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RESEARCH ARTICLE

Impact of Bacterial Endophytes of *Phyllanthus emblica* L. in Developmental Magnification of Crop for Sustainable Agriculture

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Abstract

Background/Objectives: In the search of sustainable and eco-friendly replacement of chemical fertilizer, we have isolated endophytes from *Phyllanthus emblica* L. and checked their ability as Plant Growth Promoting Rhizobacteria (PGPR). **Methods:** Based on their positive result in vitro, a total of 5 bacterial endophytes were selected and tested on seeds of legume and non-leguminous plants *Cicer arietinum* L. and *Triticum aestivum* L. respectively. Differences in plant growth and the impact of these endophytes on seed growth in terms of seed germination, seed Vigor index (SVI) and seedling length were analysed against untreated control seeds. All the data were statistically analysed by deriving average mean value and standard deviation, further it was analysed by performing ANOVA. **Findings:** Seeds coated with YR1 isolate have shown 30.31% increase in seed germination while endophyte YR2-coated seeds have shown up to 20% chickpea seed germination. In seedling growth, coating has shown 234.1% and 444.4% increases in the growth of wheat and chickpea seeds respectively. **Novelty :** *Phyllanthus emblica* L. commonly known as Indian gooseberry is a medicinal plant having beneficial effects on human health. In the current study, Indian gooseberry is screened for the presence of endophytes. A total of 25 endophytes were isolated from different parts of the amla plant and were tested for plant growth-promoting (PGP) traits. These isolates have shown appreciable direct and indirect PGP traits.

Keywords: Sustainable agriculture; PGPR; Biofertilizers; Seed coating; Medicinal plants; Endophytes

1 Introduction

Endophytes are microorganisms that successfully invade and reside within the internal tissues of their plant host, without causing any noticeable damage or adverse symptoms.

These microorganisms (including actinomycetes, fungi, and bacteria) proliferate without leading to any detrimental effects. Bacterial endophytes are mutualistic bacteria that grow inside and between plant cells without causing any chief morphological changes in plants. The positive interaction between plant parts and endophytes incorporates various plant species and bacterial classes. Their ability to multiply into plant tissues without the establishment of any infection or disease distinguishes them from plant pathogens. Endophytes are located within diverse plant structures, comprising roots, stems, leaves, and seeds, and display tissue specificity, wherein certain endophytes possess a wide host spectrum, while others demonstrate a more specialized affinity. These microorganisms are recognized for their role in enhancing plant growth via various mechanisms [1].

Various studies have emphasized the importance of endophytes in enhancing plant vitality by helping plants in biotic and abiotic stress tolerance, nutrient uptake, and soil health promotion which increases crop productivity and in the long run contributes to sustainable agriculture [2].

The enduring and direct interaction of endophytes with the plants gives them special benefits to their host over the rhizospheric microorganisms and epiphytes. This distinguished character of endophytes is nowadays making them the most significant candidate of the group of Plant Growth-Promoting Microorganisms (PGPM) for developing bioinoculants and therapeutics for plant and soil health improvement [3].

Endophytes as a ubiquitous associate of the plant are considered as a promising candidate for sustainable agriculture. These organisms play a pivotal role in the regulation of the primary and secondary metabolism of their host plant. The direct and long-lasting interaction of endophytes with the host enables them to escape from harsh environmental conditions. Especially, their endophytic nature makes them better candidates over epiphytes and rhizospheric microbes in interaction with plants. Current research findings revealed that endophytes help plants in making nutrient acquisition from the soil, nitrogen fixation, phosphate availability, phytohormone and antimicrobial production. There is a huge potential for developing novel products like endophytes-based microbial formulations and elicitors to improve plant health, ameliorating stress tolerance in plants and source of therapeutically important secondary metabolites. The present study specifically dealt with attributes such as host-tissue specificity of endophytes, the importance of seed-associated endophytes, endophyte-parasite plant-host plant interaction as well as their applications in plant in-vitro systems and as microbial consortium [4]. In addition, the conserved endophytic microbial communities in different plants are also looked upon possibly to understand the plant-endophytic microbiome on similar lines of the animal-gut microbiome. Primarily, the purpose of this review is to implicate the endophytic flora as probiotics influencing overall plant health and their survival under extreme environmental conditions [5].

