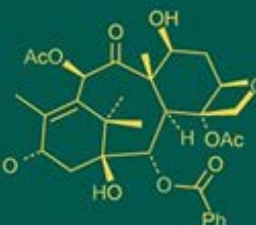
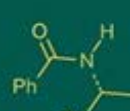
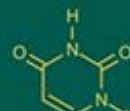
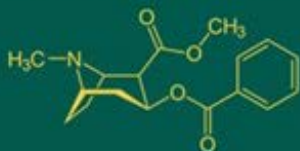


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Influence of seed coat colour on enzyme activity in storage of sorghum genotypes (*Sorghum bicolor* (L.) Moench)

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Abstract

Seed coat is the main modulator of interaction between internal structure of seed and external environment. Seed coat attains specific colour at physiological maturity and plays important role in maintaining the seed quality. A laboratory experiment was conducted in the Department of Seed Science and Technology, UAS Raichur to study the effect of seed coat colour on longevity of sorghum genotypes during 2021-2022. The study consists of 20 sorghum genotypes of different seed coat colour which were stored for ten months in cloth bag. Results revealed that, among different genotypes, black seed coat (IS25040) coloured genotype recorded significantly highest germination (80 %), total dehydrogenase activity (0.200 OD value), alpha amylase activity (16 mm) and lowest electrical conductivity (0.24 dSm^{-1}) at the end of ten months of storage period. whereas lowest seed quality parameters were observed in white seed coat coloured genotype (DJ6514) (69 %, 0.145 OD value , 10.5 mm , 0.39 dSm^{-1} respectively). Hence genotypes with dark seed coat colour are best in maintaining seed quality parameters and can be stored for longer period of time.

Keywords: Seed coat colour, longevity, sorghum, seed quality

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], a cultivated diploid ($2n = 20$) tropical cereal C_4 grass plant. *Sorghum* is also called as camel crop due to its high tolerance to water and temperature stress and also high photosynthesis efficiency. It is an important grain and forage crop of semiarid regions due to its high adaptability and suitability to rain-fed low input agriculture (Anagholi *et al.*, 2000) [1]. It is one among the most efficient crops in conversion of solar energy and use of water and is known as a high-energy and drought tolerant crop that is environmentally friendly. The sorghum covering an area of about 41.97 million ha with production of 65.21 million metric tonnes and productivity of 1.45 metric tons per ha. India is a major sorghum growing country in the world, ranks third in acreage and seventh in production next to United States of America. It is cultivated in 4.24 million ha with an annual production of 4.78 million metric tons of grain with productivity of 1130 kg/ha (Anon., 2022 a) [2]. In India, it is cultivated in Maharashtra, Karnataka and Andhra Pradesh as a rainfed crop. In Karnataka, sorghum production is about 1.41 million ha with a production of 1.13 million metric tons and productivity of 974 kg/ha . Major sorghum growing districts of Karnataka are Kalaburgi, Raichur, Koppal, Belagavi and Vijayapur (Anon., 2022 b) [3].

Seed is one of the most crucial elements in the livelihoods of agriculture communities. Quality of seed is one of major constraints for increasing the productivity of the crop. Usually farmers use their own seed. Seed coat is vital input in seed production and it attains their specific colour at physiological maturity. Seed coat colour is simple and excellent indicator of seed quality parameters. One of the unique features of sorghum seed is its variation in seed colour (Tiriyaki *et al.*, 2016). The colour of seed coat is most obvious physical characteristic which varies widely with white, cream, red, black etc. Differences in seed coat colour have been associated with differences in the composition of chemical compounds in the seed coats. Seed storage and retention of seed viability is an important post-harvest operation that decides the success of seeds in the next generation. Seed deterioration begins immediately after physiological maturity and is reflected in terms of loss in viability and vigour. Seed coat act as a major defence against harmful environmental conditions and protect embryo from mechanical stress and from temperature

and humidity fluctuation during storage. Seed coat is the main modulator of interaction between the internal structures and the external environment. It not only preserves integrity of seed parts but also protects the embryo from mechanical injuries and attacks of pest and diseases. So seed coat is having the central role in seed longevity as it is the primary defence against adverse environmental condition.

