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Seed Germination Enhancement through Plant Growth Promoting Rhizobacteria (PGPR) isolated from Papaya Rhizosphere

Jyoti Bajeli[1], P.N. Rai[1], Ranjan Srivastava[1], Ajay Veer Singh[2], Aakansha Verma[3] and Akhilesh Negi[3]*
[1]*Department of Horticulture,*
G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India.
[2]*Department of Microbiology,*
G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India.
[3]*Department of Biological Sciences,*
G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India.

(Corresponding author: Jyoti Bajeli)*
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ABSTRACT: The soil around plant roots is known as the rhizosphere, and it is directly impacted by soil microbes. Plant root exudation plays a crucial role in both soil fertility and plant health. Papaya rhizosphere is a very rich zone supplemented with a variety of beneficial bacteria and the soil samples were collected from this rhizosphere for the isolation of the bacterial isolates. The serial dilution of soil samples was carried out in laminar air flow to maintain aseptic conditions. The colony forming unit (CFU) count of samples ranged from 1.17 × 10^3 to 1.75 × 10^3. Isolation and purification of morphologically different bacteria were done in 60mm petri-plates. The functional activity test was carried out for purified bacteria for plant growth promoting activity such as phosphorus solubilization, potassium solubilization, siderophore production, and Indole acetic acid (IAA) production. Out of twenty bacterial isolates, 11potassium solubilizing, 6phosphorus solubilizing and 2 Siderophore producing bacterial isolates were obtained. All the isolated were capable of IAA production. Moreover, there was a positive correlation between IAA production, siderophore production, phosphorus and potassium solubilization efficiency of the bacterial isolates. Among the 11 bacterial isolates used in the treatment, PR2d isolate was found to take the minimum days taken for first seed germination (11 days), minimum days taken for 50% seed germination (21 days), per cent of seed germination (97.67 %), minimum mortality rate (4 %) and maximum IAA production (13.32 µg/mL) that promoted the growth and seed parameters of papaya plant, showing a potential use in horticulture production systems.

Keywords: Papaya, seed germination, rhizosphere, PGPR.

INTRODUCTION

The rhizosphere is the site of organic deposition, generator of habitat, and resource heterogeneity for soil organisms. The capacity of plants to alter or modify the rhizosphere as they grow is a crucial characteristic. A plant's rhizosphere can be altered by ion absorption, the formation of root exudates, rhizo-deposition (the loss of carbon from roots), and variations in the rhizosphere's pH. Rhizobacteria are a class of bacteria living in the rhizosphere, and certain microorganisms are capable of augmenting the plant growth and development. Through their capacity to solubilize phosphate, fix nitrogen, and create growth hormones, rhizobacteria serve as a catalyst for plant development (Sutariati *et al.,* 2014). Plant growth-promoting rhizobacteria (PGPR) can have a direct effect on a plant's metabolism by controlling the production or breakdown of phytohormones or by boosting plant growth. They cancolonize the surface of the root, endure, and proliferate in microhabitats close to the surface in competition with local microbiota, at least to express their plant-promotion activities. Several mechanisms for bacterial activity have been put up, including the generation of phytohormones, mineral solubilization, and availability, along with biological control of soil-borne diseases (María *et al.*, 2006). Papaya (Carica papaya Linn.) is a tropical plant well-known for its culinary and nutritional benefits. The medicinal advantages of fruit and other ingredients were well known to the ancient systems of medicine. The chemical groups to which the contents of the papaya plant belong include alkaloids, flavonoids, terpenoids, saponins, steroids, tannins, vitamins, quinones, minerals, and more. These families of chemicals have been shown via experimental research to be effective treatments for inflammatory, cytotoxic, microbial infections, diabetes, and liver problems (Kaur *et al.*, 2019). Moreover, the plant is a quick growing herbaceous perennial 2-10 m in height and begins to bloom in the third or fourth month. Fruits develop in seven to nine months under warmer climatic conditions, and in nine to eleven months in cooler climates. For this reason, papaya have a rhizosphere that is full of microorganisms that encourage plant development. The auxin hormone, sometimes referred to as IAA (Indole Acetic Acid) hormone, is a significant member of the auxin group that regulates key physiological processes, such as cell growth and division, tissue differentiation, and reaction to light and gravity (Larekeng *et al*., 2020). Exogenous IAA, or auxin hormone produced by species other than plants, is distinct from endogenous IAA, or auxin hormone produced by plants. A modest dose of exogenous IAA is required during multiplication to promote budding and root hair production, but auxin also helps to increase cell flexibility. The presence of IAA-producing rhizosphere bacteria promotes the formation of root hair (Liu *et al*., 2019).

