

## Effect of *Pseudomonas striata* and *Piriformospora indica* on seed germination in genotypes of Maize (*Zea mays* L.) and Moong (*Vigna radiata* L.)

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Bioinoculants increase the ability of plants to acquire nutrients from soil by either increasing the extent of the root system (e.g., fungal hyphae) or solubilizing macronutrients such as phosphorus or sulfur [1]. In fact, plant growth in nutrient-poor environments is linked to the formation of mutualistic associations with soil microorganisms such as arbuscular mycorrhizal (AM) fungi [1]. *Piriformospora indica* (Hymenomycetes, Basidiomycota) a cultivable endophyte promotes plant growth and biomass production [2]. *P. indica* resembles AM fungi in several functional and physiological characteristics [3, 4] and it has been documented to improve plant growth. *Pseudomonas sp.* is one of the most extensively used PGPR which has been reported to improve plant growth when used alone or in combination with other microorganisms [5, 6]. Seed inoculation by *Pseudomonas striata* or by AM fungi can greatly affect the uptake of mineral nutrients especially phosphorus and their potential for plant growth promotion has been well established [7-11].

Numerous biotic and abiotic factors influence the performance of the added bioinoculants on crop productivity. Among the biotic components, plant species appears to be a determinant, governing the performance of a bioinoculant. Very few attempts have been made so far to evaluate the influence of plant genotype (cultivars) on the performance of the added bioinoculant. Therefore, an experiment was conducted under controlled conditions to see the

interactive effect of single and combined inoculation of *Pseudomonas striata* and *Piriformospora indica* on per cent seed germination of different maize (*Zea mays* L.) and moongbean (*Vigna radiata* L.) cultivars.

An experiment was conducted to determine the influence of single inoculation with *P. striata*, or *Piriformospora indica* and their combined inoculation on seed germination of different cultivars of maize (*Zea mays* L.) and moongbean (*Vigna radiata* L.). *Piriformospora indica* an axenically cultivable mycorrhiza-like fungus (Patented) was maintained on modified *Aspergillus* medium [12]. The plant growth promoting rhizobacteria (PGPR) *Pseudomonas striata* was maintained on Pikovaskaya medium [13]. The affinity of *P. striata* and *P. indica* in different cultivars of maize and moongbean was assessed under axenic conditions. Seeds of different maize varieties were procured from Directorate of Maize Research (DMR), IARI, New Delhi and moongbean seeds were procured from the Pulse Laboratory, Division of Genetics, IARI, New Delhi. Contrasting agronomic characters was the selection criteria for the different cultivars in the two crops. The characters selected were time of maturity and disease resistance.

Crop	Cultivar
<i>Zea mays</i> L.	PRO-311, Him-129, Seed tech, Bio-9681, Mahikanchan, Kiran
<i>Vigna radiata</i> L.	Pusa-Vishal, Pusa-Ratna, P-9531, P-105, PS-16

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The seeds were surface sterilized with 4 per cent sodium hypochlorite (v/v) for 15 min. followed by 70% ethanol (v/v) for 30 sec. After rinsing several (8-10) times with sterile distilled water, they were transferred aseptically on water agar (0.7% agar) in 90 mm Petri dishes for germination. Seeds of each variety of moongbean and maize received following treatment on the water agar Petri plates prior to germination. A total of 400 seeds in four replicates were sown per variety for each treatment. The experiment was repeated three times under identical conditions and the results were analyzed statistically.

- (i) No microbial inoculation - control.
- (ii) Lac-Z tagged *Pseudomonas striata* broth culture having  $6.2 \times 10^6$  cfu/ml.
- (iii) *P. indica* agar disc, fortified with actively growing fungal propagules (hyphae and spores), grown for 4 weeks on modified *Aspergillus* medium [12].

Germination was carried out at 25°C temperature and evaluation was done following ISTA guidelines [14].

The results presented in table 1 represent the mean values of three different experiment conducted under identical laboratory conditions. It revealed the effect of microbial inoculation with *P. striata* or *P. indica* and their co-inoculation on the percentage germination of seeds of six different maize varieties. Single as well as the combined inoculation significantly ( $P = 0.05$ ) improved the per cent germination over their respective uninoculated controls in all the varieties tested. In general, among the treatments studied inoculation with *P. indica* was the best (32% to 10%) followed by dual inoculation and single inoculation with *P. striata* (23 to 10%) in promoting the per cent germination of maize seeds. Statistically significant differences were also noticed among the six maize varieties in per cent seed germination. The improvement of seed germination following inoculation with *P. striata* and *P. indica* was identical in the two maize varieties namely Bio-9681 and Mahikanchan. In case of cultivar Seedtech, *P. indica* improved

germination by 24 per cent whereas an improvement of 10 per cent was recorded by inoculation with *P. striata* as compared to uninoculated control. The maize variety Kiran was highly responsive showing 32 per cent and 23 per cent improvement in seed germination with individual inoculation of *P. indica* and *P. striata* respectively over the uninoculated control. Pro-311 was least responsive to microbial inoculations showing 10 and 11 per cent improvement following inoculation with *P. indica* and combined inoculation respectively. Whereas, *P. striata* inoculation failed to increase the germination. *P. indica* appears to be more effective than *P. striata* in maize because it improved germination by 24 per cent and 10 per cent in the varieties Seedtech and Pro-311 over their respective uninoculated control.

