Seed Research, Vol. 45(1): 65-69, 2017

Seed Vigor as Influenced by Rhizospheric Bacterial Isolates in Soybean (*Glycine max* L. Merril)

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ABSTRACT: Plant growth promoting rhizobacteria (PGPR) have been reported to influence the seed germination and subsequent seedling growth by an array of mechanisms, the specific traits by which PGPR promote growth and yield were limited to the expression of one or more of the traits expressed at a given environment of plant-microbe interaction. The present investigation was carried out to study the influence of bacterial isolates on seed germination and seedling vigour in soybean, under control condition. The experimental material consists of two variety of Soybean *viz.*, PRS-1 and PS-1092 which were inoculated with 10 different isolates of PGPR namely RGB-1, RMV-1, RMV-2, RMV-3, RMV-4, RPB-1, RPB-3, RPB-4, RPB-5 and RPV-4, obtained from the chickpea, mustard and pea rhizosphere and Pf-102 was used as standard check along with un-inoculated control. Under laboratory conditions, out of eleven bacterial isolates, RMV-4 exhibited greater number of germination with respect to standard germination and first count for both the cultivars (PRS-1 and PS-1092). However, root and seedling length, fresh and dry weight as well as vigour index were found to be better for RPB-1, while RMV-2 produced longer shoot.

Key words: Seed vigor, Rhizosphere, Bacterial isolates, Seed inoculation, Soybean

Soybean [*Glycine max* (L.) Merril.] being a potential oil and protein crop for narrowing the oil and nutrition gap, occupies an important place in agricultural economy of India [1]. Soybean is a member of family Papilioniacae and is supposed to have originated in China. In order to improve the yield and for disease suppression, farmers resort to inorganic fertilizers and possible pesticides, which often have hazardous effect and also do not fit into the frame work of organic farming. An alternative approach is to use biological seed treatments which are known to augment plant health through increasing the planting value of seed, better germination rate, increasing the uptake of inorganic phosphorous, better seed yield and quality [2]. Some bacteria that are associated with roots of crop plants can exert beneficial effect on their roots and they are often collectively referred to as Plant Growth Promoting Rhizobacteria [3]. The term rhizobacteria was coined for those bacteria having the ability to colonize roots aggressively.

Seed vigour has been recognized as one of the important aspects of the seed quality that provides reproducible results which are more closely correlated with emergence in the field [4]. It is an examination of seed under specific environmental conditions so as to provide means of evaluating the planting value of seed in the laboratory. Seed vigour is an inherent character and an important attribute of seed quality indicating the degree of aliveness. Through seed vigour studies we can identify the level of potentiality of bacterial isolates for soybean seed, which may be estimated in laboratory before planting in the field. Thus the present investigation was carried out to study the influence of bacterial isolates on seed germination and seedling vigour in soybean under controlled condition.

Received : January 2017 Revised : March 2017 Accepted : March 2017

MATERIALS AND METHODS

The present investigation was carried out in laboratory of department of Seed Science & Technology, College of Forestry and Hill Agriculture, GBPUAT, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand. The experimental material consists of two varieties of soybean viz; PRS-1 and PS-1092 which were inoculated with 10 different isolates of PGPR namely RGB-1, RMV-1, RMV-2, RMV-3, RMV-4, RPB-1, RPB-3, RPB-4, RPB-5 and RPV-4 obtained from the chickpea, mustard and pea rhizosphere and Pf-102 was used as standard check along with un-inoculated control, to test their potentiality with respect to seed vigor parameters in laboratory. Broth culture were prepared by taking a loop of the respective rhizobacterial isolates and transferred to the liquid medium of 100 ml which were incubated for 24 hrs on a rotary shaker. When the culture in the flask showed dense milky white growth, the broth cultures were considered ready for seed inoculation. For seed treatment, all the seeds of both soybean cultivars PRS-1 and PS-1092, were surface sterilized with 90 per cent alcohol for 30 second, followed by immersion in 32 per cent H_2O_2 for one minute followed by thorough washing in sterile distilled water. Afterwards, the seeds were immersed in the rhizospheric bacterial isolate broth for 4 hrs, with continuousagitation. The entire vigor test comprised of 100 seeds of each treatment replicated thrice. To evaluate the standard germination, seeds were kept in between paper (B.P.) media at 25°C in germinator and normal seedlings were counted on the 5thand 8th day respectively. To measure the root, shoot and seedling length, 20 normal seedlings were randomly selected on 8th day of the start of germination test. The length of root, shoot and seedling length (cm) were measured with the help of measuring scale. Seedling fresh weight was recorded in the seed germination test on 8th day. The normal seedlings were weighed to measure the fresh weight while dry weight was recorded after seedlings were dried in the oven at 80^{0}C for 24 hrs. The seedling vigour index I was calculated as a product of germination and seedling length while, vigor index II was worked out by multiplying germination per cent with seedling dry weight [5]. In order to test the validity of each treatment, statistical analysis was done following Completely Randomized Design (CRD). The critical difference at 5 per cent level of significance was calculated to compare the mean of different treatment.