Material and Methods

A laboratory experiment was conducted in *Department of Seed Science and Technology, College of Agriculture, Raichur* during 2020-21, to know influence of seed coat colour on longevity of sorghum. The experiment was laid in a completely randomised design. There were 20 genotypes, G₁ : IS37224 : White, G₂ : M-35-1 : White, G₃ : DJ6514 : White, G₄ RSL46 : Pearly white, G₅ : RSL54 : Pearly white, G₆ RSL49 : Chalky white, G₇ : IS3572 : Whitish purple, G₈ IS16310 : Purple, G₉ IS16006 : Yellow, G₁₀ : IS37247 : Red, G₁₁ : RSL69 : Red, G₁₂ : RSL 57 : Red, G₁₃ : IS37349 : Light red, G₁₄ : IS37304 : Light red, G₁₅ IS37355 : Light brown, G₁₆ : IS6508 : Light brown, G₁₇ : IS37225 : Dark brown, G₁₈ IS22436 : Dark brown, G₁₉ : IS2618 : Grey, G₂₀ : IS25040 : Black. The seeds were harvested during rabi (February) 2021 and were dried at 10 per cent moisture level and one kg seeds were packed in cloth bag in the month of August and kept for storage under ambient condition for ten months. These seeds were used for testing seed quality parameters.

Seed germination (%)

Hundred seeds in four replications was taken from each treatment and placed on germination paper uniformly. The roll towels were kept in a germination chamber maintained at 25±2 °C temperature and 90±5 per cent relative humidity. The first count was taken on 4th day and final count on 10th day. The number of normal seedlings from each replication was counted and the mean germination was expressed in percentage (ISTA., 2013) [4].

$$\text{Seed Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Electrical conductivity (DSM⁻¹)

Five gram of seeds in four replications were soaked in acetone for half a minute and thoroughly washed in distilled water for three times. Then the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25 °C±1 for twelve hours. The seed leachate was collected and volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in digital conductivity bridge (WENSAR) with a cell constant 1.0 and the mean values were expressed in deci Siemens per meter (dSm⁻¹) (Milosevic *et al.*, 2010) [5].

Total dehydrogenase activity (OD value)

Twenty five representative seeds from each treatment in four replications were taken and pre conditioned by soaking in water overnight at room temperature. The half cut seeds was steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride and kept in dark for 2 hours at 40 °C for staining. The stained seeds was thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using spectrophotometer using blue filter at 620 nm wave length and methyl cellosolve was used as a blank. The OD value obtained was reported as dehydrogenase activity (Kittock and Law, 1968) [6].

Alpha amylase activity (MM)

The α -amylase activity was analysed as per the method suggested by Simpson and Naylor (1962) [7]. Two gram of agar shreds and one gram of potato starch was mixed together in water to form paste and the volume is made up to 100 ml with distilled water. The homogenous solution of agar-starch mixture after boiling was poured into sterilized petri-dishes and allowed to settle in the form of gel after cooling. The pre soaked (for 8 hours) and half cut seeds (with their half and embryo portion intact) were placed in the petri-dishes in such a way that the endospermic part remained in contact with agar-starch gel. The petri-dishes were closed and kept in dark at 30 °C. After 48 hours, the petri-dishes were uniformly smeared with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium iodide in 500 ml distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seed was measured in millimeter and reported as α -amylase activity.

Results and Discussion

At initial month of storage, among the genotypes significantly highest germination was recorded in black seed coat colour genotype G₂₀ (95.50 %) and the lowest seed germination was noticed in white seed coat colour genotype G₃ (84.53 %) (Table 1). Similar trend was recorded throughout the storage period and at the end of tenth month of storage, significantly higher germination was found in black seed coat colour genotype G₂₀ (IS25040) (80.00 %) which was on par with G₁₇ (78.25 %) G₁₈ : (77.00 %) G₁₅ (76.25 %), G₁₆ (76.00 %), G₁₂ (75.75 %), G₁₀ (75.50 %). The lowest seed germination was noticed in white seed coat colour genotype G₃ (DJ6514) (69.00 %). This might be due to the presence of some various phenolic compounds in unpigmented seed that block the metabolism preparatory to germination and prevent the access of oxygen to embryo and release of carbonic acid which make it deteriorate faster than the pigmented genotypes. Black seed coat colour genotypes were more resistant to mechanical damage, such resistance means less nutrient leakage from the seed during germination. These findings are supported by Abdullah *et al.* (1988) [8] in long bean, Mokashi (2017) [9] in cowpea.

