Original Research Article
Influence of priming on seed quality and seed health in sorghum

ABSTRACT

Aims: The realization of sustainable agriculture requires continuous research which can reduce the use of chemical fertilizers. Seed priming alone or in combination with low dosage of fungicides and/or biocontrol agents has been used to improve the rate and uniformity of seed emergence and to overcome the diseases. In order to enhance the seed quality and to reduce the seed infection this laboratory experiment was conducted.
Study design: Completely Randomized Design
Place and Duration of Study: Seed Unit, UAS, Raichur, between March 2022 and 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 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: The results showed that, statistically, significant 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), seedling vigour index-I (3183), seedling vigour index-II (32234), lower electrical conductivity (0.35 dsm-1) and seed infection (0.6 %) was observed due to seed priming with *Bacillus amyloliquefaciens*. Control had recorded significantly lowest 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), seedling vigour index-I (1967), seedling vigour index-II (27656), higher electrical conductivity (0.35 dsm-1) and seed infection (6.8 %).
Conclusion: Sorghum seeds priming with *Bacillus amyloliquefaciens* increased the seed quality parameters and reduced seed infection.

1. INTRODUCTION

Sorghum (*Sorghum bicolour* L.) is an important nutritive cereal constituting staple diet in the country. Sorghum is the fifth major cereal crop in the world and occupies fifth position in acreage after wheat, rice, maize and barley. It is grown as a staple food crop throughout the Asian and African regions, besides as a forage and fodder crop for livestock in the developed countries like USA, Europe and Japan. Major producers of sorghum in the world are Nigeria, USA, India, Mexico, Argentina, Sudan, Ethiopia, Brazil, China and Australia. It has been classified under family poaceae, tribe Andropogonae and genus sorghum. It is also used in production of starch, biscuits, sugar and alcohol. It is rich in phytochemicals, including phenolic acids, tannins, anthocyanins, phytosterols and policosanols all of which have great value in health industry. Sorghum is helpful in reducing several kinds of chronic diseases such as cancers, cardiovascular diseases, type II diabetes and various gastrointestinal disorders. India contributes about 16 per cent of the world's sorghum production. It is the fourth most important cereal crop in the country. Sorghum is a dual-purpose crop, widely cultivated for grain and husbandry feed stock purpose in worldwide, especially in Asian countries.

Seed is considered as one of the important basic agricultural inputs for obtaining higher yield. Good quality seed acts as a catalyst for realizing the potential of all other inputs in agriculture. Without good seed, the investment on fertilizer, water, pesticides and other inputs will not play the desired dividends. Its importance has been realized with the passage of time and greater realization that efficiency is the key factor to be competitive in all the agricultural ventures. Therefore, the availability of quality seed to the farmer at an affordable price and in time is considered crucial for enhancing and sustaining the agricultural productivity. Therefore, production of quality seed and maintenance of high seed germination is of utmost importance in a seed programme. Seeds are practically worthless if upon planting they fail to germinate and give adequate plant stand in the field in addition to healthy and vigorous plants. The great quality seed is pre-essential to improve the production and yield

Seed priming is used commercially as a means to boost speed and uniformity of germination and advance final stand. It is a method for controlling major seed and soil borne pathogens. Seed priming

alone or in combination with low dosage of fungicides and/or biocontrol agents has been used to improve the rate and uniformity of seed emergence and to overcome the diseases. [1] reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions. Seed priming has presented promising and even surprising results, for many crop seeds. Primed seeds usually show improved germination parameters.

Modify the host physiological characteristics, induced by the plant hormone and synthesize other biological compounds which are of great potential application in controlling phytopathogen and promoting the plant growth. Plant diseases and insect pests have been one important factors restraining agriculture development and it have already caused a great loss to agriculture products. Although chemical pesticides can decrease the pests and the hazards of pathogen, it threatens the environment and the health of human beings, which also induce the disorder of ecological environment. Certain biocontrol agents, endophytes, Entomopathogenic microorganisms or individuals that reduce the other pathogenic microorganisms to plants used to control diseases and pests are a substitute to reduce the use of chemical products in agriculture. Endophytes are microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes [2,3]. Endophytes inhabit majority of healthy and symptomless plants, in various tissues, seeds, roots, stems and leaves. Plants benefit extensively by harbouring these endophytic microbes; they promote plant growth and confer enhanced resistance to various pathogens [4,5,6] by producing antibiotics [7].

2. MATERIAL AND METHODS

Seeds and culture collection

Sorghum var. M35-1 seeds were collected from Seed Unit, UAS, Raichur. Bioagents like *Azospirillum* and *Trichoderma harzianum* (5g/kg) were collected from Bio-input Entrepreneurship Centre, College of Agriculture, Raichur. Three endophytes were collected from ICAR-National Bureau of Agriculturally Important Microorgannisms, Kushmaur, Mau, Uttar Pradesh and Carboxin 37.5 % + thiram 37.5 % (vitavax power) (2g/kg) were collected from 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 [8] 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 [9] and further used for priming of seeds. The different priming agents at the rate of 1 x 10^{3} conidia for fungal culture and 1 x 10^{8} cfu for bacteria culture were counted using a haemo-cytometer under the light microscope.

Seed priming protocol

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 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 by following standard procedure of International Seed Testing Association [10] Each treatment was maintained with 4 replications, each replication with 100 seedlings. The final germination percentage, shoot length, root length, seedling length, seedling dry weight, seedling vigour index I and II and seed infection percentage were recorded at the end of the tenth day. Completely Randomized Design was used for statistical analysis.