RESULTS AND DISCUSSION

Under laboratory condition, results suggested that seed germination and seedling vigour of soybean were greatly affected by rhizospheric bacterial isolates, suggesting thereby the importance of PGPR in seed germination and subsequent seedling growth for achieving appropriate plant stand in field. Results in Table 1 depicted significantly higher germination at first count for RMV-4 and RMV-4 along with RPB-5 bacterial

Table1. Influence of bacterial isolates on mean values of first count and standard germination of soybean (cv. PRS -1, PS - 1092)

Treatment	First count (%)		Standard germination (%)	
	PRS-1	PS-1092	PRS-1	PS-1092
RGB-1	72.67	66.00	92.00	89.67
RMV-1	64.00	59.00	89.00	88.00
RMV-2	56.33	51.67	82.67	79.00
RMV-3	56.66	51.67	84.00	84.00
RMV-4	80.00	74.00	94.67	92.00
RPB-1	61.67	53.33	86.00	85.67
RPB-3	51.33	49.00	86.00	87.00
RPB-4	63.67	57.33	81.67	84.33
RPB-5	72.67	64.00	92.33	92.33
RPV-4	69.33	63.33	90.00	89.67
Pf-102	70.00	56.00	86.33	86.63
Control	51.00	46.00	77.33	75.67
GM	64.11	57.61	86.83	86.14
SE(m)±	0.82	0.77	0.89	0.99
CD at 1%	3.27	3.07	3.55	3.92
CD at 5%	2.41	2.26	2.61	2.89
CV (%)	2.33	2.33	1.79	1.99

isolates. Least germination was recorded for control. The PGPR promotes germination in number of crops and hence might be referred to as bio-stimulants. Similar findings have been reported by [6], in lentil and also by [7], who found that inoculation of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* L.) seed with a siderophore producing plant growth promoting rhizobacteria (fluorescent pseudomonads) resulted in increased seed germination.

Bacterial isolates markedly increased the root length in both cultivars of soybean (Table 2). The response of RMV-1 produces highest root length in both PRS-1 and PS-1092. The influence of RMV-2 with respect to shoot length was exhibited better for both cultivar and least shoot length was measured in un-inoculated control in PRS-1 while in PS-1092 recorded the lowest value.

Maximum seeding length was observed with RPB-1 while significantly lowest length of seedling was measured for inoculated control for both the cultivars. The effect of all isolates on seedling length was found significant. The possible reason for induction of root, shoot and seedling elongation might be growth response attributed to IAA production by rhizobacteria. Similar findings were also reported by [2, 8]. Studies by [9, 10] in lentil and by [11] in rice seedlings, under laboratory conditions, has also given similar results. The maximum mean fresh weight of 20 seedlings was observed with RPB-1 treatment in both soybean cultivars. Significantly high dry weight of seedlings was measured with RPB-1 for both cultivars (Table

Table 2. Influence of bacterial isolates on mean value of root, shoot and seedling length of soybean (cv. PRS -1, PS - 1092)

Treatment	Root length (cm)		Shoot length (cm)		Seedling length (cm)	
	PRS-1	PS-1092	PRS-1	PS-1092	PRS-1	PS-1092
RGB-1	18.00	20.40	15.77	17.33	33.77	37.73
RMV-1	20.70	22.50	17.53	20.17	38.23	42.67
RMV-2	19.26	21.10	18.10	20.63	37.37	41.73
RMV-3	18.23	20.67	15.90	18.43	34.13	39.10
RMV-4	18.73	21.40	16.13	17.43	34.87	38.83
RPB-1	20.46	25.93	16.40	18.63	39.86	44.57
RPB-3	18.23	21.20	16.10	17.90	34.33	39.10
RPB-4	18.10	20.37	16.27	18.77	34.36	39.13
RPB-5	17.83	19.93	15.66	17.93	33.50	37.87
RPV-4	18.86	20.70	16.20	18.20	34.73	38.90
Pf-102	18.16	21.43	16.60	18.87	34.77	40.30
Control	15.57	16.37	15.23	18.13	30.80	34.50
GM	18.76	20.00	16.32	18.54	35.06	39.54
SE(m)±	0.25	0.26	0.28	0.26	0.44	0.35
CD at 1%	1.01	1.04	1.11	1.03	1.74	1.38
CD at 5%	0.75	0.76	0.82	0.76	1.29	1.01
CV (%)	2.37	2.16	2.98	2.44	2.18	1.52

3). However, statistically lowest value was recorded with un-inoculated control. Similar studies have been reported by [12] in barley; Sushlow and Schroth [13] in sugarbeet and by [14] in spring wheat (*Triticum aestivum* L.) and Pea (*Pisum* spp.).

