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Comparative study of physical, physiological, biochemical and histological changes due to accelerated and natural ageing of blackgram seeds

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Abstract
Freshly harvested seeds of blackgram (TNAU Blackgram VBN 6) obtained from National Pulses Research Centre (Vamban) were kept under accelerated ageing condition by exposing the seeds to 40^0C temperature and 100 per cent relative humidity conducted at the Department of Seed Science and Technology, TNAU, Coimbatore. Simultaneously, the seeds were packed in cloth bag and kept under ambient condition for natural ageing for 10 months in order to assess the physical, physiological biochemical and histological changes taking place during storage. The evaluation of seed vigour was obtained by comparing accelerated aged and natural aged seeds. However, reduction of germination percent was coincided with the reduction of seed quality parameters. As a conclusion, six days of accelerated ageing was equivalent to ten months of natural storage at the time maintained the germination above Indian Minimum Seed Certification Standards.

Keywords: Blackgram seeds, seed storage, natural and accelerated ageing

Introduction
Seed deterioration is inevitable, irreversible and inexorable which causes loss of seed quality with time. It is a natural process which involves cytological, physiological, biochemical and physical changes in seeds during storage. These changes reduce vigour followed by viability and ultimately cause death of the seed. Furthermore, the losses of vigour and viability in seeds differ with species and cultivars (Chauhan, 1984) [5]. Seed longevity is one of the components of seed quality. The speed at which ageing processes taken place depends on the seed's ability to resist degradation changes as well as its protection mechanisms. Accelerated ageing is one of the vigour tests widely used to determine the quality of seed lots (Thant *et al*. 2010) [35]. Under such storage conditions, seeds typically lose their viability within a few days or weeks. To cope with the current and future demand of the increasing population for the food grains, it is emphasized to reduce the loss of seeds during and after harvest. Seeds are stored for varying periods to ensure a proper and balanced public distribution throughout the year. Post harvest losses in India are estimated to be around 10 per cent of which the losses during storage alone are estimated to 6 per cent (Singh and Satapathy, 2003) [26]. Hence, an investigation to predict the relative storability of a particular seed lot under accelerated ageing by exposing the seeds to high temperature (40^0C) and relative humidity (100 per cent) and comparing with storability of natural ageing will be useful in retention or disposal of a particular variety or seed lot.

Materials and Methods.
Freshly harvested seeds of blackgram (TNAU Blackgram VBN 6) obtained from National Pulses Research Centre (Vamban) were kept under accelerated ageing condition by exposing the seeds to 40^0C temperature and 100 per cent relative humidity. Simultaneously, the seeds were packed in cloth bag and kept under ambient condition for natural ageing for 10 months. The experiment was designed in Completely Randomized Design (CRD) with four replications. For accelerated ageing, the samples were drawn at daily interval and for natural ageing, samples were drawn at monthly intervals and analyzed for physical, physiological, biochemical and histological observations were recorded *viz.,* seed moisture content, germination per cent, root length, shoot length, dry matter production, vigour index, electrical

conductivity, α-amylase, catalase, peroxidase activity, protein content and free amino acid were made. The storability period was calculated based on the time taken to reach the germination as per the IMSCS. In order to assess the pattern of deterioration, the accelerated ageing was continued when seed reaches the germination of about 50 per cent. The data collected from the various experiments were analysed statistically adopting the procedure described by Rangaswamy (2002), wherever necessary, the per cent values were transformed to angular (arcsine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test expressed is non-significant of variant it was indicated by the letter NS.

Results and discussion

Moisture content was increased with increase of storage period both accelerated and natural ageing. The moisture content increased from 8.4 per cent to 12.6 per cent (accelerated ageing on 10th day) and 11.1 per cent in 10 months stored seeds. The possible reason could be the hydrophilic nature which provided continuous and slow supply of moisture to the seed and increased the moisture. It might be due to the prevention of moisture equilibration between the seed and storage atmosphere at higher frequency under accelerated ageing as dealt by Warham (1986) [39] in wheat. The increase in moisture content under natural ageing might be due to the increase in relative humidity of the storage environment.

