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Influence of Priming on Seed Quality and Seed Health in Sorghum

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To achieve sustainable agriculture, it is essential to conduct ongoing research aimed at reducing the reliance on chemical fertilizers. This study investigates the effectiveness of seed priming, either alone or in combination with low doses of fungicides and/or biocontrol agents, to enhance seed quality and to reduce seed infection by improving the rate and uniformity of seed emergence and overcoming diseases.
Study Design: Completely randomized design.
Place and Duration of Study: Seed Unit, UAS, Raichur, from March 2022 to September 2022**.**
Methodology: Sorghum seeds were surface sterilized with sodium hypochlorite (1 %) for five minutes and then washed thrice with sterile water. Later the seeds were primed with *Trichoderma hamatum, Bacillus amyloliquefaciens, Paenibacillus polymyxa,* Carboxin 37.5 % + thiram 37.5 % (vitavax power), *Trichoderma harzianum, Azospirillium* and control with the seed to solution ratio of 1:5 for eight hours and then the primed seeds were dried back to original moisture content (24 hrs.) and then used to assess the seed quality parameters**.**
Results: Seed priming with *Bacillus amyloliquefaciens* resulted in statistically significant improvements in seed germination (95.0%), shoot length (16.9 cm), root length (16.6 cm), seedling length (33.5 cm), speed of germination (20.1), seedling dry weight (339.3 mg), seedling vigour index-I (3183), seedling vigour index-II (32234), lower electrical conductivity (0.35 dS/m) and reduced seed infection (0.6%). In contrast, the control group exhibited significantly lower seed germination (83.0%), shoot length (12.6 cm), root length (11.1 cm), seedling length (23.7 cm), speed of germination (15.2), seedling dry weight (333.2 mg), seedling vigour index-I (1967), seedling vigour index-II (27656), higher electrical conductivity (0.65 dS/m) and increased seed infection (6.8%).
Conclusion: Sorghum seeds primed with *Bacillus amyloliquefaciens* increases the seed quality parameters and reduces seed infection, supporting its use as an effective strategy for enhancing seed performance and promoting sustainable agricultural practices.

Keywords: Sorghum; seed priming; Bacillus amyloliquefaciens; seed quality; seed infection.

1. INTRODUCTION

Sorghum is the fifth most important cereal crop globally, following wheat, rice, maize, and barley. It is cultivated across tropical and subtropical regions, with major producers including Nigeria, the USA, India, Mexico, Argentina, Sudan, Ethiopia, Brazil, China, and Australia. In India, sorghum is a critical food crop, contributing approximately 16% of the world's sorghum production and ranking as the fourth most important cereal crop in the country. Sorghum is a nutritionally rich grain, offering substantial benefits for human health. It is packed with essential nutrients, including proteins, vitamins, and minerals. Sorghum grains are a good source of dietary fiber, which aids in digestion and contributes to overall health. It is also noted for its high content of phytochemicals, such as phenolic acids, tannins, anthocyanins, phytosterols, and policosanols. These compounds have antioxidant properties and are linked to a reduced risk of chronic diseases, including cancers, cardiovascular diseases, and type II diabetes. Seed is a fundamental agricultural input crucial for achieving higher yields. Quality seed acts as a catalyst, enhancing the effectiveness of other agricultural inputs such as fertilizers, water, and pesticides. Without good quality seed, investments in these resources may not yield the desired results. The importance of seed quality has become increasingly recognized over time, as efficiency is key to maintaining competitiveness in agriculture. Consequently, ensuring the availability of high-quality seed to farmers at an affordable price and in a timely manner is essential for boosting and sustaining agricultural productivity.

Seed priming is used commercially to enhance the speed and uniformity of germination and improve final stand establishment. This technique helps in controlling major seed and soil-borne pathogens. When applied alone or in combination with low doses of fungicides and/or biocontrol agents, seed priming improves the rate and uniformity of seed emergence and aids in overcoming diseases. According to Heydecker [1], seed priming is one of the most significant advancements for achieving rapid and uniform seed germination and increasing tolerance to adverse environmental conditions. It has shown

promising and even surprising results across various crop seeds with primed seeds typically demonstrating improved germination parameters. Seed priming is a key aspect of agronomic research for crop production. While extensive research has been conducted on crops such as rice, maize, wheat, lentil, and chickpea, there has been comparatively little research on major millets like sorghum and bajra. Exploring these areas is crucial, as it aligns with modern agronomic approaches aimed at improving crop production. Seed bio-priming in sorghum is a promising approach to enhance seed performance and crop establishment. By improving germination rates and seedling growth, bio-priming contributes to better early crop development and increased resilience to stress conditions. The use of beneficial microorganisms, such as *Trichoderma* and *Bacillus* species, provides additional advantages, including disease resistance and enhanced nutrient uptake. These benefits result from the microbial activity that supports seedling vigour and stress tolerance [2,3].

