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Effect of micronutrient seed priming and biopriming on germination and seedling vigour of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*)

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Abstract
Yard long bean (*Vigna unguiculata* subsp. *sequipedalis*) is an economically important and nutritionally rich vegetable widely cultivated in Kerala, where its long tender pods form an essential component of local diets. The present study, conducted during 2024-2025 at the Department of Plant Pathology, College of Agriculture, Vellayani aimed to evaluate the effects of seed priming with zinc, boron and biopriming with bioagents i.e. *Bacillus amyloliquefaciens* VLY24 and *Bacillus velezensis* PCSE10 in yard long bean under *in-vitro* conditions. The experiment was laid out in a Completely Randomized Design with seven treatments replicated thrice. Results indicated significant enhancement in germination, shoot length, root length and seedling vigour due to both priming and biopriming treatments. Maximum shoot length (17.49 cm) was observed in seeds bioprimed with *B. amyloliquefaciens* VLY24. The highest root length (20.87 cm) and germination percentage (90%) were recorded in zinc primed seeds. Borax priming also improved germination and root development. Seedling Vigour Index-I was highest in Carbendazim treated seeds (3378.85), followed by $ZnSO_4$ priming (3272.23) and *B. amyloliquefaciens* biopriming (3136.58). From the study integrating micronutrient priming $ZnSO_4$ and microbial biopriming effectively enhances early seedling growth and vigour. These approaches offer promising, eco-friendly strategies for establishing a strong crop stand and improving the sustainable production of yard long bean.

Keywords: *Bacillus amyloliquefaciens*, biopriming, borax, germination, seed priming, yard long bean, zinc sulphate

1. Introduction

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt), a trailing variety of vegetable cowpea with a chromosome number of 2n = 24, is the most widely cultivated and economically important vegetable crop traditionally grown in Kerala. Although the crop is valued for its high content of vitamins, minerals and dietary fibre, its productivity is often constrained by poor seedling establishment and early stage stresses. Sustainable methods that enhance germination and seedling vigour are therefore essential, especially in the context of rising disease incidence and the negative impacts of over reliance on chemical inputs documented in traditional cropping systems. Seed priming and biopriming have been reported as effective strategies to improve uniform germination and early growth by stimulating metabolic processes (Bradford, 1986; Basra *et al*., 2005) [1, 2] and enabling beneficial microorganisms to colonize seeds before sowing. Micronutrients such as zinc and boron further contribute to early seedling performance by supporting key physiological and signalling functions (Tripathi *et al*., 2022) [3].

In addition to improving germination, these treatments can enhance root development, early plant vigour and resilience under stress conditions, thereby contributing to better crop stand in the field. Research on cowpea and other legumes demonstrates that early seedling vigour increased the yield and the importance of seed treatment as a important management strategy. Biopriming with *Bacillus* spp. and *Pseudomonas fluorescens* has shown their ability to colonize seeds and stimulate plant growth. Therefore, the present study was undertaken to evaluate the effects of zinc and borox priming along with biopriming using selected bioagents on germination, seedling vigour and early growth attributes to their effective, eco-friendly seed treatment strategies for enhancing crop establishment.

2. Materials and Methods

The experiment entitled "Effect of micronutrient seed priming and biopriming on germination and seedling vigour of yard long bean (*Vigna unguiculata* subsp. *sequipedalis*)" was conducted during 2024-2025 at the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University. The work was carried out under *in- vitro* conditions suitable for germination testing. Healthy seeds of the variety Vellayani Deepika were used for all treatments. All containers, tools and materials used for seed treatment were sterilized to maintain experimental hygiene and consistency.