In the present investigation, deterioration in seed quality was associated with decrease in germination, seedling length, dry matter production and vigour index become evident with advancement of time in storage under both accelerated and natural ageing. The initial germination was 93 per cent and showed significant reduction with advancing period of accelerated ageing. The germination on 2nd day of accelerated ageing was recorded as 89 per cent and reached 78 per cent on 6th day of accelerated ageing. The minimum germination for blackgram as per the IMSCS is 75 per cent. At last it was reached 54 per cent on 10thday of accelerated ageing. A declining trend was observed with germination which was falling from 93 per cent to 80 per cent at the end of 10th month of storage under natural ageing and it is above IMSCS of 75 per cent (Table 1 and 2). The decline in germinability during storage could be attributed to the irreversible ageing characteristics of all living biological organism causing deteriorative changes in the physical, physiological and biochemical characters of seeds (Abdul-Baki and Anderson, 1973) [1]. The decline in germination over the period of storage might be due to depletion of food reserves coupled with decline in synthetic activity as reported by Pal and Basu (1988) [20] and Ravichandran (1994) [24] in paddy, Ananthi *et al*. 2015 [2] in greengram and Gomathi *et al.* 2017 [8]. in blackgram. Sundaralingam *et al.* (2001) [27] observed similar decrease in germination with advances in days of accelerated ageing while Natarajan (1998) [15] found that it also correlated well with viability rating of seed.

Table 1: Physical and physiological changes due to accelerated ageing of blackgram seeds

Accelerated ageing (days)	Moisture content (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})	Vigour index
0 (control)	8.4	93 (74.66)	26.2	19.5	0.216	4250
1	8.6	92 (73.57)	25.9	19.4	0.210	4168
2	8.7	89 (70.63)	23.8	18.6	0.199	3774
3	9.1	86 (68.02)	22.5	17.4	0.192	3431
4	9.4	83 (65.65)	20.5	17.1	0.189	3121
5	9.7	80 (63.43)	18.5	16.8	0.178	2824
6	10.5	78 (65.02)	18.1	15.8	0.165	2566
7	10.6	74 (59.34)	17.9	14.9	0.159	2427
8	10.8	72 (58.05)	17.6	14.5	0.157	2311
9	11.5	61 (51.35)	17.2	14.4	0.145	2244
10	12.6	54 (47.29)	15.4	12.0	0.141	1479
Mean	9.9	78 (62.02)	20.3	16.4	0.177	2963
SEd	0.15	0.81	0.33	0.27	0.002	49.59
CD (P=0.05)	0.31	1.66	0.68	0.55	0.005	100.90

(Figures in parenthesis indicate arcsine values)

Table 2: Physical and physiological changes due to natural ageing of blackgram seeds

Natural ageing (months)	Moisture content (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})	Vigour index
0 (control)	8.4	93 (74.66)	26.2	19.5	0.216	4250
1	8.6	92 (73.57)	25.9	19.3	0.214	4158
2	8.8	92 (73.57)	24.7	18.9	0.209	4011
3	9.1	91 (72.54)	24.5	18.4	0.194	3904
4	9.3	90 (71.56)	23.3	17.9	0.191	3708
5	9.6	89 (70.63)	22.1	17.8	0.187	3551
6	9.9	88 (69.73)	21.2	17.6	0.185	3406
7	10.3	86 (68.02)	19.6	17.1	0.179	3156
8	10.7	85 (67.21)	18.9	16.7	0.171	3026
9	10.9	83 (65.65)	18.5	15.8	0.169	2847
10	11.1	80 (63.43)	17.9	14.7	0.155	2608
Mean	9.7	88 (69.73)	22.1	17.6	0.188	3511
SEd	0.18	1.22	0.36	0.25	0.003	47.88
CD (P=0.05)	0.37	2.48	0.73	0.51	0.006	97.41

(Figures in parenthesis indicate arcsine values)

The vigour parameters of the stored seeds in terms of seedling length, dry matter production and vigour index were also in decreasing order with increase in ageing period storage period (Plate 1). The decline in shoot length, root length and seedling vigor index might be attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination (Kapoor *et al.*2011) [11] and also effect the lipid peroxidation on the protein content important for seedling growth with decrease in germination percentage and seedling length, lead to decrease in vigour index. Rame Gowda (1992) [22] reported that the use of aged seeds of rice caused rapid decline in germination, root and shoot length, seedling dry matter and vigour index. It might be due to depletion of food reserves in aged seeds.

Control (fresh seeds) **10 days accelerated ageing** **10 months natural ageing**

Plate 1: Physiological quality of control, accelerated aged and natural aged seeds of blackgram

Electrical conductivity of seed leachate is a good index of seed deterioration. Intensity of membrane damage during storage was measured by electrical conductivity of the seed leachate (Matthews and Bradnock, 1968) [13]. The electrical conductivity was increased to 25.6 µS cm^{-1} from 15.7 µS cm^{-1} (control). Further, it was increased to 60.7 µS cm^{-1} at the end of 10 days of accelerated ageing. At the same time, the electrical conductivity also showed an increasing trend under natural ageing and measured as 24.9 µS cm^{-1} in 10^{th} month of storage (Table 3 and 4). The electrical conductivity of seed leachate increased with increase in period of accelerated and natural ageing. The increase in electrical conductivity might be due to auto oxidation of polyunsaturated fatty acids in the membrane liquid compound involving free radical chain reactions (Doijode, 1988) [7].

