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Effect of Phosphate Solubilizing Bacteria on Growth and Development of Pearl Millet and Ragi

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Authors' contributions

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

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ABSTRACT

In the present investigation, the effect of *Bacillus* sp. (C2) and *Pseudomonas* sp. (C7) isolated from *Calotropis gigantea* and *Cyanodon dactylon* from Shenbagathoppu hills, Srivilliputhur, Tamil Nadu, India on growth and development of pearl millet and ragi were studied. The effect of these isolates was recorded for morphological characters like plant height, fresh and dry weight. In the present study, maximum fresh weight and dry weight of pearl millet and ragi were recorded when treated with isolate C2 compared to C7 on all schedules of 30 and 45 days. Plant height recorded maximum in pearl millet on 45 days when treated with isolate C7 while ragi recorded maximum growth when treated with C2 isolate. Physiological characters like total chlorophyll, IAA, starch, fresh and dry weight were recorded on 45 day after sowing both in pearl millet and ragi treated with C2 and C7 isolates. It was found that, the isolate C2 treated plants recorded maximum total chlorophyll, IAA, starch, fresh and dry weight compared to C7 isolate treated plants both in pearl millet and ragi. Among the parameters tested, ragi plants recorded better total chlorophyll, IAA, starch, fresh and dry weight compared to pearl millet plants.

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Keywords: Phosphate solubilizing bacteria; Bacillus sp. (C2); Pseudomonas sp. (C7); ragi and pearl millet.

1. INTRODUCTION

Phosphorus (P) is an essential nutrient for biological growth. Absence of this element in the soil could limit the plant growth and development. A greater part of soil phosphorus is in the form of insoluble phosphates and cannot be utilized by the plants. To increase the availability of phosphorus for plants, large amount of fertilizers are being applied to soil. But a large proportion of phosphate fertilizer applied is quickly transformed to the insoluble forms which decrease the efficiency of fertilizers. So, there is a need for microbes which have the capacity to solubilize phosphorus.

Phosphorus plays a very important role in photosynthesis, respiration, energy storage, cell division and other metabolic activities in plantlets. Additionally, it improves the quality of vegetables, fruits and grains and also it is very important for seed germination and root formation for plant growth and development [1]. Deleterious environmental impact upon increasing the use of chemical fertilizer, pesticides and supplements leads to affect the plant growth promoting bacteria (PGPB). PGPB are defined as free living soil, rhizosphere, rhizoplane, endophytic and phylosphere bacteria that are beneficial for plants under certain conditions [2]. PGPB generally provide natural and harmless means of improving the growth, yield of crops and minimize the use of agrichemicals.

Phosphate solubilizers are economical, ecofriendly and have greater agronomic utility to compensate the expensive inorganic sources of P fertilizers. Phosphate solubilizing microorganisms (PSM) have attracted attention in semi arid regions and endowed to enhance the crop yields [3,4]. Phosphate solubilizing microorganisms have established their role under nutrient inequity conditions [5,6]. Phosphate solubilizing bacteria (PSB) are the group of PGPR in rhizosphere. Secretion of organic acids and phosphatases to solubilize insoluble phosphate to soluble forms are common in this group [7]. Although several phosphate solubilizing bacteria occur in soil, their numbers are not adequate to compete with other bacteria commonly established in the rhizosphere [8].

Phytohormones play an important role as signals and regulators of plant growth and development in plants. The capacity to produce phytohormone is often considered as trait of the plant kingdom. However, production of phytohormone is also widespread among soil and plant associated prokaryotes [9]. The production of phytohormonal substances such as auxins and gibberellins by different PGPB has been proposed as one of the mechanisms, besides N_2- fixation, to explain plant growth promotion. IAA (Indole-3-acetic acid) is naturally occurring auxin with broad physiological effects [10]. The objective of the present study was an attempt to investigate the effect of phosphate solubilizing bacteria on growth and development of ragi and pearl millet.