Vigor indices (germination per cent × seedling length and germination per cent × seedling dry weight) is an actual reflection of seedling vigor of any seed sample or seed lot. The result of Table 4 demonstrated markedly increased vigour index-I and II over the un-inoculated control in both cultivars through bacterial inoculation. The bacterial isolate RPB-1 exhibited maximum vigour index-I in cultivar PRS-1 and PS-1092 respectively, however significantly minimum value was observed with control. The influence of RPB-1 with respect to vigour index-II was found better in PRS-1 and least vigour index-II was computed in un-inoculated control. For PS-1092 the maximum vigour index-II was recorded for RMV-1 treatment and the least value was recorded with un-inoculated control. The increase in vigour index with bacterial inoculation is due to the mechanism of IAA production by rhizobacteria. The results were in accordance with the findings of [15] in banjok; [16] in lentil, [17] in pea and [18] in maize.

Table 3. Influence of bacterial isolates on mean value of fresh and dry weight of 20 seedling of soybean (cv. PRS -1, PS - 1092)

Treatment	Fresh weight of seedling (g)		Dry weight of seedling (g)	
	PRS-1	PS-1092	PRS-1	PS-1092
RGB-1	17.81	19.29	1.90	2.24
RMV-1	20.02	22.57	2.47	2.69
RMV-2	21.19	20.93	2.31	2.37
RMV-3	17.81	20.38	1.89	2.25
RMV-4	18.10	19.65	1.99	2.15
RPB-1	23.82	23.88	2.87	2.72
RPB-3	17.21	21.45	1.78	2.35
RPB-4	17.09	21.43	1.80	2.33
RPB-5	16.15	19.62	1.74	2.13
RPV-4	17.23	21.22	1.83	2.42
Pf-102	18.10	21.30	2.09	2.37
Control	14.96	15.58	1.52	1.65
GM	18.29	20.61	2.02	2.30
SE(m)±	0.40	0.48	0.80	0.72
CD at 1%	1.60	1.90	0.32	0.28
CD at 5%	1.18	1.41	0.23	0.21
CV (%)	3.84	4.05	6.87	5.44

Table 4. Influence of bacterial isolates on mean value of vigor index of soybean (cv. PRS -1, PS - 1092)

Treatment	Vigor index-I		Vigor index-II	
	PRS-1	PS-1092	PRS-1	PS-1092
RGB-1	3106.33	3383.30	175.44	200.07
RMV-1	3402.16	3806.87	219.57	236.84
RMV-2	3089.00	3297.20	190.99	187.06
RMV-3	2867.13	3284.50	158.28	185.45
RMV-4	3301.46	3572.27	188.77	227.81
RPB-1	3428.06	3819.07	246.59	233.17
RPB-3	2924.26	3401.10	153.22	204.40
RPB-4	2807.33	3300.13	147.32	195.86
RPB-5	3091.50	3496.53	160.14	195.32
RPV-4	3125.63	3488.20	164.93	217.38
Pf-102	3001.43	3478.93	183.36	204.65
Control	2380.90	2610.90	117.49	124.77
GM	3043.77	3411.58	175.43	201.06
SE(m)±	42.28	51.44	7.84	8.85
CD at 1%	167.23	203.47	31.03	35.01
CD at 5%	123.40	150.15	22.90	25.84
CV (%)	2.40	2.61	7.74	7.62

CONCLUSION

Under laboratory condition, results suggested that seed germination, seedling vigour and earlier germination of soybean were greatly affected by rhizospheric bacterial isolates, suggesting thereby the importance of PGPR in seed germination and subsequent seedling growth for achieving appropriate plant stand in field. Out of eleven bacterial isolates, RMV-4 and RPB-1 predominantly influenced most of the seed germination and seedling vigour parameters. These two isolates facilitated greater vigour under laboratory condition. However RGB-1, RPV-4, RPB-5, RMV-2 and RMV-1 also exhibited superior results in certain vigour tests conducted in this study. Hence, the PGPR, in general, promote seed germination and seedling vigour and hence may be referred to as biostimulants.