Table 3: Biochemical changes due to accelerated ageing of blackgram seeds

Accelerated ageing (days)	Electrical conductivity (µS cm^{-1})	α-amylase (mg maltose min^{-1})	Catalase (m mol H_2O_2 reduced min^{-1} g^{-1} seed)	Peroxidase (m mol tetraguaiacol formed min^{-1} g^{-1} seed)	Protein (%)	Free amino acid (µg 50 $seeds^{-1}$ 100 ml^{-1})
0 (control)	15.7	16.10	0.568	0.692	24.97	0.112
1	16.2	15.98	0.561	0.688	24.78	0.121
2	17.8	14.98	0.461	0.667	22.42	0.133
3	18.4	13.85	0.390	0.634	22.11	0.152
4	20.6	13.10	0.378	0.607	20.64	0.168
5	22.4	12.69	0.345	0.510	20.59	0.172
6	25.6	11.69	0.335	0.506	20.15	0.175
7	27.9	11.00	0.329	0.482	20.08	0.188
8	31.8	10.23	0.218	0.316	19.15	0.197
9	53.2	9.17	0.188	0.259	18.63	0.203
10	60.7	7.98	0.115	0.171	17.36	0.223
Mean	28.2	12.43	0.353	0.503	20.98	0.167
SEd	0.50	0.17	0.010	0.011	0.16	0.001
CD (P=0.05)	1.02	0.35	0.021	0.024	0.32	0.003

Table 4: Biochemical changes due to natural ageing of blackgram seeds

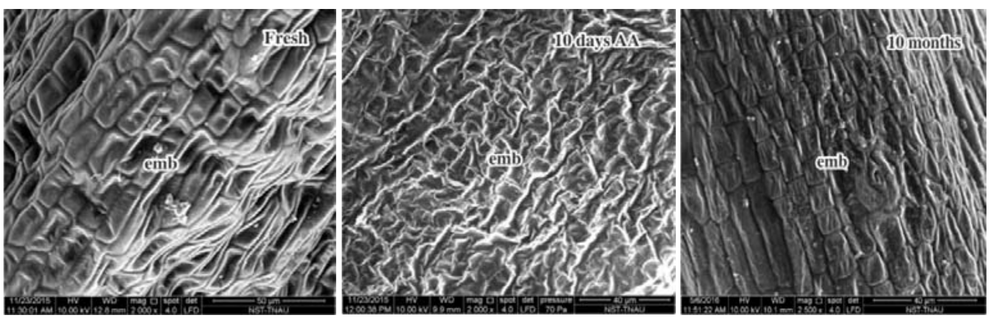
Natural ageing (months)	Electrical conductivity (µScm^{-1})	α-amylase (mgmaltosemin^{-1})	Catalase (m mol H_2O_2reduced min^{-1} g^{-1}seed)	Peroxidase (m mol tetraguaiacol formed min^{-1} g^{-1} seed)	Protein (%)	Free amino acid (µg 50$seeds^{-1}$ 100 ml^{-1})
0 (control)	15.7	16.10	0.568	0.692	24.97	0.112
2	18.6	15.47	0.529	0.681	24.85	0.115
4	19.8	14.98	0.510	0.670	23.59	0.125
6	22.3	13.99	0.426	0.620	22.68	0.148
8	23.9	12.75	0.409	0.598	21.73	0.165
10	24.9	12.64	0.390	0.570	20.97	0.174
Mean	20.8	14.32	0.473	0.639	23.13	0.140
SEd	0.36	0.19	0.007	0.008	0.33	0.002
CD(P=0.05)	0.74	0.40	0.016	0.017	0.70	0.005

The α-amylase activity was decreased with increasing period of storage both under accelerated and natural ageing. It was reduced to 7.98 mg maltose min^{-1} over the period of 0 to 10 days of accelerated ageing from 16.10 mg maltose min^{-1} where in case of natural ageing it was 12.64 mg maltose min^{-1} over the period of 10 months. Decreased activity of α-amylase during storage was reported by Rame Gowda (1992) [22] in rice. Similar results of decrease in α-amylase activity was also reported by Norastehnia *et al*. (2007) [19]. It might be due to the accumulation of aldehyde compounds especially methyl jasmonte which act as potential inhibitor of α-amylase by arresting the biosynthesis of gibbrellic acid which stimulates α-amylase synthesis and its secretion from aleurone layers in cereals.