2. MATERIALS AND METHODS

2.1 Isolation and Screening of Phosphate Solubilizing Bacteria from Rhizosphere Soil Sample

The rhizosphere soil samples were collected and transferred under sterile conditions to laboratory. 0.1 ml/mg of soil sample was spread on modified Pikovskaya agar plates for phosphate solubilization assay as described [11]. Bacterial colonies forming halo zones around the bacterial growth were considered to be phosphate solubilizers. Then it was identified by standard procedures described in Bergy's manual of determinative bacteriology. The identified organism was utilized for further experimental analysis.

2.2 Pot Experiment

2.2.1 Preparation of sand soil mixture

River sand, garden soil and red soil were sieved through mesh sieve separately to remove the coarse particles. They were mixed in the preparation of 2: 1: 1 (River sand, Garden soil and Red soil) to give a favorable medium for the growth of the root system.

2.2.2 Sterilization

The sand soil mixture was moistened with water and sterilized in the autoclave. The sterilization was carried out at 121℃ for 2 hours to destroy the various bacterial and other pathogenic organisms and their spores. After this process, the sand soil mixture was aerated overnight and transformed into a container to prevent dust contamination from air. The selected seeds of plant species (*Pennisetum glaucum* [L.] R. Br.

and *Eleusine indica* [L.]) were treated with candidate bacterial isolate. The uninoculated plantlet was considered as control.

2.3 Effect of PSB on *Pennisetum glaucum* and *Eleusine indica* Growth and Development

Sporadically after 30 and 45 days the plants were removed and washed with tap water carefully and then with distilled water without causing any damage to the plants for further experimental analysis.

2.3.1 Shoot length (cm)

Periodically after 30 days and 45 days the treated and control plants were uprooted without causing any damage to the seedlings and were thoroughly washed with tap water in order to remove soil and debris particle. Then the shoot length was measured with the help of a meter scale. (10 cm,18 cm,15 cm, 24 cm) in 30 and 45 days.

2.3.2 Root length (cm)

Periodically after 30 days and 45 days both the treated and control plants were uprooted without causing any damages to the seedlings and was thoroughly washed well with tap water in order to remove soil and debris particle. Then the root length was measured with the help of a meter scale. (7 cm, 9 cm, 11 cm, 16 cm) in 30 and 45 days.

2.3.3 Fresh weight of plants (g)

The plants were removed gently from the soil without disturbing the root system and then the roots were washed with tap water to remove the soil particles. The plants were weighed separately using an electrical balance.

2.3.4 Dry weight of plant (g)

Fresh plants from each treatment and control were cut into pieces and dried in an oven at 80℃ for 24 hours. The dried sample was weighed and the yield was recorded.

2.4 Biochemical Changes in Crop Plants

2.4.1 Estimation of chlorophyll

100 mg leaf sample was homogenized with 10 ml of 80% pre-chilled acetone. The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected. This procedure was repeated until the residue was colorless. The volume of supernatant was made up to 100 ml with 80% acetone and the absorbance was read at 645 nm and 663 nm. 80% acetone was served as blank. Total chlorophyll content was calculated by using the formula, Total chlorophyll = 20.2 (A_{645}) + 8.02 (A_{663}) x V/1000x W [12]. A - OD at specific nm, V - Final volume of plant extract in 80 % acetone, W - Fresh weight of leaf tissue used.

2.4.2 Estimation of IAA (Indole-3 Acetic Acid)

The plant leaves were crushed by using distilled water and centrifuged at 5000 rpm for 5 minutes. Then, 2 to 3 drops of O-phosphoric acid was added in to 2 ml of the supernatant. To the above mixture, 4 ml of $FeCl_3$ – $HCl.O_4$ reagent was added and incubated at room temperature for 25 minutes. Absorbance was read at 530 nm [13].

2.4.3 Estimation of soluble sugar

Estimation of soluble sugar was performed by [14].

