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ENHANCING CROP GROWTH AND SUSTAINABILITY THROUGH ENDOPHYTIC BACTERIA APPLICATION IN FINGER MILLET (*ELEUSINE CORACANA* L.)

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ABSTRACT

This study investigates the growth-promoting potential of endophytic bacteria isolated from finger millet (*Eleusine coracana* L.) landraces, focusing on their ability to produce plant hormones, solubilize phosphate, and exhibit urease activity. Eight bacterial endophytes were evaluated for their phytohormone production using high-performance liquid chromatography (HPLC). Among them, the GDEL1 isolate demonstrated the highest levels of indole acetic acid (IAA) (53.76 µg/mL) and gibberellic acid (GA3) (36.42 µg/mL), along with notable production of abscisic acid (ABA) (4.16 µg/mL) and salicylic acid (SA) (3.44 µg/mL). Additionally, KER1 and KER2 exhibited effective phosphate solubilization, while GDEL1 and KEL1 displayed significant urease activity. GDEL1 was further assessed through seed treatment, seedling root dip, and soil application under greenhouse conditions. Seed treatment resulted in the most pronounced improvements in shoot length (61.33 cm), root length (31.60 cm), and biomass production (1.01 g). These findings suggest that seed treatment with the GDEL1 endophytic strain could serve as an effective strategy to enhance finger millet growth, reduce reliance on chemical fertilizers, and promote sustainable agricultural practices.

Keywords : Endophytes, Finger millet landraces, Phytohormones, PGP traits, Method of application

Introduction

Millet, minor cereals of the Poaceae family, thrive in poor soil and harsh environments. Finger millet (*Eleusine coracana* L.), or ragi, is a key crop in India, contributing 60% of global production (Bhat *et al.*, 2019). It offers essential nutrients, with 81.5% carbohydrates, 9.8% protein, and superior fiber and mineral content compared to wheat or rice (Ravindran, 1991; Sripriya *et al.*, 1997). Rich in calcium, iron (Rathore *et al.*, 2019), and essential amino acids (Vachanth *et al.*, 2010), it has a low glycemic response beneficial for diabetics (Ramanathan and Gopalan, 1957). Karnataka leads in production, followed by Tamil Nadu and Uttarakhand (MoAFW Report, 2023). Increasing environmentally friendly finger millet production can reduce agricultural chemical use.

Endophytes are beneficial microorganisms that form mutualistic relationships with plants, residing within plant tissues without causing harm. These microorganisms offer significant potential for disease management as they can colonize plant tissues effectively, boosting plant growth (Martinez-Medina *et al.*, 2016). As endophytes promote plant health through mechanisms such as nutrient enhancement, pathogen suppression, and stress tolerance, they provide a sustainable and eco-friendly alternative to chemical treatments (Kumar, 2022).

Using host-associated microbial inoculums presents an eco-friendly alternative to enhance crop growth while reducing the adverse effects of commercial fertilizers. Bacterial endophytes play a significant role in promoting plant growth and development by supplying external phytohormones

(Shahzad *et al.*, 2016). These beneficial microorganisms support plant growth through various mechanisms, including phosphate solubilization (Wakelin *et al.*, 2004), phytohormone production (Lee *et al.*, 2004), and nitrogen fixation (Compant *et al.*, 2005). Standardizing application methods ensures consistent, reliable results by minimizing variability and enabling fair comparisons of bacterial effectiveness. It enhances reproducibility in research and optimizes agricultural use, improving crop yields while reducing reliance on chemical fertilizers (Chai *et al.*, 2022). This research highlights the significance of choosing suitable inoculation methods for effective bacterial colonization and enhanced plant growth. Endophytes isolated from finger millet landraces were evaluated for their growth-promoting potential, and the most promising isolate was applied using various methods to standardize the application process for optimal plant growth promotion.

Material and Methods

Sources of endophytes

The endophytic bacteria used in this study were isolated from finger millet landraces (Priyadarshini *et al.*, 2024). Seeds for the experiment were collected from All India Coordinated Research Programme on Millets (AICRP), Zonal Agricultural Research Station (ZARS), V.C. Farm, Mandya, Karnataka.