Phyllanthus emblica L. (Indian gooseberry) commonly recognised as "Amla" is a medicinal plant which have been documented to possess various medicinal attributes. Till date, the search for endophytes within this plant has been quite limited. Isolation and characterization of endophytes from various parts of Amla plants could result in the discovery and identification of potent isolates with significant metabolic and biochemical and PGPM activity. In 2012, Nath et. al. [6] reported the existence of endophytic fungi from the genera *Phomopsis* sp., *Xylaria* sp. and *Diporthe* sp., isolated from stem, roots and leaves of the Amla plant.

In 2018, Sharma and Sharma [7], documented isolation of endophytic fungi from fruits of Amla plant, belong to the group Ascomycetes and Deuteromycestes. Amongst these two groups, Deuteromycetes had showed greatest diversity, encompassing nine species of *Aspergillus (A. flavus, A. japonicus, A. terreus, A. niger, A. parasiticus, A. ochraceous, A. sydowii, A. nidulans, A. fumigatus)*; two species of *Fusarium (F. solani, F. verticilloides); three species of Penicillium (P. funiculom, P. crysogenum, P. waksmanii)*, in addition to species of *Acremonium, Trichothecium and Curcularia*.

Seed coating is an emerging technology in agricultural microbiology that involves the application of microbial inoculants on seeds that promote plant growth by accelerating pre-germination metabolic activities [8]. These bioinoculants can enhance the seed vigour index by increasing the germination percentage of seeds. Seed-coating can help plant growth by enhancing root development, improving the mobilisation of biomolecules, and strengthening plant defence mechanisms [9]. In the present investigation, we examined the prospective bacterial endophytes associated with *Phyllanthus emblica* L., which exhibit beneficial traits for promoting plant growth in vitro, with a focus on their effect on seed germination and vigor index. We conducted an examination of these bacterial endophytes on two widely cultivated crops in India, *Cicer arietinum* and *Triticum aestivum*, to evaluate potential enhancements in overall seed yield and the promotion of seed growth. Potent endophytes showing markable improvement can be investigated further to develop bioinoculant and can be checked for crop improvement and sustainable agriculture at higher scale.

2 Methodology

2.1 Isolation of bacterial endophytes

Plant parts of *Phyllanthus emblica* L. (commonly known as Amla) were collected from the north region of Gujarat state, India [Latitude: 23.48444 Longitude: 72.398375 (Ganpat University, Kherva, Mehsana)]. All the samples from different plant parts were prepared for the study by washing them with tap water, followed by treatment of 70% (v/v) ethanol for 30 sec and 2% sodium hypochlorite solution for 5 min for surface sterilization, and washing with sterile distilled water after each step. Surface sterilized samples were cut into small pieces of 0.5 cm, and then 1 g of each sample was macerated using a sterile mortar pestle. An aliquot of 0.2 ml supernatant (after centrifugation at 2200×g for 5 min) was inoculated on a nutrient agar plate using the spread plate method[(10)].

2.2 PGPR attribute study of endophytes

Various plant growth-promoting traits were studied using nitrogen fixation, phosphate solubilization, siderophore production[(11)], Indole acetic acid (IAA) production[(12,13)], chitinase production[(14)], ammonia production[(15)], HCN production[(16)] and EPS production[(17)] to determine the potential of isolates.

2.3 Germination study

2.3.1 Seed surface sterilization and coating

The seeds were surface sterilized with 2.5% sodium hypochlorite solution for 20 min, followed by 70% ethanol for 30 sec. Subsequently, the seeds were washed thrice with sterile distilled water[(18)]. Surface sterilized seeds were coated with inoculum of selected potent endophytes with proper cell density of 10^9 cfu ml^{-1} (adjusted to 1 optical density at 600 nm).

2.3.2 Inoculum preparation and Bacterization of seeds

For the germination study, five potent endophytic isolates of Amla were used for the seed of *Triticum aestivum* L. (Wheat) and *Cicer arietinum* L. (Chickpea). A pure isolated colony of each bacterial endophyte was grown in 50 ml nutrient broth [containing yeast extract (2.0 g l^{-1}), peptone (5.0 g l^{-1}), and sodium chloride (5.0 g l^{-1}) pH 7.4 $\pm$ 0.2] and kept on a shaking incubator at 25$\pm$2 °C till the cell density of 10^9 cfu ml^{-1} was achieved[(19)]. After seed inoculation, sterilised filter paper was moistened with 5 ml of sterile distilled water and placed in autoclaved petri plates under aseptic conditions[(20)]. For the coating of seeds, bacterial growth of 10^9 cfu ml^{-1} was used as an inoculant to coat seeds under aseptic conditions. Negative control was maintained by 'mock-inoculated' seeds (coated with uninoculated growth media)[(21)]. After coating with respective endophytic inoculant, all the plates were kept in a seed germination chamber with 60% relative humidity at 25 °C / 20 °C; day/night temperature with 16 h photoperiod[(22)].