REFERENCES

1. PRASAD B, R PRASAD AND S PRASAD (2010). Seed quality variation in relation to pod and seed position on the mother plant of soybean (*Glycine max* L. Merrill) seed lot. *J. Crop & Weed*, **6(2)**: 1-5.
2. SINGH AV, S SHAH AND B PRASAD (2010A). Effect of phosphate solubilizing bacteria on plant growth promotion and nodulation in soybean (*Glycine max* (L.)Merr.). *J. Hill Agri.*, **1(1)**: 35-39.
3. KLOEPPER JW (1993).Plant growth promoting rhizobacteria as biological control agents.*In*: Meeting Jr. F.M. ed. Soil microbial ecology: application in agriculture and environment management. Marcel Dekker, Inc. New York. pp. 255-274.
4. KUMAR R AND R CHANDRA (2008). Influence of PGPR and PSB on *Rhizobium leguminesarum* BV. Viciae Strain Competition and symbiotic performance in lentil. *World J. Agri. Sci.*, **4(3)**: 297-301.
5. ABDUL-BAKI AA AND JD ANDERSON (1973).Vigour determination in soybean seeds by multiple criteria. *Crop Sci.*, **13**: 630-632.
6. SINGH AV AND B PRASAD (2014). Enhancement of plant growth, nodulation and seed yield through Plant Growth Promoting Rhizobacteria in Lentil (*Lens culinaris* Medik cv. VL125). *Int. J. Curr.Micro. Appl. Sci.*, **3(6)**: 614-622.
7. KUMAR BSD AND HC DUBE (1992). Seed bacterization with fluorescent pseudomonads for enhanced plant growth, yield and disease control. *Soil Biol. Biochem.*, **24**: 539-542.
8. PRASAD B, R PRASAD, AV SINGH AND S PRASAD (2009). Presowing seed inoculation of wheat (*Triticum aestivum* L. cv. VL 832) for seed yield and quality enhancement in North-West Himalayan agriculture system. *J. Crop and Weed*, **5(2)**: 80-86.
9. SINGH AV, B PRASAD AND S SHAH (2010B). Screening plant growth promoting rhizobacteria for improving seed germination and seedling vigor of lentil (*Lens culinaris* Medik). *Env.and Eco.*, **28(3B)**: 2055-2058.
10. SINGH AV, B PRASAD AND S SHAH (2011). Influence of phosphate solubilizing bacteria for enhancement of plant growth and seed yield in lentil. *J. Crop and Weed*, **7(1)**: 1-4.
11. ASHRAFUZZAMAN M, FA HOSSEN, MR ISMAIL, MA HOQUE, MZ ISLAM,SM SHAHIDULAB AND S MEON (2009).Efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afri. J. Biotechnol.*, **8(7)**: 1247-1252.
12. PRASAD B, R PRASAD, S PRASAD AND S KUMAR (2012). Effect of pre-sowing seed bio-inoculation on seed yield, its contributing characters and subsequent seed quality of barley (*Hordeum vulgare*). *Ind. J. Agri.Sci.*, **82(11)**: 946-951.
13. SUSLOW TV AND MN SCHROTH (1982). Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. *Phytopathol.*, **72**: 199-206.
14. ASTROM B AND B GERHARDSON (1988). Differential reaction of wheat and pea genotype to root inoculation with growth affecting rhizosphere bacteria. *Plant and Soil*, **109**: 263-269.
15. PRASAD B, R PRASAD AND VK SAH (2011). Pre-sowing seed treatment effect of bio-agents on seed germination and seedling vigor of banjok (*Quercus leucotrichophora* A. Camus). *Ind. J. Forestry*, **34(1)**: 71-74.
16. RAWAT NS, B PRASAD AND S SACHAN (2010). Enhancement of plant growth, seed yield and quality through pre-sowing microbial seed inoculation in Lentil (*Lens culinaris* Medik) cv. VL125. *Env. and Eco.*, **28(4A)**: 2515-2518.
17. MURALI G, A GUPTA AND SP MAGU (1999). Effect of neem based granules on growth attributes of soybean on inoculation with rhizobium and fluorescent Pseudomonads. *Ind. J. Agril Sci.*, **69**: 509-512.
18. NEZARAT S AND A GHOLAMI (2009). Screening plant growth promoting rhizobacteria for improving seed germination seedling growth of maize. *Pak. J. Biol.Sci.*, **12(1)**: 26-32.