Extraction and estimation of IAA, GA₃, ABA and SA production

The production of indole-3-acetic acid (IAA), gibberellic acid (GA₃), abscisic acid (ABA), and salicylic acid (SA) by selected endophytic bacterial isolates was assessed using high-performance liquid chromatography (HPLC). For IAA estimation, nutrient broth flasks supplemented with a sterilized L-Tryptophan solution (100 µL) were inoculated with 2 mL of bacterial culture and incubated for 7 days, with a control maintained without L-Tryptophan. The incubated broth was centrifuged at 6000 rpm for 20 minutes, the pH of the supernatant was adjusted to 2.8 using 0.1N HCl, and the acidified supernatant was extracted with diethyl ether. After phase separation and solvent evaporation, the residue was dissolved in 2 mL of HPLC-grade methanol and analysed using a C18 column with a methanol: water (80:20) mobile phase, a 1 mL/min flow rate, and detection at 270 nm. For GA₃, ABA, and SA estimations, bacterial cultures were grown in nutrient broth without L-Tryptophan. After incubation, similar centrifugation, pH adjustment, solvent extraction, and evaporation steps were followed. GA₃ was analysed using a methanol: water

(70:30) mobile phase at a 0.8 mL/min flow rate with detection at 208 nm. ABA estimation involved an acetonitrile:0.5% acetic acid (80:20) mobile phase with a 0.8 mL/min flow rate and detection at 254 nm. SA analysis was conducted with an acetonitrile:0.5% acetic acid (90:10) mobile phase at a 1 mL/min flow rate, with detection at 302 nm. All HPLC analyses were performed using a Shimadzu system with a C18 column, maintained at a consistent temperature of 30°C (Cosoveanu *et al.*, 2021).

Phosphate solubilisation

The growth of bacterial isolates was analysed by spot inoculation of cultures on the Pikovskaya's (PVK) media plates and incubated at 30 °C for 7 days. The ability of the bacteria to solubilize insoluble phosphorus and form clear halo zones around them was considered as positive result for their phosphate solubilization potential (Shah *et al.*, 2021).

Urease production

Urease activity was determined by using Stuart's urea broth containing the following in one litre: 20g urea, 9.5g K₂HPO₄, 9.1g KH₂PO₄, 0.1g yeast extract, and 0.01g phenol red having the pH of 6.8. Ten millilitres of broth were distributed in test tubes and each test tube was inoculated with 10 µl of an 18h culture. These were incubated at 35°C for 48h and the color change to bright pink indicated a positive urease reaction (Mutungi *et al.*, 2022).

Standardising the method of application of endophytes to plants under greenhouse experiment

The selected potential endophytes were applied to the plants in different methods *viz.*, seed treatment, seedling root dip and soil application to test its efficacy in plant growth promotion. This experiment was conducted under greenhouse condition in the Department of Agricultural Microbiology.

Mass multiplication of endophytic isolates

Bacterial isolates were grown in sterile nutrient broth. One hundred ml of nutrient broth was inoculated with 24 hrs old endophytic bacterial culture. The flasks were incubated in shaker at 150 rpm for seven days at ambient temperature of 28 °C. The bacterial inoculum containing 10⁶ cfu / ml was used for experiment (Rini and Sulochana, 2007).

Seed treatment

For seed treatment, seeds of finger millet var. INDAF-5 were surface sterilized with 70 per cent ethanol for 1 min, 3 per cent Sodium hypochlorite for 5 min followed by again 70 per cent ethanol wash for 1 min, then rinsed in sterile distilled water for three times

and dried for overnight under sterile air stream. Pre-germinated seeds were soaked in ten millilitres of 10^8 CFU of bacterial suspension for 12 hrs and dried under laminar air flow. The seeds which were soaked in sterile distilled water served as control (Feng *et al.*, 2021).

Seedling root dip

For seedling root dip method, the seedlings were transplanted 15 DAS. Before transplanting, roots of seedlings were dipped in endophytic bacterial suspension for 30 mins to 1 hour and then transplanted to pots containing sterile pot mixture (Nagendran *et al.*, 2013).