Seed coating and seed soaking are two of the most popular bioinoculation methods used to promote plant development, while foliar sprays can be used to prevent disease. Phosphate-solubilizing bacteria (PSB) hydrolyze organic and inorganic insoluble P molecules into soluble P forms that plants may readily use (Singh *et al*., 2014). Many microbes and certain plant species may produce siderophores, which are low molecular-weight peptides or iron chelators (Santos-Villalobos *et al*., 2012). Microorganisms that might produce siderophores are beneficial to plants because they improve the iron availability to the plant (Ahmed and Holmström, (2014). Moreover, they could also be linked with an increase in the production of pathogen-protecting antifungal compounds (Laslo *et al*., 2012).

Bioinoculants are carrier-based preparations containing live micro-organisms in a viable form. The success of establishment and crop performance depend on uniform germination and strong seedling vigour, which are crucial components of current cultivation systems. Beneficial microorganisms can be added to seeds prior to planting to improve germination. Early interactions formed by their presence with plants result in bio stimulant effects such enhanced plant growth, greater nutrient absorption, and improved plant resistance to abiotic stress. The use of microbial inoculation in seed germination has attracted research in this area. In the present study, the effect of bacterial isolates from papaya rhizosphere were investigated on papaya seedlings and their combined effect on plant growth promotion under protected condition.

MATERIALS AND METHODS

Soil sampling. A total of sixsoil samples were collected for the isolation of bacteria from the rhizosphere of papaya from different locations of Pantnagar, Udham Singh Nagar, Uttarakhand, India. The purpose of the sampling was to isolate and screen the efficient bacteria which are capable of solubilizing phosphate and IAA production which could further be utilized to check their efficiency to improve the growth and quality of papaya.

Isolation and purification of bacteria. For the isolation of bacteria, 1 g of soil sample was weighed and mixed with10 ml distilled water under aseptic conditions. From this solution, further serial dilutions were done by taking 1 ml of aliquot from 10-4, 10-5, 10-6 dilutions and plated on petri plates in nutrient agar media. The growth was observed after 24 hours at 28°C. The bacteria were isolated from the plates based on their visual appearances such as colony colour, size, and growth pattern. Further, these bacteria were streaked on different functional media such as Chromazuraol S (CAS) medium, and Pikovskya agar incubated at 28°C for 2-3 days to check their potential of siderophore and P solubilization, respectively. Plates were examined periodically for the appearance of bacterial colonies in the respective medium. The colonies showing positive functional activity were picked up and streaked in fresh agar plates for further purification.

Characterization of PGPB for plant growth promoting (PGP) traits

Indole acetic acid (IAA) production. The Gordon and Weber technique was used to determine the amount of IAA produced by bacteria (1951). Individually infected tubes containing 10 ml of succinate broth with 100 g/ml tryptophan were incubated at 28°C for 48 hours while being shaken at a rate of 120 rpm. The cultures were centrifuged at room temperature for 10 minutes at a speed of 7,500 rpm after incubation. After that, 1 ml of culture supernatant was combined with 2 ml of Salkowski reagent, and the combination was incubated at 30°C for 25 min. The test microorganisms produced IAA, as evidenced by the development of pink colour. Using a UV/VIS spectrophotometer, absorbance was measured at 530 nm (Ray-Leigh UV 2601).

