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SEED DETERIORATION IN PEARL MILLET: CHANGES IN GERMINATION, VIGOUR AND ANTIOXIDANT ENZYME ACTIVITY DURING STORAGE

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ABSTRACT

The study investigated the effects of seed ageing on vigour, viability and biochemical parameters in three pearl millet hybrids (RHB 223, AHB 1200 and AHB 1269). Seeds were subjected to accelerated ageing for three and six days and subsequently stored under ambient conditions. Seed quality was assessed periodically for germination, electrical conductivity, seedling vigour index (SVI-I), field emergence and enzyme activities (dehydrogenase, peroxidase and catalase). Results revealed a significant decline in germination, vigour, field emergence and enzyme activities (dehydrogenase and peroxidase) with storage and ageing, while catalase activity increased, indicating an adaptive antioxidant response. Non-aged seeds consistently outperformed aged treatments, with RHB 223 maintaining superior storability across traits.

Keywords: Ageing, Vigour, Genotypes and seed quality.

Introduction

Pearl millet (*Pennisetum glaucum* R. Br.) is the sixth most important cereal crop globally after rice, wheat, maize, barley and sorghum. It is predominantly cultivated in arid and semi-arid regions, serving as a staple food in the tropical drylands of Asia and Africa, accounting for nearly 50% of the global millet area (Satyavathi *et al.*, 2021). Being grown under marginal soils and hostile environments, the seed quality of pearl millet is of prime importance for successful crop establishment (AOSA, 1983). However, seed deterioration during storage often results in reduced vigour and weak seedlings, limiting survival and productivity (Atici *et al.*, 2007).

Seed ageing is a natural process of quality decline over time, leading to loss of vigour and viability (Verma *et al.*, 2003). This deterioration involves complex physiological and biochemical changes, such as reduced metabolic efficiency and damage to cellular constituents, ultimately reducing germination and crop

yield (Sundareshwaran *et al.*, 2023). Most investigations on seed ageing have been carried out using accelerated ageing techniques, where seeds are exposed to high temperature and humidity. While this method is considered a good predictor of seed storability, the mechanisms of deterioration under accelerated conditions may differ from those under natural storage environments (McDonald, 1999; Lehner *et al.*, 2008).

The present study was undertaken to evaluate the impact of seed ageing on vigour, viability and associated biochemical parameters in pearl millet. Insights gained from this research will help in better understanding of the deterioration process and in developing strategies for improving seed storage, ensuring sustainable productivity in this important dryland cereal.

Materials and Methods

The experimental material consisted of three pearl millet (*Pennisetum glaucum* R. Br.) genotypes, RHB 223, AHB 1200 and AHB 1269, procured from the

ICAR–Indian Institute of Millets Research (IIMR), Rajendranagar, Hyderabad. Seeds of each hybrid were subjected to accelerated ageing at $40 \pm 1^\circ\text{C}$ and 100% relative humidity for 3 days (A_2) and 6 days (A_3). Non-aged seeds served as the control (A_1). After ageing, seeds from each treatment were stored under ambient conditions in cloth bags.

Seed quality assessments were carried out initially and subsequently at trimonthly intervals (0 month, 3 month and 6 month). Germination percentage was determined according to ISTA (2022), while electrical conductivity of seed leachates was measured as per ISTA (2023). Seedling vigour index I (SVI-I) was calculated following Abdul-Baki and Anderson (1973) and field emergence was estimated using the method of Shenoy *et al.* (1990). Dehydrogenase activity (OD values) was assayed according to Kittock and Law (1968), peroxidase activity (units ml^{-1}) was determined using the protocol of Malik and Singh (1980) and catalase activity was estimated according to the method of Barber (1980). The experiment was laid out in a two-factorial Completely Randomized Design (CRD) and the data were subjected to statistical analysis to assess the significance of main effects and interactions.

