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Evaluation and SEM analysis of plant endophytic bacteria Isolated from rain tree *Samanea saman* (Jacq) Merr on the growth performance of foxtail millet *Setaria italica*

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Abstract

Due to overpopulation, there is an increasing demand for more food crops and nutrients. Consequently, the world requires a greater production of grains and millets, as millets are a highly nutritious food source. To meet the nutritional needs we are cultivating larger quantities of millet Foxtail Millet *Setaria italic*. Recognizing the harmful effects of chemical fertilizers on human health, there is a pressing need to shift towards bio-fertilizers. This study investigated the plant growth promotion activities of two endophytes (SRP1 and SRP2) isolated from Pods of Rain tree (*Samanea saman* (Jacq) Merr). Comprehensive analysis revealed that SRP1 and SRP2 positively influenced various parameters associated with enhanced plant growth. SRP1 and SRP2 exhibited significant potential as a plant growth-promoting Endophytes (PGPE) enhancement in Foxtail millet plants, efficacy testing under field conditions, they are essential to elucidate the role of PGPE as biofertilizers that confer beneficial effects on host plant growth and development.

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Introduction

Endophytes pose a crucial challenge in addressing India's food production security by maximizing the utilization of land resources. Traditionally, these organisms have been perceived as having a positive influence on promoting crop growth and overall productivity. A sustainable strategy for enhancing both crop production and growth involves harnessing the potential of plant growth-promoting endophytes (PGPE). The primary routes for the colonization of plants by endophytic bacteria are elucidated in the main text. Notably, certain soil bacteria can infiltrate plants through various root zones, as detailed later. The distinction between rhizospheric bacteria, depicted in red and yellow, lies in their inability to colonize inner plant tissues (Malfanova *et al.,* 2013). Virtually all plants are inhabited by diverse bacteria known as endophytes. Endophytic bacteria are referred to as those which can be detected at a particular moment within the tissues of apparently healthy plant hosts (Hallmann *et al.,* 1997) and (Schulz and Boyle, 2006). Some are capable of colonizing the reproductive organs of plants, such as flowers, fruits, and seeds. Since the first reliable reports about the isolation of endophytic bacteria from surface- sterilized plants (Samish *et al.,* 1960) and (Mundt and Hinkle, 1976) Over 200 bacterial genera spanning 16 phyla have been documented as endophytes. Once established within a plant, these endophytes have the capacity to enhance both plant growth and its resilience to various stresses. For comprehensive insights into their advantageous effects, readers are directed to detailed overviews (Ryan *et al.,* 2008). Numerous studies suggest that rice, with its high glycemic index, may contribute significantly to elevated diabetes and weight gain levels. In contrast, millets, a group of gluten-free cereals, have experienced a resurgence in recent years, driven by an increased awareness of embracing traditional foods. In certain regions like Rayalaseema in Andhra Pradesh and Karnataka, millets often take precedence over rice, being widely consumed for their diverse health benefits. Both rice and millets, including Foxtail, offer unique benefits, and incorporating a variety of grains into your diet can contribute to a well-rounded and nutritious eating plan. In this present study, we have attempted to use the endophytes isolated from the Pods of *Samanea saman* (Jacq) Merr for the enhancement of growth parameters of Foxtail millet as a novel approach. This work primarily aimed to study the influence of some PGPE on promoting crop growth under greenhouse condition and the effects of PGPE on foxtail millet under field condition.

Materials and methods

Sample collection and isolation of endophytes

Pod sample (*Samanea saman* (Jacq) Merr) was collected from Sadakathullah Appa College, (8.72° N 77.76° E), Tirunelveli, Tamil Nadu, India. Further experiments, Pods were washed with tap water; Subsequently, the pods were immersed in 70% ethanol in 3 min, washed with fresh sodium hypochlorite solution (2.5% available Cl^-) for 5 min, finally washed five times with sterile distilled water. Pod sample that were not contaminated as detected by culture-dependent sterility test were used for further analysis. Sample (pod) were macerated with a sterile mortar and pestle, then plated on Nutrient Agar and Actinomycete Isolation Agar media (AIA,). The plates were examined for bacterial growth after incubation at 28°C for 3 - 5 days.

In vivo screening of endophytes for their plant Growth promotion activities surface sterilization of seed

Foxtail millet seeds used in this study were procured. Seeds were surface sterilized by soaking in 70% (v/v) ethanol for 2 min followed by 0.2% (v/v) sodium hypochlorite and rinsed five times in sterile, distilled water, a modification of the method used by Ryu *et al.* (2003). The seeds were dried overnight under sterile condition.

Seed bacterization

Seed bacterization was done according to Dileep Kumar *et al.* (2001). Endophytic isolates namely SRP1 and SRP2 were grown on nutrient agar for 24 hours at 28 ± 2° C, were scrapped from the plates, and finally suspended in sterile 1%

Carboxymethylcellulose (CMC) to a concentration approximately 10^7 CFU/ml. Five grams of surface sterilized Foxtail millet seeds were steeped into each endophytic bacterial suspension in CMC for 30 min and dried overnight in sterile petri plates. Seeds treated with 1% CMC served as the control.

