination (%)	Seedling vigour index	Germination (%)	Seedling vigour index
EEB-C consortium	79.11±1.25^{a}	1700.249±56.13^{b}	100±0^{a}	2752±41.01^{a}
Hydropriming	62.665±3.76^{b}	1063.331±11.42	76.665±4.72^{c}	1110.176±70.46^{c}
Control	67.5±3.53^{b}	1354.688±104.91^{bc}	80±0^{b}	1183.2±78.07^{c}

**Mean of 3 replications having 10 plants. Values with same letters in a column are not significantly different. LSD (P<0.05)*

Table 7. Germination percentage and seedling vigour index of cowpea seeds at different NaCl concentration in roll towel and protray assay

Treatments	Roll towel assay				Protray assay			
	Germination %		Seedling vigour Index		Germination %		Seedling vigour Index	
	50mM	100mM	50mM	100mM	50mM	100mM	50mM	100mM
EEB-C consortium	96.0±5.66^{a}	86±5.66^{a}	1656±126.39^{a}	967.5±47.73^{a}	92.5±3.54^{a}	59.16±5.89^{a}	2243.27±166.14^{a}	883.63±57.12^{a}
Hydro priming	57.7±3.15^{b}	35±7.07^{b}	637.63±6.17b^{b}	178.5±7.64^{b}	47.5±10.61^{b}	35.00±0^{c}	538.63±150.24^{b}	190.48±1.61^{c}
Control	45.0±7.07^{b}	10±00^{c}	427.5±130.82^{c}	15.00±00^{c}	40±7.07^{c}	40.00±7.07^{b}	452.97±70.89^{c}	345.5±63.78^{b}

Mean (± SD) of 5 replications. Values with same letters in a column are not significantly different. LSD (p<0.05)

Table 8. Germination percentage and seedling vigour index of cowpea seeds under moisture stress in roll towel and protray assay

Treatments	10% PEG		Percentage survival of plants 10 days after withdrawal of water	Percentage survival of plants 20 days after withdrawal of water
	Germination (%)	SVI		
EEB-C consortium	85.00	1899.50	100 ± 00[a]	92 ± 10.95[a]
Hydropriming	30.00	204.00	72 ± 10.95[b]	64 ± 8.94[b]
Control	57.77	459.01	60 ± 00[c]	32 ± 10.95[c]

** Mean (± SD) of 5 replications. Values with same letters in a column are not significantly different. LSD (P<0.05)*
**SVI: Seedling Vigor Index*

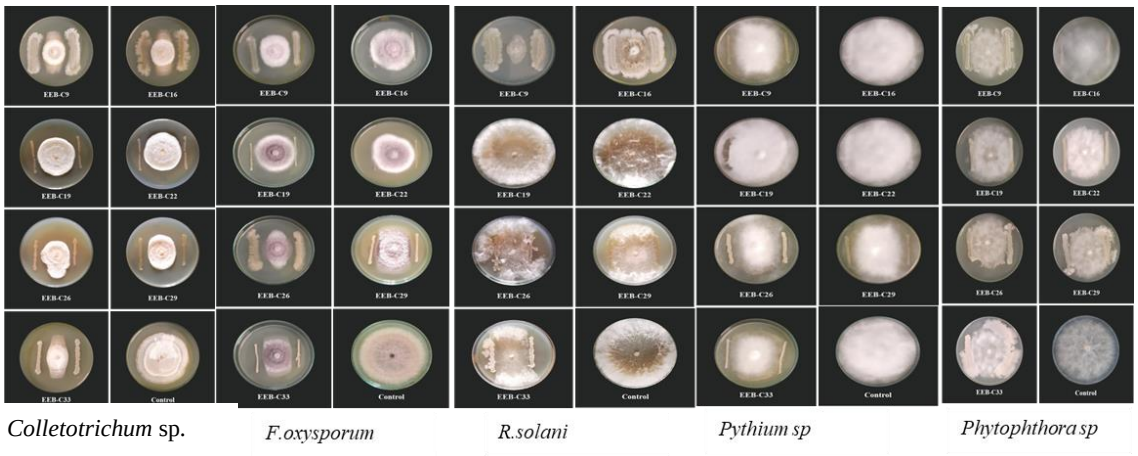

Fig 1. Antagonistic activity of EEB cowpea isolates against fungal pathogens in dual culture plate assay

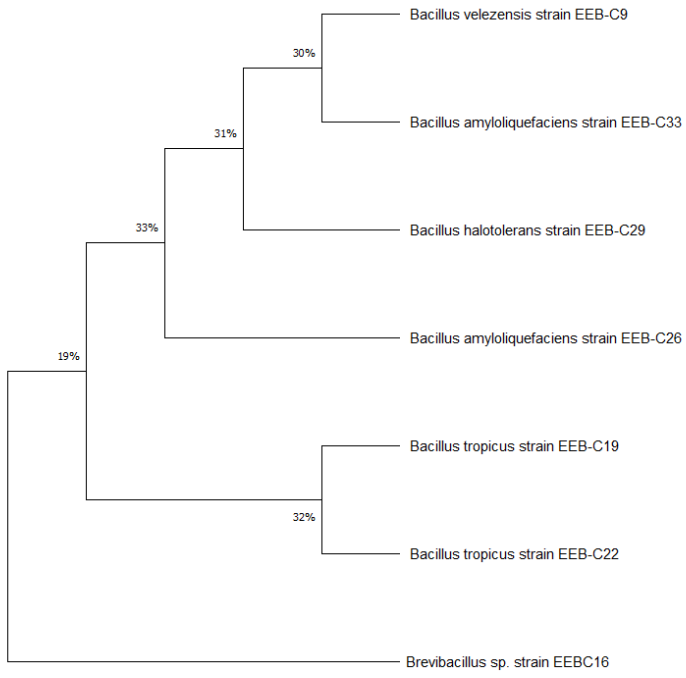

Fig. 2. Phylogenetic relatedness of EEB isolates of cowpea after 16SrRNA sequence analysis