3. RESULTS AND DISCUSSION

Currently, phosphate solubilising bacteria (PSB) have been attracted the attention of agriculture scientists to reduce the application of chemical fertilizers. When PSB is used with rock phosphate, it can save about 50% of the crop requirement of phosphatic fertilizer. Given the negative environmental impacts of carcinogenic chemical fertilizers and their increasing costs, the use of PSB is advantageous in the sustainable agricultural practices. Phosphate solubilizing bacteria especially are slowly emerging as an important organisms used to improve soil health. PSB can be used as biofertilizers, which is ecofriendly, pollution free and is a possible way to increase the efficiency of P fertilizers. This study was therefore undertaken to investigate the effect of PSB on growth and development of millet crops.

In the present investigation, eight isolates of PSB were isolated from the rhizosphere soil of local weed plants from Senbaga thoppu hills in Srivilliputtur, Tamilnadu, India. Isolated organisms were further screened for phosphate solubilization by measuring the zone formation around the bacterial growth in pikovskaya agar. Among the four phosphate solubilizing bacterial strains, two bacterial strains were finally selected for further experimental analysis due to high amount of phosphate solubilizing capacity.

Based on the morphological, physiological and biochemical characteristics, the suspected bacterial strains were identified as *Pseudomonas* sp. (C7) and *Bacillus* sp. (C2) by the following standard keys of Bergey's Manual of Determinative Bacteriology (Table 1). The present study is matching to the earlier study and screened the PSB from the Asian elephant (*Elephas maximusindicus*) dung sample [1].

Table 1. Biochemical tests of phosphatase degrading strain *Pseudomonas aeruginosa* and *Bacillus sp.*

Tests	*Pseudomonas sp.*	*Bacillus sp.*
Indole	Negative	Positive
Methyl red	Negative	Negative
Voges – proskauer	Negative	Positive
Citrate	Positive	Positive
Catalase	Positive	Positive
Oxidase	Positive	Negative
Urease	Positive	Negative

Effect of PSB on growth and development of ragi and pearl millet was studied after 30 and 45 days. The plant growth parameters were analyzed from the two crop plants by the inoculation of potential strain isolated from weed plants. The estimation of fresh weight, dry weight, starch, chlorophyll and IAA were done in the PSB inoculated and control plantlets. The inoculation of PSB in ragi and pearl millet proved the potential to increase plant height, numbers of tillers, root length and shoot length [Figs. 1, 2, 3 and 4]. This report is similar to the earlier report of Sankaralingam et al. [15], who stated that the inoculation of PSB on *Sesbania grandiflora* and *Moringa oleifera* showed excellent growth, plant height, numbers of tillers, root length and shoot length. Afsal and Bano [16] have also stated that 30-40% more efficiency of PSB strains as biofertilizer to increase the growth and grain yield of wheat (*Triticum aestivum*) and dual inoculation of microorganisms without P fertilizer improved 20% more grain yield.

In the present investigation, there was a significant improvement of dry biomass and fresh biomass recorded in PSB inoculated *Pennisetum glaucum* and *Eleusine indica* after 30 and 45 days [Plate 1]. The present report is in the line of previous report of Mohan and Ayswarya [17], who have reported that the positive effect on growth parameters of *Tectona grandis* that was dual inoculated with *Bacillus subtitlis* and *Pseudomonas fluorescens* under pot experiment. Hoberg et al. [18] also endorsed our results that the PSB solubilize inorganic P due to the secretion of organic acids for the growth and development of required plants.

The maximum amount of chlorophyll contents on leaf samples were obtained from the PSB inoculated plantlets while compared with uninoculated plants [Fig. 5]. This report is similar to the earlier report of Shankar et al. [1], who reported the significant improvement of chlorophyll and carotenoid contents in maize and chilli plants due to the inoculation of *Pseudomonas aeruginosa.* The dual inoculation of *Pseudomonas putida* along with mycorrhiza which showed an increase in leaf chlorophyll content in barley is in support of our finding. These effects may also influence other physiological functions and agronomical parameters in the plant [19].

