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Rathod Parshuram
Ph.D. Scholar Department of Plant Pathology, Indira Gandhi Krishi Vishwavidhyalaya, Raipur, Chhattisgarh, India

AS Kotasthane
Professor and Head Department of Plant Pathology, Indira Gandhi Krishi Vishwavidhyalaya, Raipur, Chhattisgarh, India

Kishan Kumar Sharma
Ph.D. scholar Department of Plant Pathology, Indira Gandhi Krishi Vishwavidhyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Rathod Parshuram
Ph.D. Scholar Department of Plant Pathology, Indira Gandhi Krishi Vishwavidhyalaya, Raipur, Chhattisgarh, India

Evaluation of fluorescent *pseudomonas* on *in-vitro* seed germination and seedling growth of soybean

Rathod Parshuram, AS Kotasthane and Kishan Kumar Sharma

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Abstract
Bacterization of soybean seeds with 10 isolates of fluorescent *Pseudomonas* isolated from rhizospheric soil of different crop plants derived from different locations of Bastar and Bilaspur districts of Chhattisgarh resulted in increased seed germination and growth (in terms of length of root and shoot) over control. Fluorescent *Pseudomonas* 9704 increased seed germination reduced seedling mortality and improved plant growth promotion in soybean (CG-SOYA-1) seedlings on seed germination paper under lab conditions.

Keywords: fluorescent *Pseudomonas*, seed treatment, Soybean seedlings.

Introduction
Seed germination is the major issue in soybean. Poor germination or failure in germination may be due to poor quality of seed, unavailability of sufficient moisture in the soil and many other biotic and abiotic factors acts as a hindrance to germination [1]. Many studies state that seed inoculation with plant growth-promoting rhizobacteria (PGPR) before sowing will enhance germination and seedling establishment [2, 3]. Rhizobacteria Group of *Pseudomonas* (*P. fluorescens, P. putida, and P. aeruginosa*) is known to be beneficial to plants. Seed bacterization or seed inoculation with PGPR bacteria helps them to establish in soil and rhizosphere [4]. Nevertheless, due to the poor survival of bio inoculants, the large-scale commercial production of biofertilizer or biocontrol agents is hindered [5]. Thus, improving the formulation of microbial inoculum that can survive, establish, and develop in the soil to provide nutrients to plant. Through seed treatment with PGP microbes, complete protection solutions can be provided to seeds against different environmental stresses, which can be cost-effective and nature friendly which leads to precision agriculture [6]. Seed treatment with PGP microbes can be handled more easily and used to protect the plant from biotic stress (like plant pathogens) and abiotic stress (like drought and salinity). Recent developments reveal that coating seed with microbes can efficiently help to inoculate and develop its population in the soil, which significantly produce the microbes coated seed to meet higher safety measure and efficiency standard [7, 8]. Any successful strategy aimed at enhancing crop productivity with microbial products ultimately relies on the ability to scale at regional to global levels. Microorganisms that show promise in the lab may lack key characteristics for widespread adoption in sustainable and productive agriculture systems. To make a significant impact on global sustainable agriculture, the implementation of plant beneficial microorganisms will require a more seamless transition between laboratory and farm applications. This study was conducted to obtain information on the speed of germination of the seeds of soybean after seed bacterization with *Pseudomonas* isolates on seed germination paper under lab conditions.

Materials and Methods
The 10 fluorescent *Pseudomonas* isolates were grown in 100 ml Kings B base broth in 250ml conical flasks on a rotary shaker (150 rpm/min) for 48hrs at room temperature. The culture broth was used as bacterial inoculum. The bacterial suspensions were prepared and tested for their plant growth-promoting activity following seed bacterization. Soybean seeds (CG SOYA-1) were used for testing the efficacy of 10 fluorescent *Pseudomonas* isolates. The slurry for seed bacterization was prepared by adding bacterial culture and talcum powder to the seeds of the soybean.

Care was taken for uniform coating of all seeds, which were blot dried, placed in wet blotters, and two-third of the blotter was immersed in water and incubated in a growth chamber for 7 days. Untreated seeds were kept as control. Observations were recorded on the 7th day. Ten seedlings were taken randomly from each bacterial treatment and their shoot length and root length were recorded.

