

RHIZOBACTERIA MEDIATED GROWTH ENHANCEMENT IN PEARL MILLET**ANUJ SAXENA^{a1}, NIRANJAN RAJ^b, B. R. SAROSH^c, RAMACHANDRAN KINI^d
AND H. S. SHETTY^e**^aDepartment of Botany, Sacred Heart Degree College, Sitapur, U.P., India^{bcd}Department of Studies in Applied Botany and Biotechnology, University of Mysore, Mysore, Karnataka, India**ABSTRACT**

Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that colonize plant roots and promote plant growth. Present study fairly demonstrates the PGPR (*Bacillus subtilis*) treatment as an eco-friendly, cost effective, simple, easy deployable and potential tool for productivity enhancement in pearl millet. The increased growth and vigour on PGPR treatment is evident by early germination, increased vigour index, plant height, leaf area, number of tillers, fresh weight and dry weight.

KEYWORDS : Pearl Millet, Plant Growth Promoting Rhizobacteria (PGPR), Vigour index

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), a crop of marginal land, provides staple food for poor in semiarid regions of Asia and Africa. Pearl Millet, the fifth major crop of India is grown as a rain-fed or irrigated crop on 10 million hectares producing 7.01 million tons (Bhatnagar et al., 2002).

In recent years, the concept of PGPR mediated plant growth promotion is gaining worldwide importance and acceptance (Heil and Bostock, 2002; Saharan and Nehra, 2011). The utilization of native PGPR strains for promotion of plant growth and as inducers of plant defense response may increase their chance of applicability and offer a practical way to deliver growth promotion and disease management. The present study was undertaken to evaluate the effect of PGPR on growth promotion in pearl millet.

MATERIALS AND METHODS**Host and Inducers**

Seeds of pearl millet cultivar HB 3 were obtained from International Crop Research Institute for Semi Arid Tropics (ICRISAT), Hyderabad, India. Four strains of *Bacillus subtilis* viz. ISR 5, ISR 12, ISR 13 and ISR 17 were obtained from culture collection of Department of Applied Botany, University of Mysore. All bacterial strains were maintained at 80 °C in Tryptic Soy Broth (TSB) amended with 20% glycerol.

Preparation of PGPR for seed treatment

The bacteria were grown on Tryptic Soy Agar

(TSA) for 24 hours at 37 °C. Subsequently, the bacterial lawn was scraped into sterile distilled water and centrifuged at 6000 rpm for 5 min and the pellet obtained was suspended in sterile distilled water. The optical density of the suspension was adjusted using a UV visible spectrophotometer (Hitachi U-2000, Japan) following the method of Mortensen, (1992) to obtain a final density of 1×10^6 , 1×10^8 and 1×10^{10} cfu/ml.

Seed Treatment with PGPR suspension

Seeds of pearl millet were surface sterilized with 0.02% mercuric chloride for 5 min and rinsed thoroughly with sterile distilled water. Bacterization of the seeds was achieved by soaking 1g of seeds in 10 ml of bacterial suspension, using 0.2% sterilized carboxy methyl cellulose (CMC) as sticker. They were incubated at 26 °C in a rotary shaker for 3, 6, 8 h to facilitate attachment of bacterial cells to the seed coat. Later, the seeds were allowed to dry in an incubator at 30 °C for 15 min. Seeds treated with sterile distilled water followed by CMC served as control. Seeds were sown in earthen plastic pots filled with autoclaved soil and sand at the ratio of 2:1. Seedlings were watered daily.

Effect of seed treatment with PGPR isolates on growth promotion

For germination test paper towel method was followed (ISTA, 1993). Vigour index was analyzed at the end of 7 days of incubation following the method of Abdul Baki and Anderson, (1973).

Vigour Index = (Mean root length + Mean shoot length) X Percentage germination X 100

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Number of seeds germinated was noticed on the 7th day of sowing. At the end of vegetative growth (30 days), different growth parameters viz., height, fresh weight, dry weight and leaf area were determined. Height of the plant was measured from the base to the tip of the plant. Fresh weight was determined by uprooting the plant carefully, washing them thoroughly to remove remnants of soil particles and weighed. Dry weight was determined by drying the plants in an oven at 65°C until the weight remained constant. The leaf area was measured using the instrument ∇ T area meter MK 2 Burwell, Cambridge, England.

The experiment was carried out with four replicates of one hundred seeds each. The data presented are the average of all the replicate with standard error.

RESULTS

Effect of PGPR treatment on vigour index and optimization of concentration and duration of seed treatment with PGPR

It was found that the concentrations 1×10^6 and 1×10^8 cfu/ml increased the germination percentage and vigour index at 3 and 6 hours duration, while a decrease in these parameters was recorded at 9 hours incubation. The concentration of 1×10^{10} cfu/ml did not affect the seeds at 3 hours incubation but exhibited negative effect on germination and vigour index at 6 and 9 hours of incubation (Table,1). Thus, 1×10^8 cfu/ml concentration at 6 hours

incubation period was found to be optimum for seed treatment.

Treatment with all the isolates of *Bacillus subtilis* showed considerable enhancement in germination and vigour index varying from 70 to 97% in germination and 390 to 1319 in vigour index over the control. Highest enhancement of 97% germination and 1319 vigour index was recorded by treatment with ISR 5. This was followed by ISR 13 with 92% germination and 1028 vigour index. Control showed 93% germination and 888 vigour index (Table,1).