Initially significantly lowest electrical conductivity was noticed in G₂₀ (IS25040) black seed coat colour genotype (0.10dSm⁻¹) and significantly highest electrical conductivity was noticed in white seed coat colour genotype (G₃) (0.15dSm⁻¹). The similar trend was noticed throughout the storage period and at the end of ten months of storage. The lowest electrical conductivity was recorded in both black (G₂₀) and brown (G₁₇) seed coat colour genotypes (0.24dSm⁻¹) (Table 2). The lowest electrical conductivity in dark or pigmented genotypes may be due to the presence of phenolic compounds coupled with antioxidant activity which may delays ageing process in seeds and in turn results in less solute leakage. While the highest electrical conductivity was observed in two genotypes G₁ and G₃ (0.39dSm⁻¹). The highest electrical conductivity in white or unpigmented genotypes was attributed to thinner seed coat leading to rapid water uptake into the outer cells of cotyledons that might disrupts the cell membranes and resulting in high solute leakage from the embryo. These observations are in accordance with Capeleti *et al.* (2005) [10] in soybean, Ibrahim *et al.* (2011) [11] in red clover, Mokashi (2017) [9] in cowpea and Kumar *et al.* (2020) [12] in soybean.

In the initial month of storage, significantly highest total dehydrogenase activity was found in black seed coat colour genotype G₂₀ (0.208 OD value) and the lowest dehydrogenase activity was found in white seed coat colour genotype (G₃) (0.179 OD value). At the end of storage period, the black seed

coat colour genotype (G₂₀) exhibited significantly highest dehydrogenase activity (0.200 OD value) followed by dark brown colour genotype G₁₇ (0.188 OD value) and least was noticed in white seed coat colour genotype G₃ (0.145 OD value) (Table 3). The least dehydrogenase activity in white seed coat colour genotype might be due to its highest moisture content which reduces all the seed quality parameters and thus the cotyledon of the seeds fails to stain 2, 3, 5- tri phenyl tetrazolium chloride leading to the reduced respiration and decline in both the transfer of food reserves from cotyledons to growing axis and growth rate of surviving seedlings. These findings are in accordance with Saxena and Maheswari (1980) [13] in soybean and Mokashi (2017) [9] in cowpea.

During initial storage period, significantly highest alpha amylase activity was confirmed in black seed coat colour genotype G₂₀ (17.40 mm) which was on par with G₁₇ (17.20

mm), G₁₈ (16.90 mm), G₁₆ (16.70 mm), G₁₅ (16.60 mm), G₁₂ (16.40 mm), and G₁₀ (16.30 mm). While the white seed coat genotype G₃ recorded the lowest alpha amylase activity (14.20 mm). Significantly highest alpha amylase activity was noticed in G₂₀ black seed coat colour genotype (16.00 mm) after the end of ten months of storage and it was on par with dark brown seed coat colour genotype G₁₇ (15.80 mm). Lowest alpha amylase activity was recorded in white seed coat colour genotype (G₃) (10.50 mm) (Table 4 and Fig.1). The lowest alpha amylase activity in unpigmented genotypes is probably due to ageing of seeds upon storage were aluerone layer and scutellum gets deteriorated resulting increase of alpha amylase inhibitors indicating lowest alpha amylase activity. These findings are in line with Akazawa and Beck and Ziegler, (1989) [14] in paddy and Thevenot *et al.* (1992) [15] in maize.

Table 1: Effect of seed coat colour on seed germination (%) during storage in sorghum genotypes