Results and Discussion

In the present investigation effect of seed bacterization with ten *Pseudomonas* isolates on the germination and growth of soybean seedlings was studied. In comparison with the untreated control, all the *Pseudomonas* isolates significantly enhanced both germination and growth of soybean (CG SOYA-1) seedlings on the 3rd 4th, and 7th day (Plate: 1). Earlier germination of soybean seeds treated with *Pseudomonas* isolates was recorded (Plate: 1a). Besides, the highest root (21.3 ± 0.30 cm) and shoot (14.05 ± 0.48 cm) growth were recorded on the 7th day in soybean seeds treated with isolate 9704 (Table: 4.10) which led to a 63.61% and 13.02% increase over control respectively (Plate: 1c). Significant increase in root length was observed with isolates 9809 (61.72%) > BSP-23 (61.05%) > 9829 (58.10%) > BSP-14 (58.10%) > BS-4 (55.45%) > BSP-19 (52.30%) > BS-1(51.41%) >BS-2 (49.83%) > BS-3 (33.18%) over control in respective order. Similarly significant increase in shoot length was observed with isolates 9809 (13.02%) > BSP-23 (11.6%) > 9829 (7.34%) > BS-2 (6.58%) > BSP-14 (6.58%) > BS-4 (3.4%) over control in respective order. Whereas *Pseudomonas* isolates BS-1, BS-3 and BSP-19 showed a decreased shoot growth over control (Figure: 1). The results suggest that *Pseudomonas* isolate 9704 is a plant growth-promoting rhizobacteria which have the potential for use in seed bacterization for higher germination and growth of soybean seedling. This phenomenon might be attributed to the production of auxins, lower levels of ethylene, or mineralization of nutrients by *Pseudomonas* isolate. Seed inoculated with PGPR showed an increase in the number and length of lateral roots and stimulates root hair elongation [9]. Similar results were also obtained by [10] fluorescent *Pseudomonas* RBT 13, isolated from tomato rhizoplane, resulted in increased seed germination (in terms of root and shoot length and weight) and yield of plants.

Seed bacterization with *Pseudomonas* isolate had enhanced seed germination in soybean (Table: 1). The results suggest that *Pseudomonas* isolate 9704 is a plant growth-promoting rhizobacteria that have the potential for use in seed bacterization for higher germination and growth of soybean seedling. These findings may be due to the increased synthesis of hormones by *Pseudomonas* isolate 9704. Gibberellins trigger the activity of specific enzymes such as amylase, which increases the availability of starch that promoted early germination [11]. [12] Studied that seed inoculation initiates physiological processes of germination and helps in the proliferation of bioagent in the spermosphere. Therefore using *Pseudomonas* isolates as seed inoculants useful in enhancing germination in soybean.

Conclusion

Plant growth-promoting abilities of ten *Pseudomonas* isolates were evaluated in soybean (CG SOYA-1) cultivar following seed treatment. The effect of seed bacterization with ten *Pseudomonas* isolates on the germination and growth of soybean seedlings were studied. Following seed treatment with ten *Pseudomonas* isolates significantly enhanced both germination and growth of soybean seedlings. Early germination of soybean seeds treated with *Pseudomonas* isolates was recorded. Besides, the highest root and shoot growth was recorded on the 7th day in soybean seeds treated with *Pseudomonas* isolate 9704.