In moongbean, a highly significant improvement in per cent seed germination in all the six varieties was recorded following single or combined inoculation with *P. striata* and *P. indica* over their respective uninoculated control (Table 1). P-Vishal, P-105 and P-Ratna varieties of moongbean were highly responsive to individual inoculation of *P. striata* and *P. indica* whereas the least response was recorded in P-9531 and PS-16. The moongbean variety Pusa Vishal was highly responsive to inoculation as it showed an improvement of 50 per cent, 50 per cent and 49 per cent following inoculation with *P. indica*, *P. striata* and their combined inoculation, respectively over the uninoculated control. A synergetic response to the combined inoculation was seen in moongbean variety P-9531 (23%) which was lacking in the variety PS-16 (15%).

A comparison of different genotype of moongbean with respect to their response to the two bioinoculants used showed that in cultivars Pusa-Vishal, P-9531 and PS-16 the increase in per cent seed germination was statistically identical. Therefore, the two inoculants resulted in an identical promotion of seed germination in these varieties. However, in case of moongbean cultivar P-105, *P. striata* increased seed germination (36%) in comparison to an enhancement of 18 per cent by *P. indica* over their uninoculated control.

Table 1. Effect of inoculation of *Piriformospora indica* and *Pseudomonas striata* on the seed germination (percentage) of different Moongbean (*Vigna radiata* L.) Maize (*Zea mays* L.) varieties.

Treatments/crop varieties	Control	<i>P. indica</i>	<i>P. striata</i>	<i>P. indica</i> + <i>P. striata</i>	S.Em±	CD (5%)
Moong bean ( <i>Vigna radiata</i> L.)						
P-9531	80	91(13.75)	91(13.75)	98(22.5)	0.601	2.440
P-Vishal	66	99(50.00)	99(50.00)	98(48.88)	0.577	2.313
P-105	73	86(17.80)	99(35.61)	98(34.24)	0.913	3.660
P-Ratna	73	98(34.24)	86(17.80)	98(34.24)	0.913	3.660
PS-16	85	99(16.47)	98(15.29)	98(15.29)	0.500	2.000
S.Em±	0.931	0.632	0.632	0.577		
CD (5%)	3.230	2.174	2.174	1.984		
Maize ( <i>Zea mays</i> L.)						
Bio-9681	81	90(11.11)	90(11.11)	89(9.88)	0.882	3.589
Kiran	66	87(31.82)	81(22.72)	81(22.72)	0.972	3.897
Him-129	83	97(16.86)	91(9.63)	91(9.63)	0.913	3.661
Mahikanchan	83	91(9.63)	91(9.63)	98(18.07)	0.577	2.313
Pro-311	83	91(9.63)	83(0.00)	92(10.84)	0.764	3.063
Seedtech	67	83(23.88)	74(10.44)	75(11.94)	0.577	2.313
S.Em±	1.054	0.758	0.577	0.577		
CD (5%)	3.277	2.357	1.794	1.794		

Whereas, in Pusa-Ratna germination of the seeds treated with *P. indica* inoculation (34%) was nearly two fold over those treated with *P. striata* inoculation (18%). The germination response observed in the different moongbean varieties to the added bio-inoculants was variable indicating the influence of plant genotypes. A number of workers [15-17] reported the influence of plant species on the abundance and activity of PGPR in the rhizosphere.

A comparison of the response of two crops namely maize and moongbean showed that the beneficial effect of the bioinoculants manifested as per cent improvement in seed germination was more pronounced in moongbean, a leguminous crop than in the cereal (*Zea mays* L.). The results presented in table 1 revealed a significant ( $P = 0.05$ ) improvement in per cent seed germination

by the single and combined inoculation in all cultivars of maize and moongbean crops. Baldani and Dobereiner [18] recorded similar observations in cereals. In case of maize the synergetic effect of PGPR, *Pseudomonas striata* and the symbiotic fungus, *P. indica* was not pronounced. However, in case of moongbean cultivars, performance of combined inoculation was significantly either higher or identical to the individual inoculants. This may be attributed to the differences in biomolecules released as seed exudates and rhizodeposits and their interaction with the microorganisms [6]. PGPR like *Pseudomonas* sp. exerts positive influence on various plant growth parameters by release of secondary metabolites namely phytohormones and biologically active substances [19]. Several Workers have reported an increased seed germination or seedling emergence of cereals due to inoculation with

PGPR [19-21]. Increased seed germination by fluorescent *Pseudomonas* sp. has been reported in groundnut [11, 10]; Chilli [22] and Okra [23]. The ability to produce secondary metabolites such as siderophores, antibiotics, enzymes, phytohormones have been implicated for the observed plant growth promotion. These biomolecules may be responsible for suppression of deleterious inhibitors and/or increase the proteolytic activity to break seed dormancy during the germination process [24-26].

Seed germination response to inoculation with *P. striata* and/or *P. indica* involves cultivar/variety specificity as seen in this experiment conducted under controlled conditions. Plant cultivars differ in their ability to provide suitable substrates through seed/root exudates. The importance of plant cultivars/species and their interaction with the bioinoculants in enhancing plant growth parameters have been emphasized by several workers [18, 27, 28]. The observed differential stimulation of plant genotypes to the applied bioinoculants may be explained on the basis that plant cultivars differ in the biochemical characteristics of the seed/root exudates. The biochemical basis of the plant-microbe interactions demands further investigations.

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