Seed coat colour	Genotypes	Storage period					
		Initial (Aug)	2 (Sep)	4 (Nov)	6 (Jan)	8 (Mar)	10 (May)
White	G ₁ -IS37224	86.59	86.25	85.50	81.75	77.25	69.50
	G ₂ -M35-1	87.85	87.25	86.25	82.00	78.75	69.75
	G ₃ -DJ6514	84.53	84.25	83.50	81.00	76.75	69.00
Pearly white	G ₄ -RSL46	88.92	88.75	87.50	83.75	79.00	71.50
	G ₅ -RSL54	88.25	88.00	87.25	82.50	78.75	70.75
Chalky white	G ₆ -RSL49	87.98	89.75	87.75	82.00	78.75	69.50
Whitish purple	G ₇ -IS3572	90.12	94.60	92.25	84.25	79.25	72.25
Purple	G ₈ -IS16310	90.75	89.75	88.00	84.29	79.50	72.25
Yellow	G ₉ -IS16006	90.82	90.50	89.50	84.75	79.75	72.50
Red	G ₁₀ -IS37247	93.45	93.00	92.00	86.25	82.25	75.50
	G ₁₁ -RSL69	92.75	92.50	90.50	86.00	82.00	74.50
Light red	G ₁₂ -RSL57	93.56	93.25	92.50	87.25	83.25	75.75
	G ₁₃ -IS37349	92.69	92.00	90.25	85.75	81.00	73.75
	G ₁₄ -IS37304	91.48	86.25	85.50	84.75	79.75	73.25
Light brown	G ₁₅ -IS37355	93.86	91.25	89.50	87.25	83.50	76.25
	G ₁₆ -IS6508	93.88	93.25	93.00	87.25	83.75	76.00
Dark brown	G ₁₇ -IS37225	94.86	93.50	93.00	88.25	84.75	78.25
	G ₁₈ -IS22436	94.76	95.00	94.50	88.28	84.25	77.00
Grey	G ₁₉ -IS2618	89.99	90.25	88.25	84.00	79.25	72.00
Black	G ₂₀ -IS25040	95.50	87.00	86.00	89.75	85.75	80.00
Mean		91.13	90.77	89.59	85.05	80.86	73.46
SEm±		1.42	1.45	1.49	1.55	1.58	1.76
CD @1%		4.01	4.10	4.21	4.39	4.49	4.98

Table 2: Effect of seed coat colour on electrical conductivity (dSm⁻¹) during storage in sorghum genotypes

Seed coat colour	Genotypes	Storage period					
		Initial (Aug)	2 (Sep)	4 (Nov)	6 (Jan)	8 (Mar)	10 (May)
White	G ₁ -IS37224	0.15	0.18	0.22	0.27	0.32	0.39
	G ₂ -M 35-1	0.14	0.17	0.21	0.26	0.31	0.36
	G ₃ -DJ6514	0.15	0.18	0.22	0.27	0.33	0.39
Pearly white	G ₄ -RSL46	0.14	0.17	0.21	0.26	0.30	0.34
	G ₅ -RSL54	0.14	0.17	0.21	0.26	0.31	0.34
Chalky white	G ₆ -RSL49	0.15	0.18	0.22	0.27	0.31	0.36
Whitish purple	G ₇ -IS3572	0.13	0.16	0.20	0.25	0.29	0.31
Purple	G ₈ -IS16310	0.13	0.16	0.20	0.25	0.28	0.30
Yellow	G ₉ -IS16006	0.13	0.16	0.20	0.25	0.26	0.31
Red	G ₁₀ -IS37247	0.12	0.15	0.19	0.24	0.25	0.28
	G ₁₁ -RSL69	0.12	0.15	0.19	0.24	0.25	0.29
Light red	G ₁₂ -RSL57	0.12	0.15	0.19	0.24	0.25	0.28
	G ₁₃ -IS37349	0.12	0.15	0.19	0.24	0.25	0.30
	G ₁₄ -IS37304	0.12	0.15	0.19	0.24	0.26	0.30
Light brown	G ₁₅ -IS37355	0.12	0.15	0.19	0.24	0.25	0.27
	G ₁₆ -IS6508	0.11	0.14	0.18	0.23	0.24	0.26
Dark brown	G ₁₇ -IS37225	0.10	0.13	0.17	0.22	0.23	0.24
	G ₁₈ -IS22436	0.10	0.13	0.17	0.22	0.23	0.25
Grey	G ₁₉ -IS2618	0.13	0.16	0.20	0.25	0.29	0.31
Black	G ₂₀ -IS25040	0.10	0.13	0.17	0.22	0.23	0.24
Mean		0.13	0.16	0.20	0.25	0.27	0.31
SEm±		0.003	0.004	0.005	0.006	0.006	0.007
CD @1%		0.008	0.01	0.013	0.016	0.018	0.02

Table 3: Effect of seed coat colour on total dehydrogenase activity (OD value) during storage in sorghum genotypes