The experiment was laid out in a Completely Randomized Design (CRD) with seven treatments replicated thrice. The treatments consisted of T_1: Seed priming with $ZnSO_4$ @ 0.05%, T_2: Seed priming with Borax @ 0.025%, T_3: Seed biopriming with *Bacillus amyloliquefaciens* VLY24, T_4: Seed biopriming with *Bacillus velezensis* PCSE10, T_5: Seed treatment with *Pseudomonas fluorescens* @ 2% (talc formulation), T_6: Seed treatment with Carbendazim @ 0.05% and T_7: Untreated control. The bioagents *Bacillus amyloliquefaciens* VLY24 and *Bacillus velezensis* PCSE10 used for biopriming were obtained from the Department of Microbiology, College of Agriculture, Vellayani. The strains were maintained under standard laboratory conditions and used for the preparation of bacterial suspensions for seed biopriming. Micronutrient seed priming was carried out following the hydration-dehydration technique of (Abdul-Baki and Anderson 1973) [4]. Seeds were soaked in $ZnSO_4$ (0.05%) and Borax (0.025%) solutions for 4 hours, ensuring uniform hydration of seeds. Seeds were shade-dried to restore their original moisture level, enabling easy handling and preventing premature germination. Biopriming was performed using freshly prepared suspensions of *Bacillus amyloliquefaciens* VLY24, *Bacillus velezensis* PCSE10 and *Pseudomonas fluorescens* was used as a commercial talc-based formulation at 2% concentration. Seeds were immersed in the bacterial suspensions and mixed to ensure uniform coating over the entire seed surface and evaluated using the paper towel method. A uniform number of seeds from each treatment were placed on moistened germination sheets, rolled carefully and kept upright under controlled indoor conditions. Moisture levels were monitored regularly and distilled water was added when required to maintain uniform hydration. The following parameters were recorded at the end of the germination test Shoot length (cm), Root length (cm), Germination percentage (%), Seedling Vigour Index-I (SVI-I)

SVI-I was calculated using the formula:

SVI-I = Germination (%) × (Shoot length + Root length)

The data were subjected to analysis of variance (ANOVA) under CRD. Treatment means were compared at the 5% level of significance. All values were expressed as mean ± standard deviation.

3. Results and Discussion

Growth attributes of yard long bean seedlings were significantly influenced by the different seed priming and biopriming treatments evaluated under *in-vitro* conditions (Table 1). Among the treatments, the highest shoot length was recorded in (T_3) seed biopriming with *Bacillus amyloliquefaciens* VLY24 (17.49 cm), which was on par with carbendazim @ 0.05% (T_6) (17.29 cm), whereas the lowest shoot length was recorded in the control (T_7) (13.99 cm). The enhanced shoot growth in T_3 may be attributed to the ability of *B. amyloliquefaciens* to produce growth-promoting metabolites such as IAA, cytokinins and siderophores that stimulate cell elongation and nutrient uptake, thereby accelerating early seedling vigour. Similar findings were reported by Idris *et al*. (2004) [5].

Seed priming with $ZnSO_4$ @ 0.05% (T_1) recorded the highest root length (20.87 ± 0.60 cm), followed by T_2 (borax priming), whereas the untreated control recorded the lowest. The superior root growth in zinc primed seeds is due the effect of zinc in auxin biosynthesis, enzyme activation and root meristem development. Similar effects of zinc priming on root elongation and seedling vigour have been documented in various leguminous crops, supporting the enhanced root proliferation observed in the present study. The boron primed treatment (T_2) also produced the longer root length and maximum germination, as a boron's role in cell wall formation, membrane stabilization and sugar transport during early seedling development. These results are in agreement with Tripathi *et al*. (2022) [3] who reported zinc deficiency restricts root meristem activity, while zinc supplementation significantly enhances root elongation and root tip growth The higher root length recorded in zinc primed seeds aligns with the physiological roles of zinc in auxin biosynthesis, enzyme activation and improved structural integrity of root tissues.

