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Effect of seed priming on quality of accelerated aged seed of sorghum

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Abstract
The seed samples with different levels of initial germination and seed quality parameters obtained through different duration (24, 36, 48 and 60 hours) of accelerated ageing was subjected to seed priming with different chemicals *viz.*, 50 ppm GA_3, 2% KH_2PO_4, 2% $CaCl_2$ and 2% KNO_3 for 10 h of duration. The seed was tested for germination, seed quality traits to study the effect of seed priming on accelerated aged seed. The seed priming significantly improved the germination and all the seed quality parameters except electrical conductivity of leachates of accelerated aged seed. The electrical conductivity of seed leachates was reduced with the priming. The improvement in initial seed quality was found to be highest with seed priming of accelerated aged seed with 2% KH_2PO_4 followed by 2% $CaCl_2$.

Keywords: accelerated ageing, seed priming, seed quality

1. Introduction

In India, sorghum is grown in an area of 5.62 million hectares with an annual production of 4.56 million tonnes with an average productivity of 812 kg per hectare in 2016-17 (https://www.indiastat.com). In Andhra Pradesh, sorghum is grown in an area of 0.97 million hectares with an annual production of 1.98 million tonnes with an average productivity of 2041 kg per hectare in 2016-17 (https://www.indiastat.com) [11].

Seed enter an ageing process after physiological maturity. Seed ageing is associated various alterations at cellular level, reduction in energy metabolism, impairement of RNA, protein synthesis and DNA degradation result in detrimental effects on seed quality (Kibinza *et al.*, 2006 and Jyoti and Malik, 2013) [16, 15]. Studying effect of seed ageing on seed viability and vigour through natural aging of seeds is a long-duration and costly procedure. Accelerated ageing test is employed as a common method to simulate seed aging in a short time compared to natural ageing for research purpose. The principle involved is acceleration of the rate of seed deterioration, by exposing the seed to high temperature (40-45 °C) and relative humidity (greater than 95%) for different durations (Delouche and Baskin, 1973) [8]

Seed priming improves the seed vigor with quick and uniform germination over a range of environmental conditions (Nawaz *et al*., 2013) [18]. Priming of seed reversed some of the ageing-induced deteriorative events and thus improved the seed performance through repair of the age-related cellular and sub-cellular damage (proteins, RNA and DNA) of low vigor seed (Clarke and James, 1991) [5]. Many seed priming treatments have been used to reduce the damage of ageing and enhance their performance in many crops (Basra *et al*., 2003) [4]. Therefore present investigation was conducted to study the influence of seed priming with different chemicals in improving the seed quality of accelerated aged seed of sorghum.

2. Materials and Methods

The present investigation was carried in the Department of Seed Science and Technology, Advanced Post Graduate Center, Acharya N.G. Ranga Agricultural University, Lam, Guntur, Andhra Pradesh with freshly harvested (*Rabi*, 2017-18) foundation seed of sorghum variety, NTJ-4.

Seed with initial germination of 97% was subjected to accelerated ageing in water jacketed accelerated ageing (AA) chamber by the procedure described by the Tekrony (2005) [30]. For accelerated ageing the sorghum seed was placed on a screen tray in uniform layer, which was inserted into a small plastic box containing 40 ml of water. The plastic box was placed into a water-jacketed AA chamber and the seed was aged at a temperature 45 °C and relative humidity 95±5 % for 24, 36, 48 and 60 h.

Then the seed aged for different duration was subjected to seed priming with 50 ppm GA_3, 2 % KH_2PO_4, 2 % $CaCl_2$ and 2 % KNO_3 respectively using 1:1 seed weight to solution volume (w/v) ratio for 10 hours. After treatment the seed was dried back to 12 % moisture content under shade at room temperature. The experiment was conducted in Factorial completely randomized design with four replications.

Data on germination, seedling length, seedling vigour index, electrical conductivity and field emergence were recorded as mentioned below

2.1 Germination: Four replicates of 100 seeds from each treatment were taken at random and placed at uniform spacing in between two wetted germination paper towels. The paper towels were rolled and secured with rubber bands on both sides and were placed in plastic tray in upright position and the trays were placed in germinator at 25 ± 2 °C and 95 % RH for 10 days.