Results and Discussions

1. Seed germination %

Seed ageing significantly reduced germination in pearl millet, with a highly significant interaction effect. Non-aged (control) seeds showed the highest germination, while three and six-day aged seeds exhibited progressive declines. Initially, germination ranged from 80% (AHB 1200) to 84% (AHB 1269). Mean germination dropped from 81% at 0 months to 72% after six months, with RHB 223 retaining the highest (74%) and AHB 1200 the lowest (70%). The maximum reduction was observed in AHB 1269 (13.9%), while RHB 223 showed the least decline (8.64%). The decline in germination may be attributed to seed deterioration, particularly mitochondrial membrane degradation, which reduces energy supply for germination (Gidrol *et al.*, 1998). Similar reductions have been reported in maize (Mansouri-Far *et al.*, 2015) and rice (Kapoor *et al.*, 2011) under accelerated ageing conditions (Table 2).

2. Electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$)

Electrical conductivity (EC) of seed leachates in pearl millet increased significantly with ageing and storage, reflecting loss of membrane integrity. Mean EC rose from $107.65 \mu\text{S cm}^{-1}\text{g}^{-1}$ (initial) to $190.48 \mu\text{S cm}^{-1}\text{g}^{-1}$ at three months and $243.01 \mu\text{S cm}^{-1}\text{g}^{-1}$ at six

months. Non-aged seeds showed the lowest EC, while six-day aged seeds recorded the highest increase (106.6%). Among genotypes, AHB 1200 exhibited the highest EC initially, whereas after six months RHB 223 showed maximum leakage ($278.87 \mu\text{S cm}^{-1}\text{g}^{-1}$). The genotype \times ageing interaction was significant. Electrical conductivity (EC) of seed leachate is a reliable indicator of seed deterioration, as it increases with storage due to metabolite leaching. In rice, a progressive rise in EC with storage was reported by Prakash *et al.* (2020) and similar results were observed by Deshpande and Mahadevappa (1994) under extended ageing conditions (Table 3).

3. Seedling vigour index (SVI-I)

Seedling vigour index (SVI-I) in pearl millet was significantly affected by genotype, ageing treatments and storage duration, with a highly significant interaction effect. Non-aged seeds consistently showed the highest vigour, while six-day aged seeds recorded the lowest. Initially, RHB 223 had the maximum SVI (1576), followed by AHB 1269 (1462) and AHB 1200 (1323). After six months, the mean vigour declined to 1332, with RHB 223 retaining the highest (1611) and AHB 1200 the lowest (1140). The maximum reduction was observed in AHB 1269 (14.8%). Overall, vigour decreased progressively with storage and ageing, with RHB 223 maintaining superior storability. Similar declines with prolonged ageing have also been reported in rice (Deshpande and Mahadevappa, 1994; Kapoor *et al.*, 2011), while Bharathi *et al.* (2024) also observed a gradual decline in seedling vigour index-I during the storage period (Table 4).

4. Field Emergence (%)

Field emergence percentage in pearl millet declined significantly with genotype, ageing treatments and storage duration, with a notable interaction effect. Initially, RHB 223 recorded the highest emergence (77%) followed by AHB 1200 and AHB 1269 (74%). After six months, mean emergence declined to 58%, with RHB 223 maintaining the highest (63%) and six-days aged seeds showing the lowest (49%). The maximum reduction occurred in AHB 1269 (25.7%), whereas RHB 223 showed the least decline (18.2%). These results confirm that field emergence decreases progressively with storage and ageing, which is in agreement with earlier findings of reduced seed vigour and viability over time (Murali *et al.*, 2002; Saha and Sultana, 2008) (Table 5).