Soil application

For soil application method, bacterial suspension was mixed with sterile soil mixture with FYM and allowed to incubate for 15 days. At the time of transplanting, the mixture was applied to the soil and followed by transplanted to main pot. Observations were taken 40 DAS.

Observation of plant growth promotion

Plant growth promotion was assessed by measuring shoot length, root length, and plant biomass at 40 days after sowing (DAS) (Horo *et al.*, 2018; Kushwah *et al.*, 2014).

Statistical Analysis

The observations collected on various parameters both in laboratory and greenhouse condition were

subjected to appropriate statistical analysis. CRD analysis was taken up both lab and greenhouse experiments, by using the Wasp 2.0 statistical tool.

Results and Discussion

Estimation of phytohormone produced by endophytic bacterial isolates

The study investigated the capacity of endophytes to synthesize plant growth-promoting hormones such as indole acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and salicylic acid (SA). The levels of these hormones were measured using high-performance liquid chromatography (HPLC) (Table 1). The highest IAA production was recorded in the BMEL1 isolate at 60.25 $\mu\text{g/mL}$, followed by GDEL1 at 53.76 $\mu\text{g/mL}$. In contrast, KER1 and KER2 showed significantly lower IAA production at 23.82 $\mu\text{g/mL}$ and 20.94 $\mu\text{g/mL}$, respectively. For gibberellins, GDEL1 produced the most at 36.42 $\mu\text{g/mL}$, with HKES and KEL1 following at 27.34 $\mu\text{g/mL}$ and 26.42 $\mu\text{g/mL}$, respectively. The lowest production was noted in GES at 4.91 $\mu\text{g/mL}$. In terms of salicylic acid, isolates GES (3.35 $\mu\text{g/mL}$), HKES (3.44 $\mu\text{g/mL}$), and KER2 (3.32 $\mu\text{g/mL}$) exhibited similarly high levels of production, while AES (2.16 $\mu\text{g/mL}$) and KER1 (2.22 $\mu\text{g/mL}$) had the lowest amounts. Regarding abscisic acid, GDEL1 had the highest production at 4.16 $\mu\text{g/mL}$, closely followed by BMEL1 at 3.98 $\mu\text{g/mL}$ and HKES at 3.72 $\mu\text{g/mL}$. The lowest ABA production was observed in KER2 (1.05 $\mu\text{g/mL}$) and GES (1.14 $\mu\text{g/mL}$).

Table 1 : Phytohormone produced by endophytic bacterial isolates

Isolates	Source	IAA ($\mu\text{g/mL}$)	GA ($\mu\text{g/mL}$)	SA ($\mu\text{g/mL}$)	ABA ($\mu\text{g/mL}$)
AES	Seed	34.16 ^d	11.76 ^d	2.16 ^d	2.56 ^c
BMEL1	Leaf	60.25 ^a	16.02 ^c	3.04 ^{bc}	3.98 ^a
GDEL1	Leaf	53.76 ^b	36.42 ^a	2.98 ^c	4.16 ^a
GES	Seed	34.20 ^d	4.91 ^f	3.35 ^a	1.14 ^f
HKES	Seed	39.24 ^c	27.34 ^b	3.44 ^a	3.72 ^b
KEL1	Leaf	27.32 ^e	26.42 ^b	3.23 ^{ab}	2.28 ^d
KER1	Root	23.82 ^f	6.63 ^e	2.22 ^d	1.45 ^e
KER2	Root	20.94 ^f	7.88 ^e	3.32 ^a	1.05 ^f

Note: Means with same letter in column, do not differ significantly at $P < 0.05$ as per Duncan Multiple Range Test (DMRT)