2.2 Seedling length (cm): The total distance from the tip of primary leaf to root tip of 10 randomly selected seedlings in each treatment in each replication was measured with scale and their mean was expressed as seedling length in centimeter.

2.3 Seedling vigor index: It was computed by adopting the following formula as suggested by Abdul-Baki and Anderson (1973) [2] and was expressed in whole number.

Seedling Vigor index = Germination (%) x Seedling length (cm)

2.4 Electrical Conductivity of Seed Leachates: Fifty randomly selected seed from each treatment and each replication were soaked in 75 ml of deionized water for 24 h at room temperature. The seed steep water was decanted and referred to as seed leachate. The electrical conductivity of the seed leachate was measured with a digital conductivity meter (Model: Conductivity TDS meter-307) with a cell constant of one and expressed as dSm^{-1}.

2.5 Field Emergence (%): One hundred seed from each treatment in each replication were counted and sown in well prepared soil at 3 cm depth. The field emergence was recorded on the 15^{th} day after sowing and the field emergence percentage was calculated as per the formula:

$$\text{Field emergence (\%)} = \frac{\text{Number of normal seedlings emerged}}{\text{Total number of seeds sown}} \times 100$$

3. Results and Discussion

The analysis of variance of the data revealed that accelerated ageing duration, seed treatment had showed significant difference in germination, seedling length, seedling vigour index, electrical conductivity and field emergence. While interaction between accelerated ageing and seed treatment had exhibited significant variation among all seed quality parameters expect germination and field emergence.

3.1 Germination

Germination followed decreasing trend with increasing the duration of accelerated ageing (Table 1). Highest mean germination (95.60%) was recorded in 24 h of accelerated ageing and lowest mean germination (93.05%) in 60 h of accelerated ageing. The reduction in germination might be due to changes in biochemical activities of seed during the accelerated ageing. Radha *et al.* (2014) [19] noticed decreased activity of dehydrogenase, α- amylase and peroxidase after natural and accelerated ageing treatments and concluded that inactivation of free radical scavenging enzymes (*i.e*., SOD) during ageing showed direct relationship with germination efficiency of seed.

The seed treatment caused significant improvement in germination of accelerated aged seed of sorghum. The mean germination of accelerated aged seed subjected to seed treatment ranged from 92.06% to 96.5% (Table 1). Highest enhancement in mean germination was observed with 2% KH_2PO_4 treatment (96.50%) and 2% $CaCl_2$ (96%), while lowest improvement in mean germination was realized with 2% KNO_3 (93.56%) over the untreated accelerated ageing treatment (92.06%). The interaction effect of accelerated ageing duration and seed treatment caused significant increase in germination. Seed treatment with 2% KH_2PO_4 resulted in highest per cent increment in germination (8.19%) of 60 h accelerated aged seed (Fig.1).

These results are in support of the findings of many earlier researchers *viz*., Chauhan *et al*. (2016) [6] in sorghum and Hussein (2016) [12] in maize. The increase in seed germination upon priming may be due to increase in various free radical scavenging enzymes, such as superoxide dismutase, catalase and peroxidase and decreased lipid peroxidation (Bailly *et al*., 2002) [3].

3.2 Seedling length (cm)

The seedling length decreased significantly with increase in the duration of accelerated ageing (Table 1). Highest mean seedling length was noticed in 24 h of ageing (31.37 cm), while the lowest mean seedling length was obtained with 60 h of accelerated ageing (27.51cm).

Seed treatment of accelerated aged seed resulted in significant improvement in seed length (Table 3). Maximum increment in mean seedling length after seed treatment over the untreated (25.55 cm) seed was noticed with 2 % KH_2PO_4 (31.91 cm), whereas minimum improvement was obtained with KNO_3 (28.51 cm).

Interaction between accelerated ageing duration and seed treatment resulted in significant enhancement in seedling length. Longer seedlings were obtained with 24 h accelerated aged seed treated with 2% KH_2PO_4 (34.95cm), whereas, highest per cent (30.08%) increment over untreated seed was observed in 60 h accelerated aged seed treated with 2 % KH_2PO_4 (Table 1).

Ilse *et al*. (2012) [14] stated that priming activates the repair processes such as damaged DNA, proteins, membranes and mitochondria via stored mRNA and stored protein, which in turn increases the energy production (ATP) required for germination and seedling growth.