Germination percentage was mainly influenced by the seed priming treatments. (T_1) $ZnSO_4$ @ 0.05% and (T_2) Borax @ 0.025% both recorded the highest germination percentage (90%), while the lowest was observed in the control. The improved germination in micronutrient-primed seeds is likely due to enhanced enzymatic repair and activation during controlled hydration, which leads to faster and more synchronized emergence. Similar findings were reported by Granata *et al*. (2024) [7] who reported the priming treatments increased the seed germination.

Table 1: Effect of seed priming and biopriming on shoot length, root length and germination percentage of yard long bean seeds var. Deepika

Treatments	Shoot length (cm)*	Root length (cm)*	Germination percentage (%) *
T_1- Seed priming with $ZnSO_4$ @ 0.05%	15.49 ± 0.36^{e}	20.87 ± 0.60^{ab}	90.00 ± 3.92^{a}
T_2- Seed priming with Borax @ 0.025%	15.99 ± 0.34^{cd}	19.89 ± 0.42^{b}	90.00 ± 3.97^{a}
T_3- Seed biopriming with *B. amyloliquefaciens* VLY24	17.49 ± 0.66^{a}	18.84 ± 0.92^{c}	86.30 ± 2.88^{ab}
T_4- Seed biopriming with *B. velezensis* PCSE10	16.49 ± 0.56^{bc}	18.67 ± 0.43^{cd}	80.00 ± 3.39^{c}
T_5- Seed treatment with *P. fluorescens* @ 2%	15.79 ± 0.68^{cd}	17.88 ± 0.67^{cd}	88.89 ± 3.92^{ab}
T_6- Seed treatment with Carbendazim @ 0.05%	17.29 ± 0.48^{ab}	21.53 ± 0.18^{a}	86.99 ± 1.49^{ab}
T_7- Control	13.99 ± 0.38^{e}	17.70 ± 0.50^{d}	83.30 ± 2.63^{bc}
SE(m) ±	0.29	0.33	1.89
CD (0.05)	0.900	1.006	5.748

*mean of three replications ± SD

The highest seedling vigour index was recorded in seed treatment with (T_6) carbendazim @ 0.05% (3378.85), which was on par with seed priming using $ZnSO_4$ @ 0.05% (T_1) (3272.23). Seed priming with Borax @ 0.025% (T_2) (3231.80) and seed biopriming with *Bacillus amyloliquefaciens* VLY24 (T_4) (3136.58) were on par with each other. Whereas, the other treatments recorded comparatively lower vigour index values. These findings are supported by Fiodor *et al.* (2023) [6] who observed plant growth promoting bacteria can directly stimulate germination and early seedling growth. Granata *et al.* (2024) [7] who reported the priming treatments also increases the plant vigor.

Table 2: Effect of seed priming and biopriming on Seedling vigour index (SV-I) of yard long bean seedlings var. Deepika

Treatments	Seedling vigour index I (SV-I)*
Seed priming with $ZnSO_4$ @ 0.05%	3272.23 ± 120.75^{a}
Seed priming with Borax @ 0.025%	3231.80 ± 196.43^{ab}
Seed biopriming with *B. amyloliquefaciens* VLY24	3136.58± 188.72^{ab}
Seed biopriming with *B. velezensis* PCSE10	2815.80± 196.02^{cd}
Seed treatment with *P. fluorescens* @ 2%	2994.30 ± 144.98^{bc}
Seed treatment with Carbendazim @ 0.05%	3378.85 ± 111.84^{a}
Control	2639.40 ± 61.20^{d}
SE(m) ±	85.87
CD (0.05)	260.486

*mean of three replications ± SD

4. Conclusion

From the study it could be inferred that biopriming with *Bacillus amyloliquefaciens* VLY24 is most effective in enhancing shoot elongation and overall seedling vigour, while micronutrient priming with $ZnSO_4$ and Borax significantly improves germination and root development. These findings collectively highlight that integrating beneficial microbes and essential micronutrients through seed treatment is an efficient, eco friendly strategy to enhance early seedling growth and establish a strong crop stand in yard long bean.

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