

Influence of Pre-Sowing Bio-Agent Seed Treatment on Seed Yield, its Contributing Characters and Subsequent Seed Quality in Barley (*Hordeum vulgare* L.) cv VLB-1 under Rainfed Conditions

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Abstract

Impact of pre-sowing seed treatment on seed yield, its contributing characters and subsequent seed germination and seedling vigor of barley was evaluated through bio-agent inoculation. The seeds were inoculated by *Pseudomonas fluorescens* (pf-173), *Trichoderma harzianum* at 4 g per kg seed and mixture of both bio-agents for (2g + 2 g) each for the same rate. All the inoculated treatments significantly affected the field germination, root length, plant height, length of the ear, number of grain/ear, 1,000-seed weight and ultimately yield at field level. However, seed produced through these inoculated treatments also significantly influenced the seed quality i.e. germination, root, shoots and seedling length, fresh and dry weight of seedlings, electrical conductivity, absorbance and transmittant of seed leachates and also vigor index at laboratory conditions. Seed inoculated with mixture of bio-agents performed the best as indicated by seed yield, its contributing character and most of the seed quality parameters. In addition *Pseudomonas fluorescens* and *Trichoderma harzianum* were also capable of enhancing the seed yield and quality at field and in controlled condition.

Key words : Bio-agents, Seed treatment, Seed yield, Quality, Barley.

Barley *Hordeum vulgare* L. (Vernacular-Jau or Jav), has six rows of grain and their arrangement, evolved from a wild species *Hordeum spontaneum* (1). It is the major source of food for large number of people living in a cooler, semi-arid areas of the world where wheat and other cereals are less well adapted. It is a staple food of the poor countries people like Tibet, Nepal and Bhutan (2). In India the chief barley growing region are mid to higher Himalayas, central parts of eastern Uttar Pradesh, eastern part of Rajasthan and north western parts of north Bihar. Plants resemble the wheat plant and usually grows 0.75 to 1.0 meter in height and belongs to family Poaceae. It requires cool weather during early growth and warm and dry weather at maturity and grows fairly well in temperate and in sub-tropical region of the earth. The crop has low water requirement, also name drought resistant suits areas with scanty rainfall. In Uttarakhand, barley is a major crop along with rice, wheat and small millets and importance of barley cultivation in higher Himalayas goes up in most of the

rainfed areas. This is the main crop livelihood and economy of Uttarakhand farmers. But the soils in most farming situations are poor in organic matter, low in pH and phosphorus content. These poor soil conditions do not support a better yield and disease resistance. To improve the yield and disease suppression farmers resort to inorganic fertilizers and pesticides, which often have hazardous effects and also do not fit into the frame work of organic farming. An alternative approach is to use bio-control agents which are known to augment plant health through increasing the planting value of seed, better germination, vigor, and increasing the seed yield and yield contributing character with better seed quality. Some bacteria associated with the roots of crop plant can exert beneficial effects on their roots and they are often collectively referred to as plant growth promoting rhizobacteria (PGPR). Bio-control of phyto-pathogens by PGPR appears to be major mechanism of plant growth promotion and in addition to bio-fertilization which may contribute to enhance plant growth as

Table 1. Influence of bio-agent pre-sowing seed treatment on seed yield and its contributing characters in barley (*Hordeum vulgare* L.) cv VLB-1 under rainfed conditions. T₁= *P. fluorescens* (Pf-173) at 4 g/kg. seed, T₂= *T. harzianum* at 4 g/kg. seed, T₃= *P. fluorescens* + *T. harzianum* each at 2 g/kg. seed, T₄= Control (uninoculated).

