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Research article

Influence of bio-priming on seed quality and yield in berseem

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Abstract

An experiment was conducted in berseem with nine treatments and three replications *viz*., T_1 (Control), T_2 (Hydro-priming), T_3 (*Rhizobium*), T_4 (Phosphate solubilising bacteria), T_5 (Endophyte), T_6 (*Azotobacter*), T_7 (Enterobacter), T_8 (*Rhizobium* + Phosphate solubilising bacteria), T_9 (Phosphate solubilising bacteria + *Azotobacter*) to record its seedling vigour and crop establishment in combination with microbial inoculants. Seeds were soaked in water (hydro-priming) and microbial solution (bio-priming) for 12 hours. The results revealed that bio-priming of berseem seeds significantly impacted seed yield and quality. Seed bio-priming with *Azotobacter* at 2 g/kg of seeds for 12 hours recorded significantly higher values for germination percentage (97.00%), shoot length (6.02 cm) and seedling vigour index (987) when tested for initial seed quality parameters before sowing. Whereas seed bio-priming with phosphate solubilising bacteria at 2 g/kg of seeds for 12 hours (T_4) had significantly increased plant growth traits such as plant height (87.23 cm), branches per plant (52.33), leaf-to-stem ratio (0.74 g), green biomass at 60 DAS (232.62 g) and same treatment (T_4) also recorded maximum values for seed yield contributing traits like number of heads (4.70), number of seeds (50.20), 1000-seed weight (3.05 g), seed yield per plant (0.67 g), net plot yield (0.15 kg) and seed yield per ha (193.11 kg). The change in seed yield/ plot over control was 66.67% during *Rabi* season at Dharwad.

Keywords: Berseem clover, Bio-priming, Phosphate solubilising bacteria, *Pseudomonas*, Seed quality, Seed yield

Introduction

Berseem (*Trifolium alexandrinum* L.) 2n=2x=16, known as 'King of forage crops', is a low-input and highly productive annual winter forage legume crop grown on an area of 2 million hectares (Vijay *et al*., 2017; Kantwa *et al*., 2019). It is well adapted to Central, Northern and North-Western parts of India. Berseem is a highly esteemed fodder crop with a special place in animal husbandry programmes throughout the country. Berseem has high digestibility (up to 65%), high palatability and good forage quality with 20% crude protein (Bahukhandi *et al*., 2017). As a multicut annual forage crop, berseem provides high-quality green forage for longer durations. This crop offers re-growth after every cut at 25-30 days. Hence, it is very popular among small and dairy farmers due to its high green fodder yield (65-80 t/ha) and faster regeneration (Vijay *et al*., 2017).

Bio-priming of seeds has been used in many leguminous crops, which has shown a good effect on crop establishment and growth, apart from giving resistance against biotic and abiotic factors. As a leguminous forage crop, berseem fixes the atmospheric nitrogen through symbiotic association with *Rhizobium* bacteria. The symbiotic association is affected by many environmental factors, especially soil temperature. However, information on *Rhizobium* diversity in Indian soil, berseem-*Rhizobium* association under high soil temperature and different growth stages are not available for developing sustainable agriculture. The information on the response of berseem to microbial inoculants in general and *Rhizobium*, phosphate solubilising bacteria, *Azotobacter*, is meagre. Therefore, an experiment was conducted to assess berseem's seedling vigour and crop establishment in combination with microbial inoculants at Dharwad, Karnataka, India.

Materials and Methods

Location of the experiment and climate: The experiments were conducted in the laboratory and field from 2020 to 2021 at Southern Regional Research Station, ICAR-Indian Grassland and Fodder Research Institute, Dharwad. Geographically, the station is situated in the North Transition Zone (Zone-8) of Karnataka state at 15°27′ N latitude and 75°00′ E longitude and at an altitude of 750.0 meters above mean sea level. During the experiment, the maximum rainfall (5.37 mm) was received in the month of April. The minimum temperature during the winter months (*Rabi* season) of December ranged around 12.56°C. A maximum temperature (36.07°C) and minimum relative humidity (23.29%) were observed in the month of March. Meanwhile, the maximum relative humidity (85.14%) was noticed in February.