Phytohormones play a crucial role in regulating various physiological processes in plants, encompassing growth, development, nutrient distribution, and adaptation to environmental conditions. As a result, all biological activities within the plant, as well as its interactions with external factors, are influenced directly or indirectly by these hormones (Fahad *et al.*, 2015). Auxins, particularly indole acetic acid (IAA), are essential phytohormones that influence various aspects of plant growth, such as

cell elongation, the development of vascular tissues, and maintaining apical dominance. Gibberellins (GAs) are vital for processes including seed germination, flower initiation, leaf expansion, stem elongation, and the maturation of flowers and fruits. Abscisic acid (ABA) plays a significant role in how plants respond to abiotic stresses like drought, heat, chilling, and salinity, as well as in regulating dormancy. ABA causes stomata closure in plant leaves, thereby reducing water loss. Additionally, salicylic acid (SA) is involved in

several physiological processes, especially in defense mechanisms against biotic stresses, including attacks from necrotrophic pathogens and pests (Fahad *et al.*, 2015; Poveda, 2020).

Phosphate solubilization and urease production

The eight potential endophytic isolates were assessed for plant growth-promoting traits, including

urease production and phosphate solubilization (Table 2). Urease activity was observed in GDEL1 and KEL1, as indicated by a color change in the broth to pink, while the other isolates showed negative results (Fig. 1A). Among the eight isolates, only KER1 and KER2 exhibited phosphate solubilization, forming clear zones around the bacterial colonies on petri dish (Fig. 1B).



Fig. 1 : Estimation of growth promoting traits. A) Urease production B) Phosphate solubilisation

Phosphorus is often unavailable in soils due to its presence in insoluble forms like iron, aluminium, or calcium phosphates. Phosphate-solubilizing bacteria (PSB) convert these into bioavailable forms for plant uptake, enhancing crop yields. In return, they receive carbon compounds from plant roots. PSB inoculation can cut phosphate fertilizer use by up to 50% without reducing yields (Walia *et al.*, 2017). Urease production enhances the availability of nitrogen for plant growth by converting urea, whether naturally occurring or added, into ammonia and carbonate. This ammonia is subsequently transformed into nitrite and then nitrate, which plants can readily absorb. The activity of urease significantly improves the efficiency of nitrogenous fertilizers (Nosheen and Bano, 2014).

Table 2 : Biochemical Traits of Endophyte Isolates including Urease Production and Phosphate Solubilization

Isolates	Urease production	Phosphate solubilisation
AES	-	-
BMEL1	-	-
GDEL1	++	-
GES	-	-
HKES	-	-
KEL1	+	-
KER1	-	+
KER2	-	+

Note: (-)-Negative, (+)-good, (++)-very good

The most efficient culture in phosphate solubilization was KER1 and KER2, which formed

clear zones around their colonies. GDEL1 and KEL1 were the top performers in urease activity, showing a color change in the broth. BMEL1 exhibited the highest production of IAA, and GDEL1 produced the highest levels of gibberellins. In terms of salicylic acid production, GES, HKES, and KER2 were the most efficient, while GDEL1 stood out for its high abscisic acid production. Two best isolates from each assay were chosen to identify the most effective culture that performed well across nearly all growth promotion tests. GDEL1 isolate stood as a potential isolate in four assays among the six tested assays (Fig. 2). Thus, this bacterial isolate was used to standardise the method of application of endophytes to plants.

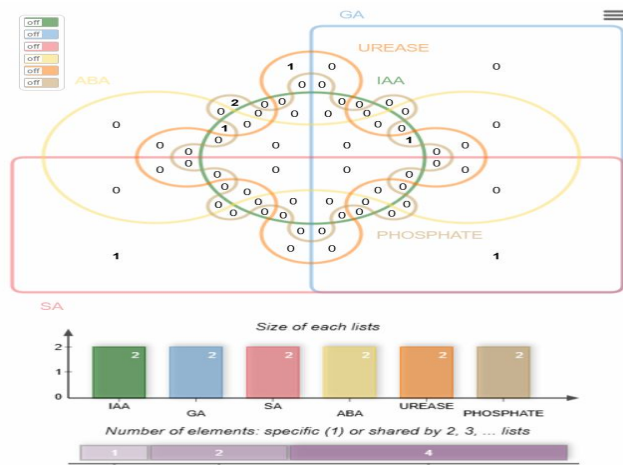


Fig. 2 : Growth promotion activities exhibited by endophytic isolates

Standardization of method of application of endophytic bacteria to the plants

The endophytic strain GDEL1 was applied to plants using various methods, including seed treatment, seedling root dip, and soil application, to assess its efficacy in promoting plant growth under greenhouse condition.

