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Screening of bioagents for seed biopriming in French bean (*Phaseolus vulgaris* L.) under Laboratory condition

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Abstract
An investigation was carried out during (2016-17) at laboratory of Department of Seed Science and Technology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-273230 (H. P.). Nine indigenous isolates of bioagents including three plant growth promoting rhizobacteria (PGPR-1, PGPR-2 & PGPR-3), three rhizobial biofertilizers (*Rhizobium* strain- B_1, *Rhizobium* strain- B_2 & *Rhizobium* strain- P_1) and three biocontrol agents (*Trichoderma harzianum, T. viride & T. virens*) were screened for their efficacy as seed biopriming under laboratory conditions following standard methods of seed quality and health testing as prescribed by ISTA. On the basis of these tests, PGPR-1 with maximum germination (), *Rhizobium* strain- B_1 with highest germination () and *T. viride* with maximum germination () were performed best among their respective isolates.

Keywords: Biopriming, PGPR, *Rhizobium*, *Trichoderma*, *Phaseolus vulgaris*, germination

Introduction
Biopriming is a technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects of disease control. It is recently used as an alternative method for controlling many seed and soil borne pathogens. It is an ecological approach using selected fungal antagonists against soil and seed borne pathogens. Biological seed treatments provide an alternative to chemical control with additional benefits of induced diseases resistance, eco friendly nature and sustainable disease management. Biopriming has great promise for enhancing the efficacy, shelf life, and consistent performance of bioagents (Callan *et al*., 1997) [3]. The most commonly studied genera of growth-promoting rhizobacteria include *Bacillus, Pseudomonas, Azospirillum* and rhizobia. Similarly, biopriming with *Trichoderma* spp. has yielded promising results for maintaining quality while reducing the need for chemical products that can harm the environment and non target organisms. Plant growth promoting rhizobacteria (PGPR's) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion and disease suppression. The PGPR's have been demonstrated to increase growth and productivity of many commercial crops (Saharan and Nehra, 2011) [9]. Their association is distributed to many plant species and is commonly present in varied environments. Effect on enhancement in plant growth by root-colonizing species of *Rhizobium* and *Pseudomonas* is well documented in literature (Sahni *et al*., 2008) [10]. *Trichoderma* has been exploited as biocontrol agent against a range of plant pathogenic fungi because it antagonizes a number of plant pathogens. Many strains of *Trichoderma* have been widely used as biological control agents as well as plant growth promoters (Harman, 2000) [7]. Keeping in view, the importance of the crop and the benefits of the seeds biopriming with beneficial microbes, the present investigation were done. Under which evaluate the efficacy of plant growth promoting rhizobacteria (PGPR's), rhizobial biofertilizers and biocontrol agents (BCA's) for seed biopriming on different seed quality parameters in French bean under laboratory conditions and also standardize the dose and duration of seed biopriming were worked out.

Materials and Methods

Source of material

Three isolates of PGPRs (PGPR-1, PGPR-2, PGPR-3), rhizobial biofertilizers (*Rhizobium* strain- B_1, *Rhizobium* strain- B_2, *Rhizobium* strain- P_1) and *Trichoderma* spp (*Trichoderma harzianum, Trichoderma viride, Trichoderma virens*) were used for seed biopriming. The seeds of French bean cv. Contender were collected from the Department of Seed Science and Technology, Nauni, Solan (HP).

Seed Biopriming

The seeds of French bean with no cracks were selected and surface sterilized with 1.5 % NaOCl solution for 5 minutes. Seeds were than rinsed thrice with sterilized distilled water and dried under laminar air flow on sterilized blotting paper. The surface sterilized and dried seeds were bioprimed by soaking in the spore suspensions of PGPR, Rhizobial fertilizer and *Trichoderma* spp. for 8 hrs separately. The seeds were shade dried to bring down their moisture content to original moisture content.

This experiment was conducted in year the 2016-2017 at Department of Seed Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The first experiment was conducted in Complete Block Design (CBD) with four replications. Ten treatments *viz.*, PGPR-1 (T_1), PGPR-2 (T_2), PGPR-3 (T_3), *Rhizobium* strain- B_1 (T_4), *Rhizobium* strain- B_2 (T_5), *Rhizobium* strain- P_1 (T_6), *Trichoderma harzianum* (T_7), *Trichoderma viride* (T_8), *Trichoderma virens* (T_9) and untreated control (T_{10}) were used for seed biopriming.