Seed coat colour	Genotypes	Storage period					
		Initial (Aug)	2 (Sep)	4 (Nov)	6 (Jan)	8 (Mar)	10 (May)
White	G ₁ -IS37224	0.180	0.179	0.175	0.172	0.169	0.149
	G ₂ -M35-1	0.182	0.181	0.178	0.173	0.171	0.153
	G ₃ -DJ6514	0.179	0.179	0.174	0.162	0.154	0.145
Pearly white	G ₄ -RSL46	0.183	0.182	0.179	0.172	0.165	0.158
	G ₅ -RSL54	0.183	0.182	0.180	0.173	0.171	0.155
Chalky white	G ₆ -RSL49	0.181	0.180	0.179	0.172	0.162	0.150
Whitish purple	G ₇ -IS3572	0.186	0.185	0.183	0.175	0.172	0.162
Purple	G ₈ -IS16310	0.187	0.186	0.185	0.175	0.172	0.164
Yellow	G ₉ -IS16006	0.191	0.189	0.188	0.182	0.179	0.168
Red	G ₁₀ -IS37247	0.196	0.195	0.192	0.190	0.186	0.175
	G ₁₁ -RSL69	0.194	0.192	0.187	0.182	0.176	0.173
Light red	G ₁₂ -RSL57	0.197	0.196	0.191	0.186	0.182	0.179
	G ₁₃ -IS37349	0.193	0.192	0.189	0.184	0.179	0.172
	G ₁₄ -IS37304	0.193	0.191	0.187	0.184	0.181	0.169
Light brown	G ₁₅ -IS37355	0.200	0.200	0.198	0.191	0.189	0.185
	G ₁₆ -IS6508	0.205	0.202	0.200	0.194	0.190	0.186
Dark brown	G ₁₇ -IS37225	0.206	0.204	0.202	0.196	0.192	0.188
	G ₁₈ -IS22436	0.206	0.202	0.200	0.195	0.192	0.187
Grey	G ₁₉ -IS2618	0.185	0.184	0.181	0.174	0.172	0.160
Black	G ₂₀ -IS25040	0.208	0.207	0.206	0.204	0.202	0.200
Mean		0.192	0.190	0.188	0.182	0.178	0.169
SEm±		0.005	0.004	0.004	0.004	0.004	0.004
CD @1%		0.014	0.013	0.012	0.012	0.012	0.011

Table 4: Effect of seed coat colour on alpha amylase (mm) during storage in sorghum genotypes

Seed coat colour	Genotypes	Storage period					
		Initial (Aug)	2 (Sep)	4 (Nov)	6 (Jan)	8 (Mar)	10 (May)
White	G ₁ -IS37224	14.30	14.10	13.80	13.60	13.00	10.70
	G ₂ -M 35-1	14.80	14.80	14.40	14.10	13.40	11.00
	G ₃ -DJ6514	14.20	14.00	13.60	13.30	12.50	10.50
Pearly white	G ₄ -RSL46	15.30	15.00	14.80	14.40	13.40	11.30
	G ₅ -RSL54	15.10	14.80	14.50	14.10	13.40	11.20
Chalky white	G ₆ -RSL49	14.80	14.70	14.20	13.70	13.30	10.80
Whitish purple	G ₇ -IS3572	15.60	15.30	15.00	14.50	13.50	11.70
Purple	G ₈ -IS16310	15.60	15.40	15.10	14.60	13.60	12.10
Yellow	G ₉ -IS16006	15.70	15.40	15.10	14.70	13.70	12.70
Red	G ₁₀ -IS37247	16.30	16.00	15.70	15.10	14.30	13.50
	G ₁₁ -RSL69	16.20	16.00	15.40	14.80	14.10	13.20
Light red	G ₁₂ -RSL57	16.40	16.10	15.80	15.20	14.40	13.70
	G ₁₃ -IS37349	15.80	15.60	15.40	14.80	14.10	13.00
	G ₁₄ -IS37304	15.80	15.50	15.20	14.70	13.90	12.90
Light brown	G ₁₅ -IS37355	16.60	16.40	16.10	15.30	14.70	13.90
	G ₁₆ -IS6508	16.70	16.40	16.20	15.40	14.70	14.10
Dark brown	G ₁₇ -IS37225	17.20	17.00	16.70	16.20	16.00	15.80
	G ₁₈ -IS22436	16.90	16.80	16.20	15.50	15.10	14.90
Grey	G ₁₉ -IS2618	15.30	15.10	14.80	14.50	13.50	11.50
Black	G ₂₀ -IS25040	17.40	17.20	16.90	16.50	16.20	16.00
Mean		15.80	15.60	15.30	14.80	14.00	12.70
SEm±		0.38	0.37	0.36	0.35	0.34	0.30
CD @1%		1.07	1.06	1.04	1.00	0.96	0.87

