



RESEARCH ARTICLE

Seed invigoration treatments as a tool for enhancing the vigour and performance of stored sorghum seeds

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Abstract

Seed deterioration during storage adversely affects vigour and subsequent field performance of sorghum (*Sorghum bicolor* L. Moench), necessitating effective post-storage interventions to restore seed quality. The present study was conducted to evaluate the efficacy of different seed invigoration treatments in enhancing vigour, physiological performance, antioxidant activity and field establishment of stored sorghum seed lots differing in initial vigour status. Seeds subjected to various invigoration treatments were evaluated under laboratory and field conditions. The results indicated that all seed invigoration treatments improved germination, seedling vigour, biochemical attributes and field establishment compared with the untreated control, although the magnitude of improvement varied among treatments. Among the evaluated treatments, seed priming with BioNPK + drought alleviating bacteria (DAB) at 0.5 % (B6) significantly improved germination (83.83 %), mean germination time (33.30 hr), seedling vigour index I and II (1790 and 918 respectively) and improved antioxidant activity i.e. catalase, peroxidase, superoxide dismutase, proline content and reduced malondialdehyde content under laboratory conditions. Other priming treatments also exhibited positive effects but were comparatively less effective than B6. Under field conditions, all invigoration treatments improved field emergence, plant growth and yield-related traits over the untreated control, with BioNPK + DAB at 0.5 % (B6) outperforming other treatments by enhancing field emergence (71.78 %), plant growth parameters and yield parameters compared with the untreated control, with pronounced benefits observed in medium- and low-vigour seed lots. Enhanced early seedling establishment translated into improved crop growth and physiological performance under field conditions. The study demonstrates that appropriate seed invigoration treatments can effectively mitigate storage-induced deterioration, improve seed lot performance and serve as a practical tool for enhancing productivity in sorghum cultivation.

Keywords: seed invigoration; seed priming; seed quality; sorghum

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a versatile cereal crop that belongs to the grass family Poaceae. It is one of the top five cereal crops globally and is a multifunctional plant because all parts of the plant can be used either as primary product (such as seeds, leaves and stems are used as food sources, animal feed and industrial raw materials for handicrafts) or as derivatives such as sugar, starch and bioethanol, which possess industrial value (1). Sorghum contains various vitamins, including 0.09 mg of vitamin B1, 0.14 mg of vitamin B2, 2.8 mg of vitamin B3 and minerals such as 4.4 mg of iron, 7 mg of sodium, 249 mg of potassium, 28 mg of calcium and 287 mg of phosphorus. It also relatively high levels of carbohydrates (73.10–79.12 %) and protein (7.98–11.77 %) (2). In India, sorghum is cultivated on approximately 3.97 Mha, with an annual production of 4.4 Mt and an average productivity of 1.0 t/ha. Karnataka accounts for 22 % of the national area (1.09 Mha) and 22.9 % of production (1.13 Mt), achieving a productivity of 1040 kg/ha (3).

Sorghum often referred to as the king of millets, plays a

vital role in food, feed and nutritional security, particularly in semi-arid and arid regions. However, the successful establishment of sorghum crops largely depends on the physiological quality of the seeds used for sowing. During storage, sorghum seeds undergo inevitable ageing processes that lead to a decline in seed vigour, germination capacity and field emergence, primarily due to membrane disintegration, oxidative damage and metabolic dysfunction. Such deterioration is more pronounced in low-vigour seed lots, resulting in poor and non-uniform crop establishment, ultimately affecting productivity (4). Deteriorated sorghum seed lots exhibit low field emergence. Reducing the difference between germination in laboratory condition and field emergence has become one of the primary goals in addition to increase yield (5). An array of seed quality enhancement techniques is available for invigoration of seeds. These techniques are used to increase viability of deteriorated seed or to increase their ability to grow under wide range of environmental conditions or to get high, fast and homogeneous percentages of germination and field emergence, strong seedling and good field establishment.

Seed priming is an effective invigoration technique that can improve the performance of plants under abiotic stresses, in terms of physiological and biochemical changes. Seed priming is generally defined as the controlled hydration of seeds, which allows pre-germinative metabolic activities while avoiding the surfacing of the radicle (6). Seed priming comprises hormonal priming, osmopriming, nutrient priming, hydropriming and biopriming are known to improve germination rate, seedling vigour and field emergence, particularly in aged or deteriorated seed lots. These treatments function by improving membrane integrity, enhancing antioxidant activity and facilitating early metabolic processes essential for seedling establishment (7). Despite these reported benefits, the effectiveness of seed priming often varies depending on the type and concentration of priming agent, seed age, genotype, soil characteristics and prevailing environmental conditions (8). Previous studies have shown inconsistent responses across crops and storage conditions, highlighting the need to identify treatment-specific protocols that provide reliable improvements in seed performance. Moreover, most investigations have focused on individual microbial inoculants, osmotic agents, or chemical priming treatments, while limited information is available on integrated microbial formulations combining nutrient-solubilising and stress-alleviating microorganisms.

BioNPK supplemented with drought-alleviating bacteria (BioNPK + DAB) represents a promising microbial consortium that may offer synergistic benefits beyond conventional priming treatments by enhancing nutrient mobilisation, promoting antioxidant activity, improving membrane stability and stimulating early metabolic activation during germination (9). However, its potential as a post-storage seed invigoration treatment for naturally aged sorghum seed lots has not been adequately investigated under laboratory and field conditions, representing an important research gap. Therefore, the present study was undertaken to evaluate the response of naturally aged sorghum seed lots to BioNPK + DAB seed priming under laboratory and field conditions and to assess its effectiveness as a sustainable seed invigoration strategy.