3.3 Seedling vigour index

Duration of accelerated ageing exhibited significant negative impact on seedling vigour index of sorghum seed. Seedling vigour index was more in shorter duration of accelerated ageing (24 h) (3001) compared to higher duration of accelerated ageing (60 h) (2565) (Table 2). The reduction in seedling vigour index from control to 60 h of accelerated ageing treatment might be due to increase in solute leakage.

The mean seedling vigour index in accelerated aged seed was significantly improved with seed treatment, the maximum mean seedling vigour was observed in 2% KH_2PO_4 treatment (3072) followed by 2% KNO_3 (2672) treatment over the untreated accelerated aged seed (2358) (Table 2).

Interaction between accelerated ageing duration and seed treatment had significant impact on seedling vigour index. Highest seedling vigour index was observed in 24 h accelerated aged seed treated with 2% KH_2PO_4 (3381) and lowest in 60 h accelerated aged seed treated with 2% KNO_3 (2560).

Such Improvement in seedling vigour index up on seed treatment was observed in maize (Sathish *et al.*, 2011 and Hussien, 2016 and sorghum (Chauhan *et al.,* 2016) [21, 12, 7].

3.4 Field emergence (%)

The trend of variation in field emergence due to duration of accelerated ageing was similar to that of germination (Table 2). The mean field emergence decreased from 85.50% in 24 h of accelerated ageing to 67.50% in 60 h of accelerated ageing. However, the impact of decline in mean field emergence from 24 h to 36 h (81.28%) was comparatively less. Thereafter further increase in duration of accelerated ageing resulted in more pronounced negative influence on field emergence.

Such decline in field emergence with accelerated ageing was reported by Ibrahim *et al*. (1993) [13] in sorghum, Samarah and Al-Kofahi (2008) [20] in barley and Ghassemi-Golezani and Dalil (2011) [9] in maize.

The field emergence of accelerated aged seed was significantly improved with the seed treatment (Table 2). The mean field emergence improved from 72.81% (untreated) to 80.50% with KH_2PO_4 treatment and followed by 78.37% with 2% $CaCl_2$.

Interaction between accelerated ageing duration and seed treatment showed non-significant effect on field emergence. However, the highest (13.89%) per cent increase was observed in 60 h aged seed treated with 2% KH_2PO_4 seed over the untreated 60 h accelerated aged seed (Fig. 2).

According to Mir-Mahmoodi *et al*. (2011) [17] seed priming of maize seeds with KH_2PO_4 resulted in higher emergence and establishment in field.

3.5 Electrical conductivity (dSm^{-1}) of seed leachates

The results revealed that duration of accelerated ageing had negative effect on electrical conductivity of seed leachates (Table 3). The highest mean electrical conductivity of seed leachates was recorded in 60 h of accelerated aged seed (0.17 dSm^{-1}) and lowest in seed subjected accelerated ageing for 24 h (0.11 dSm^{-1}). The increase in electrical conductivity might be due to autoxidation of polyunsaturated fatty acids in the membrane by the free radicle chain reaction (Doijode, 1988) [8]. Gupta *et al.* (2005) [10] also reported increase in electrical conductivity due to membrane deterioration and metabolic changes in accelerated aged seed.

Seed treatment with different chemicals had resulted in significant reduction in mean electrical conductivity of seed leachates of accelerated aged seed. Highest reduction in mean electrical conductivity of seed leachates was noticed with 2% KH_2PO_4 (0.11 dSm^{-1}) when compared with the untreated accelerated aged seed (0.17 dSm^{-1}) (Table 3).

Interaction between accelerated ageing duration and seed treatment showed significant decline in mean electrical conductivity of accelerated aged seed. More pronounced decline was observed in 60 h accelerated aged seed treated with 2% KH_2PO_4 (0.13 dSm^{-1}) compared to 60 h accelerated aged untreated seed (0.23 dSm^{-1}).

The decrease in electrical conductivity might be due to the positive effect of KH_2PO_4 the membrane stability and reduced the negative effects of accelerated ageing (Abdolahi *et al*., 2012) [1]. Siri *et al.* (2013) [22] also reported enhanced defensive antioxidant enzyme system in aged seed due to priming and consequently reduction in the cell membrane damage caused by the accumulation of ROS.