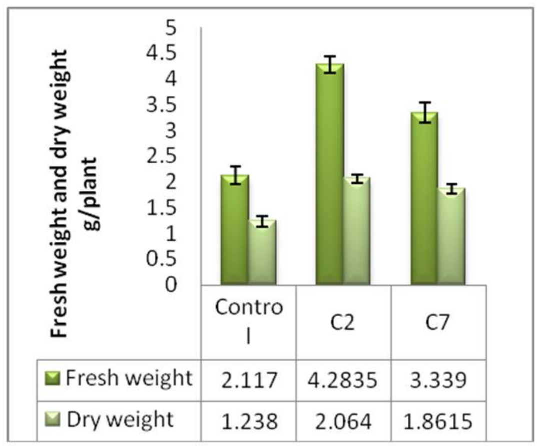

Fig. 1. Effect of PSB on fresh weight and dry weight of pearl millet after 30 days

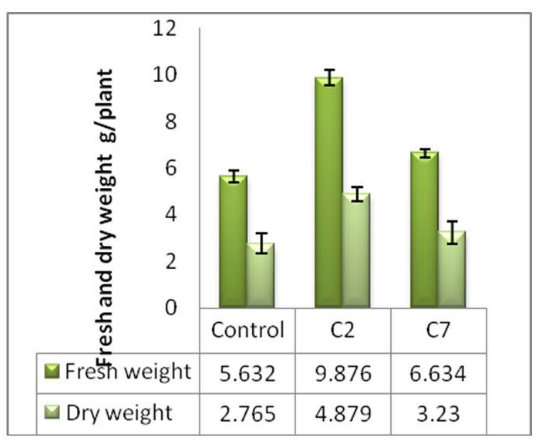

Fig. 2. Effect of PSB on fresh weight and dry weight of ragi after 30 days

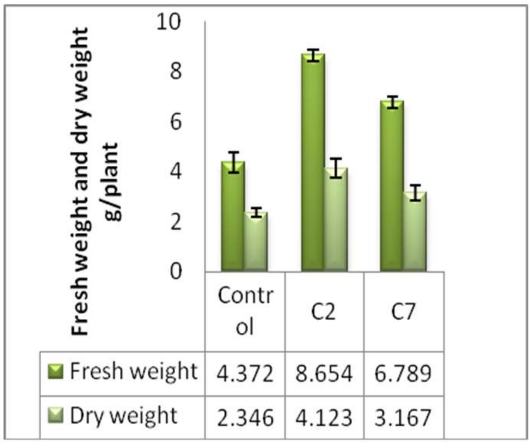

Fig. 3. Effect of PSB on fresh weight and dry weight of pearl millet after 45 days

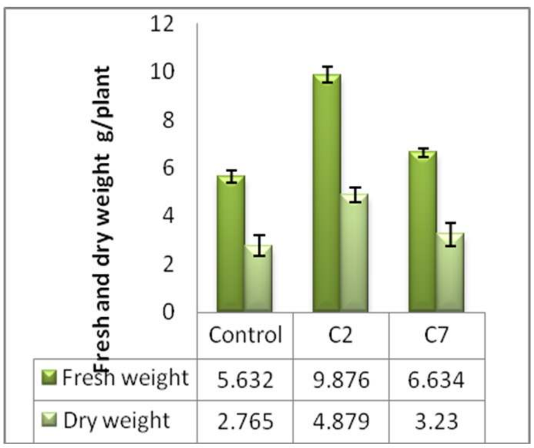

Fig. 4. Effect of PSB on fresh weight and dry weight of ragi after 45 days

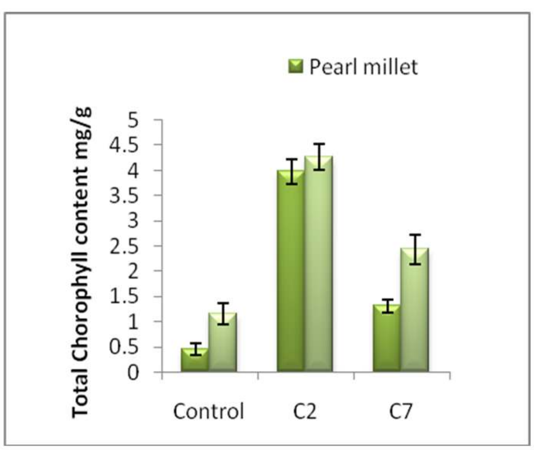

Fig. 5. Effect of PSB on total chlorophyll content in pearl millet and ragi after 45 days