2.4 Treatment and experimental design

The experiment was set up with a completely randomised design (CRD) in a triplicate set consisting of 5 endophytic inocula as a treatment along with control plates as: T1: Mocked inoculated control; T2: Endophyte SN4; T3: Endophyte SY1; T4: Endophyte SY12; T5: Endophyte YR1 and T6: Endophyte YR2.

2.5 Growth parameter study

All the plates were incubated for 14 days and examined daily for the development of radicles, cotyledons, root apex, and shoot apex. At the end of the experiment, various parameters, namely shoot length, root length, and the number of seeds germinated, were measured. Using these measurements, seed germination percentage (% germination) (Equation (1))[(23)] and seed vigour index (SVI) (Equation (2))[(18)] were calculated using the following formula,

$$\text{Percent germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds primed}} \times 100 \tag{1}$$

$$\text{SVI} = (\text{ Mean shoot length } + \text{ Mean root length }) \times \%\text{ germination} \tag{2}$$

3 Results and Discussion

3.1 PGPR traits

In our previous study[24], it was reported that all the bacterial endophytic isolates have good plant growth-promoting characteristics. Isolates YR1, YR2, SY1, and SY12 can fix nitrogen, while SN4, YR1, and SY12 show phosphate solubilization ability, all the isolates have the potential to produce ammonia and exopolysaccharides (Figure 1). Isolate YR1 produces the highest indole-3 acetic acid at 14.50±0.30 μg ml^{-1}, followed by other isolates. Endophyte YR2 (26.7±2.42 mg ml^{-1}) produces a reasonably good amount of EPS, followed by isolates SN4, YR1, SY1, and SY12. In the case of ammonia, among these isolates, SY1 produces the highest amount, 8.13±0.015 mg ml^{-1}, followed by 8.07±0.008 mg ml^{-1} by YR1, and so on (Table 1).

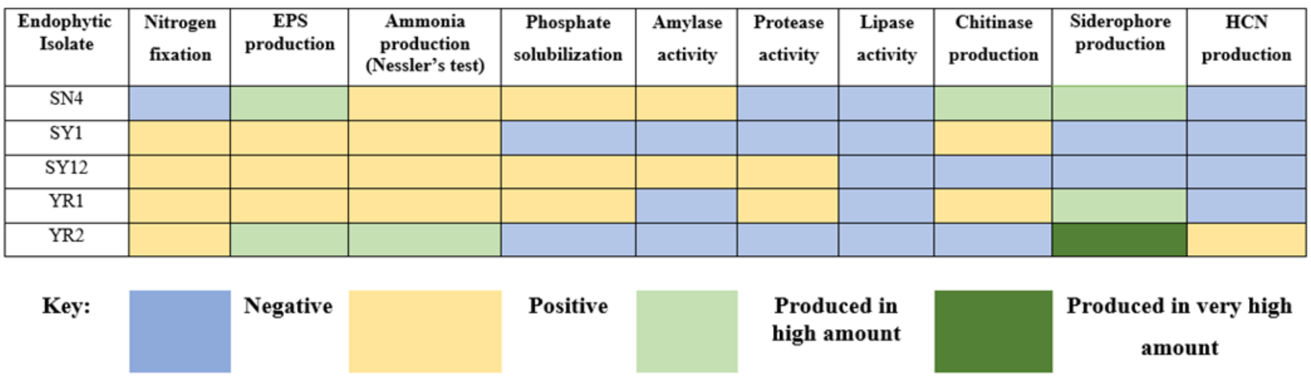

Fig 1. Qualitative direct and in-direct PGPR traits of Amla endophytes

Table 1. Quantitative direct and in-direct PGPR traits of Amla endophytes

Endophytic isolate	IAA production μg ml^{-1}	EPS (wet weight) mg ml^{-1}	Ammonia production mg ml^{-1}
SN4	12.64 ±0.46	71.2 ± 1.1	1.97 ± 0.034
SY1	1.10 ± 0.10	24.06 ± 0.75	8.13 ± 0.015
SY12	1.46 ± 0.10	23.3 ± 0.95	5.89 ± 0.007
YR1	14.50 ± 0.30	33.53 ± 0.51	1.86 ± 0.013
YR2	3.83 ± 0.30	106.9 ± 2.42	8.07 ± 0.008

3.2 Potential of isolates in accelerating seed germination and seed vigour index

3.2.1 Potential of isolates in growth magnification of seeds

Isolates showing good PGPR attributes were primed on wheat and chickpea seeds, which were observed for magnification of plant growth. It was observed that the seed coating with bacterial inoculum showed a positive effect on various parameters like seed germination, shoot length, root length, and seed vigour index.