Materials and Methods

The present investigation on seed invigoration in sorghum seeds was conducted at the ICAR-National Institute of Seed Science & Technology, Regional Station, Bangalore, India. The experiment was laid out in a factorial randomised complete block design (FRCBD) with 2 factors and 3 replications under laboratory and field conditions. Factor A consisted of three sorghum seed lots differing in vigour levels which were stored in cloth bags under ambient conditions ($25 \pm 1^\circ\text{C}$) for 1 year (May 2024–May 2025). A1: CSV 48 (high vigour: >80 % germination), A2: CSH 24 MF (medium vigour: >75 %), A3: CSV 32 F (low vigour: <75 %). Factor B comprised seven seed invigoration treatments, including B1: control, B2: hydropriming (1 : 1.5 w/v ratio) (10), B3: seed priming with KNO_3 (0.5 %) (11), B4: seed priming with salicylic acid (400 ppm) (12), B5: seed priming with azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL kg^{-1} seed), B6: seed priming with BioNPK + DAB (0.5 %) (ICAR-NBAIM). BioNPK is a unique kind of liquid bioformulation comprising nitrogen (N_2) fixing (*Azotobacter chroococcum*), phosphorus (P)-solubilising (*Paenibacillus tylophil*) and potassium (K)-solubilising (*Bacillus decolorationis*) bacteria. The DAB is a microbial consortia (13) and B7: seed priming with seaweed extract (0.5 %) (28 % total soluble solids red and brown algae) (14).

The seeds were subjected to invigoration treatments by soaking for 24 hr at ambient temperature ($25 \pm 1^\circ\text{C}$) and then shade-dried at room temperature. The treatment combinations, comprising different seed lot vigour levels and seed invigoration treatments, were randomly allocated within each replication to evaluate their individual and interactive effects on various seed performance parameters. The experimental site was characterised by red sandy loam soil with good drainage and moderate fertility. The crop was raised following the recommended package of practices for sorghum cultivation. Irrigation was provided as required to maintain adequate soil moisture throughout the crop growth period, particularly during germination, vegetative growth, flowering and grain filling stages. Seeds were sown at a spacing of 45 cm between rows and 15 cm between plants, maintaining the recommended plant population for sorghum. Standard agronomic practices, including nutrient management, intercultural operations and plant protection measures, were followed uniformly across all treatments to minimise experimental variation.

Observations recorded

Evaluation of physiological quality of seed lots

The physiological quality of the seed lots was determined by evaluating germination (%), mean seedling length (cm), mean seedling dry weight (mg), seed vigour index I and II, mean germination time (MGT) (hr).

The germination percentage (%) was evaluated using the standard germination test with 4 replications of 100 seeds each (15). The seeds were placed on Germitest® paper previously moistened with distilled water, arranged in rolls and kept in a Mangelsdorf germinator at 25°C . Germination percentage (%) was recorded after 10 days using the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Total no. of seeds germinated}}{\text{Total seeds kept}} \times 100$$

Mean seedling length and mean seedling dry weight was recorded by selecting 10 normal seedlings from the germination test. Seed vigour indices were calculated using the following formula (16):

$$\text{SVI I} = \text{Germination percentage (\%)} \times \text{mean seedling length (cm)}$$

$$\text{SVI II} = \text{Germination percentage (\%)} \times \text{mean seedling dry weight (mg)}$$

Mean germination time (MGT) was calculated using 400 seeds in 4 replicates of 100 seeds each. The seeds were incubated at 25°C using the top paper method. Radicle emergence (just emerged) counts were recorded at 2 hr intervals after 18 hr and MGT was calculated in hours (17).

Mean germination time

$$(\text{MGT}) = \Sigma \frac{G_t \times D_t}{\Sigma G_t} \times 100$$

where G_t = Number of seeds newly germinated at time D_t .

Evaluation of biochemical parameters of seed lots

Alpha-amylase activity was determined using the 3,5-dinitrosalicylic acid (DNS) method (18), with modifications. Enzyme extraction was performed using 0.5 g of previously macerated seeds, along with 10 mL of sodium acetate buffer 240 mmol L^{-1} pH 5.4 with 10 mmol L^{-1} of CaCl_2 and 0.005 % of Triton X-100. The samples were stirred for 60 min in the presence of ice and then centrifuged. The enzyme extract was incubated in a water bath for 10 min at 70°C and then 250 μL of

enzymatic extract and 250 μL of 2.0 % (w/v) starch solution were incubated in a water bath for 20 min at 38 °C. The reaction was stopped by adding 500 μL of DNS solution and the samples were kept in a water bath for 6 min at 95 °C. At the end of this procedure, 4 mL of distilled water was added, followed by homogenisation. The readings were performed at 540 nm and the results were expressed in mMol/min/mL.

The peroxidase (POX) assay was carried out according to the previous study (19). The reaction mixture was prepared in cuvette by adding 2 mL of 0.1 M phosphate buffer (pH 7.0), Guaiacol (200 μL) and 12.3 mM H_2O_2 (200 μL). The mixture was brought to 25 °C and then the cuvette was placed in the spectrophotometer set at 436 nm. Subsequently, 100 μL of enzyme extract was added. The initial absorbance at 436 nm and the increase in absorbance for 3 min at interval of 30 s recorded using enzyme kinetics. Enzyme activity was expressed as the change in absorbance per minute per gram of seed. Catalase (CAT) activity was measured based on an assay of hydrogen peroxide through the formation of its stable complex with ammonium molybdate (20). A 200 μL aliquot of seed enzyme extract was incubated in 1 mL of reaction mixture containing 65 mM hydrogen peroxide in 60 mM sodium potassium phosphate buffer (pH 7.4) at 25 °C for 4 min. The enzymatic reaction was stopped by adding of 1 mL of 32.4 mM ammonium molybdate and the concentration of the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. The results were expressed as mMol H_2O_2 degraded per minute per gram of seed.