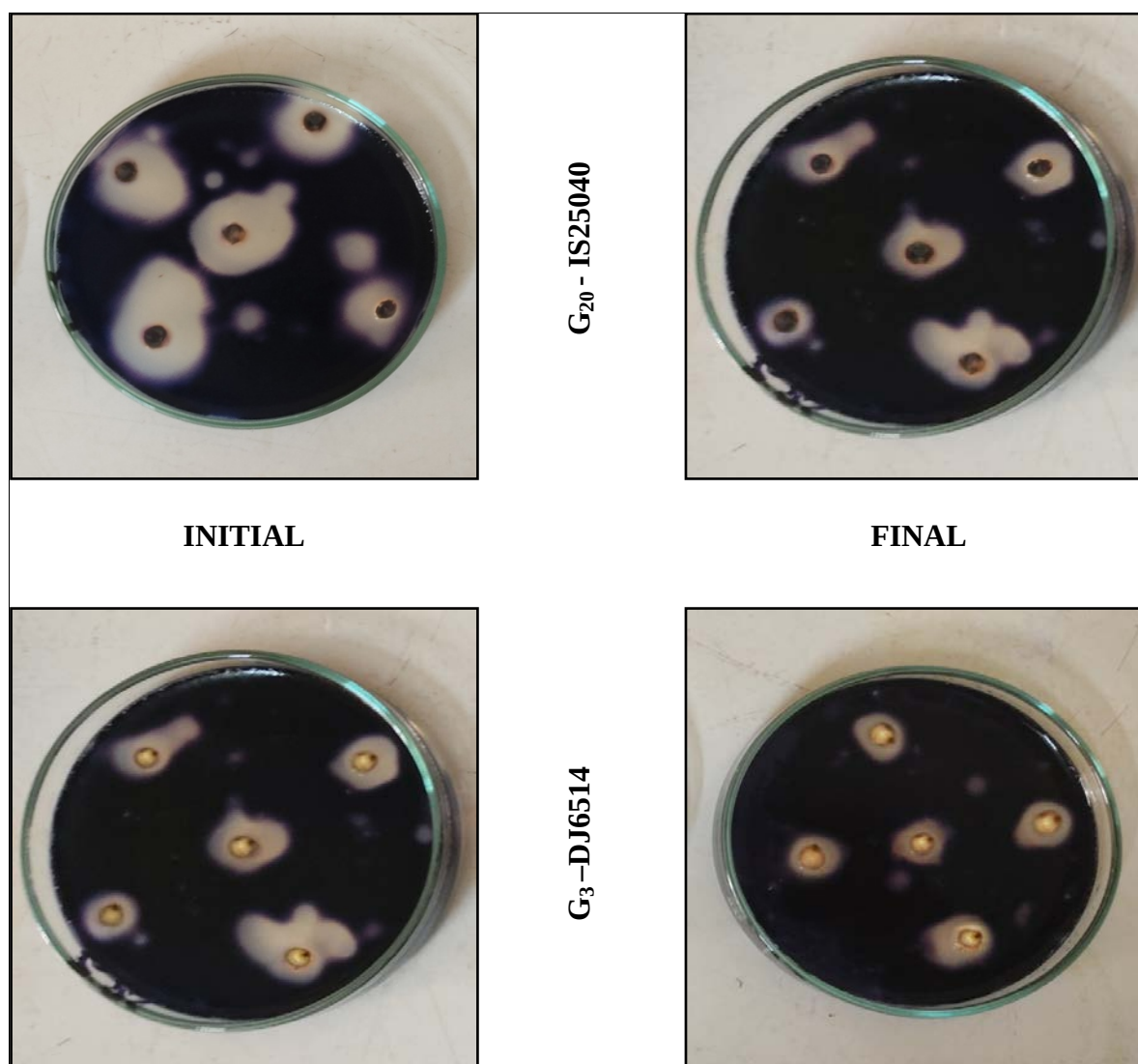


Fig 1: Alpha amylase activity of different seed coat colour sorghum genotypes at initial and at the end of the storage period

Conclusion

All the pigmented seeds recorded significantly higher enzyme activities, indicating better seed quality attributes. Among the genotypes, black seed coat colour genotype G₂₀-IS25040 and the dark brown colour genotype G₁₇-IS37255 maintained its seed quality up to the ten months of storage which is above the Indian minimum seed certification standards. Hence these two genotypes were found to be superior in maintaining seed quality during storage.

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