In chickpea, 14 days after sowing (DAS), the most extended seedling shoot of 2.72±1.08 cm was recorded in seed inoculated with endophyte SY1, showing a significant increase of 444.4% against uncoated control seeds (0.5±1.65 cm) and magnificent increase up to 179.9% in root length of seeds treated with culture YR2 (8.21±1.69 cm) against control seeds (2.9±1.77 cm) (Figure 2**b**). In wheat, at 14 DAS, the highest shoot length (8.7 ± 2.19 cm) of seeds has been observed in seeds coated with culture SY1 and YR2 against untreated control (3.7 ± 0.17 cm). Meanwhile, endophyte SY1 gave a 234.1% increase in root length (5.44 ± 0.38 cm) relative to control seeds with root length 1.6 ± 0.65 cm (Figure 2 **d**).

3.2.2 Seed germination

In the case of seed germination, all the isolates have shown a positive impact on wheat and chickpea seeds in terms of per cent seed germination. *Cicer arietinum* L. has demonstrated a positive increase of 100% seed germination when coated with endophyte YR2 against 83.33±4.71% germination of untreated control seeds. After seed coating treatment of wheat seeds, up to

86.67±4.71% seed germination has been seen in seeds primed with endophyte YR2 compared to 66.33±4.71% seed germination by untreated control seeds.

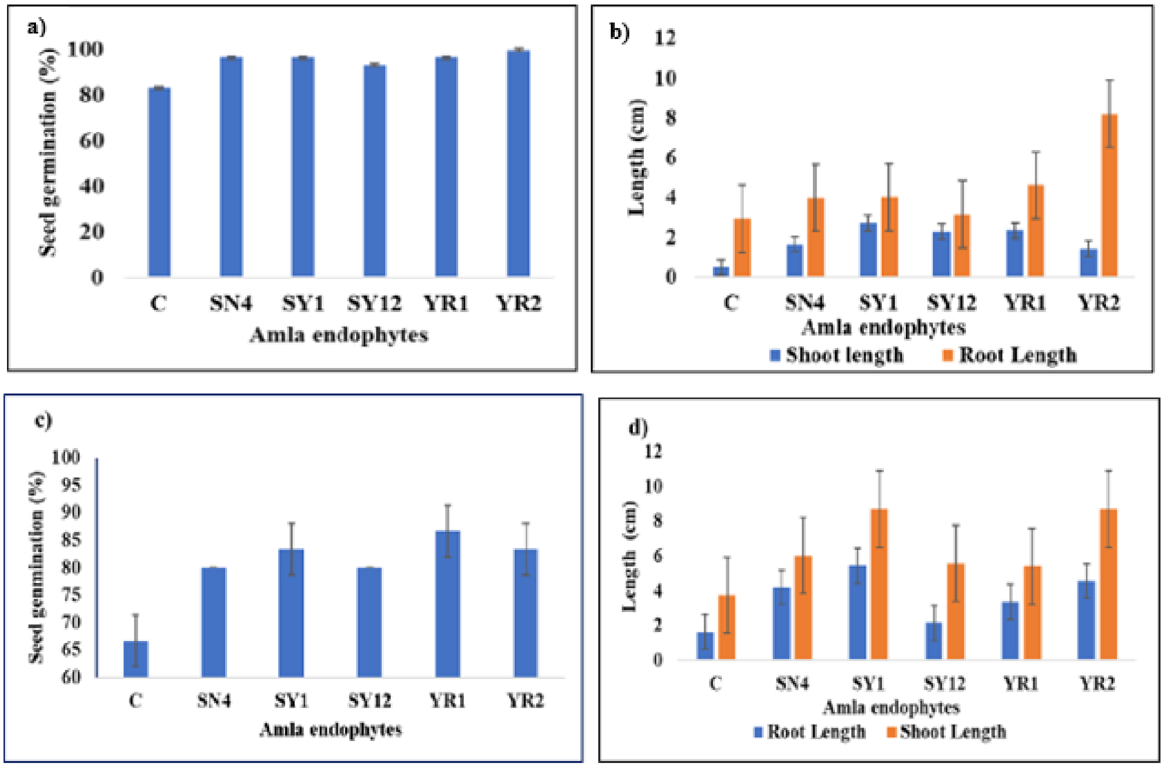

Fig 2. a) Seed germination study of *Cicer arietinum* L. seeds, b) Plant growth parameters study of *Cicer arietinum* L. seeds, c) Seed germination study of *Triticum aestivum* L. seeds, d) Plant growth parameter study of *Triticum aestivum* L.