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) using the method (21). One gram of sorghum seed was soaked overnight in distilled water. It was then homogenised in 6 mL of 50 mM phosphate buffer (pH 7). The homogenate was centrifuged at 16000 rpm for 30 min. The supernatant obtained was used as the enzyme extract. To each vial, 0.2 mL each of 200 mM methionine, 1.125 mM NBT, 1.5 mM ethylenediaminetetraacetic acid (EDTA) were added. Then, 0–40 μL of enzyme extract was added and the final volume in each vial was made up to 2.8 mL with 50 mM phosphate buffer (pH 7.8). Subsequently, 0.2 mL of 75 μM riboflavin was added as the last component. The tubes were shaken and kept 30 cm below a light source consisting of two 15 W fluorescent tubes. The vials were exposed to light for 10 min, after which they were removed from the light source and immediately covered with a black cloth. The absorbance of the reaction mixture was measured at 560 nm.

The malondialdehyde assay was carried out according to the previous study (22). The supernatant was removed and the absorbance was read at 532 nm. The value for nonspecific absorption at 600 nm was also recorded and subtracted from the absorbance value at 532 nm. Proline was extracted and determined according to the previous study (23). Briefly, the tissue (800 mg) was pulverised using a liquid nitrogen-chilled mortar and pestle and homogenised in 5 mL of 3 % (w/v) sulfosalicylic acid. The extract was centrifuged for 15 min at 8000 g. The pellet was extracted again with 10 mL of 3 % (w/v) sulfosalicylic acid. The pooled supernatant was used for proline determination.

Field observations

Growth parameters such as field emergence percentage at 20 days after sowing (DAS), plant height (cm), chlorophyll content (measured using a Spad 502 Plus compact SPAD meter in five randomly selected plants per plot at fully expanded leaves), leaf area index,

days to initiation of flowering and 50 % flowering were recorded. Panicle weight (g) and length (cm) was measured by randomly selecting five plants per plot at maturity. Yield parameters such as seed yield (kg/ha), biological yield (kg/ha) and harvest index (%) were assessed.

Statistical analysis

The data collected from the experiment were statistically analysed using R statistical program version 3.5.0 (R Development Core Team, 2021). Analysis of variance (ANOVA) was performed using the agricolae package and treatment means were compared using the appropriate post hoc test at the 5 % level of significance. Whenever the 'F' test was found significant, the critical difference (CD) values were calculated and the means were compared at the 1 % level of significance.

Results

Effect of seed quality enhancement strategies on sorghum seed quality parameters under laboratory conditions

Among the seed lots (Factor A), CSV 48 recorded the highest germination (86.14 %), shoot length (12.66 cm) and root length (12.58 cm), along with the lowest mean germination time (23.83 hr), indicating faster germination. In contrast, CSV 32 F showed the lowest germination (75.64 %), seedling growth (7.64 cm shoot length and 6.97 cm root length) and the highest MGT (42.82 hr) (Table 1). Among the invigoration treatments (Factor B), B6 was the most effective, recording the highest germination (83.83 %), maximum seedling growth (10.86 cm shoot length and 10.24 cm root length) and the lowest MGT (32.30 hr), while B1 showed the poorest performance. The interaction effect (Factor A $\times$ B) was significant, with A1 $\times$ B6 showing the best results (89.00 % germination, superior seedling growth and the lowest MGT), whereas A3 $\times$ B1 recorded the poorest performance with reduced germination and delayed germination time.

Seedling dry weight, vigour index I (SVI I) and vigour index II (SVI II) were recorded as the highest in seed lot CSV 41 (117.92 mg, 2175 and 1016 respectively) (Table 2), while the lowest values were recorded in CSV 32 F (94.07 mg, 1106 and 712, respectively). The B6 treatment exhibited superior performance, with the highest seedling dry weight (108.95 mg), SVI I (1790) and SVI II (918), followed by B5 and B3. In contrast, B1 recorded the lowest values across all parameters. The interaction effect (A $\times$ B) was significant. The combination A1 $\times$ B6 produced the highest seedling dry weight (121.99 mg), SVI I (2283) and SVI II (1086). Conversely, A3 $\times$ B1 recorded the lowest values, indicating poor seed vigour under this combination.

α -Amylase activity, which is an important biochemical indicator of seed vigour, showed prominent differentiation in our results (Table 3). The activities of different antioxidant enzymes also showed significant variation among seed lots and seed treatments. Among seed lots, CSV 48 recorded the highest mean α -amylase activity, catalase activity, peroxidase activity and superoxide dismutase activity (0.67 mMol/min/g, 0.24 mMol H_2O_2 /min/g, 0.71 A_{436} /min/g of seed and 0.55 units/mL/g respectively) followed by CSH 24 MF, while CSV 32 F displayed the lowest values (0.47, 0.11, 0.22 and 0.31 respectively). Among the treatments, α -amylase, CAT, POX and SOD activities were highest in B6 (0.67, 0.18, 0.51 and 0.48 respectively) followed by B3, while the lowest values were observed

Table 1. Effect of different seed treatments on germination (%), mean germination time (hr), shoot length (cm) and root length (cm) of sorghum seed lots

Treatments	Germination (%)				Mean germination time (hr)				Shoot length (cm)				Root length (cm)			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	82.00	79.00	73.50	78.17	24.53	33.45	43.54	33.84	12.44	11.24	7.44	10.37	12.34	10.26	6.73	9.77
B2	85.50	81.00	74.50	80.33	24.23	33.32	43.23	33.59	12.60	11.49	7.56	10.55	12.45	10.42	6.85	9.91
B3	88.50	83.50	78.00	83.33	23.56	32.45	42.34	32.78	12.77	11.79	7.62	10.72	12.82	10.65	7.10	10.19
B4	86.50	82.00	76.00	81.50	24.02	33.13	43.02	33.39	12.56	11.61	7.67	10.61	12.48	10.58	7.00	10.02
B5	86.00	82.00	75.00	81.00	23.24	33.11	43.00	33.12	12.74	11.80	7.75	10.76	12.65	10.60	7.08	10.11
B6	89.00	85.00	77.50	83.83	23.11	32.03	41.76	32.30	12.84	11.92	7.82	10.86	12.82	10.72	7.19	10.24
B7	85.50	81.50	75.00	80.67	24.10	33.15	42.83	33.36	12.69	11.60	7.62	10.64	12.55	10.49	6.89	9.98
Mean	86.14	82.00	75.64		23.83	32.95	42.82		12.66	11.63	7.64		12.58	10.53	6.97	
	SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)	
Factor A	0.368	1.439	1.69		0.022	0.115	2.16		0.032	0.124	1.12		0.033	0.130	1.24	
Factor B	0.562	2.198			0.039	0.170			0.049	0.190			0.051	0.198		
A × B	0.974	3.807			0.073	0.239			0.084	0.329			0.088	0.343		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean.