Phosphate solubilization on solid medium. The Pikovskaya assay of phosphate solubilization was applied to the bacterial isolates (1948). The isolate was spot-inoculated on Pikovskaya's medium and incubated at 28°C for 4-7 days, which contains insoluble tri-calcium phosphate (TCP). The emergence of a halo zone surrounding the colony revealed the ability of the test organisms to solubilize phosphate.

Siderophore production. Separately placed on ChromazurolS (CAS) medium, the bacterial isolates were cultured for 3-4 days at 28°C. The presence of an orange to yellow halo around the bacterial colonies proved that siderophores were being produced (Schwyn and Neilands 1987).

Potassium solubilization. The study of microbes on the growth and K solubilization was done by using Aleksandrov agar medium (Alexandrov et al., 1967). The isolate was spot-inoculated and incubated at 28°C for 4-7 days using Alexandrov’s agar, containing potassium aluminosilicate. The emergence of a halo zone surrounding the colony revealed the ability of the test organisms to solubilize potassium.

Seed sterilization. The papaya seeds were sterilized through surface sterilization method by first washing in tap water for 5 minutes followed by tween 20 solution for 5 minutes, 1% Bavistin for 1 hour, 1% sodium hypochlorite for 3 minutes, 90% ethanol for 1 minute and finally with distilled water for 1 minute. Seed germination test. The dried seeds were taken and the seed were inoculated with the bioinoculants prepared by mixing in talcum powder and carboxymethyl cellulose (CMC). The seed germination test was undertaken using paper towel method and kept inside the incubator at 28˚C.

Experimental Design and Statistical Analysis. Experiments followed a completely randomized design (CRD). Analysis of variance (ANOVA) was performed with OPSTAT software and mean separation with Duncan's multiple range test. The level of significance was recorded at $p \leq 0.05$.

RESULTS AND DISCUSSION

In this study, it was found that papaya rhizosphere is a rich source of IAA producing bacteria which are involved in direct IAA production. The CFU count of the samples was taken by counting the total colony units in the media.

A total of twenty bacteria were isolated from the four samples collected from different locations of Pantnagar. The selection of the potential bacterial isolates was done based on functional tests on three different mediums such as CAS agar, Pikovaskya agar, Alekxendrovagar and IAA production activity. Out of these twenty bacterial isolates, eleven isolates showed positive results in Alekxendrov agar and showing potassium solubilization ability, seven bacterial isolates showed phosphate solubilization in Pikovaskya agar and two isolates showed siderophore activity.

Effect of bioinoculant isolates on seed germination in papaya. Table 3 depicts the data related to days to seed germination, germination percentage, mortality rate and IAA production as impacted by various bacterial isolates. Out of twenty bacterial isolates, 11 showing positive functional activity were used for seed germination experiment in papaya. Significant variations between the treatments were seen regarding the number of days needed for seed germination in treatments using various isolates. The first seed germination in the control treatment took the longest time (17 days) while the early germination was taken place in PR2d strain treatment (11 days). The data relating to days to 50% seed germination represents that PR2d took the least time of 21 days and maximum days were noticed in the control treatment (31 days). Moreover, PR2b, PR3b, PR3c, PR1b, and PR4aisolates also showed early germination at a significant level as compared to control treatment. This may be due to the fact that under regulated environmental conditions, the growth conditions prevailed for seed germination. Consequently, the shorter time needed for seed germination may be attributable to the bio-inoculants that are responsible for fostering favorable circumstances, including optimal moisture retention, temperature, vitamin sequestration, growth-promoting agents, and water absorption. An essential component of a seed's planting value is seed germination. Radicle and plumule separate from the seed coat during this procedure (Roshni *et al*., 2020). Similar to other bacterial isolates, precocious germination with the isolated bacterial isolates was also seen and related to favorable environmental factors such as ideal moisture retention, temperature, and the release of plant growth regulators such as gibberellins, vitamins, and water absorption. Similar results were obtained by Roshni *et al*. (2020) in wheat where early germination took place due to bacterial consortia.