Treat- ments	Field germi- nation (%)		Root length (cm)		Plant height (cm)		Length of ear (cm)		No. of grain/ear		1000-seed wt (g)		Seed yield (q/ha)	
	2005- 06	2006- 07	2005- 06	2006- 07	2005- 06	2006- 07	2005- 06	2006- 07	2005- 06	2006- 07	2005- 06	2006- 07	2005- 06	2006- 07
T ₁	67.33	71.66	6.93	7.03	66.63	70.86	7.00	8.23	62.40	71.80	44.75	46.53	19.49	21.99
T ₂	68.66	73.33	6.83	6.90	68.46	72.86	6.90	8.09	61.60	69.06	45.11	46.59	19.73	21.88
T ₃	69.00	71.00	6.90	7.10	67.76	71.46	6.83	8.00	63.90	72.50	45.25	47.23	20.08	22.71
T ₄	62.00	63.33	6.26	6.33	62.20	64.33	6.26	7.13	57.56	60.96	41.63	42.72	17.79	18.15
Gm	66.75	69.83	6.73	6.84	66.26	69.88	6.75	7.86	61.36	68.58	44.18	45.77	19.27	21.18
SE	0.81	0.31	0.79	0.60	0.75	0.29	0.51	0.53	0.40	0.72	0.31	0.26	0.16	0.34
CD at														
5%	2.80	1.10	0.27	0.21	2.62	1.00	0.17	0.18	1.41	2.50	1.07	0.92	0.56	1.19
CV	2.10	0.79	2.04	1.54	1.98	0.72	1.32	1.17	1.15	1.82	1.21	1.01	1.47	2.82

suggested earlier (3—5). Here two groups of PGPRs i.e. *Pseudomonas fluorescens* pf-173 and *Trichoderma harzianum* have recorded considerable attention and have been tested on seed yield and quality of barley. This present study was designed to address the following issues : To assess the seed yield and their contributing characters, and to assess the subsequent seed quality parameters.

Methods

The experiment were conducted during 2005-06 and 2006-07 of two successive *rabi* seasons at the Seed Science and Technology Research Block, GBPUA & T, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India. The breeder seed of barley cv VLB-1 was obtained from Vivekanand Parwariya Krishi Anushandhan Sansthan, Almora, Uttarakhand, India and both the bio-agents i.e. *Pseudomonas fluorescens* pf-173 and *Trichoderma harzianum* were received from Plant Pathology Division of Hill Campus, Ranichauri, Uttarakhand, India. The seed quality parameters were assessed at Seed Science and Technology Laboratory, GBPUA & T, Hill Campus, Ranichauri, Uttarakhand, India.

The talc based formulation of *P. fluorescens* pf-173 and *T. harzianum* were used for seed treatment. Seeds to be treated were weighed and moistened with distilled water. Any excess water was drained. Bio-agents i.e. *P. fluorescens* pf-173 and *T. harzianum* were added at 4 g per kg of seed and made treatment first and second (T₁ and T₂) while third treatment (T₃) was

made by mixing of these bio-agents in 50 : 50 ratio and used at 4 g (2 g + 2 g each) per kg of seed. However, untreated seed was considered as a control i.e. T₄. Added bio-agents to the seed were mixed gently so that the bacterial cells in the formulation get absorbed on seed surface and subsequently seeds were air dried in shade. The experiments were laid out in a randomized block design with three replications. Each row of 3.5 meter with row to row spacing of 25 cm and plant to plant of 5 cm were kept.

To know the field germination per cent, proportion of germinated seed to the total number of seed sown in a row was counted after 20 days of sowing. Observations were recorded for every row on 20 randomly selected ear heads/plant. Before harvesting selected plants were uprooted for recording root length, plant height and length of the ear. Number of grain/ear was counted through randomly selected 20 ear head harvested from each row of each plot. Crops were threshed, cleaned, sun dried and weighed to assess the seed yield (q/ha). For 1,000-seed weight, random sample of 1,000 well filled grain from the bulk produce of each plot was weighed.