Treatment Details:

T_1: *Trichoderma hamatum* (Fungal culture 1 x 103 conidia ml-1)

T_2: *Bacillus amyloliquefaciens* (Bacterial culture 1 x 108 cfu ml-1)

T_3: *Paenibacillus polymyxa* (Bacterial culture 1 x 108 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 108 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 [11].

Seedling vigour index-I and II

Seedling vigour were calculated by using the following formulae and expressed in whole number [12].

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 [13].

$$\text{Speed of germination} = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \frac{G_n}{T_n}$$

Where,

G_1, G_2 and G_n are germination counts on the respective days.

T_1, T_2 and T_n are the number of days on which the counts are 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}) [14].

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 priming is influenced by a number of factors such as ventilation, light, temperature, time and seed quality. This experiment was designed to evaluate the role of priming on seed quality and seed health in chickpea. The seeds were bio primed for eight hours duration. Seed priming with water was taken as control and seeds were air dried to bring back to original moisture content. Observations were recorded, results obtained from the present investigation were analysed. Seed priming had shown a significant effect on 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.

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* recorded maximum seed germination, might be due to different characteristics of bacterial endophytes. Bacillus group of microbes possess extra advantages over the other microbes due to endospore formation which can tolerate adverse conditions and also various pathogens. *Bacillus amyloliquefaciens Bacillus subtilis, Bacillus licheniformis* and *Bacillus pumilis* are being utilized as bio-fertilizer and bio-control agents for controlling phytopathogens [15]. A bacterial strain, *Enterobacter cloacae* was reported to produce IAA in higher amount [16]. Similar results were also reported by Baldani *et al.* (2000) [17] in rice.

Speed of germination was attributed to higher per cent germination of seeds over time. From the results it was observed that *Bacillus amyloliquefaciens* found having higher speed of germination, this might be due to secretion of certain harmones such as cytokinin and auxin, which stimulates for better absorption of water which in turn helps in germination of seeds [18] thus the rate of germination might have improved. Similar results were also observed by Shukla et al. (2015) [19] in wheat; Piri et al. (2019) [20] in cumin

Increase in shoot and root length by the endophytes may have been caused by nitrogen fixation, phosphate solubilization ability of endophytes and also increase in the uptake of shoot nitrogen, phosphorus and potassium (NPK). Increased nutrient uptake associated with seed primed plants may be the results of more root-shoot ratio resulting in enhanced nutrition because of seed priming with bioagents. Endophytes have been referred to have great impact on nitrogen fixation by increasing N03 uptake capacity, indirectly as a consequence of stimulated lateral root development and possibly directly by stimulating N03 transport systems [21]. Plant growth promotion provided by endophytes isolates was related to improved root development and enhanced nodulation, resulting in better nutrient uptake capability and increased N supply in the plant. An increase in root length and the volume of soil foraged by the roots, which leads to an enhanced nutrient uptake is the most commonly proposed explanation for the beneficial effects of endophytes on plant growth. This result is in conformity with Shahzad *et al*. (2016) [22] reported that significantly higher root length was found in *Bacillus amyloliquefaciens* inoculated plants (5.69 ± 1.37 cm) as compared to plants treated with water (3.54 ± 1.01 cm) in rice. Significantly high seedling vigour index-I and germination rate was observed with the combined inoculation of PSB with *Pseudomonas* due to improved auxin biosynthesis in chilli [23]. The better seedling vigour index-II due to seed priming with *Bacillus amyloliquefaciens* was mainly due to higher seed germination and seedling dry weight registered in the present study. This might be due to the compatibility and antagonistic reaction in endophyte inoculated seed. Similar findings were also observed by Jagadeesh *et al.* (2018) in pigeonpea [24].

The endophyte *Bacillus amyloliquefaciens* showed significantly minimum seed infection might be because of bacterial endophytes characterized by strong antifungal activity, broad spectrum antimicrobial activities and also to produce various metabolites with strong antibiotic activities. The results are in line with Lastochkina *et al*. (2020) [25] showed *B. subtilis* 10^{-4} exerts antagonistic activity against phytopathogenic fungus *Fusarium culmorum* growth due to production of various metabolites with strong antibiotic activities and also produce LPs surfactin C14, C15, and C15 which are characterized by strong antifungal activity in wheat. The presence of surfactin is a strong antibacterial and antifungal and bacillomycin D genes were confirmed in the genome of *Bacillus subtilis* [26] in rice. The electrical conductivity in seeds bio primed with *Bacillus amyloliquefaciens* was less over control this may be due to protection of the seeds by the endophyte from infecting pathogens, thus reduces the seed infection, cracks and aberrations of the seed coat and reduce the leaching of the electrolytes. Similar results were also observed by [27].

Table 1: Influence of priming on seed germination (%), shoot length (cm), root length (cm) and seedling length (cm) 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*	3183	339.3	32234	0.35	0.6

amyloliquefaciens (1×10^8 cfu ml^{-1})					
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

5. CONCLUSION

From the present investigation, it is concluded that sorghum seeds primed with endophyte *Bacillus amyloliquefaciens* showed better results in terms of seed germination, shoot length, root length, seedling length, speed of germination, seedling dry weight, seedling vigour index-I, seedling vigour index-II, lower electrical conductivity (0.35 dsm^{-1}) and seed infection (0.6 %) compared to all other treatments and control. Hence, this endophyte can be utilised for seed bio priming to enhance the seed quality and reduce the seed infection.

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