Green house study

The bacterial isolates, alone were assessed for their efficiency in plant growth promotion under greenhouse conditions. Bacterized seeds were transferred to plastic pots (20 seeds per pot) containing non-sterilized soil and cow dung in the ratio of 4:1 and kept in a greenhouse. A control pot without bacterized seeds was also maintained. The pots were maintained at a temperature of 26 ± 2°C, RH of 90% and a photoperiod of 16 h for 60 days. Every day the plants were irrigated with tap water. Shoot and root lengths, fresh and dry weights were determined in 7th, 14th days intervals. All experiments were carried in 3 replicates.

SEM images of distribution of endophytes in foxtail millet root cells

To prepare scanning electron microscopy (SEM) samples, the 28d cultivated Foxtail millet plant roots were washed and cut into 1 cm long pieces, and examined under the scanning electron microscope (Zeiss Evo 40 EP). The gold coated metal stub was viewed on the SEM at an accelerating voltage of 20 KV, a probe diameter of 102 pA, to obtain secondary electron images. The field was scanned at low magnification until the line of growth was detected. Areas with clear, intact root structures were then selected for the examination at higher magnification. Suitable fields in the preparation were photographed.

Results and discussion

Sample collection and isolation of endophytes

Pod sample (*Samanea saman* (Jacq) Merr) was collected from Sadakathullah Appa College, (8.72° N 77.76° E), Tirunelveli, Tamil Nadu, India. Two endophytic bacteria namely SRP1 and SRP2 were isolated from pods of Rain tree.

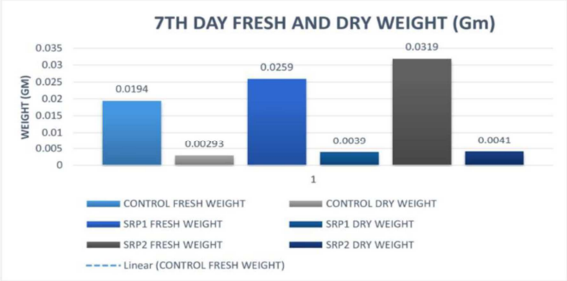

Fig. 1. 7th day fresh and dry weights of foxtail millet

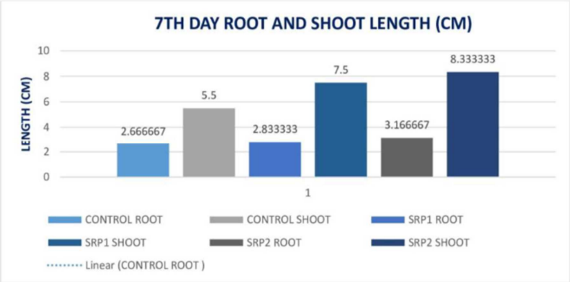

Fig. 2. 7th day root and shoot length of foxtail millet

Fig. 3. 14th day fresh and dry weight of foxtail millet

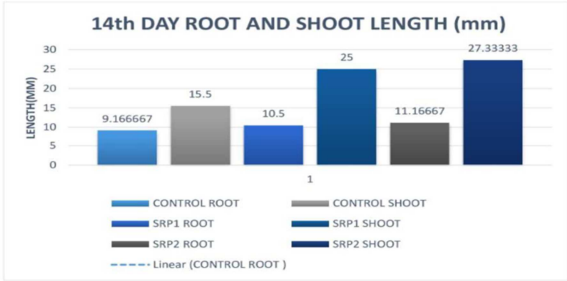

Fig. 4. 14th day root and shoot length of foxtail millet

Plant growth promotion studies in vivo

In vivo plant growth promotion studies of these endophytes showed a great impact on the shoot length and fresh weight of Foxtail millet samples. 7th days highest shoot length was observed in the organism SRP2 which showed 8.3 cm and lowest was for SRP1 7.5 cm (Fig. 2). 14th days highest shoot length was observed in the organism SRP2 which showed 27 cm and lowest was for SRP1 25 cm (Fig. 4).

The effect of SRP2 showed a significant difference in shoot length when compared to control after 7, 14 days. In the case of root length, 7th days highest root length was exhibited by the organism SRP2 which showed 3.16 cm and lowest one was SRP1 which showed 2.8 cm. 14th days highest root length was exhibited by the organism SRP2 which showed 11 cm and lowest one was SRP1 which showed 10 cm.

7th days highest fresh weight was observed for the organism SRP2 had 0.031 gm weight and SRP1 had an average weight of 0.025 gm (Fig. 1). 14th days highest fresh weight was observed for the organism SRP2 had 0.223 gm weight and SRP1 had an average weight of 0.161 gm (Fig. 3). In the case of dry weight also SRP2 showed a 7th days highest weight of 0.112 gm and SRP1 showed a lowest of 0.0.082 gm weight.