Table 2. Effect of different seed treatments on seedling dry weight (mg), seedling vigour index I and II of sorghum seed lots

Treatments	Seedling dry weight (mg)				SVI I				SVI II			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	113.33	102.86	92.50	102.89	2032	1698	1041	1590	929	813	680	807
B2	116.25	103.55	92.92	104.24	2141	1775	1073	1663	994	839	692	842
B3	119.80	107.05	94.55	107.13	2264	1873	1148	1762	1060	894	737	897
B4	117.41	105.95	93.95	105.77	2165	1819	1114	1699	1016	869	714	866
B5	119.95	107.95	95.01	107.64	2183	1837	1112	1711	1032	885	713	877
B6	121.99	108.85	96.02	108.95	2283	1924	1163	1790	1086	925	744	918
B7	116.76	106.41	93.55	105.57	2158	1800	1087	1682	998	867	701	856
Mean	117.92	106.09	94.07		2175	1818	1106		1016	870	712	
	SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)	
Factor A	0.283	1.106	0.99		7.038	27.505	1.55		4.647	18.160	2.01	
Factor B	0.432	1.689			10.751	42.015			7.099	27.740		
A × B	0.749	2.925			18.622	72.772			12.295	48.048		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean.

Table 3. Effect of different seed treatments on α-amylase activity (mMol/min/mL), catalase (mMol H₂O₂/min/g of seed), peroxidase (A₄₃₆/min/g of seed) and super oxide dismutase (unit/mL/g) of sorghum seed lots

Treatments	α-Amylase activity				Catalase				Peroxidase				Superoxide dismutase			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	0.55	0.49	0.33	0.46	0.23	0.14	0.09	0.15	0.69	0.47	0.20	0.45	0.53	0.42	0.27	0.40
B2	0.62	0.52	0.45	0.53	0.24	0.14	0.09	0.16	0.70	0.48	0.21	0.46	0.55	0.42	0.30	0.42
B3	0.75	0.63	0.51	0.63	0.25	0.16	0.14	0.18	0.72	0.50	0.23	0.48	0.56	0.44	0.36	0.45
B4	0.65	0.56	0.48	0.56	0.24	0.15	0.10	0.16	0.71	0.48	0.21	0.46	0.55	0.43	0.29	0.42
B5	0.67	0.56	0.48	0.57	0.24	0.15	0.10	0.16	0.70	0.48	0.24	0.47	0.55	0.47	0.32	0.44
B6	0.79	0.68	0.55	0.67	0.27	0.16	0.13	0.18	0.73	0.51	0.27	0.51	0.59	0.51	0.35	0.48
B7	0.67	0.59	0.49	0.58	0.25	0.14	0.10	0.16	0.70	0.47	0.22	0.47	0.56	0.46	0.30	0.44
Mean	0.67	0.58	0.47		0.24	0.15	0.11		0.71	0.48	0.22		0.55	0.45	0.31	
	SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)		SEM (±)	CD (1 %)	CV (%)	
Factor A	0.007	0.027	4.91		0.003	0.011	4.11		0.004	0.017	3.54		0.004	0.016	3.52	
Factor B	0.010	0.041			0.004	0.016			0.007	0.027			0.006	0.025		
A × B	0.018	0.071			0.007	0.028			0.012	0.046			0.011	0.043		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean

in the control (0.46, 0.15, 0.45 and 0.40 respectively). Among the interactions, A1B6 recorded the highest α -amylase, CAT, POX and SOD activities (0.79, 0.27, 0.73 and 0.59 respectively), whereas the lowest values were observed in seed lot CSV 32 F(R23) under the control treatment (0.33, 0.15, 0.20 and 0.27 respectively).

The lowest MDA content and highest proline content were recorded in CSV 48 (0.31 μ M/g and 0.76 μ mol/g) followed by CSH 24 MF, whereas CSV 32 F displayed the highest MDA content (Table 4 and Fig. 1) and lower proline content (0.74 μ M/g and 0.50 μ mol/g) (Fig. 2). Among the treatments, mean MDA content was lowest in B6 (0.49), followed by B3, while the highest was observed in the control (0.56). Mean proline content was highest in treatment B6 (0.69), followed by B3 (0.67), whereas the lowest was observed in the control (0.62). Among the interactions, A1B6 recorded the lowest MDA content and highest proline content (0.26 and 0.81 respectively), whereas the highest MDA content and lowest proline content were found in CSV 32 F under the control treatment (0.77 and 0.47 respectively).

Effect of different seed treatments on growth parameters of sorghum seed lots under field conditions

Field emergence (20 DAS), plant height, leaf area index and chlorophyll content (60 DAS) varied significantly among seed lots

and seed treatments. Among the seed lots, CSV 48 significantly recorded the highest field emergence, plant height, leaf area index and chlorophyll content i.e. 83.19 %, 83.61 cm, 7.02 and 130.16 respectively, followed by CSH 24 MF, while CSV 32 F recorded the lowest values (55.65 %, 57.64 cm, 4.39 and 65.89 respectively) (Table 5). Among the interaction between seed lots and seed treatments, A1B6 recorded the highest field emergence, plant height, leaf area index and chlorophyll content (85.33 %, 114.35 cm, 7.87 and 153.35 respectively), whereas the lowest values were found in seed lot CSV 32 F under the control treatment (66.88 %, 49.48 cm, 3.33 and 49.55 respectively).