5. Dehydrogenase activity (OD value)

Dehydrogenase activity (OD value) in pearl millet seeds declined significantly with storage duration and ageing treatments, showing a strong genotype \times ageing

interaction. At the initial month, AHB 1269 recorded the highest activity (1.317), while RHB 223 maintained the highest (0.455) after six months of storage. Non-aged seeds consistently exhibited superior OD values compared to aged genotypes. The reduction was most prominent in AHB 1269 (69.6%), followed by AHB 1200 (65.3%) and RHB 223 (59.9%). The findings indicate that extended storage and accelerated ageing markedly decreased enzyme activity, reflecting a progressive loss of seed vigour and viability, consistent with the earlier reports of Malarkodi and Dharmalingam (1998) and Sundareswaran *et al.* (2009) (Table 6).

6. Peroxidase activity (units/ml)

Peroxidase activity in pearl millet seeds declined significantly with genotype, ageing and storage duration, with a notable genotype \times ageing interaction. Initially, RHB 223 recorded the highest activity (1.108 units/ml), while AHB 1200 and AHB 1269 were lower. After six months, mean activity decreased to 0.383 units/ml, with RHB 223 retaining the highest (0.623) and AHB 1200 the lowest (0.246). The greatest reduction occurred in AHB 1200 (72.4%), whereas RHB 223 showed better stability (43.8%). Non-aged seeds consistently maintained higher activity than aged treatments, confirming the adverse effect of ageing on enzymatic function and seed vigour. Similar declines in peroxidase activity during storage and ageing were reported in radish (Scialabba *et al.*, 2002), sunflower (Pallavi *et al.*, 2003) and pearl millet (Sundareswaran *et al.*, 2009) (Table 7).

7. Catalase Activity (CAT)

Catalase activity varied across genotypes, ageing treatments and storage periods, showing a significant effect of ageing stress. At the initial stage, RHB 223 recorded the highest activity (113.108), while AHB 1200 recorded lowest (100.182). Activity increased with ageing, being highest in six-day aged seeds. After six months of storage, catalase activity reached its peak with a of mean 470.241, with AHB 1200 showing the maximum (507.372). Unlike peroxidase, catalase activity increased with storage and ageing, indicating an adaptive antioxidant defense mechanism against oxidative stress. Similar induction of catalase with prolonged storage was also observed in pearl millet seeds (Kamble *et al.*, 2018) (Table 8).

Conclusion

The present study clearly demonstrated that seed ageing negatively influences the quality of pearl millet, as evidenced by significant reductions in germination, vigour, and field emergence, along with increased electrolyte leakage. The progressive decline in dehydrogenase and peroxidase activities established their sensitivity as indicators of seed deterioration, while the enhanced catalase activity reflected an adaptive antioxidant response to oxidative stress. Among the genotypes tested, RHB 223 consistently exhibited higher vigour and greater stability of enzymatic activity, thereby confirming its superior storability over AHB 1200 and AHB 1269. Overall, these findings highlight the detrimental effects of ageing on seed quality and underscore the potential utility of biochemical markers as reliable tools for monitoring seed deterioration during storage.

Table 1 : ANOVA for seed quality parameters of stored Pearl millet seed.

Source of variation	df	Seed germination (%)			Electrical Conductivity ($\mu\text{S}/\text{cm}/\text{g}$)			Seedling vigour index – I		
		Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
G	2	39.593***	64.111***	52.000***	983.731***	1,406.001***	8,987.757***	144,864.593***	287,834.037***	548,455.444***
A	2	771.815***	660.333***	775.000***	7,479.730***	14,429.820***	15,244.406***	502,420.593***	749,507.704***	575,454.778***
G X A	4	14.370***	30.278***	34.667***	172.002***	458.061***	2,212.796***	15,684.259***	26,576.759***	84,299.389***
Error	18	1.333	1.444	5.111	7.605	17.210	27.917	969.481	494.889	9,457.926
Total	26	65,549.846	61.384	72.487	682.762	1,300.525	2,223.769	52,875.310	84,226.866	1,05,971.564

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 –AHB 1200, G3- AHB 1269 ; A1 –Control variety, A2 – 3 days aged, A3 – 6 days aged; MAS – Months After Storage