Modifying host physiological characteristics through plant hormones and synthesizing other biological compounds hold significant potential for controlling phytopathogens and promoting plant growth. Plant diseases and insect pests are major factors impeding agricultural development and have caused substantial losses to agricultural products. While chemical pesticides can reduce pests and pathogen hazards, they pose environmental and health risks and disrupt ecological balance. Biocontrol agents, such as endophytes and entomopathogenic microorganisms, offer alternatives to reduce reliance on chemical products in agriculture. Endophytes are microorganisms that reside within plant tissues for all or part of their life cycle without causing visible symptoms of their presence [4,5]. These microorganisms inhabit various parts of healthy, symptomless plants, including seeds, roots, stems, and leaves. By harboring endophytic microbes, plants benefit from enhanced growth and increased resistance to pathogens [6,7,8]. Endophytes contribute to this resistance by producing antibiotics, which further supports their role in disease and pest management [9].

2. MATERIALS AND METHODS

Seeds and culture collection: Sorghum var. M35-1 seeds were obtained from the Seed Unit at UAS, Raichur. Bioagents, including *Azospirillum* and *Trichoderma harzianum* (5 g/kg), were sourced from the Bio-input Entrepreneurship Centre at the College of Agriculture, Raichur. Three endophytes were collected from the ICAR-National Bureau of Agriculturally Important Microorganisms in Kushmaur, Mau, Uttar Pradesh. Additionally, Carboxin 37.5% + Thiram 37.5% (Vitavax Power) (2 g/kg) was procured from the Department of Plant Pathology, University of Agricultural Sciences, Raichur, Karnataka.

Preparation of endophyte inoculums: Potato dextrose broth for fungus and nutrient broth for bacteria was prepared and fungal cultures were inoculated to potato dextrose broth and bacterial cultures were inoculated to nutrient broth and incubated at 25 ± 2 ^{o}C for 14 days for fungus and two days for bacteria [10] After incubation, the culture filtrates were filtered into pre sterilized conical flasks using Whatman no. 1 filter paper. The filtrates were stored in a refrigerator at 4^{o}C [11] and further used for priming of seeds. The different priming agents at the rate of 1 x 10^3 conidia/ml for fungal culture and 1 x 10^8 cfu/ml for bacteria culture were counted using a haemo-cytometer under the light microscope.

Seed priming protocol: Sorghum seeds were surface sterilized using a 1% sodium hypochlorite solution for five minutes, followed by washing three times with sterile water. The seeds were then primed in a seed-to-solution ratio of 1:5 for eight hours. After priming, the seeds were dried back to their original moisture content over 24 hours. Seed quality parameters were assessed following the standard procedures of the International Seed Testing Association [12]. Each treatment was replicated four times, with each replication consisting of 100 seedlings. At the end of the tenth day, the following parameters were recorded: final germination percentage, shoot length, root length, seedling length, seedling dry weight, seedling vigour index I and II, speed of germination, electrical conductivity and seed infection percentage. Statistical analysis was performed using a Completely Randomized Design**.**

Treatment Details:

T_1: *Trichoderma hamatum* (Fungal culture 1 x 10^3 conidia ml^{-1})
T_2*: Bacillus amyloliquefaciens* (Bacterial culture 1 x 10^8 cfu ml^{-1})
T_3: *Paenibacillus polymyxa* (Bacterial culture 1 x 10^8 cfu ml^{-1})

T_4: Carboxin 37.5 % + thiram 37.5 % (vitavax power) (2g/kg)
T_5: *Trichoderma harzianum* (5g/kg)
T_6: *Azospirillium* (Bacterial culture 1 x 10^8 cfu ml^{-1})
T_7: Control

Seed quality parameters:

Germination percentage (%):

The standard germination test was conducted in four replications of 100 seeds each by following between paper method and the rolled towels were incubated in the walk in seed germination chamber maintained at 25 ± 2 ^{0}C temperature and 90 ± 5 per cent relative humidity. The germination counts were taken on fourth day for first count and tenth day for final count in sorghum. The numbers of normal seedlings from each replication were counted and the mean germination was calculated and expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings obtained}}{\text{Number of seeds used for germination}} \times 100$$

Shoot length: Shoot length was measured from collar region to the point of attachment of cotyledons. Average of ten normal seedlings was expressed as shoot length in centimeters.