Table 1: Effect of seed priming of accelerated aged seed on germination (%) and seedling length (cm) in sorghum

Treatments	Germination (%)						Seedling Length (cm)					
	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
A_1	94.75 (76.90)*	95.00 (77.08)	96.75 (79.72)	96.50 (79.40)	95.00 (77.08)	95.60 (78.03)^a	29.46	30.27	34.95	32.18	29.99	31.37^a
A_2	93.50 (75.30)	95.25 (77.64)	96.50 (79.33)	96.25 (78.87)	94.25 (76.20)	95.15 (77.47)^a	26.04	30.04	31.59	31.31	27.31	29.32^b
A_3	91.50 (73.10)	95.75 (78.35)	96.00 (78.55)	95.75 (76.82)	94.00 (76.77)	94.60 (76.72)^{ab}	24.00	29.23	31.60	30.40	28.75	28.79^b
A_4	88.50 (70.19)	94.25 (76.20)	95.75 (78.13)	95.25 (77.47)	91.50 (73.09)	93.05 (75.01)^b	22.70	28.49	29.53	28.85	27.99	27.51^c
Mean	92.06 (73.87)^D	95.00 (77.24)^B	96.50 (79.30)^A	96.00 (78.59)^{AB}	93.56 (75.45)^C		25.55^E	29.63^C	31.91^A	30.64^B	28.51^D	
	A		T		A × T		A		T		A × T	
SEm±	0.45		0.50		1.01		0.29		0.33		0.65	
CD (5%)	1.28		1.43		NS		0.82		0.92		1.84	
CV (%)	2.63						4.44					

*Values in the parenthesis indicate arc-sine transformed values

The values in the same column / row with the same alphabet are not significantly different ($P < 0.05$) by DMRT.

NS: Non-significant

Factor–I

A1– 24 h of accelerated ageing

A2– 36 h of accelerated ageing

A3 – 48 h of accelerated ageing

A4 – 60 h of accelerated ageing

Factor – II

T0 – No seed treatment

T1 – Seed treatment with GA3 @ 50 ppm for 10 h

T2 – Seed treatment with 2% KH2PO4 for 10 h

T3 – Seed treatment with 2% CaCl2 for 10 h

T4 – Seed treatment with 2% KNO3 for 10 h

Table 2: Effect of seed priming of accelerated aged seed on seedling vigour index and field emergence (%) in sorghum

	Seedling vigour index						Field Emergence (%)					
Treatments	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
A_1	2793	2876	3381	3105	2850	3001^a	81.50 (64.52)*	86.5 (68.51)	87 (68.87)	86.75 (68.75)	85.75 (68.01)	85.50 (67.73)^a
A_2	2434	2909	3049	2995	2574	292^b	75.75 (60.53)	81.25 (64.38)	84.75 (67.07)	83.00 (65.76)	81.25 (64.32)	81.28 (64.41)^b
A_3	2195	2798	3032	2911	2702	2728^b	71.00 (57.44)	75.50 (60.37)	78.50 (62.36)	76.00 (60.68)	73.75 (59.20)	74.59 (60.01)^c
A_4	2007	2683	2827	2747	2560	2565^c	63.00 (52.55)	67.25 (55.10)	71.75 (57.89)	67.75 (55.38)	67.75 (55.38)	67.50 (55.26)^d
Mean	2358^E	2817^C	3072^A	2940^B	2672^D		72.81 (58.76)^C	77.62 (62.09)^B	80.50 (64.05)^A	78.37 (62.65)^{AB}	77.12 (61.73)^B	

	A	T	A × T	A	T	A × T
S Em ±	27.57	30.82	61.65	0.55	0.62	1.23
CD (5%)	7.98	87.19	174.38	1.57	1.75	NS
CV (%)	4.44			3.96		

*Values in the parenthesis indicate arc-sine transformed values
The values in the same column / row with the same alphabet are not significantly different (P < 0.05) by DMRT.
NS: Non-significant
Factor–I

A1– 24 h of accelerated ageing
A2– 36 h of accelerated ageing
A3 – 48 h of accelerated ageing
A4 – 60 h of accelerated ageing