After the field trial, harvested seeds were subjected to laboratory test for evaluating the seed quality parameters in a completely randomized block design. To assess the seed germination percent, 100 seeds of three replications for each treatment were tested. The seeds were kept between papers and then placed for germination at 20 C. Normal seedlings were recorded on day 7 and germination was expressed as the percentage of seed which produced normal seed-

lings (6). After the germination count, ten normal seedlings from every replication of each treatment were used to measure the root, shoot, seedling length, fresh weight and dry weight of seedlings. Seedling vigor index (SVI) I and II were derived by multiplying percent germination with total seedling length and dry weight of seedling respectively (7). To measure the electrical conductivity, absorbance and transmittant of seed leachates, 5 g seeds were surface sterilized with 0.1% mercuric chloride solution in three replicates of each treatment. After washing the seeds were soaked in 25 ml distilled water for 24 hours. The leachates were measured for electrical conductivity with the help of electrical conductivity meter, while absorbance and transmittance were measured with spectrophotometer at 480 nm wave length at room temperature.

Results and Discussion

The influence of pre-sowing bio-agent seed treatment on seed yield and its contributing characters for barley is presented in Table 1, while seed quality parameters are given in Table 2. The results were subjected to analysis of variance and tested for significant differences at 5% level of significance (8).

Field Parameter

The seeds treated with *P. fluorescens*, *T. harzianum* and mixture of both the bio-agents had significantly more number of plants in the field as compared to the control treatment in both the years.

Among the treatments difference in terms of field stand was non-significant. Seed treatment with *P. fluorescens* increased seedling emergence rate in winter wheat was reported by Freitas and Germida (9) and with *T. harzianum* increased percentage of emergence as compared to control. Similar observation was found in tomato by Ahmad and Baker (10).

Significant impact of the bio-agents was observed on root length and on plant height over control in both the years. The maximum root length (6.90 and 7.10 cm) was found in T₃ (mixture of both bio-agents), which non-significantly differed with other treatments, while the maximum plant height (68.46 and 72.86 cm) was observed for *T. harzianum* in two successive years and the results of 2006-07 were significantly greater over other treatments. Manoranjitham et al. (11) reported that application of talc based formulation of *T. viride* and *P. fluorescens* increased the root, shoot and biomass production of tomato seedlings.

All the treatments induced significantly more length of the ear and number of grain per ear was recorded compared to the control (Table 1). *P. fluorescens* (pf-173) was found to be more affective on ear length while the significantly higher number of grain per year (63.90 and 72.50) was recorded for T₃ in both the years over other treatments. Similar of findings have been obtained in tomato by Amara et al. (12).

Significant increase in 1,000-seed weight and seed yield per hectare was observed for all the treatments over control and greatest influence had recorded for T₃ (45.25 g and 47.23 g) for test weight and

Table 2. Influence of bio-agents on subsequent seed quality in barley (*Hordeum vulgare* L. Cv. VLB-1). T₁= *P. fluorescens* (Pf-173) at 4 g/kg seed, T₂ = *T. harzianum* at 4 g/kg seed, T₃ = *P. fluorescens* +*T. harzianum* each at 2 g/kg seed, T₄ = Control (uninoculated).

Treatments	Germination (%)		Seedling root length (cm)		Shoot length (cm)		Total seedling length (cm)		Fresh weight of 10 seedlings (g)		Dry weight of 10 seedlings (g)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
T ₁	89.33	91.00	17.06	18.00	15.26	15.90	32.33	33.90	3.71	3.95	0.30	0.31
T ₂	87.66	89.33	16.23	17.33	13.63	13.96	30.86	31.30	3.23	3.45	0.27	0.28
T ₃	90.33	92.66	17.46	18.03	15.10	15.36	32.56	33.40	3.54	3.68	0.29	0.29
T ₄	81.33	82.00	14.13	14.66	12.16	12.30	29.23	26.96	2.97	3.06	0.23	0.24
Gm	87.16	88.75	16.22	17.00	14.04	14.38	31.24	31.39	3.36	3.53	0.272	0.282
SE	0.78	0.68	0.25	0.21	0.15	0.11	0.37	0.21	0.04	0.04	0.004	0.005
CD at 5%	2.54	2.23	0.81	0.71	0.51	0.38	1.22	0.68	0.14	0.12	0.013	0.016
CV	1.55	1.34	2.67	2.21	1.95	1.41	2.09	1.16	2.30	1.94	2.57	3.065

Table 2. Continued.