Root length: Ten normal seedlings in each replication were randomly selected for the measurement root length on the day of final count. The root length was measured from the collar region to the tip of the primary root. Average of ten normal seedlings was expressed as root length in centimeters.

Seedling dry weight: Ten normal seedlings used for measurement of shoot and root length in germination test were placed in butter paper bag separately for each treatments and for four replications and dried in hot air oven maintaining 70 ± 1 ^{0}C temperature for 24 h. Later, they were removed and allowed to cool in a desiccator for 30 minutes before weighing on electronic weighing balance. Mean dry weight of seedlings were recorded and expressed in grams in chickpea and groundnut and milligrams in sorghum [13].

Seedling vigour index-I and II: Seedling vigour were calculated by using the following formulae and expressed in whole number [14].

Seedling vigour index - I = Germination (%) × [(Root length (cm) + Shoot length (cm)]

Seedling vigour index - II = Germination (%) x dry weight of seedling (mg)

Speed of germination: Speed of germination indicates a population of fast germinating seeds. The daily germination count was made up to final count. Speed of germination was calculated by using the following formula [15].

$$\text{Speed of germination} = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \frac{Gn}{Tn}$$

Where,

G_1, G_2 and G_n are the number of seeds germinated on each successive count day.

T_1, T_2 and T_n are the corresponding days on which these counts were made..

Electrical conductivity (EC): Five grams of seeds in four replications were soaked in 25 ml distilled water and kept in an incubator maintained at 25°C ± 1°C for twelve hours. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in deci simons per meter (dSm^{-1}) [16].

Seed infection (%) test by blotter paper method: In blotter paper method to know the pathogen infection, three pieces of filter paper were properly soaked in sterilized water and was placed on plastic petri dishes. Twenty five seeds per petri dish were placed using a pair of forceps and making sure that seeds were placed equidistantly under aseptic conditions. The petri dishes containing seeds were incubated at room temperature (25 ± 2 ºC) for 8 days under alternating cycles of light and darkness of 12 hours each. On the day of final count, that is on 8th day. The number of infected seeds were counted and expressed in percentage.

3. RESULTS

The efficiency of seed priming is influenced by several factors, including ventilation, light, temperature, time and seed quality. This experiment was designed to evaluate the impact of priming on seed quality and seed health in sorghum. The seeds were bio-primed for eight hours, with water-primed seeds serving as the control group. After priming, the seeds were air-dried to return to their original moisture content. Observations were recorded and the results were analyzed. The findings from this investigation

demonstrated that seed priming significantly affected the selected crop.

Influence of priming on seed germination (%) and speed of germination of sorghum: The effect of priming had significantly influenced the seed germination (%) and speed of germination which is depicted in Table 1. Statistically, significant seed germination (95.0 %) and speed of germination (20.1) was observed due to seed priming with *Bacillus amyloliquefaciens*, which was on par with *Trichoderma hamatum* (90.3%) and (19.6), *Paenibacillus polymyxa* (90.0%) and (19.2) followed by *Azospirillum* (89.3%) and (18.9), *Trichoderma harzianum* @ 5g/kg (88.6%) and (18.2) and Carboxin 37.5% + thiram (37.5%) vitavax power @ 2g/kg (87.0%) and (17.5). While, significantly lowest seed germination (83.0%) and speed of germination (15.2) was recorded by control.

Influence of priming on shoot length (cm), root length (cm) and seedling length (cm) of sorghum: Effect of priming had significantly influenced the shoot length (cm), root length (cm) and seedling length (cm) which is presented in Table 1. Results revealed that significantly highest shoot length (16.9 cm), root length (16.6 cm) and seedling length (33.5 cm) was recorded due to seed priming with *Bacillus amyloliquefaciens*. Which was on par with *Trichoderma hamatum* (16.8 cm), (16.1 cm) and (32.9 cm) followed by *Paenibacillus polymyxa* (16.1 cm), (15.0 cm) and (31.1 cm), *Azospirillum* (15.6 cm), (14.8 cm) and (30.4 cm), *Trichoderma harzianum* @ 5g/kg (14.5 cm), (13.7 cm) and (28.2 cm) and Carboxin 37.5 % + thiram (37.5 %) vitavax power @ 2g/kg (13.7 cm), (13.0 cm) and (26.7 cm). Control showed lowest shoot length (12.6 cm), root length (11.1 cm) and seedling length (23.7 cm).