Results and Discussion

In-vitro screening of bioagents

Seed biopriming with the bioagents, especially PGPR-1, *Rhizobium* strain B_1 and *Trichoderma viride* resulted in significantly higher germination and vigour as compared their respective isolates of bioagents and untreated control. Out of a total of nine bioagents including 3 isolates each of plant growth promoting rhizobacteria, rhizobial biofertilizers and biocontrol agents used for biopriming of French bean seeds one best isolate was selected under each category on the basis of their effects on seed germination, speed of germination and seedling vigour index length (SVI-L) and mass (SVI-M).

Seedling Germination

Amongst PGPRs, PGPR-1 (T_1) resulted significantly higher germination (94.00%) and speed of germination (6.76) than other isolates. Amongst rhizobial biofertilizers, *Rhizobium* strain- B_1 (T_4) resulted significantly higher germination (95.50 %) and speed of germination (7.19) than other isolates. In biocontrol agents (*Trichoderma* spp.), biopriming with *Trichoderma viride* (T_8) resulted in significantly higher germination (93.00%) and speed of germination (6.08) than other *Trichoderma* isolates. Similar, results on seed germination upon biopriming with bioagents have been observed by earlier workers (Entesari *et al.*, 2013 and Kumar *et al.*, 2014) [5, 8] in crops like chickpea.

Seedling Vigour

PGPR-1 (T_1) resulted significantly higher seedling vigour index-length (2735.63) and seedling vigour index- mass (188066.00) from their respective isolates. Amongst rhizobial biofertilizers, *Rhizobium* strain- B_1 (T_4) resulted significantly higher seedling vigour index- length (2959.79) and seedling vigour index- mass (198792.60) than other isolates. biocontrol agents (*Trichoderma* spp.), biopriming with *Trichoderma viride* (T_8) resulted in significantly higher seedling vigour index- length (2676.24) and seedling vigour index- mass (146713.50) than other *Trichoderma* isolates. These results were accordance to Vishwa *et al.*, 2017 [12] in different crops.

The bioagents promote synthesis of growth regulatory substances like gibberellins, which trigger the activity of α-amylase enzyme and other germination specific enzymes like proteases and nucleases involved in hydrolysis and assimilation of the stored material upon seed imbibation (Bench and Sanchez, 2004) [1]. The present findings are, therefore, in accordance with the findings of Kumar *et al.* (2014) [8] and Vishwa *et al.* (2017) [12] who have reported that seed germination and vigour was significantly influenced by biopriming with PGPR and *Rhizobium* in chickpea. Biopriming of seeds not only increases rate and uniformity of germination, but also protects seeds against the soil and seed-borne pathogens. The variation of germination may be attributed to the difference in efficacy of bioagents which are used for biopriming. These workers have attributed this increase in speed of germinate onto the accelerated rate of cell division and also to the increased synthesis of hormones like gibberellins upon biopriming of seeds. The increased synthesis of GA would have triggered the activity of specific enzyme such as α- amylase, which might have brought an increase in availability of starch assimilation and influenced early germination (Bose and Mishra, 1992) [2]. Gholami *et al.* (2009) [6] have also reported the same results on speed of germination in maize upon seed biopriming with PGPRs and they have also attributes this increase to the physiological changes occurring in the seeds during biopriming.

Table 1: *In vitro* screening of PGPR, rhizobial fertilizer and BCA's for seed biopriming in French bean for seed germination and vigour

Treatment	Germination* (%)	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seedling vigour index-L (SVI-L)	Seedling vigour index-M (SVI-M)
T_1: PGPR-1	94.00 (9.74)	6.76	29.10	2000.70	2735.63	188066.00
T_2: PGPR-2	89.50 (9.51)	5.73	25.34	1920.60	2267.65	171918.50
T_3: PGPR-3	87.00 (9.38)	5.16	21.49	1579.690	1871.21	137461.00
T_4: *Rhizobium* strain B_1	95.50 (9.82)	7.19	30.99	2081.75	2959.79	198792.60
T_5: *Rhizobium* strain B_2	86.50 (9.35)	5.74	27.96	1655.71	2419.26	142942.40
T_6: *Rhizobium* strain P_1	87.50 (9.40)	6.26	26.03	1295.19	2278.21	113203.90
T_7: *Trichoderma harzianum*	87.00 (9.38)	5.21	26.83	1180.98	2334.52	102664.90
T_8: *Trichoderma viride*	93.00 (9.69)	6.08	28.78	1578.04	2676.24	146713.50
T_9: *Trichoderma virens*	86.50 (9.35)	5.14	27.78	1030.30	2402.72	89137.84
T_{10}: Untreated control	86.00 (9.32)	5.02	25.65	983.00	2206.73	84548.70
C.D. (0.01)	2.84 (0.15)	0.27	0.95	148.40	117.50	12259.50