Screening techniques play a crucial role in the effective identification and selection of bacterial strains with potential Plant Growth-Promoting (PGP) activity [29,30]. Various methods are employed to assess the capabilities of EEB isolates in promoting plant growth. Some common screening techniques include nutrient uptake assay, nitrogen fixation tests, indole 3 acetic acid (IAA) production, phosphate solubilization assays, siderophore production tests, ammonia production, antagonistic activity against pathogens, tolerance to abiotic stress and green house or field trials [31,32]. Green house or field trials are more expensive and time consuming. Hence less expensive and quick *in vitro* screening techniques help to identify bacterial strains that possess desirable traits for promoting plant growth.

Seed germination and seedling vigour play pivotal roles in productive capacity of a crop, exerting substantial influence on both crop yield and quality. Seed vigour encompasses the overall health and robustness of seeds, indicative of their potential for successful germination and subsequent seedling establishment. This characteristic serves as a measure of the seed's capacity to thrive diverse environmental conditions [33]. Inoculation of plant growth promoting bacteria on pre-sowing seeds improve seed germination and increase rate of germination, seedling vigour, growth and yield of many crops [34,35]. In the current study biopriming with the isolates EEB-C9 and EEB-C16 showed maximum germination percentage. Similarly, Devi et al. [36] reported the benefit of tomato seeds inoculated by endophytic *Bacillus* sp. improved the germination percentage, shoot length and root length of tomato seedlings. It is proved that *Bacillus* spp. have the capability to produce gibberellins and cytokinins. This biochemical activity contributes to enhanced root growth, an increased number of root hairs, and the establishment of a close and intimate association between the seeds and the bacteria thereby resulting in resilience of the seedlings [37].

The inhibitory effect of EEB isolates were observed against various bacterial and fungal pathogens such as *Fusarium oxysporum, Rhizoctonia solani, Colletotrichum* sp., *Phytophthora* sp., and *Pythium* sp. in dual culture plate assay. *In vitro* assessment for antifungal activity is one of the pre-requisites to select potential antagonist for the development of biocontrol strategies [22,38]. The isolates EEB-C9 and EEB-C33 exhibited the highest level of inhibitory activity in the assay. The results align with the previously findings by Athira and Anith, [39] and Kollakkodan et al. [40] that many of the EEB have antagonistic activity against phytopathogenic fungi. Endophytic bacteria are recognized for their ability to improve plant health through the modulation of the plant immune system by inhibition of pathogens. The biocontrol mechanism of plant pathogens comprises a multifactor interplay to suppress or inhibit pathogens by competition for space and nutrients, producing active soluble substances such as antibiotics and lytic enzymes, as well as volatile compounds and induction of plant defense mechanism known as induced systemic resistance [41,42]. Many of the EEB exhibit such multiple traits and thus contribute to plant health.

The introduction of endophytes to plants showcased their inherent potential to mitigate salinity stress and augment stress tolerance. In the present experiments, bioprimed seeds of cowpea under salinity of 50mM, 100mM, 150mM and 200mM NaCl concentration had better performance than those unprimed. Bioprimed cowpea seeds tolerated stress level up to 100mM NaCl concentration. Similar aspects of endophytic mediated salinity stress tolerance were reported by Al-*Shwaiman* et al. [43] in wheat plants, and it was also interpreted that endophytic *Beijerinckia fluminensis* imparted tolerance to NaCl at 10% concentration.

Endophytic microorganisms have been reported to alter plant responses to drought and can be used as an alternative and rapid way to enhance crop productivity under moisture stress [44]. Our study to evaluate the performance of bioprimed seeds under moisture stress condition also proved the same. In cowpea with 10% PEG addition, seeds treated with the endophytic isolates showed higher performance including germination percentage and seedling vigour index. In protray assessment the bioprimed seeds showed 100 percentage survival of plants 10 days after withdrawal of water and 92 percentage survival of plants 20 days after withdrawal of water. This may be attributed to the production of plant growth substances produced by the bacterial endophytes [41]. Jeong et al. [45] reported that endophytic *Pseudomonas* sp. and *Pantoe*a sp. improved the seedling length and leaf biomass under water deficient conditions. It has been reported that endophytic bacterial isolates could produce IAA, ACC deaminase, and some osmolytes which help to maintain the

metabolism and demonstrated the growth promotion under stress conditions [46].

5. CONCLUSION

The current findings suggest that endospore forming endophytic bacterial consortia had better efficiency in plant growth promotion and abiotic stress tolerance by either direct and indirect mechanisms. It is advisable to have a consortium made with different isolates obtained from the same crop plant than the individual isolates for the use in plant health management as many with varying PGP traits act together and perform in a better way. The biopriming treatment enhanced the plant growth through various direct mechanism such as production of IAA, GA, ammonia, HCN, siderophore, ACC deaminase activity, nitrogen fixation and phosphorus solubilization. These findings further contribute to possibility of application of the endospore forming endophytic bacteria for plant growth promotion under stress condition as well. Moreover, it is concluded that, a consortium of cowpea EEB isolates could give better results on plant growth promotion and stress tolerances.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.30498/IJB.2020.137279.2308
PMID: 33542934; PMCID: PMC7856403

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/112658