***Treatment details*:** Berseem variety 'Wardan' seeds and microbial inoculants were collected from Indian Grassland and Fodder Research Institute, Jhansi. Suspension of microbial agents was prepared by mixing 2 g of lignite formulation of microbial agents in 100 ml of water to get two per cent of concentration. Berseem seeds were soaked in this microbial suspension for 12 hours. After soaking, seeds were spread on the floor to drain off excess water. Later seeds were shade dried and kept ready for sowing. Seeds were soaked in distilled water for 12 hours for hydropriming treatment. These seeds were sown in the field using a randomised block design with three replications in *Rabi* season to evaluate the performance of these microbial inoculants on seed quality and seed yield. Seeds without microbial inoculant priming were used as a control. The experiment consisted of 9 treatments *viz*., T_1 (Control), T_2 (Hydro-priming), T_3 (*Rhizobium*), T_4 (Phosphate solubilising bacteria), T_5 (Endophyte), T_6 (*Azotobacter*), T_7 (Enterobacter), T_8 (*Rhizobium* + Phosphate solubilising bacteria), T_9 (Phosphate solubilising bacteria + *Azotobacter*).

***Land preparation and manure application*:** The field was prepared by ploughing and levelling operations, and the layout was prepared in three blocks. In each block, nine plots were accommodated with two irrigation channels. Nitrogen @ 20 kg ha^{-1}, Phosphorus @ 60 kg ha^{-1} and Potassium @ 40 kg ha^{-1} were uniformly applied for all the plots after layout preparation and mixed into soil manually. After the basal application of fertilizers, seeds were sown manually and impounded the water in all the plots. The treated berseem seeds were sown in the plots as per the treatment with a specified seed rate on 14th December 2020.

***Data recording and analysis*:** Data were recorded on initial seed quality parameters (immediately after treatment but before sowing) such as germination, speed of germination, shoot length, root length, seedling vigour index, thousand seed weight and field parameters like field emergence percentage, plant height, tillers per plant, branches per plant, leaf to stem ratio, days to 50 per cent flowering, days to maturity, green biomass, dry biomass, number of nodules per plant, chlorophyll content, number of heads per plant, number of seeds per head, seed yield per plant and seed yield per ha. Seed quality parameters were also recorded with the harvested seeds. Data obtained were analysed using analysis of variance (ANOVA). The significance of treatment effect was analysed by following a randomized block design (RBD) analysis (Gomez and Gomez, 1984) whereas laboratory data were subjected to a completely randomized design (CRD) analysis.

Results and Discussion

***Initial seed quality parameters*:** The highest germination (97.0%), shoot length (6.02 cm) and seedling vigour index (987) were observed in seeds bio-primed with *Azotobacter* (T_6). *Enterobacter* (T_5) recorded higher values for speed of germination (25.73) and root length (4.57 cm). At the same time, unprimed control (T_1) seeds recorded the least germination (89.0%), speed of germination (22.33), shoot length (5.15 cm), root length (3.61 cm) and seedling vigour index (819; Fig 1-3). The mean thousand seed weight of 3.2 g was observed in unprimed control seeds before sowing in berseem. Seed bio-priming with different microbial inoculants showed a significant difference in all seed quality parameters. Seed priming might have initiated the important metabolic processes favouring increase in germination per cent, shoot length, root length and seedling vigour index in berseem. Similar results were reported by Adnan *et al*. (2020) in forage sorghum with hydro-priming, which significantly increased dry weight, root length, shoot length and vigour index. Priming of seeds led to an increase in cell division, which in turn increased speed of emergence of the forage sorghum and better performance of bean seeds was recorded due to hydro-priming (Singh *et al*., 2015). Soaking seeds in bacterial suspension initiated the seed's physiological processes, but plumule and radicle emergence was arrested (Damalas, 2019).