*Figures in parentheses are square root transformed values

Seed mycoflora (%): Biopriming of seeds with different PGPRs, rhizobial biofertilizers and biocontrol agents showed minimum seed mycoflora (%) as compared to untreated control. Amongst all the PGPRs, minimum incidence of seed mycoflora (2.00 %) was recorded when seeds were bioprimed with PGPR-1 (T_1).Within rhizobial biofertilizers, *Rhizobium* strain- B_1 (T_4) recorded minimum incidence of seed mycoflora (1.00 %). Biopriming with *T. viride* (T_8) observed minimum incidence of seed mycoflora (2.00 %) from rest of the biocontrol agents. However, the maximum seed mycoflora incidence (5.00 %) was recorded in untreated control (T_{10}). In the present study, seed biopriming with strains PGPR-1, *Rhizobium* strain- B_1 and *Trichoderma viride* resulted in decrease of seed mycoflora incidence. The present findings are in accordance with the findings of Elad *et al.* (1980) [4] and Van Loon (2007) [11] who have reported reduction in the incidence of seed storage fungi due to the application of bioagents. Bioagents showed antagonistic activity against storage pathogens and contaminants i.e. *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp. Several cell wall degrading enzyme like chitinase and glucanase play an important role in antagonistic action of *Trichoderma* against a wide range of fungal pathogens (Elad *et al*., 1980; Van Loon, 2007) [4, 11]. However, PGPRs also synthesize hydrolytic enzymes, such as proteases and lipases that lyse pathogenic fungal cells.

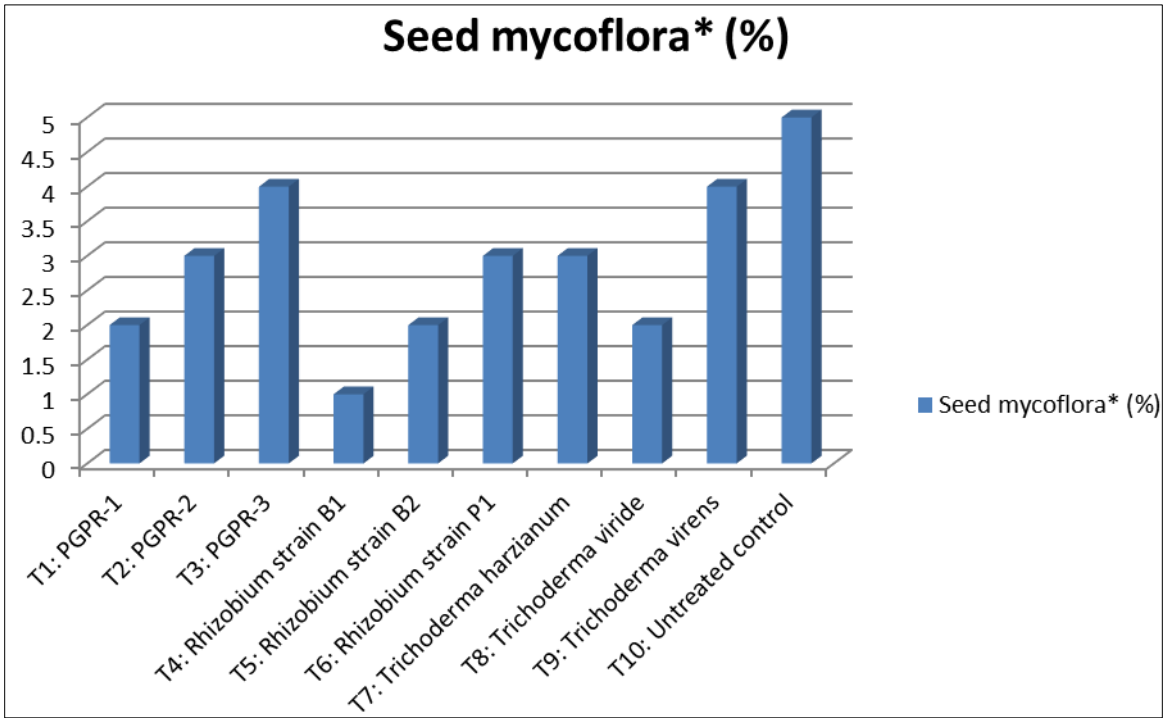

Fig 1: Incidence of seed mycoflora (%) in different treatments

Conclusion

On the basis of *in vitro* experiments, it is concluded that seed biopriming of French bean with PGPR-1, *Rhizobium* strain-B1 and *T. viride* provided better seed quality, germination and health parameters.

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