Influence of priming on seedling vigour index-I, seedling dry weight (mg) and seedling vigour index-II of sorghum: The data regarding seedling vigour index-I and seedling vigour index-II presented in Table 2 indicated statistically significant differences among the treatments but non-significant differences with respect to seedling dry weight (mg). The maximum increase in seedling vigour index-I (3183) and seedling vigour index-II (32234) was observed in seed primed with endophyte *Bacillus amyloliquefaciens* followed by *Trichoderma hamatum* (2971) (30594), *Paenibacillus polymyxa* (2799) (30474), *Azospirillum* (2715) (30085), *Trichoderma harzianum* @ 5g/kg (2499) (29778) and Carboxin 37.5 % + thiram (37.5 %) vitavax power @ 2g/kg (2323) (29206). Significantly, minimum seedling vigour index-I (1967) and seedling vigour index-II (27656) was recorded in control.

Table 1. Influence of priming on seed germination (%), shoot length (cm), root length (cm) seedling length (cm) and speed of germination of sorghum

Treatments	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Speed of germination
T_1: *Trichoderma hamatum* (1×10^3 conidia ml^{-1})	90.3	16.8	16.1	32.9	19.6
T_2: *Bacillus amyloliquefaciens* (1×10^8 cfu ml^{-1})	95.0	16.9	16.6	33.5	20.1
T_3: *Paenibacillus polymyxa* (1×10^8 cfu ml^{-1})	90.0	16.1	15.0	31.1	19.2
T_4: Carboxin 37.5 % + thiram 37.5 % (vitavax power) (2g/kg)	87.0	13.7	13.0	26.7	17.5
T_5: *Trichoderma harzianum* (5g/kg)	88.6	14.5	13.7	28.2	18.2
T_6: *Azospirillum* (1×10^8 cfu ml^{-1})	89.3	15.6	14.8	30.4	18.9
T_7: Control	83.0	12.6	11.1	23.7	15.2
Mean	89.0	15.2	14.3	29.5	18.3
S.Em ±	2.1	0.2	0.2	0.5	0.2
CD @ 1%	6.5	0.6	0.7	1.7	0.9

Table 2. Influence of priming on seedling vigour index-I, seedling dry weight (mg), seedling vigour index-II, electrical conductivity (dsm^{-1}) and seed infection (%) of sorghum

Treatments	Seedling vigour index-I	Seedling dry weight (mg)	Seedling vigour index-II	Electrical conductivity (dsm^{-1})	Seed infection (%)
T_1: *Trichoderma hamatum* (1×10^3 conidia ml^{-1})	2971	338.8	30594	0.39	1.6
T_2: *Bacillus amyloliquefaciens* (1×10^8 cfu ml^{-1})	3183	339.3	32234	0.35	0.6
T_3: *Paenibacillus polymyxa* (1×10^8 cfu ml^{-1})	2799	338.6	30474	0.42	2.3
T_4: Carboxin 37.5 % + thiram 37.5 % (vitavax power) (2g/kg)	2323	335.7	29206	0.57	5.3
T_5: *Trichoderma harzianum* (5g/kg)	2499	336.1	29778	0.51	3.3
T_6: *Azospirillum* (1×10^8 cfu ml^{-1})	2715	336.9	30085	0.48	2.6
T_7: Control	1967	333.2	27656	0.65	6.8
Mean	2637	336.9	30004	0.48	2.9
S.Em ±	43	6.4	518	0.009	1.3
CD @ 1%	133	NS	1589	0.027	5.4

Influence of priming on electrical conductivity (dsm^{-1}) and seed infection (%) of sorghum: The data regarding electrical conductivity (dsm^{-1}) and seed infection (%) presented in Table 2 indicated significant differences among the different treatments. Significantly minimum electrical conductivity (0.35 dsm^{-1}) and seed infection (0.6 %) was observed in seed priming with endophyte *Bacillus amyloliquefaciens* followed by *Trichoderma hamatum* (0.39 dsm^{-1}) (1.6%), *Paenibacillus polymyxa* (0.42 dsm^{-1}) (2.3%), *Azospirillum* (0.48 dsm^{-1}) (2.6%), *Trichoderma harzianum* @ 5g/kg (0.51 dsm^{-1}) (3.3%) and Carboxin 37.5% + thiram (37.5%) vitavax power @ 2g/kg (0.57 dsm^{-1}) (5.3%). Control showed maximum electrical conductivity (0.65 dsm^{-1}) and seed infection (6.8%).