***Plant growth and yield parameters*:** Amongst different seed bio-priming treatments, maximum plant height (87.23 cm), number of tillers per plant (5.83) and number of branches per plant (6.13) were observed with seeds treated with phosphate solubilising bacteria (T_4) when compared to control (T_1) which recorded 72.23 cm of plant height, 4.17 of tillers per plant and 5.37 of branches per plant, respectively (Tabe 1). The highest leaf-to-stem ratio of 0.74 was exhibited by the plants treated with phosphate solubilising bacteria (T_4) and endophyte (T_5), whereas control (T_1) seeds showed the least leaf-to-stem ratio value (0.40). Bio-priming with phosphate solubilising bacteria (T_4) recorded maximum green biomass of 232.67 g and dry biomass of 45.33 g at 60 days

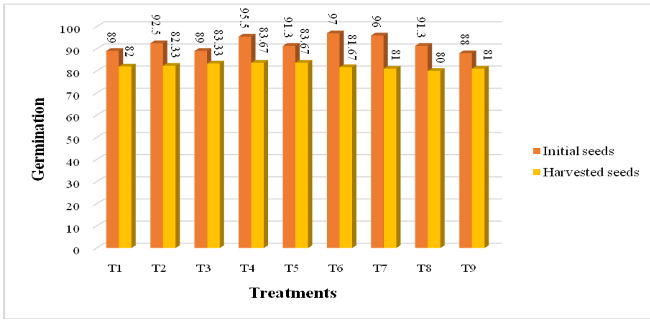

Fig 1. Effect of seed biopriming on germination (%)

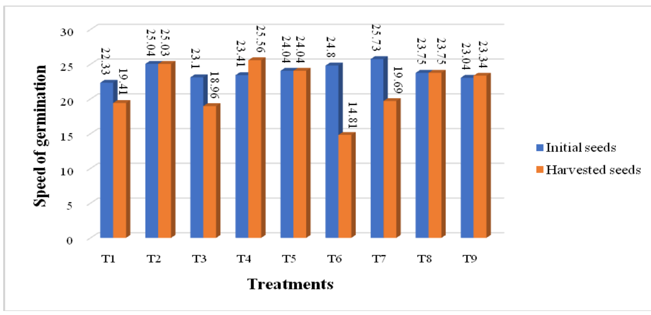

Fig 2. Effect of seed biopriming on speed of germination

after sowing, while 126.67 g of green biomass and 27.15 g of dry biomass were recorded in control (T_1; Table 1). Maximum chlorophyll content was obtained for seeds treated with phosphate solubilising bacteria (T_4) (52.33), and the minimum was recorded in control (T_1)

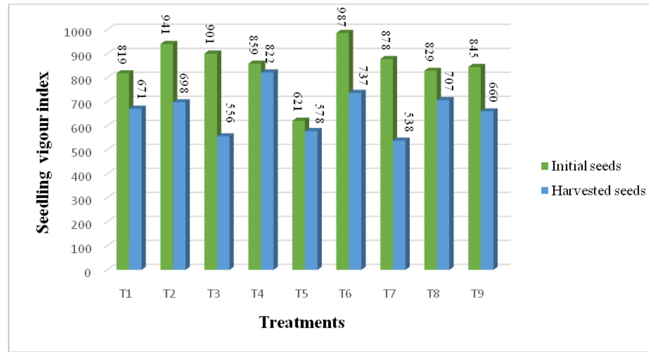

Fig 3. Influence of seed biopriming on seedling vigour index

(43.33). Significant differences in the above attributes were observed due to seed bio-priming with different microbial inoculants. Inoculation with phosphate-solubilising bacteria on phosphorus uptake by plants could be attributed in part to microbial alteration of one or more of the critical factors influencing the concentration of phosphorus in soil solution and the absorption of phosphorus by plants. The prime source of phosphatase activity, which mineralizes organic phosphorus in soils, is typically considered to be of microbial origin (Tian *et al.*, 2021). These phosphorus-solubilising microorganisms could release organic acids and solubilize soil insoluble phosphorus. They increase the mobility of phosphorous elements in soil by changing the surrounding acidity and by enzymatic processes (Puchalka *et al.*, 2008). Increased phosphorus absorption improved plant