Days to initiation of flowering and days to 50 % flowering were significantly lower in CSV 48, with 66.05 and 74.29 days respectively which were on par with CSH 24 MF (66.33 and 77.29 respectively), while CSV 32 F(R23) recorded the highest values (70.05 and 78.67 respectively) (Fig. 3). Among the treatments, B6 recorded fewer days to initiation of flowering and days to 50 % flowering (64.33 and 74.88 days respectively) while the maximum number of days was observed in the control (69.77 and 79.77 days respectively). Among the interactions, A1B6 recorded fewer days to initiation of flowering and days to 50 % flowering (61 and 71 days respectively), whereas the highest values were found in seed lot CSV 32 under the control treatment (71.67 and 81.67 days respectively).

Table 4. Effect of different seed treatments on malondialdehyde (μ M/g seed wt) and proline content (μ mol/g) of sorghum

Treatments	MDA (μ M/g seed wt)				Proline (μ mol/g)			
	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	0.37	0.55	0.77	0.56	0.70	0.68	0.47	0.62
B2	0.34	0.52	0.77	0.55	0.74	0.70	0.48	0.64
B3	0.27	0.51	0.72	0.50	0.79	0.73	0.50	0.67
B4	0.30	0.51	0.73	0.51	0.74	0.69	0.51	0.65
B5	0.33	0.53	0.75	0.54	0.77	0.70	0.49	0.65
B6	0.26	0.49	0.71	0.49	0.81	0.74	0.52	0.69
B7	0.28	0.50	0.76	0.51	0.77	0.70	0.50	0.66
Mean	0.31	0.51	0.74		0.76	0.71	0.50	
	SEM ($\pm$)	CD (1 %)	CV (1 %)		SEM ($\pm$)	CD (1 %)	CV (1 %)	
Factor A	0.007	0.027	4.91		0.004	0.015	2.24	
Factor B	0.010	0.041			0.006	0.023		
A $\times$ B	0.018	0.071			0.010	0.040		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean.

Table 5. Effect of different seed treatments on growth parameters of sorghum seed lots under field conditions

Treatments	Field emergence (%)				Plant height (cm)				Leaf area index				Chlorophyll (SPAD meter)			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	80.00	66.75	53.88	66.88	65.07	55.68	49.48	56.74	6.27	4.76	3.33	4.79	106.46	94.35	49.55	83.45
B2	82.00	67.33	54.67	68.00	70.70	63.05	52.43	62.06	6.63	4.97	4.10	5.23	115.35	100.34	53.21	89.63
B3	84.33	69.33	55.33	69.66	94.12	85.31	61.70	80.38	7.46	6.72	4.76	6.31	143.32	126.39	75.65	115.12
B4	83.33	67.67	54.67	68.56	84.41	76.94	61.64	74.33	7.22	6.62	4.61	6.15	134.54	122.36	72.43	109.78
B5	84.67	68.33	57.33	70.11	74.58	69.12	52.63	65.44	6.75	5.46	4.36	5.53	133.28	104.54	59.65	99.16
B6	85.33	70.67	59.33	71.78	114.35	91.15	69.20	91.56	7.87	7.06	5.12	6.68	153.35	134.65	86.44	124.81
B7	82.67	67.00	54.33	68.00	82.05	75.18	56.38	71.20	6.93	6.16	4.46	5.85	124.84	115.36	64.33	101.51
Mean	83.19	68.15	55.65		83.61	73.78	57.64		7.02	5.96	4.39		130.16	114.00	65.89	
	SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)	
Factor A	2.981	8.636	10.26		1.706	4.941	10.91		0.084	0.244	6.65		1.525	4.417	6.76	
Factor B	1.554	3.192			2.605	6.548			0.128	0.372			2.329	3.747		
A $\times$ B	1.888	2.850			2.513	3.073			0.222	0.645			2.034	2.687		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean.