The highest shoot length of 61.33 cm was recorded by the plants seed treated with GDEL1. The best root length at 31.60 cm was resulted by the plants seed treated with endophytic bacteria. Additionally, seed treatment with endophytes yielded the greatest biomass production, with 1.01 g, surpassing other methods (Fig.3&4). Therefore, among the various application techniques assessed, seed treated plants involving the endophytic bacteria GDEL1 demonstrated outperformed the alternative methods in growth promotion.

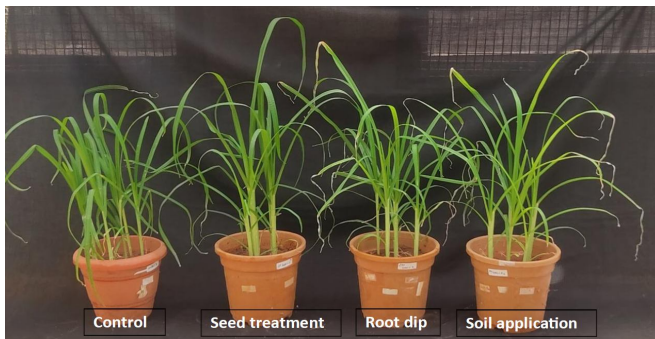


Fig. 3 : Different methods of application of endophytic bacteria GDEL1 in finger millet plants under green house condition

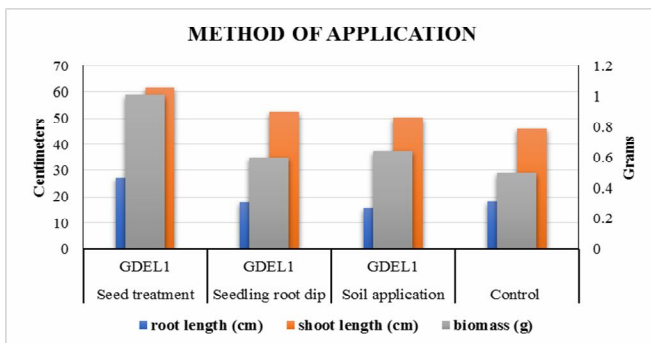


Fig. 4 : Assessing plant growth promotion through various application techniques of endophytic bacteria

Seed endophytic bacteria (SEB) play a significant role in plant development as early colonizers of roots and shoots post-germination (Verma *et al.*, 2019). Upon planting, they reactivate and enhance plant growth and resilience (Truyens *et al.*, 2015; Mitter *et al.*, 2017). SEB influence plant growth from germination through maturity, providing immediate microbial support essential for seedling establishment, especially in invasive plant species that struggle to find

compatible microbiomes (Shearin *et al.*, 2018). Extended seed treatment increases SEB colonization in root, stem, and leaf tissues (Jaber and Enkerli, 2016). Their benefits include growth promotion, pathogen biocontrol, and stimulation of genes related to root architecture and stress responses (Irizarry and White, 2018). Studies show that removing SEB from finger millet seeds impairs seedling health, while reintroduction restores growth (Kumar *et al.*, 2020). Additionally, inoculating rice seeds with *Enterobacter* sp. from rice sprouts has improved biomass in mature plants under salt stress (Liu *et al.*, 2020). From the results seed treatment with endophytic bacteria were found to be beneficial than other methods of application.

Conclusion

In conclusion, the endophytic bacterial isolate GDEL1 exhibited significant plant growth-promoting potential, excelling in the production of phytohormones, urease activity, and overall plant growth enhancement. Among the various application methods evaluated, seed treatment with GDEL1 proved to be the most effective, leading to superior shoot and root growth as well as increased biomass production under greenhouse conditions. These findings highlight the potential of GDEL1 as a promising bioinoculant for sustainable agricultural practices, offering an eco-friendly alternative to chemical fertilizers for enhancing crop productivity.

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