Table 1. Influence of microbial inoculants on growth attributes in berseem

Treatment	Plant height at maturity (cm)	Change over control (%)	Tillers/ plant at 60 DAS	Branches/ plant	Leaf to stem ratio	Days taken for 50 % flowering	Days to maturity	Green biomass at 60 DAS (g)	Change over control (%)	Dry biomass at 60 DAS (g)	Change over control (%)
T_1	72.23	-	4.17	5.37	0.40	72.00	100.67	126.67	-	27.15	-
T_2	78.07	8.085	4.03	5.80	0.66	73.00	101.33	164.00	29.47	34.85	28.36
T_3	80.27	11.131	5.60	6.04	0.61	74.00	104.00	182.67	44.21	36.76	35.40
T_4	87.23	20.767	5.83	6.13	0.74	74.33	104.67	232.67	83.68	45.33	66.96
T_5	82.33	13.983	5.77	6.07	0.74	74.33	105.33	228.67	80.52	43.55	60.41
T_6	74.20	2.727	5.00	5.86	0.44	73.00	104.00	146.00	15.26	35.76	31.71
T_7	79.97	10.716	4.33	6.01	0.62	74.67	103.00	138.67	9.47	32.38	19.26
T_8	78.20	8.265	4.67	5.72	0.70	74.67	105.67	177.33	39.99	38.25	40.88
T_9	79.13	9.553	5.17	4.87	0.45	73.67	100.67	118.67	-6.32	24.82	-8.58
SEM	2.61		0.43	0.20	0.04	0.71	1.86	16.13		4.19	
CD ($P<0.05$)	7.81		1.29	0.61	0.11	NS	NS	48.34		12.55	

T_1: Control; T_2: Hydropriming for 12 hr; T_3: *Rhizobium* 2g/kg seed for 12 hr; T_4: Phosphate solubilising bacteria 2g/kg seed for 12 hr; T_5: Endophytes (*Pseudomonas fluorescence*) 2g/kg seed for 12 hr; T_6: *Azotobacter* 2g/kg seed for 12 hr; T_7: *Enterobacter* 2g/kg seed for 12 hr; T_8: *Rhizobium* + Phosphate solubilising bacteria 2g/kg seed for 12 hr; T_9: Phosphate solubilising bacteria + *Azotobacter* 2g/kg seed for 12 hr; NS: Non-significant growth and yield (Antoun *et al.*, 1996). Phosphorus solubilising microorganisms enhances plant growth and development through various mechanisms such as the release of important growth-promoting substances, the increase in soil availability of other micronutrients as well as the enhancement of nitrogen fixation efficiency (Zaidi *et al.*, 2010; Rahman *et al.*, 2017). For natural growth and development, *Trifolium* requires an adequate and absolute source of phosphorus needed for the cell (Puchalka *et al.*, 2008). An increase in phosphorus absorption boosted plant nutrition, and possibly the further development of roots in soil is the cause of the further growth of *Trifolium alexandrinum* stalk (Janati *et al.,* 2021). Seeds treated with endophyte bio-priming (T_5) had the highest nodule count at 103.07 per plant, compared to just 7.53 nodules in unprimed control seeds (T_1). This result aligned with Hassan *et al.* (2017), who reported that phosphorus solubilising bacteria inoculation improved various growth parameters in mung beans including plant height, branches, leaves, pods, seeds, pod length, 1000-seed weight and effective nodules. Nogales *et al.* (2008) claimed that *Pseudomonas putida* can adjust the soil nitrogen for the root and supply the root with amino-acidi compounds. Betlach *et al.* (1981) reported that *Pseudomonas fluorescence* bacterium can assimilate nitrate, change it to ammonium, and transfer it actively. *Pseudomonas* species amplified the number of nodules, dry weight of nodules, nutrient availability and uptake in soybean crop (Son *et al*., 2006). *Pseudomonas* strain add to symbiotic properties measured in terms of number of nodules, weight of nodules, leghaemoglobin content and nitrogenase activity, which eventually enhances plant growth and yield (Janati *et al.,* 2021).