Regarding the percentage of seed germination, there was no significant variability between the treatments. However, the various isolates that were added to the medium improved the seed germination percentage in all treatments as compared to control (Table 3). The highest germination percentage was observed in treatment with PR2d strain (97.66%) while it was the least in the case of control (87.33%). Similarly, other isolates such as PR2b (96.00%), PR3b (95.33%), PR1b (94.33%) and PR4a (94.00%) also showed high germination percent as compared to control treatment. This may be a result of the application of bacterial isolates, which aided the media with good physical and chemical characteristics as well as nutrient and water absorption. Singh *et al.* (2011) reported increase emergence of lentils seedlings treated with phosphorus solubilizing bacteria. These results are supported by the research of Ou *et al*. (2023) in mulberry, Singh *et al*. (2010) in soybean. Similar results were obtained by Pathak *et al*. (2009) in guava where seed germination was significantly improved by the application of bioinoculants.

The seed mortality rate was also found the least in the case of PR2d (4.00%) and PR2b (4.00%) bacterial isolates while maximum in the case of the control treatment (7.67%). Other bacterial isolates also prevented mortality rate by a good amount. Speedy seed germination and a lower mortality rate may be ascribed to microbial inoculants' effectiveness in providing the necessary crop nutrients, suppressing pathogenic organisms and absorbing moisture. Application of bio-inoculants, which fix nitrogen or solubilize insoluble phosphate or secrete hormones and vitamins, is responsible for the lowest death rate. Bioinoculation using PGPR boosted the biomass content and germination rates while also giving roots of plants vital nutrients including N, P, and K as well as hormones such as auxin and gibberellin, siderophores, ammonia, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Previously, it was believed that decreasing plant pathogens through the generation of hydrogen cyanide (HCN) was a key factor in promoting plant growth (Voisard *et al*., 1989). Eventually, the theory was revised, and it is now thought that HCN synthesis indirectly drives nutrient accessibility to the rhizobacteria and host plants and enhances phosphorus accessibility by metal chelation and sequestration (Rijavec and Lapanje 2016).

Among, all the bacterial stains used in the experiment, PR2d was the highest IAA producing strain (13.31µg/mL) followed by PR2b (11.91 µg/mL), PR2a (11.74 µg/mL). It can be speculated that the capacity of selected bacteria to interfere with auxin and ethylene homeostasis through synthesis of indole acetic acid and ACC-deaminase activity could have a role in the promotion of a more robust system (Mayak *et al*., 2004) able to exploit a larger soil volume and improve water uptake which resulted in better germination activity. Auxin signalling has a role in common symbiotic pathways that affect plant-microbe interactions (Laplaze *et al*., 2015). Based on the research by Fibach-Paldi *et al*. (2012), the influence of rhizobacteria on the elongation of primary and later roots is correlated with higher levels of microbial IAA. Additionally, Belimov *et al*. (2015) demonstrated that the culture supernatants of *Pseudomonas oryzihabitants* Ep4, *Variovorax paradoxus* 5C- 2, and *Achromobacter xylosoxidans* Cm[4] contained indolic substances. Similar results were found by Rolli *et al*. (2015) in grape rootstock. Plant growth promotion is mediated by IAA producing rhizobacteria as it helps plants from seed germination to maturity.

This study concludes that the bacterial isolates PR2d obtained from papaya rhizosphere were effective in early seed germination, enhancement of seed germination percentage, increased IAA production and better retention of seedlings in papaya. In order to attain sustainability, the use of bioinoculants is a novel approach that has not been utilized to its fullest potential. Production of fruits like papaya is of urgent need due to its nutritional and medicinal value and also to reduce the toxic residues of chemicals in fruits. Through this research, it can be concluded that novel isolates present in the papaya rhizosphere have great potential to improve seed germination, earliness and reduce mortality in papaya seedlings.

Table 1: Colony forming units (CFU) count of the isolated bacterial isolates from papaya rhizosphere.

Samples	CFU g^{-1}
PR1	1.33×10^5
PR2	1.86×10^5
PR3	1.48×10^5
PR4	1.075×10^5

Table 2: Functional activity of the bacterial isolates isolated from the papaya rhizosphere.