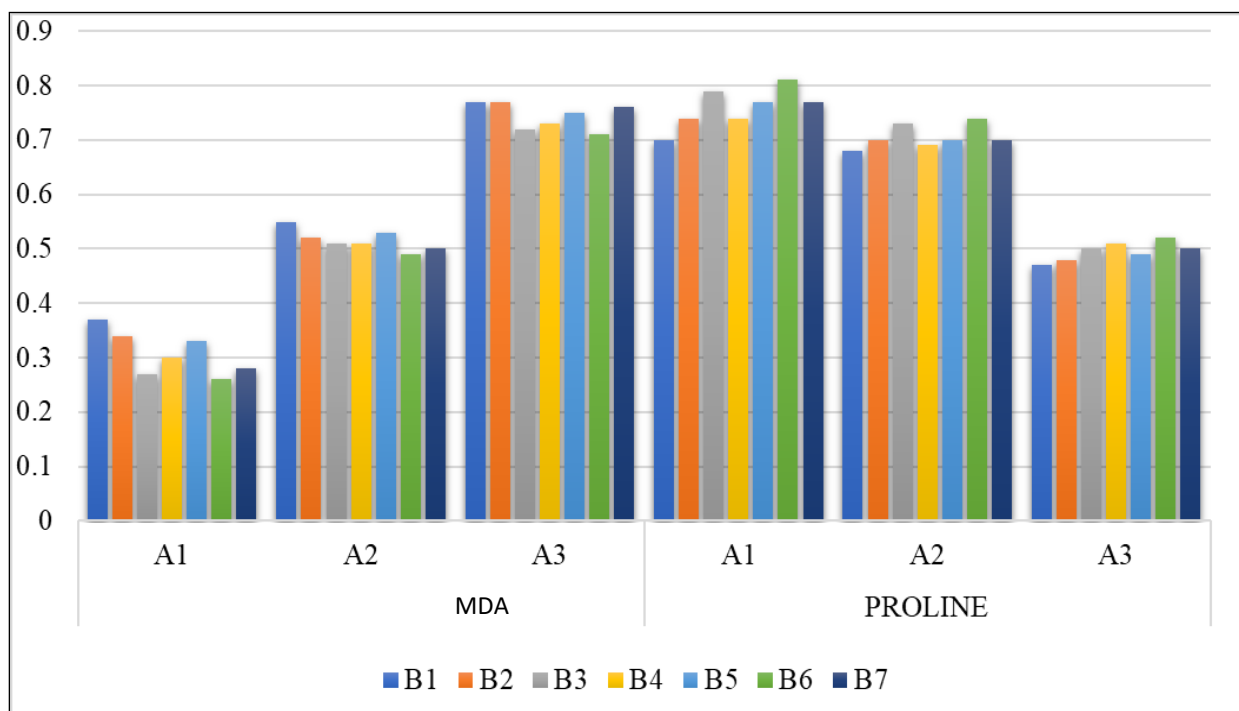


Fig. 1. Effect of different seed treatments on malondialdehyde ($\mu\text{M/g}$ seed wt) and proline content ($\mu\text{mol/g}$) of sorghum seed lots. A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. B1: control, B2: hydropriming (1 : 1.5 w/v ratio), B3: KNO_3 (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %).



Fig. 2. Effect of seed treatments on proline content of sorghum seed lots.

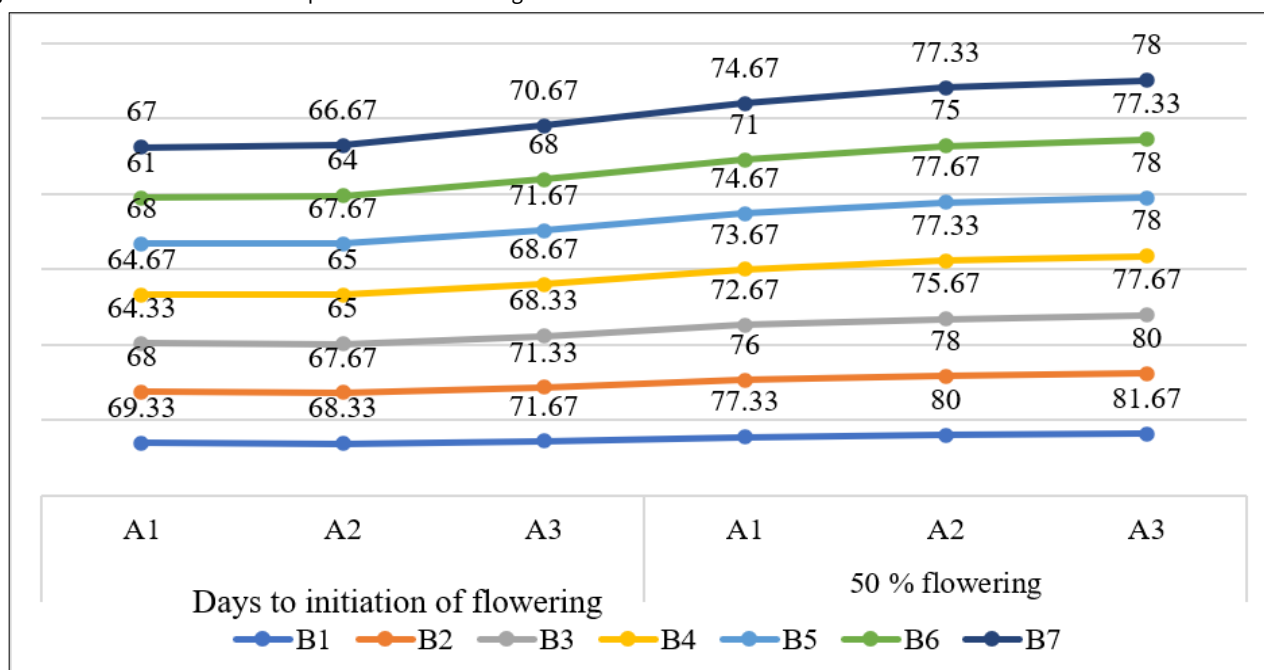


Fig. 3. Effect of different seed treatments on days to initiation of flowering and days to 50 % flowering of sorghum seed lots. A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO_3 (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %).

Effect of different seed treatments on yield parameters of sorghum seed lots

Panicle length, panicle weight and seed yield per plant were recorded during maturity stage. Among the seed lots, CSV 48 significantly recorded the highest panicle length (Fig. 4), panicle weight and seed yield per plant i.e. 18.28 cm, 58.86 g and 39.16 g respectively, while CSV 32 F recorded the lowest (14.95 cm, 46.27 g and 27.72 g respectively) (Table 6). Among the treatments, these observations were highest in treatment B6 (17.52 cm, 54.89 g and 36.10 g respectively) which was on par with B3 (17.34 cm, 53.87 g and 35.07 g respectively), while the control recorded the lowest values (15.46 cm, 50.45 g and 30.72 g respectively). Among the interactions, A1B6 recorded the highest panicle length, panicle weight and seed yield per plant (19.35 cm, 54.55 g and 41.43 g respectively), whereas the lowest values were found in seed lot CSV 32 F under the control treatment (13.58 cm, 44.46 g and 25.48 g respectively).