Effect of seed treatment with inducers on growth promotion

Seeds treated with live bacteria recorded early emergence compared to the control. ISR 5 and ISR 13 treatments showed 100% emergence on the second day after sowing while the control emerged completely only on the third day after sowing. Isolates ISR 5, ISR 13 and ISR 17 proved very efficient in growth promotion. At 30 days old stage, plants with ISR 5 treatment recorded a height of 31.7 cm, (which was 41% more over the control), 85% and 76% more fresh weight and dry weight over the control respectively. Seedlings raised from treatment with ISR 13 were 36% more in height, 64% and 59% more in fresh and dry weight respectively over the control, which recorded 22.5 cm height, 6.1 g and 1.7 g fresh and dry weight respectively. Seed treatment with ISR 5 and ISR 13 resulted in plants with 33.7% and 11.7% more leaf area than control,



Figure 1: Effect of seed treatment with *Bacillus subtilis* isolate ISR 5 on vegetative growth of pearl millet in comparison with the control under greenhouse conditions

Table 1: Effect of seed treatment with different concentrations of *Bacillus subtilis* at different time intervals on seed germination and seedling vigour of pearl millet

Bacteria	Conc. (cfu/ml)	Time (h)	Germin. (%)	Vigour Index
ISR 5	10 ⁶	3	92.85	1183.83
		6	83.87	889.02
		9	82.14	702.29
	10 ⁸	3	96.55	1124.80
		6	96.29	1319.17
		9	84.37	873.22
	10 ¹⁰	3	88.46	797.90
		6	71.42	842.75
		9	77.77	783.92
ISR 12	10 ⁶	3	86.20	663.74
		6	82.14	961.03
		9	82.35	856.44
	10 ⁸	3	85.71	919.66
		6	78.57	797.48
		9	73.07	504.18
	10 ¹⁰	3	80.00	784.00
		6	89.65	936.84
		9	67.85	390.13
ISR 13	10 ⁶	3	80.76	827.79
		6	80.76	868.17
		9	77.77	766.03
	10 ⁸	3	92.30	998.68
		6	85.71	1028.52
		9	80.00	920.00
	10 ¹⁰	3	75.00	868.50
		6	76.92	765.35
		9	68.96	762.00
ISR 17	10 ⁶	3	81.48	757.76
		6	75.86	656.18
		9	70.37	418.70
	10 ⁸	3	77.41	827.51
		6	85.18	716.36
		9	76.66	659.27
	10 ¹⁰	3	81.48	877.53
		6	82.14	915.86
		9	79.41	806.01
Control DH ₂ O		3	92.59	888.86
		6	93.75	887.81
		9	82.75	611.52

Table 2: Effect of seed treatment 1 with pure culture of *Bacillus subtilis* isolates on growth of pearl millet cultivar HB3 under greenhouse conditions

Treatment	Height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)	No. of basal tillers
Control (DW)	22.5 ± 1.9	6.1 ± 0.41	1.7 ± 0.14	25.5 ± 2.7	2 ± 0.14
ISR 5	31.7 ± 2.6 (40.8)	11.3 ± 0.61 (85.2)	3.00 ± 0.27 (76.4)	34.1 ± 3.5 (33.7)	3 ± 0.27
ISR 12	24.4 ± 1.9 (8.4)	10.5 ± 1.2 (72.1)	2.04 ± 0.16 (20.0)	26.7 ± 2.7 (4.7)	3 ± 0.22
ISR 13	30.7 ± 2.8 (36.4)	10.0 ± 0.65 (63.9)	2.7 ± 2.3 (58.8)	28.5 ± 2.4 (11.7)	3 ± 0.26
ISR 17	28.5 ± 3.1 (26.6)	10.4 ± 1.0 (70.4)	2.5 ± 2.4 (47.0)	30.3 ± 2.8 (18.8)	3 ± 0.23

Inducer treatment was achieved by treating pearl millet seeds for 6 h in the respective bacterial culture at the concentration of 10⁶ cfu / ml. Results are as on 30th day of growth based on 4 experiments with 25 plants per treatment per experiment. Values relative to control are in parenthesis.

which had 25.5 cm² leaf area (Table 2, Figure, 1). All the bacteria treated plants showed three tillers at this stage whereas control recorded two tillers.

DISCUSSION

The results showed a marked enhancement in germination percentage, growth and productivity of pearl millet by ISR treatment. Such growth promotions are studied by earlier workers in different plants with different PGPR (Raupach and Kloepper 1998, Yadegari et al., 2008).

PGPR mediated plant growth promotion may be due to (i) the ability to produce or change the concentration of the plant hormones indoleacetic acid (Mordukhova et al., 1991), gibberellic acid (Mahmound et al., 1984), cytokinins (Tien et al., 1979) and ethylene (Glick et al., 1995); (ii) asymbiotic N₂ fixation (Kennedy et al., 1997); (iii) solubilization of mineral phosphates and other nutrients (De Freitas et al., 1997). PGPR treatment exhibited the potential for downy mildew disease management in pearl millet.

Present study fairly demonstrates application of PGPR as an environmentally sustainable approach to increase crop production and health. With the advancement in our understanding of their diversity, colonization ability, mechanisms of action, formulation, and application, the PGPR can develop as reliable components in the management of sustainable agricultural systems.

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