Samples	Phosphate solubilization	Potassium solubilization	Siderophore Production	IAA Production
PR1a	-	+	-	+
PR1b	+	+	-	+
PR1c	-	+	-	+
PR1d	-	-	-	+
PR1e	-	-	-	+
PR2a	-	+	-	+
PR2b	+	+	+	+
PR2c	-	+	-	+
PR2d	+	+	+	+
PR2e	-	-	-	+
PR2f	-	-	-	+
PR2g	-	-	-	+
PR3a	-	+	-	+
PR3b	+	+	-	+
PR3c	+	+	-	+
PR3d	-	-	-	+
PR3e	-	-	-	+
PR4a	+	+	-	+
PR4b	-	-	-	+
PR4c	-	-	-	+
"+" denotes activity by isolates; "–" denoted no activity by isolates				

Table 3: Growth attributes of bacterial isolates.

Isolates	Days taken for first seed germination	Days taken for 50% seed germination	Per cent of seed germination (%)	Mortality rate (%)	IAA Production (µg/mL)
Control	17 ± 1^{a}	33 ± 1^{a}	87.33 ± 2.51^{g}	7.66 ± 0.57^{a}	-
PR1a	15 ± 1^{b}	28 ± 2^{b}	92 ± 0^{ef}	6 ± 0^{cd}	10.08 ± 0.11^{d}
PR1b	15.33 ± 0.57^{b}	31 ± 2^{a}	94.33 ± 1.15^{bcd}	4.33 ± 0.57^{e}	10.3 ± 0.3^{d}
PR1c	14.33 ± 0.57^{bc}	27.66 ± 1.52^{b}	91 ± 1^{f}	6.66 ± 0.57^{bc}	10.52 ± 0.44^{d}
PR2a	12 ± 0^{de}	24 ± 0^{de}	93.66 ± 1.15^{cde}	7 ± 0^{ab}	11.74 ± 0.24^{b}
PR2b	12 ± 0^{de}	26 ± 1^{bcd}	96 ± 0^{ab}	4 ± 0^{e}	11.91 ± 0.11^{b}
PR2c	12.66 ± 0.57^{de}	22.66 ± 0.57^{ef}	91 ± 0^{f}	6 ± 0^{cd}	10.33 ± 0.26^{d}
PR2d	11 ± 1^{e}	21 ± 0^{f}	97.66 ± 1.15^{cde}	4 ± 0^{e}	13.31 ± 0.31^{a}
PR3a	15 ± 2^{b}	26.33 ± 1.52^{bcd}	93 ± 1^{ab}	7 ± 0^{ab}	10.21 ± 0.22^{d}
PR3b	12.66 ± 0.57^{de}	28.33 ± 1.52^{b}	95.33 ± 0.57^{bc}	5.66 ± 0.57^{d}	10.19 ± 0.26^{d}
PR3c	12 ± 1^{de}	27.33 ± 1.52^{bc}	93 ± 1^{def}	6.66 ± 0.57^{bc}	11.11 ± 0.08^{c}
PR4	13.33 ± 0.57^{cd}	25 ± 1^{cd}	94 ± 0^{bcde}	5.66 ± 0.57^{d}	10.43 ± 0.66^{d}
C.D.	1.521	2.225	1.81	0.69	0.528
SE(m)	0.518	0.758	0.62	0.24	0.18
SE(d)	0.733	1.072	0.87	0.33	0.254
C.V.	6.635	4.916	1.15	6.93	3.006

CONCLUSIONS

This study concludes that the bacterial isolates PR2d obtained from papaya rhizosphere were effective in early seed germination, enhancement of seed germination percentage, increased IAA production and better retention of seedlings in papaya. In order to attain sustainability, the use of bioinoculants is a novel approach that has not been utilized to its fullest potential. Production of fruits like papaya is of urgent need due to its nutritional and medicinal value and also to reduce the toxic residues of chemicals in fruits. Through this research, it can be concluded that novel isolates present in the papaya rhizosphere have great potential to improve seed germination, earliness and reduce mortality in papaya seedlings.

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Conflict of Interest. None.

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