Seed yield per hectare, biological yield and harvest index were recorded after harvesting the seeds. CSV 48 significantly recorded the highest mean seed yield per hectare, biological yield and harvest index i.e. 943 kg/ha, 5859.43 kg/ha and 16.10 % respectively, followed by CSH 24 MF, while CSV 32 F recorded the lowest values (512 kg/ha, 5193 kg/ha and 9.87 % respectively) (Table 7). Among the treatments, these observations were highest in treatment B6 (774 kg/ha, 5590 kg/ha and 13.75 % respectively)

followed by B3, while the control recorded the lowest values (762 kg/ha, 5562 kg/ha and 13.58 % respectively). B2 and B7 treatments showed non-significant variation compared to the control, which was due to lower seed yield per plant and biological yield per plant. Among the interactions, A1B6 recorded the highest mean seed yield per hectare, biological yield and harvest index (978.61 kg/ha, 5867.34 kg/ha and 16.68 % respectively), whereas the lowest values were found in seed lot CSV 32 F under the control treatment (485.96 kg/ha, 5030.33 kg/ha and 9.66 % respectively).

Discussion

The results clearly demonstrate that both the genetic constitution of seed lots and invigoration treatments play crucial role in determining sorghum seed physiological quality. The superior performance of seed lot CSV 48 in terms of higher germination, enhanced seedling growth and reduced mean germination time indicates better inherent seed vigour and storability. In contrast, the poor performance of CSV 32 F was attributed to faster deterioration, reduced enzymatic activity and membrane damage, which are commonly associated with seed ageing (4). Seed invigoration treatments significantly improved germination and seedling growth while reducing mean germination time. Seed priming with BioNPK + DAB (0.5 %) resulted in increased α -amylase activity, which in turn promoted the mobilisation of stored carbohydrate reserves, resulting in improved germination,

Table 6. Effect of different seed treatments on panicle length (cm), panicle weight (g) and seed yield per plant (g) of sorghum seed lots

Treatments	Panicle length(cm)				Panicle weight(g)				Seed yield per plant (g)			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	17.45	15.35	13.58	15.46	56.54	50.35	44.46	50.45	36.34	30.34	25.48	30.72
B2	17.73	15.78	14.95	16.15	57.25	51.00	44.78	51.01	37.24	30.98	26.43	31.55
B3	18.94	17.35	15.74	17.34	60.14	53.46	48.00	53.87	40.65	35.43	29.13	35.07
B4	18.15	16.94	15.31	16.80	59.56	52.13	46.78	52.82	39.56	32.47	27.96	33.33
B5	18.47	16.48	14.89	16.61	59.43	51.89	45.66	52.33	39.78	33.65	27.47	33.63
B6	19.35	17.43	15.79	17.52	61.35	54.55	48.76	54.89	41.43	36.54	30.34	36.10
B7	17.89	16.13	14.41	16.14	57.73	51.46	45.45	51.55	39.13	31.38	27.24	32.58
Mean	18.28	16.49	14.95		58.86	52.12	46.27		39.16	32.97	27.72	
	SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)	
Factor A	0.233	0.675	6.44		0.736	2.133	6.44		1.284	3.139	7.47	
Factor B	0.356	1.030			1.125	2.258			1.766	2.795		
A $\times$ B	0.616	1.785			1.948	5.643			3.867	4.305		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean

Table 7. Effect of different seed treatments on seed yield (kg/ha), biological yield (kg/ha) and harvest index (%) of sorghum seed lots

Treatments	Seed yield (kg/ha)				Biological yield (kg/ha)				Harvest index (%)			
	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
B1	906.43	758.46	485.96	716.95	5771.45	5395.34	5030.33	5399.04	15.71	14.06	9.66	13.14
B2	921.99	762.54	490.54	725.02	5850.24	5404.44	5151.23	5468.64	15.76	14.11	9.52	13.13
B3	965.38	783.40	537.66	762.15	5923.00	5513.76	5250.35	5562.37	16.30	14.21	10.24	13.58
B4	950.45	780.65	506.80	745.97	5836.40	5491.87	5222.20	5516.82	16.28	14.21	9.70	13.40
B5	949.40	777.97	516.88	748.08	5830.00	5461.00	5198.93	5496.64	16.28	14.25	9.94	13.49
B6	978.61	789.63	554.21	774.15	5867.34	5577.33	5327.00	5590.56	16.68	14.16	10.40	13.75
B7	932.01	771.09	498.26	733.79	5937.56	5454.00	5176.25	5522.60	15.70	14.14	9.63	13.15
Mean	943.47	774.82	512.90		5859.43	5471.11	5193.76		16.10	14.16	9.87	
	SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)		SEM ($\pm$)	CD (1 %)	CV (%)	
Factor A	10.675	30.923	6.577		77.233	63.736	6.396		0.190	0.551	6.513	
Factor B	6.306	19.236			117.972	241.766			0.290	0.413		
A $\times$ B	18.243	21.815			104.344	191.952			0.503	0.757		

Factor A: seedlots, A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. Factor B: seed treatments, B1: control, B2: hydropriming (1: 1.5 w/v ratio), B3: KNO₃ (0.5 %), B4: salicylic acid (400 ppm), B5: azoxystrobin 2.5 % + thiophanate methyl 11.25 % + thiamethoxam 25 % FS (10 mL/kg seed), B6: BioNPK + DAB (0.5 %), B7: seaweed extract (0.5 %). CD (1 %): critical difference at 1 % probability level; treatment means differing by more than the CD value are considered statistically significant. CV (%): coefficient of variation, indicating the extent of variability in relation to the mean. SEM: standard error of the mean.



Fig. 4. Effect of different seed treatments on panicle length (cm) of sorghum seed lots. A1: CSV 48, A2: CSH 24 MF, A3: CSV 32 F. B1: control, B6: seed priming with BioNPK + DAB (0.5 %).

seedling dry matter accumulation and vigour indices (24). Similar improvements in seed performance due to priming treatments have been widely reported in sorghum, forage sorghum, wheat, soybean, French bean and sunflower (25–30). The pronounced improvement observed in A1 and A2 under B6 indicates that high-vigour seed lots respond more efficiently to invigoration treatments, whereas low-vigour seeds (A3) show comparatively limited improvement. This differential response is linked to the extent of physiological damage and the capacity for repair mechanisms within the seeds (31).