Source of variation	df	Dehydrogenase activity (OD value)			Peroxidase activity (POX) (units/ml)			Catalase activity (CAT)		
		Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
G	2	0.087***	0.170***	0.009***	0.163***	0.899***	0.390***	375.978***	1,629.173***	11,404.243***
A	2	0.114***	0.042***	0.002***	0.589***	0.034***	0.065***	6,297.537***	4,730.948***	120,436.278***
G X A	4	0.005***	0.004***	0.010***	0.055***	0.001***	0.005***	469.964***	595.303***	501.369***
Error	18	0.010	0.002	0.001	0.001	0.002	0.002	198.430	104.437	93.990
Total	26	0.023	0.018	0.003	0.067	0.073	0.036	723.024	653.128	10,283.782

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 –AHB 1200, G3- AHB 1269 ; A1 –Control variety, A2 – 3 days aged, A3 – 6 days aged ; MAS – Months After Storage

Source of variation	df	Field emergence (%)		
		Initial	3 MAS	6 MAS
G	2	32.259***	75.148***	165.148***
A	2	803.370***	428.926***	848.481***
G X A	4	10.704**	68.815***	60.870***
Error	18	2.296	1.704	1.296
Total	26	67.515	156.934	156.934

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 2 : Effect of genotypes and ageing on Seed germination (%) of pearl millet during storage

Storage duration		Seed germination (%)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
			G1	86	85	72	81	82	79	69	77	81	74
	G2	89	82	67	80	85	72	61	73	81	71	57	70
	G3	90	87	74	84	84	79	70	78	79	77	62	73
	Mean	88	85	71	81	84	77	67	76	81	74	62	72
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	1.15	1.15	2.00		1.20	1.20	2.08		2.26	2.26	3.91	
	SE (m)	0.39	0.39	0.67		0.40	0.40	0.69		0.75	0.75	1.31	
	CV%	1.42				1.59				3.13			

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 3 : Effect of genotypes and ageing on electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$) of pearl millet during storage

Storage duration		ELECTRIAL CONDUCTIVITY($\mu\text{S}/\text{cm}/\text{g}$)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
			G1	81.80	94.10	128.00	101.30	149.90	177.20	211.70	179.60	228.75	247.80
	G2	95.45	103.25	160.45	119.72	155.05	168.45	239.65	187.72	196.50	220.80	240.35	219.22
	G3	75.75	98.85	131.20	101.93	178.25	177.25	256.90	204.13	204.55	221.40	266.90	230.95
	Mean	84.33	98.73	139.88	107.65	161.07	174.30	236.08	190.48	209.93	230.00	289.10	243.01
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	2.75	2.75	4.77		4.14	4.14	7.17		5.27	5.27	9.13	
	SE (m)	0.92	0.92	1.59		1.38	1.38	2.40		1.76	1.76	3.05	
	CV%	2.56				2.18				2.17			

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 4 : Effect of genotypes and ageing on Seedling vigour index of pearl millet during storage

Storage duration		SEEDLING VIGOUR INDEX-I											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
			G1	1,779	1,712	1,238	1,576	1,818	1,669	1,259	1,582	1,891	1,535
	G2	1,545	1,376	1,048	1,323	1,610	1,115	947	1,224	1,525	1,031	865	1,140
	G3	1,599	1,509	1,278	1,462	1,664	1,423	1,155	1,414	1,327	1,453	955	1,245
	Mean	1,641	1,532	1,188	1,454	1,697	1,402	1,120	1,407	1,581	1,340	1,076	1,332
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	31.08	31.08	53.83		22.20	22.20	38.46		97.06	97.06	168.12	
	SE (m)	10.38	10.38	17.98		7.42	7.42	12.84		32.42	32.42	56.15	
	CV%	2.14				1.58				7.30			

Note: ***- Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 5 : Effect of genotypes and ageing on field emergence (%) of pearl millet during storage