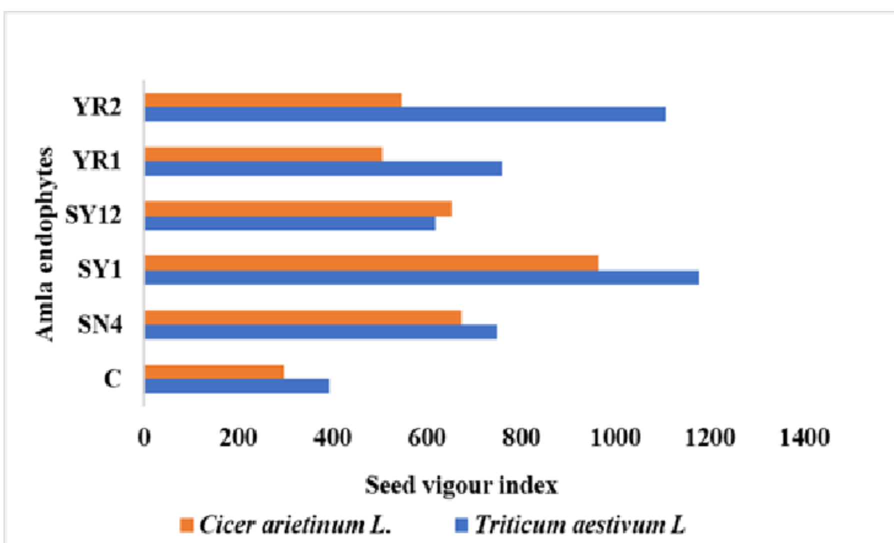

Fig 3. Seed vigour index (SVI) of coated seeds of *Cicer arietinum* L. and *Triticum arietinum* L. with various endophytic bacterial isolates of Amla

3.2.3 Seed vigour index

As shown in Figure 3, in the case of *Cicer arietinum* L., seeds treated with SY1 isolate showed a maximum increase of seed vigour - 666.8% with SVI-964.4 followed by SN4 (376.9%), SY12 (354.4%), YR2 (248.7) and YR1 (209.3%). The seed of *Triticum aestivum* L. also showed a positive impact on treatment with endophytic inoculant. Isolate SY1 has shown a 784.6% maximum increase with 1177.9 SVI followed by YR2 (713.6%), YR1 (366.4%), SN4 (355.5%), and SY12 (224.3%).

4 Discussion

Since the ancient era, agriculture has been the prime source of finance in civilisation, and almost 1.3 billion people depend on agriculture[25]. As India is a farming country, half of the population cultivates cereal, pulses, spices, and vegetables in different geographical areas, and agricultural productivity plays a vital role in the country's socio-economics[26]. Agricultural productivity has been highly dependent on chemical fertilisers in recent days. Chemical fertilisers offer visible increases in crop yield. The equation between chemical fertilisers and soil health is not eco-friendly. Biofertilisers can be a potential option for sustainable agriculture to balance soil health and agricultural productivity[27]. Plants continuously encounter various biotic and abiotic stresses during their life cycle[28]. Microbial priming of seeds is an adaptive technique that directly or indirectly benefits plants.

4.1 Plant growth-promoting traits

Endophytes serve as good PGPR as they improve plant growth by providing multiple advantages to host plants by nutrient availability[29], production of plant hormones[30], protecting plants against plant pathogens[31], and abiotic stress like soil salinity[32], metal toxicity[33], and drought[34]. Based on the best results of PGPR tests, 5 isolates YR1, YR2, SY1, SY12, and SN4 were selected for further work. As shown in Figure 1, four out of five selected endophytes can fix nitrogen biologically, and all five amla endophytes can produce exopolysaccharides and ammonia. Indole 3-acetic acid is a plant growth-promoting hormone. All five isolates have been observed to produce IAA; quantitively, YR1 produces the highest amount of IAA (Table 1). Three of all five amla endophytes can solubilize phosphate, and YR2 can produce HCN. The effect of all these plant-growth-promoting traits tested in vitro can be seen in different parameters of seed development. IAA plays crucial role in the development of leaves, roots, and growth regulation, seed germination and seedling growth and establishment of germination process with gibberellins, ethylene and abscisic acid- mediated pathways[35]. Brescia et.al[36] have reported that endophytes isolated from chickpea has shown highest production of 6.96 μg ml^{-1} IAA, in the present study bacterial endophytes isolated from amla plant YR1 has showed maximum production of 14.50$\pm$0.30 μg ml^{-1} IAA which has shown very good impact on seed germination of Chickpea and wheat seeds.