Scanning electron microscopic (SEM) analysis

The thin sections of Foxtail millet root samples were observed under SEM for the colonization of endophytes in the inner cortex. The presence of endophytes colonized inside root cortex was observed in scanning electron microscope (Fig. 5).

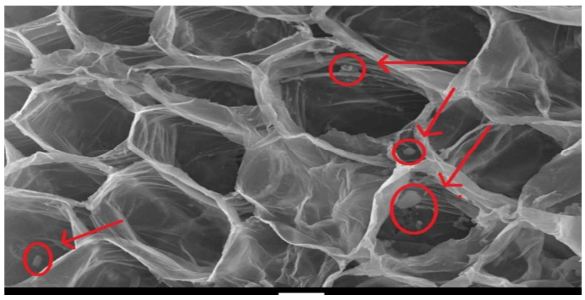

Fig. 5. SEM image of distribution of endophyte SRP2 in Foxtail millet root cells

In the present study, we isolated 2 (SRP1 and SRP2) endophytic bacteria from Rain tree pods. Isolation of endophytes from the plants was related to the work (Sang Hye Jia *et al.,* 2014) in which they have been isolated 576 endophytic bacteria from the leaves, stems, and roots of 10 Korean rice cultivars. Through 16S rDNA sequence analysis, nif H genes were confirmed in the two species of *Paenibacillus*, three species of *Microbacterium*, three *Bacillus* species, and four species of *Klebsiella*. Shankar *et al.* (2009) isolated seventy one endophytic bacteria which were screened using PDA and TSA medium. The majority of strains isolated from root tissues were totally 26 isolates, exclusively collected from 3 years organic rice farm. Phenotypic characteristics of all isolates illustrated that 34 isolates were identified as *Pseudomonas* sp., while other isolates were also identified as *Bacillus*, *Azotobacter*, and *Enterobacter* species (Phetcharat and Duangpaeng 2012). Endophytic populations were isolated from 2400 segments of *Oryza sativa* collected from Bhadra River Project Area, Southern India during December (winter) and (summer). *Streptomyces* sp., *Chaetomium globosum, Penicillium chrysogenum, Fusarium oxysporum* and *Cladosporium cladosporioides* were dominant endophytes in this study. Some other important endophytes were isolated from the plants and they have been reported as *Sphingomonas paucimobilisa* and *Azorhizobium caulinodans* (Engelhard *et al.,* 2000), *Bradyrhizobium japonicum* (Chantreuil *et al.,* 2000), *Rhizobium leguminosarum* (Yanni *et al.,* 1997), *Pantoea* sp., (Kuklinsky-Sobral *et al.,* 2004) and (Verma *et al.,* 2004), *Serratia* sp. (Sandhiya *et al.,* 2005), *Serratia marcescens* (Gyaneshwar *et al.,* 2001), *Chromobacterium violaceum* and *Sphingobacterium* sp. (Phillips *et al.,* 2000), *Streptomyces, Nocardioides* (Tian *et al.,* 2007).

Among the 2 endophytic bacterial isolates, in a review on the mechanisms of biocontrol of plant growth-promoting rhizobacteria includes rhizospheric and endophytic bacteria (Compant *et al.,* 2005). The presence of endophytic bacteria in the inner cortex of root was observed through SEM analysis. Euan *et al.* (2002) observed that bacteria (*Herbaspirillum seropedicae*) entered the roots via cracks at the points of lateral root emergence, in rice seedlings. These bacteria subsequently colonized the root intercellular spaces, aerenchyma, and cortical cells, with a few penetrating the stele to enter the vascular tissue. In this study, the plant growth promotion activity of the endophytes was observed in Foxtail millet seeds after bacterization. The increased growth and biomass in case of seedlings raised from seeds treated with SRP2

may be attributed. In the present study, SRP2 significantly increased the plant height, fresh weight and dry weight of Foxtail millet seedlings.

Conclusion

In summary, two endophytes (SRP1 and SRP2) isolated from Pods of Rain tree (*Samanea saman* (Jacq) Merr) showed significant plant growth promotion activities in Foxtail millet seeds. Among the isolates, SRP2 was found to be potential in PGPR as well as biocontrol properties. SRP2 exhibited all parameters that could be attributed to the enhancement of plant growth. The mechanisms presented by these endophytes can promote plant growth, increase plants ability to resist pathogen attack, or even directly inhibit the pathogen growth. The result of this study provides a strong basis for the procurement of the strain SRP2 as a bioinoculant to attain the desired plant growth attributes in agriculture. Further studies including the enhancement of yield in Foxtail millet plants, efficacy test under field conditions and future selection of the potent isolate as PGPR for broad host ranges for crop colonisation are needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on host plant growth and development.

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