Auxins are growth promoting hormones present in plants. It is a well-known fact that plant tissues show an increased capacity to accumulate bound auxins following auxin application [20]. The present investigation was undertaken to verify the effect of free IAA during rooting of *Pennisetum glaucum* and *Eleusine indica*. At the same time, higher auxin content was registered in PSB inoculated plants when compared with uninoculated plants [Fig. 6]. PSB inoculated plantlets produced high amount of secondary metabolites such as phytohormones, antibiotics and siderophores and aided in plant growth and development [21]. However, PSB can facilitate growth and development of plants by producing essential nutrients or by varying the concentration of plant growth promoting substances including phytohormones, such as Indole acetic acid [22].

Starch is the most abundant storage reserve carbohydrate produced in plants and the most important dietary source of energy for humans, representing up to 80% of the daily caloric intake. Starch is a cheap, natural renewable raw material whose varied structures are exploited in the agri-food sector and as a source of energy after conversion to ethanol. In the present study, the maximum amount of sugar and soluble starch was noticed in PSB inoculated plantlets after 30 and 45 days [Fig. 7]. The primary role of sugars depends not only on direct involvement in the synthesis of biochemical compounds and production of energy, but also on the stabilization of membranes. Content of soluble sugar in leaves is an important form and a sign of supply ability of leaves or a physiological index of leaf status [23,24].

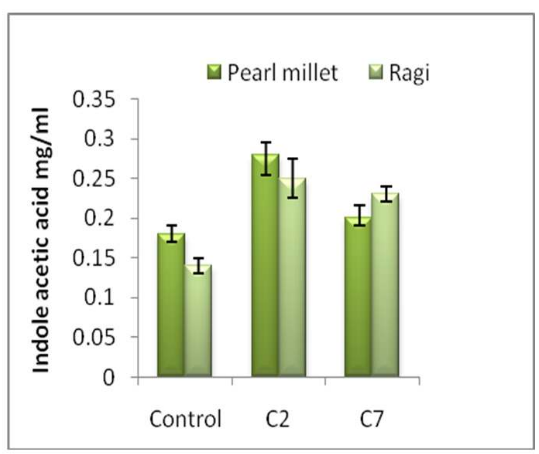

Fig. 6. Effect of PSB on Indole acetic acid in pearl millet and ragi after 45 days

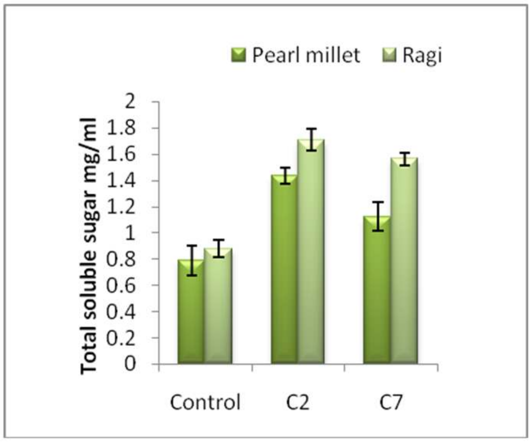

Fig. 7. Effect of PSB on starch content in pearl millet and ragi after 45 days

A B

Plate 1. Effect of PSB (C2, C7) on pearl millet (A) and (B) ragi growth and development

4. CONCLUSION

Soil fertility management by bio- fertilizers are one of the basic components of sustainable agriculture. The obtained data showed that the application of bacterial inoculums caused tremendous increase on biochemical parameters in pearl millet and ragi. The nutrients present in the soil have natural abilities of plant uptake potential, enhancing and improving the soil biodiversity, developing the bio- activities, increasing the environmental hygiene, conservation and supporting the natural and non-renewable resources are among the most important reasons to increase the utilization of biological fertilizers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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