4.2 Seed germination

Seed germination is an important parameter that increases the yield and growth of plants[37]. Seed germination is a composite process that begins with the germination of embryo radicle across seedcoat followed by degradation and utilisation of substances and energetic substances by enzymes for seed development via hormonal signals of plant hormones in seeds[38]. As shown in Figure 2**(c)**, the highest 30.13% increase has been observed in seeds primed with YR1 (p value – 0.013) in the case of *T. aestivum* L. followed by the lowest increase up to 8.70% on seeds treated with endophytes SY1 (p - value – 0.024). In the case of *C. arietinum* L., an increase in seed germination has been observed up to 20.0% by seeds coated with endophyte YR2 (p – value – 0.007) followed by other treatments up to 11.96% by seeds coated with endophyte SY12 (Figure 2 **a**).

4.3 Seed vigour index and seedling germination

Seed vigour is a multifaceted trait that helps determine multiple factors responsible for good crop plant growth and yield. It helps to determine various aspects of seed, including seed viability, dormancy, seedling establishment, and seed dormancy[39]. Seedling growth is a crucial stage of seed growth for establishing any seed in a plant[40]. It plays a vital role in the development of plants under various abiotic stress conditions[41]. Coating of wheat seeds with bacterial endophyte in the study conducted by Latochcina et al. (2020)[42] has shown up to 20-30% increase on seedling growth of wheat seeds, bacterial endophyte of chickpea *Pseudomonas aeruginosa* BHUJPCS-7 and *Enterobacter clocae* BHUJPCS-21 reported by Mukherjee et al.(2020)[43] in their study has shown maximum increase in shoot length up to 113.59% and 103.18% respectively; while our bacterial endophyte SY1 has shown 234.1% and 444.4% increase in wheat and chickpea seedling growth respectively which directly showing positive influence of seed vigour index. As shown in Figure 3, in case of *T. aestivum* L. significant increase of 784.85% in seed vigour index has been seen in seeds treated with endophyte SY1 with SVI of 1177.9 $\pm$ 66.6 (p value of 0.024) and a tremendous statistically significant increase (p value of 0.00011) has been seen on seeds primed with endophyte SN4 with SVI of 697.77±32.89 against untreated control seeds with SVI of 286.18±16.18 in the case of *C. arietinum* L.

5 Conclusion

The global landscape is experiencing an urgent demand for viable eco-friendly substitutes to chemical fertilizers in the pursuit of sustainable agricultural practices. The present investigation has demonstrated that endophytes represent a significant category of plant growth-promoting rhizobacteria (PGPR) that possess the capability to function as an effective fertilizing agent due to their beneficial influence on seed development. In current study, we have noticed that endophyte isolated from *Phyllanthus emblica* L. is showing markable positive impact on the seeds of *Triticum aestivum* and *Cicer arietinum* seeds in the parameter of seed germination percentage, seed vigour index (SVI), and seed growth parameter against their own endophytes. Bacterial endophyte of Amla-SY1 has shown a potential seed vigour index of 1178 in the seeds of *Triticum aestivum* in comparison to the highest reported 1150 SVI by wheat endophyte *Achromobacter mucicolens*[(44)]. Likewise, Patel et al.[(45)] in 2020 has reported in their study that chickpea seeds treated with chickpea endophytes have shown a maximum 93% seed germination, in our study endophyte YR2-treated chickpea seeds are showing 100% seed germination. This study provides evidence of the capability of bacterial endophytes isolated from Amla to improve seed germination percentage and SVI of seeds of wheat and chickpea leading to an increase in total crop yield and soil fertility. Further investigation of their potential for plant and soil health improvement at a natural agricultural field ecosystem is required.

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Contributory statement:

Ushma B. Joshi has carried out this whole research and drafted this manuscript, Mohammed Faisal Ansari and Devayani R. Tipre has designed this research and thoroughly verified data and this manuscript draft.

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