4. DISCUSSION

Seed priming with *Bacillus amyloliquefaciens* resulted in the highest seed germination, likely due to the unique characteristics of bacterial endophytes. The *Bacillus* group of microbes has advantages over other microorganisms, primarily due to their ability to form endospores, which allows them to tolerate adverse conditions and combat various pathogens and also due to production of plant growth hormones like GA and IAA and also production of secondary metabolites. Increased GA and IAA might trigger the enzyme activity of α-amylase which is responsible for early germination through maximizing the availability of starch assimilation, GA and IAA might have played an important role on seed germination and radical length. *Bacillus amyloliquefaciens, Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus* are commonly used as bio-fertilizers and biocontrol agents for managing phytopathogens [17]. Additionally, the bacterial strain *Enterobacter cloaca* has been reported to produce higher amounts of indole acetic acid (IAA) [18]. Similar findings were reported by Baldani et al. [19] in rice.

The speed of germination was linked to the higher percentage of seeds germinating over time. The results indicated that *Bacillus amyloliquefaciens* exhibited a higher germination speed. This improvement is likely due to the secretion of hormones such as cytokinins and auxins by the bacterium, which enhance water absorption and thereby facilitate seed germination [20]. Ghani et al. [21] found that bio-priming with specific beneficial microbes significantly improved germination speed and seedling vigour in sorghum. Similarly, Vazquez et al. [22] reported that bio-priming with *Trichoderma* species resulted in enhanced germination rates and seedling growth in sorghum, demonstrating the effectiveness of this technique in optimizing crop establishment. Comparable results were observed by Shukla et al. [23] in wheat and by Piri et al. [24] in cumin.

Increase in shoot and root length by the endophytes may have been caused by nitrogen fixation, The phosphate solubilization ability of endophytes contributes to increased uptake of

nitrogen, phosphorus, and potassium (NPK) by plants. Enhanced nutrient uptake associated with seed-primed plants may result from an improved root-to-shoot ratio, which boosts overall nutrition. This improvement is likely due to seed priming with bioagents, which increases nutrient availability. Endophytes are known to have a significant impact on nitrogen fixation by enhancing the capacity for nitrate (NO_3) uptake, potentially through stimulated lateral root development and the stimulation of nitrate transport systems [25]. The promotion of plant growth by endophyte isolates is related to improved root development and enhanced nodulation, which in turn leads to better nutrient uptake and increased nitrogen supply to the plant. Increased root length and the volume of soil explored by roots, which enhances nutrient uptake, are commonly proposed explanations for the beneficial effects of endophytes on plant growth. These findings align with Shahzad et al. [26], who reported that *Bacillus amyloliquefaciens* inoculated plants exhibited significantly greater root length (5.69 ± 1.37 cm) compared to water-treated plants (3.54 ± 1.01 cm) in rice. Similarly, a high seedling vigour index-I and germination rate were observed with combined inoculation of phosphate solubilizing bacteria (PSB) and *Pseudomonas*, attributed to improved auxin biosynthesis in chili [27]. In this study, the better seedling vigour index-II due to seed priming with *Bacillus amyloliquefaciens* was mainly due to higher seed germination and seedling dry weight. This improvement may result from the compatibility and antagonistic interactions within the endophyte-inoculated seeds. Similar results were also observed by Jagadeesh et al. [28] in pigeonpea.

The endophyte *Bacillus amyloliquefaciens* exhibited significantly lower seed infection, likely due to its strong antifungal activity, broad-spectrum antimicrobial properties and production of various antibiotic metabolites. This aligns with the findings of Lastochkina et al. [29], who demonstrated that *Bacillus subtilis* 10^{-4} has antagonistic activity against the phytopathogenic fungus *Fusarium culmorum* attributed to its production of metabolites with strong antibiotic properties, including LPs surfactin C14, C15, and C15, which exhibit potent antifungal activity in wheat.

Surfactin is known for its strong antibacterial and antifungal properties and the presence of bacillomycin D genes has been confirmed in the genome of *Bacillus subtilis* [30] in rice. In this study, seeds bio-primed with *Bacillus amyloliquefaciens* demonstrated lower electrical conductivity compared to the control. This reduction in electrical conductivity is attributed to the endophyte's protective effect against pathogens, which minimizes seed infection, reduces seed coat damage and cracks, and decreases the leaching of electrolytes [31].

5. CONCLUSION

From the present investigation, it is concluded that sorghum seeds primed with the endophyte *Bacillus amyloliquefaciens* yielded superior results in seed germination, shoot length, root length, seedling length, speed of germination, seedling dry weight, seedling vigour index-I, and seedling vigour index-II. Additionally, these seeds exhibited lower electrical conductivity (0.35 dS/m) and reduced seed infection (0.6%) compared to all other treatments and the control. Therefore, *Bacillus amyloliquefaciens* can be effectively utilized for seed bio-priming to enhance seed quality and minimize seed infection.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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