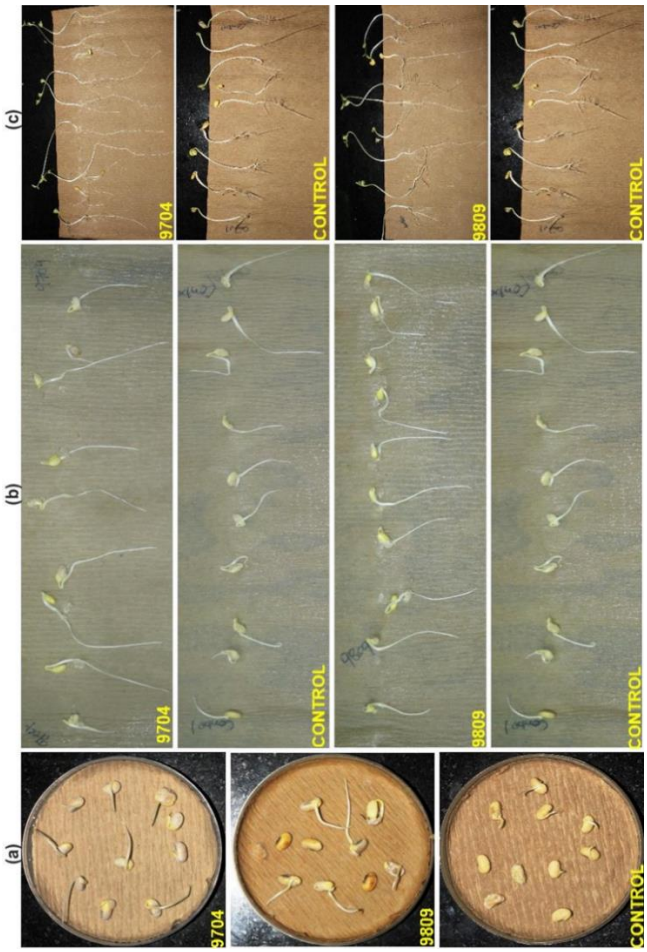

Plate 1: Effect of seed bacterization with *Pseudomonas* isolates on the germination and growth of soybean seedlings a) 3days after seed treatment b) 4days after seed treatment c) 7days after seed treatment

Table 1: Effect of seed bacterization with *Pseudomonas* isolates on the germination and growth of soybean seedlings (seven days after seed treatment)

S. NO.	Isolates	Shoot length (cm)	Root length (cm)
1	9829	12.25^{ABC} ± 0.58 (7.34 %)	18.5^{ABC}±1.19 (58.10%)
2	9704	14.05^{A} ± 0.48 (19.2%)	21.3^{A}±0.30 (63.61%)
3	9809	13.05^{AB} ± 0.63 (13.02%)	20.25^{AB}±0.58 (61.72%)
4	BS-1	10.75^{BCD} ± 0.32 (**)	15.95^{C}±0.83 (51.41%)
5	BS-2	12.15^{ABCD} ± 0.40 (6.58%)	15.45^{C}±0.96 (49.83%)
6	BS-3	8.5^{CDE}± 0.29 (**)	11.6^{D}±0.74 (33.18%)
7	BS-4	11.75^{ABCD} ± 0.47 (3.4%)	17.4^{BC}±0.90 (55.45%)
8	BSP-14	12.15^{ABCD} ± 0.78 (6.58%)	18.5^{ABC}±0.96 (58.10%)
9	BSP-19	9.8^{DE} ± 0.42 (**)	16.25^{C}±0.87 (52.30%)
10	BSP-23	12.85^{ABC} ± 0.52 (11.6%)	19.9^{AB}±0.40 (61.05%)
11	CONTROL	11.35^{E} ± 0.31	7.75^{E}±0.40
	C.D	1.39	2.22
	SE(M)	0.49	0.79
	SE(D)	0.70	1.11
	C.V	13.75	15.02

Values in the parenthesis represent percentage increase over control (**) = represent percentage decrease over control

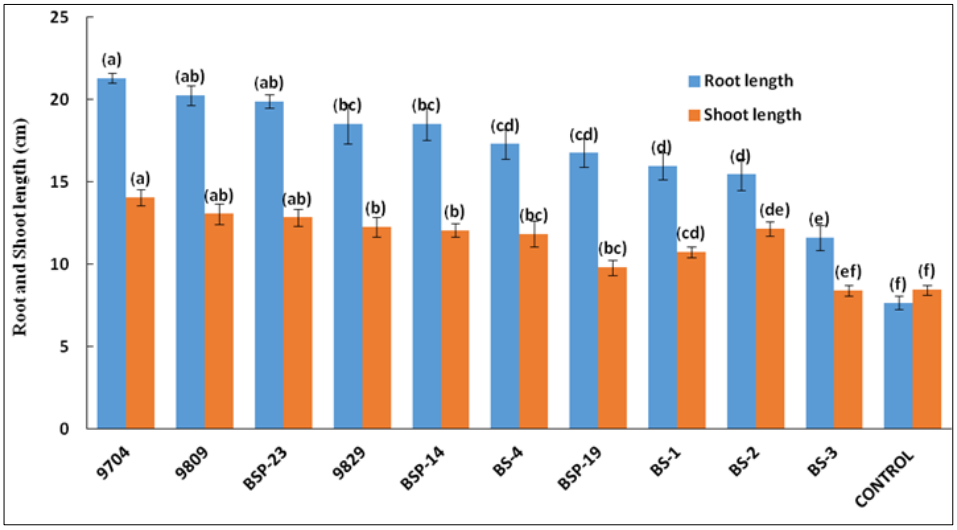

Fig 1: Effect of seed bacterization with *Pseudomonas* isolates on the root shoot growth of soybean seedlings (seven days after seed treatment)

Means followed by a common letter are not significantly different at the 5 % level
Error bars indicate one standard error of the mean
All the treatment values are average of three replications

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