Treatments	Electrical conductivity microphose/cm ²		Absorbance of seed leachates at 480 nm wave length		Transmittant sed leachates at 480 nm wave length		Vigor index-I(SG × seedling length)		Vigout index-II (SG × seedling dry weight)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
T ₁	0.28	0.28	0.033	0.030	77.03	79.86	2887.56	3084.93	26.89	28.85
T ₂	0.29	0.28	0.040	0.036	73.40	74.80	2618.13	2796.10	24.69	25.66
T ₃	0.26	0.25	0.030	0.026	82.33	83.30	2942.20	3095.26	26.68	27.67
T ₄	0.35	0.35	0.063	0.070	67.66	68.50	2377.73	2229.06	19.57	20.03
Gm	0.299	0.293	0.041	0.040	75.108	76.166	2706.408	2801.34	23.957	25.555
SE	0.002	0.002	0.002	0.002	0.766	0.509	35.697	27.533	1.069	0.494
CD at 5%	0.009	0.009	0.007	0.007	2.499	1.660	116.349	89.740	3.484	1.611
CV	1.67	1.70	9.79	9.99	1.76	1.15	2.228	1.702	7.729	3.350

for seed yield (20.08 and 22.71 q/ha) in both the years respectively. The values for these two characters differed non-significantly among the treatments. These findings have been confirmed by Dileep et al. (13) in rice, groundnut and total yield increased by 26.2% over control by seed coating with *T. harzianum* in tomato by Sivan et al. (14).

Laboratory Parameters

Seed germination significantly influenced by the bio-agent seed treatments and it is revealed that highest germination (90.33 and 92.66%) was recorded for T₃ during both the years. The subsequent growth of seedlings i.e. root, shoot and total seedling length were also significantly affected by the product of bio-agent treated seed and maximum root length and seedling length was noticed for *P. fluorescens* and *T. harzianum* bio-agent mixture (T₃), while more value for shoot length was shown in *P. fluorescens* treated seeds in both the years. The effect of bio-agent seed treatment on fresh and dry weight of seedling was positively significant over control. The greatest value was recorded for fresh and dry weight of seedling in *P. fluorescens* over all other treatments. An increased seed germination, shoot length, root height, fresh weight and dry weight have also been reported by Kumar (15) through seed bacterization with fluorescent *Pseudomonas* and through *T. viride* and *P. fluorescens* by Monoramhitham et al. (16) in tomato seedlings. The electrical conductivity and absorbance of seed leachates are negatively correlated with the seed vigor and significantly least value was shown in

T₃ over other treatments, while transmittant of seed leachates was also significantly greater for T₃ in both the years over all the treatments, which showed linear correlated with seed vigor.

Seed vigor index (germination % × seedling length) and (germination % × dry weight of seedling) exhibit seedling vigor of a seed/seed lot. *P. fluorescens* + *T. harzianum* mixture resulted in the maximum value of seedling vigor index I compared to other treatments. However, vigor index II exhibited highest in *P. fluorescens* treated seed compared to other treatments while vigor index was extremely influenced by the bio-agents inoculation.

Seed coating with biological agents is the most common strategy used in many crops for a variety of purposes. Bio-fertilizers and bio-fungicides include many types of bacteria and fungi. Out of these *P. fluorescens* and *T. harzianum* are extensively investigated for plant growth promoting activities to various agricultural crops (17). It is concluded that pre-sowing seed treatment with that *P. fluorescens*, *T. harzianum* or mixture of both bio-agents at 4 g/kg talc powder induced field germination, better seed yield, yield contributing characters and further seed quality in barley cv VLB-1. Increase in these characters by seed inoculation of bio-agent might be due to activities of plant growth promotion, biological control of various diseases activation of disease resistance in the host plant and direct or indirect way of resulting in stimulation of growth of plant by nutrients secreted by the plant root. This study is in close agreement with earlier observation in carnation (18), radish and tomato (19) with *P. fluorescens*. Similar re-

sults have also been reported by Etebarian et al. (20) and Durman et al. (21) in tomato against *Phytophthora erythroseptica* and *Rhizoctonia solani* respectively with *T. harzianum*.

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