Storage duration		FIELD EMERGENCE (%)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
		G1	89	76	67	77	77	71	69	72	70	61	57
	G2	82	76	64	74	78	75	55	69	66	53	50	56
	G3	82	74	66	74	80	77	69	75	69	55	40	55
	Mean	84	75	66	75	78	74	65	72	68	56	49	58
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	1.14	1.14	1.97		1.30	1.30	2.26		1.14	1.14	1.97	
	SE (m)	0.38	0.38	0.66		0.44	0.44	0.75		0.38	0.38	0.66	
	CV%	1.72				1.80				1.97			

Note: ***. Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 6 : Effect of genotypes and ageing on dehydrogenase activity (OD value) of pearl millet during storage

Storage duration		Dehydrogenase activity (OD value)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
		G1	1.247	1.103	1.053	1.134	0.703	0.687	0.530	0.640	0.468	0.398	0.500
	G2	1.255	1.150	1.087	1.164	0.813	0.769	0.692	0.758	0.471	0.364	0.378	0.404
	G3	1.470	1.320	1.160	1.317	0.987	0.882	0.873	0.914	0.367	0.445	0.388	0.400
	Mean	1.324	1.191	1.100	1.205	0.834	0.779	0.698	0.771	0.435	0.402	0.422	0.420
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	0.102	0.102	N/A		0.047	0.047	N/A		0.032	N/A	0.056	
	SE (m)	0.034	0.034	0.059		0.016	0.016	0.027		0.011	0.011	0.019	
	CV%	8.456				6.141				7.682			

Note: ***. Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

Table 7 : Effect of genotypes and ageing on peroxidase activity (POX) of pearl millet during storage

Storage duration		Peroxidase activity (POX) (units/ml)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
		G1	1.230	1.107	0.987	1.108	0.868	0.826	0.777	0.824	0.756	0.560	0.553
	G2	1.300	0.704	0.674	0.892	0.344	0.263	0.211	0.272	0.297	0.252	0.189	0.246
	G3	1.193	0.793	0.593	0.860	0.343	0.301	0.196	0.280	0.378	0.273	0.193	0.281
	Mean	1.241	0.868	0.751	0.953	0.518	0.463	0.395	0.459	0.477	0.362	0.312	0.383
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	0.038	0.038	0.066		0.039	0.039	N/A		0.039	0.039	0.067	
	SE (m)	0.013	0.013	0.022		0.013	0.013	0.022		0.013	0.013	0.022	
	CV%	4.035				8.485				10.116			

Table 8 : Effect of genotypes and ageing on catalase activity (CAT) of pearl millet during storage

Storage duration		Catalase activity (nmoles/unit/g)											
		Initial				3 MAS				6 MAS			
Ageing	Genotypes	A1	A2	A3	Mean	A1	A2	A3	Mean	A1	A2	A3	Mean
		G1	67.865	126.035	145.425	113.108	126.035	145.425	155.120	142.193	358.715	455.665	591.395
	G2	77.560	106.645	116.340	100.182	145.425	164.815	174.510	161.583	416.885	475.055	630.175	507.372
	G3	87.255	106.645	126.035	106.645	126.035	174.510	203.595	168.047	339.325	397.495	572.005	436.275
	Mean	77.560	113.108	129.267	106.645	132.5	161.58	177.74	157.274	371.64	442.74	597.86	470.746
		G	A	GXA		G	A	GXA		G	A	GXA	
	CD@ 5%	N/A	14.059	N/A		10.200	10.200	17.666		9.676	9.676	16.759	
	SE (m)	4.696	4.696	8.133		3.406	3.406	5.900		3.232	3.232	5.597	
	CV%	13.209				6.498				2.059			

Note: ***. Significant at 0.1%, **- Significant at 1%, *- Significant at 5%; G1 - RHB 223 , G2 -AHB 1200, G3- AHB 1269 ; A1 -Control variety, A2 - 3 days aged, A3 - 6 days aged ; MAS - Months After Storage

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