A higher number of heads per plant was obtained by the seeds bio-primed with phosphate solubilising bacteria (T_4), which recorded 4.70 heads per plant, and the lowest number of heads, 3.67 per plant, was observed in control (T_1) seeds. Seed inoculation with phosphate solubilising bacteria (T_4) and endophytes (T_5) recorded a maximum number of 50.20 seeds per head, and a significantly lower number of seeds per plant (39.53) were recorded with hydro-primed (T_2) seeds. The highest thousand seed weight was recorded with seeds treated with phosphate solubilising bacteria (T_4) with 3.05 g compared to unprimed control (T_1) seeds, which recorded 2.57 g of thousand seed weight (Table 2). Among all the treatments, the plots sown with seeds bio-primed with phosphate solubilising bacteria (T_4) performed very well and recorded 0.67 g of seed yield per plant and control (T_1) recorded least (0.57 g). Seeds treated with phosphate solubilising bacteria (T_4) showed a 17.54 per cent increase in seed yield per plant over control. Net plot yield (0.15 kg) was significantly highest in seeds treated with phosphate solubilising bacteria (T_4), and 0.09 kg of net plot yield was recorded in control (T_1). Seeds treated with phosphate solubilising bacteria (T_4) showed a 66.67 per cent increase in seed yield per net plot over control. Maximum seed yield per ha (193.11 kg) was recorded with phosphate solubilising bacteria (T_4), while control (T_1) plots recorded 115.90 kg of seed yield per ha (Table 2). Plots sown with seeds bio-primed with phosphate solubilising bacteria (T_4) showed an 83.68 per cent increase in green biomass

Table 2. Influence of microbial inoculants on seed yielding attributes in berseem

Treatment	Number of nodules/ plant	Chlorophyll content	Number of heads/ plant	Number of seeds/ head	1000 seed weight (g)	Seed yield/ plant (g)	Change over control (%)	Net plot yield (kg)	Change in seed yield/ plot over control (%)	Seed yield/ha (kg)
T_1	7.53	43.33	3.67	44.73	2.57	0.57	-	0.09	-	115.90
T_2	7.07	43.67	3.93	39.53	2.58	0.51	-10.53	0.08	-11.11	106.27
T_3	90.13	51.33	4.50	49.67	2.84	0.55	-3.51	0.13	44.44	165.47
T_4	43.09	52.33	4.70	50.20	3.05	0.67	17.54	0.15	66.67	193.11
T_5	103.07	51.67	4.60	50.20	2.27	0.64	12.28	0.14	55.56	184.96
T_6	34.93	48.33	4.43	44.07	2.75	0.38	-33.33	0.09	0.00	120.63
T_7	37.87	46.67	4.50	50.00	2.53	0.43	-24.56	0.11	22.22	140.28
T_8	39.20	49.67	4.00	50.10	2.99	0.63	10.53	0.12	33.33	158.33
T_9	8.47	47.67	3.97	39.53	2.85	0.42	-26.32	0.08	-11.11	106.21
SEM	2.30	2.02	0.46	2.00	0.12	0.02		0.004		20.73
CD (P<0.05)	6.88	6.05	1.37	5.98	0.49	0.05		0.011		62.14

T_1: Control; T_2: Hydropriming for 12 hr; T_3: *Rhizobium* 2g/kg seed for 12 hr; T_4: Phosphate solubilising bacteria 2g/kg seed for 12 hr; T_5: Endophytes (*Pseudomonas fluorescence*) 2g/kg seed for 12 hr; T_6: *Azotobacter* 2g/kg seed for 12 hr; T_7: Enterobacter 2g/kg seed for 12 hr; T_8: *Rhizobium* + Phosphate solubilising bacteria 2g/kg seed for 12 hr; T_9: Phosphate solubilising bacteria + *Azotobacter* 2g/kg seed for 12 hr at 60 DAS and a 66.96 per cent increase in dry biomass at 60 DAS over control (T_1).

Significant differences in the above attributes were observed due to seed bio-priming with different microbial inoculants. Similar observations were recorded by Hassan *et al*. (2017) in mung bean**,** who reported that inoculation with phosphorus solubilising bacteria enhanced the growth of plant and quality parameters such as number of seeds per plant (2.56 fold) and 1000 seed weight. The application of these microbial inoculants to nutrient-deficient soils, where immense amounts of nutrients are fixed in inaccessible forms, might have aided in increased crop yield via more rational use of phosphorous fertilizers besides an increase in biological nitrogen fixation, and this fact was confirmed by Belimov *et al.* (1995). The seed bio-priming in berseem with endophyte and phosphorus solubilising bacteria resulted in better nodulation, which relocated into higher shoot nitrogen accumulation and pronounced number of heads per plant. The hike in seed yield of berseem might probably be due to a rise in physiological activities, such as the synthesis of carbohydrates and amino acids and the translocation of photosynthates into developing heads in bio-primed seeds.