Factor –II

T0 – No seed treatment
T1 – Seed treatment with GA3 @ 50 ppm for 10 h
T2 – Seed treatment with 2% KH2PO4 for 10 h
T3 – Seed treatment with 2% CaCl2 for 10 h
T4 – Seed treatment with 2% KNO3 for 10 h

Table 3: Effect of seed priming of accelerated aged seed on electrical conductivity (dSm^{-1}) in sorghum seed

Electrical conductivity (dSm^{-1})						
Treatments	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**Mean**
A_1	0.13	0.11	0.11	0.10	0.10	0.11
A_2	0.15	0.12	0.11	0.11	0.12	0.12
A_3	0.16	0.11	0.11	0.12	0.12	0.12
A_4	0.23	0.16	0.13	0.14	0.19	0.17
Mean	0.17	0.12	0.11	0.12	0.13	
	A		T		A × T	
SEm ±	0.002		0.002		0.004	
CD (5%)	0.005		0.006		0.012	
CV %	6.96					

The values in the same column / row with the same alphabet are not significantly different (P < 0.05) by DMRT.
Factor–I

A1– 24 h of accelerated ageing
A2– 36 h of accelerated ageing
A3 – 48 h of accelerated ageing
A4 – 60 h of accelerated ageing

Factor –II

T0 – No seed treatment
T1 – Seed treatment with GA3 @ 50 ppm for 10 h
T2 – Seed treatment with 2% KH2PO4 for 10 h
T3 – Seed treatment with 2% CaCl2 for 10 h
T4 – Seed treatment with 2% KNO3 for 10 h

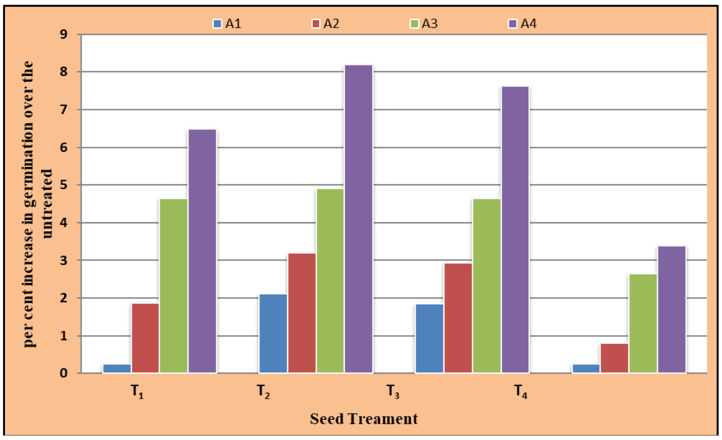

Fig 1: Per cent increase of germination in accelerated aged seed over the control (Untreated) due to seed treatment

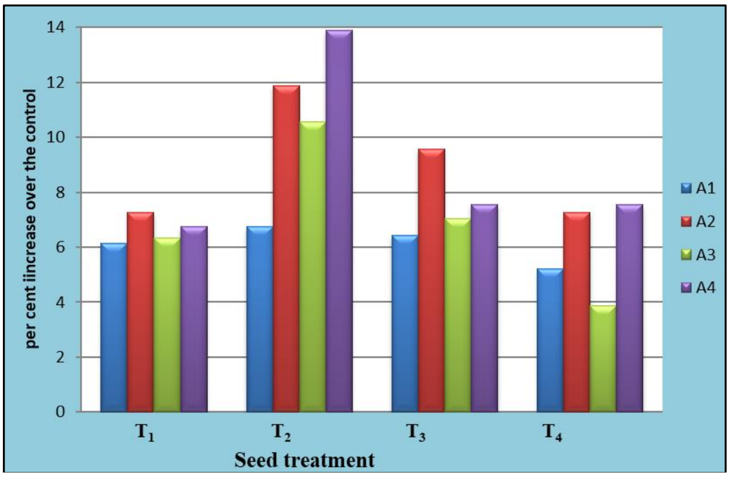

Fig 2: Per cent increase in field emergence over the control (Untreated) due to seed treatment

4. Conclusion
Significant enhancement in seed quality is possible through the invigouration of accelerated aged seed with different chemicals. Finally, it can be concluded that seed treatment with 2% KH_2PO_4 and 2% $CaCl_2$ improved the seed quality of accelerated aged.

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