The enhancement of α -amylase activity is directly associated with improved seed germination and vigour, as this enzyme plays a critical role in the degradation of starch reserves into sugars that fuel embryo growth. Seed priming act as a signal for the activation of α -amylase enzyme and further rejuvenates embryo elongation through radicle protrusion (6). The enhanced activity of various antioxidant enzymes under B6 treatment can be attributed to increased metabolic activation by microbial consortia and drought-alleviating bacteria-mediated improvement in oxidative stress regulation, which supports better membrane stability during germination (32, 33). Seed priming with KNO_3 also enhanced antioxidants activity, reflecting nitrate-induced activation of antioxidant pathways that reduce hydrogen peroxide accumulation (34). The results are in agreement with the findings of previous studies, which emphasised the effect of priming treatments on the antioxidant system in seeds (35–39). Reduced MDA levels under B6 can be attributed to enhanced antioxidant defence stimulated by microbial consortia and DAB, which helps maintain membrane stability and reduces peroxidative damage during germination (40). B6 and B3 treatments promoted osmotic adjustment and protein stabilisation. The accumulation of proline content might act as a compatible solute, regulating and reducing water loss from plant cells during water deficit and playing an important role in osmosis regulation, indicating higher lipid peroxidation due to oxidative stress in untreated seeds (41–43).

The BioNPK + DAB priming is particularly beneficial for aged seeds because it combines plant growth-promoting bacteria with a stress-tolerant microbial consortium that enhances metabolic repair during the early stages of germination. BioNPK contains nitrogen-fixing (*A. chroococcum*), phosphorus-solubilising (*P. tylophil*) and potassium-solubilising (*B. decolorationis*)

microorganisms, which improve nutrient mobilisation and stimulate seed metabolic activity. DAB, a unique liquid microbial consortium containing archaea with high thermal stability and extended shelf life, contributes to enhanced stress tolerance and resilience under adverse conditions (13). During seed priming, these beneficial microorganisms facilitate membrane repair, activate antioxidant defence systems, promote enzyme activities and improve osmotic regulation, thereby counteracting the physiological and biochemical damage associated with seed ageing. KNO_3 priming enhances nitrate uptake and accelerates cell division, leading to rapid radicle protrusion, improved seedling performance and increased plant height (44). Salicylic acid priming (B4) improved emergence due to its role in activating stress signalling pathways that enhance antioxidant responses, resulting in higher survival of emerging seedlings (45). The positive impact of seed priming on field emergence and better plant establishment of sorghum seeds is supported by studies conducted in maize (46, 47) and cowpea (48). The advancement of flowering in B6 is due to improved physiological efficiency, enhanced hormonal regulation and better nutrient mobilisation, which accelerate the transition from vegetative to reproductive growth. KNO_3 promoted early flowering by improving nitrogen availability, enzyme activity and osmotic balance, thereby supporting rapid early growth and faster attainment of reproductive maturity. Similar findings have shown that primed seeds provide an energetic start and early crop establishment thereby leads to earlier flowering in rice and maize (39, 43, 46).

Better plant stand establishment enhanced panicle length, panicle weight and consequently increased seed yield per plant. B2 and B7 treatments showed non-significant variation compared to the control, indicating that although these treatments enhanced growth parameters, they did not effectively influence yield parameters in sorghum. The interaction effect showed that significant differences exists among the different seed lots and priming treatments with respect to panicle length, panicle weight and seed yield per plant. These findings are in accordance with those reported in sunflower and spotted bean (49, 50). CSV 48 showed superior seed yield, biological yield and harvest index due to better seed vigour, crop establishment and efficient assimilate partitioning, whereas CSV 32 F performed poorly due to inferior seed quality. Among the treatments, B6 was the most effective, enhancing yield through improved germination, growth and physiological efficiency, with B3 and B5 also proving beneficial.

The significant interaction indicated that high-vigour seeds (A1) responded better (A1 × B6), while low-vigour seeds (A3) showed limited improvement (51, 52).

Conclusion

The present study demonstrated that seed invigoration treatments effectively alleviated storage-induced deterioration in sorghum seeds by improving their physiological, biochemical and field performance. Among the evaluated treatments, seed priming with BioNPK + drought alleviating bacteria (DAB) at 0.5 % (B6) consistently exhibited superior or comparable performance across most laboratory and field parameters and showed statistically significant improvements over the untreated control for several key traits, including germination, seedling vigour, antioxidant enzyme activities, proline accumulation and reduced lipid peroxidation. These physiological and biochemical enhancements were associated with improved field emergence, plant stand establishment, vegetative growth, chlorophyll content and earlier flowering. The B6 treatment also resulted in significantly higher yield and yield-attributing traits, including panicle characteristics, seed yield per plant, seed yield per hectare, biological yield and harvest index, compared with the control. Although KNO₃ at 0.5 % (B3) and some other invigoration treatments also produced beneficial effects and significantly improved certain parameters relative to the control, their overall performance was generally lower than that observed with BioNPK + DAB. Considering its simple seed-soaking procedure, compatibility with existing seed management practices and substantial improvement in seed quality and productivity, BioNPK + DAB at 0.5 % represents a practical and economically promising seed invigoration strategy for restoring the performance of stored sorghum seeds and enhancing field establishment and yield under farmers' conditions.

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Authors' contributions

SKV conceptualised the research. PD and SKV carried out experimental work according to plan including data collection, analysis, contributed to data interpretation and manuscript preparation. BNK supervised the research work. SR and P reviewed and edited manuscript. MB, GKR and BK assisted in conducting experiments and analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (OpenAI) solely for language editing and grammar improvement. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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