Further, it increased the number of seeds per head, seed yield per plant, and seed yield per ha in berseem. A strong positive correlation (0.736) was found between seed yield per plant and net plot yield. This indicated that an increase in seed yield per plant is strongly associated with an increase in overall net plot yield (Fig 4). A moderate positive correlation (0.569) was observed between the number of seeds per head and the seed yield per plant. This suggested that as the number of seeds per head increases, the seed yield per plant also tends to increase. In comparison, the positive correlation between the number of seeds per head and 1000 seed weight was found to be 0.055, and the correlation between seed yield per plant and 1000 seed weight was 0.111 (Fig 5). This increase in seed yield in bio-primed plants might also be due to uptake of major and minor nutrients along with growth regulators, enzymes and amino acids at all-important stages of plant growth and development (Ali *et al.,* 2022).

***Seed quality parameters after harvest*:** The seed quality parameters of freshly harvested crop, *viz*. seed germination, speed of germination, shoot length, root length and seedling vigour index were statistically non-significant. However, numerically higher germination of 83.7% was recorded with seeds treated with phosphate solubilising bacteria (T_4) and endophytes (T_5). Meanwhile, untreated control (T1) seeds recorded 82.0% germination. Seeds bio-primed with phosphate solubilising bacteria (T_4) recorded the highest value for speed of germination with 25.56, and un-inoculated control (T_1) seeds recorded 19.41 for speed of germination (Fig 1-3).

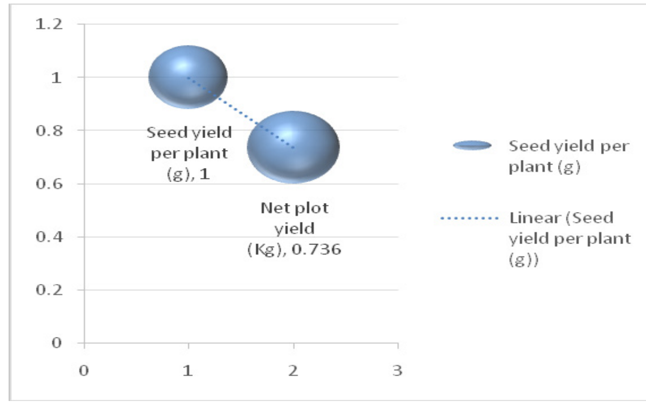

Fig 4. Correlation matrix of seed yield per plant and net plot yield

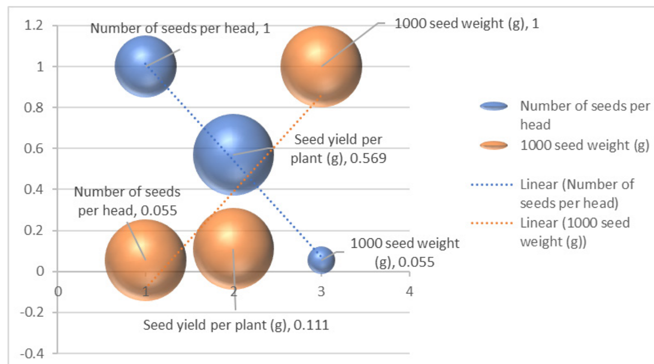

Fig 5. Correlation matrix of number of seeds per head, seed yield per plant and 1000 seed weight

Conclusion

Based on the outcome of the present investigation, it was concluded that in berseem, seed bio-priming with phosphate solubilising bacteria, endophyte, *Rhizobium* and *Rhizobium* + phosphate solubilising bacteria prior to sowing was found to be a potent treatment which refined all the growth parameters. The bio-priming with phosphate solubilising bacteria (T_4) resulted in a 20.77% increase in plant height, 83.68% increase in green biomass, 66.96% increase in dry biomass at 60 DAS, and 17.54% increase in seed yield per plant compared to the control. Seed bio-priming with phosphorus solubilising bacteria and endophyte at 2 g per/kg of seeds for 12 hours before sowing was very effective for increasing the plant available phosphorus in soil in addition to growth of plants and yield of crops. Hence, the present study indicated the potential of bio-priming in sustainably overcoming the complications of fodder and seed yields in berseem.

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