The less catalase activity was recorded on 10^{th} day (0.115 m mol H_2O_2 reduced min^{-1} g^{-1} seed) of accelerated aged seeds when compared to control seeds (0.568 H_2O_2 reduced min^{-1} g^{-1} seed). In natural ageing also the declining trend was observed and recorded (0.390 m mol H_2O_2 reduced min^{-1} g^{-1} seed) by 10^{th} month of storage under natural ageing. The peroxidase activity followed the similar trend of results as that of catalase activity under both accelerated and natural ageing. The loss of activity of enzymes in accelerated ageing might be due to the rapid oxidative stress caused by high temperature and relative humidity. Certain toxic metabolites like hydrogen peroxide might have formed as a consequence of oxidative stress. Accelerated ageing in wheat was in association with accumulation of hydrogen peroxide (Lehner *et al*. 2008) [12]. Reduction of catalase activity was also observed in onions during accelerated ageing (Demirkaya *et al*. 2010) [6]. The loss of sunflower seed viability during accelerated ageing conditions was associated with a decrease in the activities of catalase enzyme (Hussein *et al*. 2012) [9].

The protein content followed a decreasing trend while free amino acid followed an increasing trend under both accelerated and natural ageing. Decrease in protein content was observed during accelerated ageing conditions in pigeonpea (Kalpana and Madhav Rao, 1997) [10] and in rice (Kapoor *et al.* 2011) [11]. Severe loss in reserves in seed is responsible for major damage leading to loss of viability (Roberts, 1972) [25]. Loycrajjou *et al.* (2008) [13]. reported that ageing induced deterioration and increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes. The electrical conductivity and free amino acid enhanced with storage period as reported by Bewley and Black (1994) [3]. due to degradation of nutrients and loss of membrane integrity.

The anatomical structures have been studied using Scanning Electron Microscope (SEM) for control, accelerated aged and naturally aged seeds reported that, the cells of embryonic axis were clearly visible and found to be intact in fresh seeds while it was collapsed in both accelerated and natural ageing. The damage was severe when period of ageing was extended. It could be clearly seen in the 10 days accelerated aged seeds (Plate. 2).



Control (fresh seeds) **10 days accelerated ageing** **10 months natural ageing**

Emb - embryonic axis

Plate 2: Longitudinal section of blackgram seed viewed under Scanning Electron Microscope (SEM) – pattern of cell arrangement in embryonic axis

The present results thus support the 'classical' conception of Briggs (1978) [4] and Varner and Ho (1976) [38] concerning the role of the aleurone layer in the production of α-amylase in barley. This result was also in hormony with the findings of Ramanadane and Ponnuswamy (2004) [20]. in rice.

Conclusion
The results of the present study showed that, subjecting the seeds of blackgram (TNAU Blackgram VBN 6) to accelerated ageing and natural ageing, it attained minimum germination as per the IMSCS of 75 per cent on 6^{th} day of accelerated ageing and 10 months of ambient storage. As a conclusion, six days of accelerated ageing was equivalent to ten months of natural storage at the time maintained the germination above Indian Minimum Seed Certification Standards.

References
1. Abdul-Baki AA, Anderson JD. Vigour determination of soybean seeds by multiple criteria. Crop Science. 1973; 13:630-633.
2. Ananthi M, Sasthri G, Srimathi P, Malarkodi K. Prediction of storability of greengram seeds through accelerated ageing test. International journal of farm science. 2015; 5(4):326-331.
3. Bewley JD, Black M. Physiology and biochemistry of seeds: In relation to germination. Springer - Verlang, 1994; p.77-94.
4. Briggs DE. Barley. Chmann & Hall, London, 1978.
5. Chauhan KPS. The incidence of deterioration and its localization in aged seeds of Soybean and Barley. Seed Sci. & Technol. 1984; 13:769-773.
6. Demirkaya M, Dietz KJ, Sivritepe HO. Changes in Antioxidant Enzymes during Ageing of Onion Seeds. Not. Bot. Hort. Agrobot. Cluj. 2010; 38(1):49-52.
7. Doijode SD. Comparison of storage containers for storage of french bean seeds under ambient conditions. Seed Research. 1988; 16:245-247.
8. Gomathi G, Malarkodi K, Ananthi M. Evaluation of blackgram seed viability through accelerated ageing test. Environment and Ecology. 2017; 35(2):642-645

9. Hussein JH. Physiological and Biochemical Changes in Maize and Sunflower Seeds during Accelerated Ageing Conditions. Ph.D. thesis, Univ. of Babylon, Iraq. 2012.
10. Kalpana R, Madhav Rao KV. Protein metabolism of seeds of pigeonpea (*Cajanus cajan* L. Mill sp.) cultivars during accelerated ageing. Seed Science and Technology. 1997; 25:271-279.
11. Kapoor N, Arya A, Siddiqui Mohd Asif, Kumar H, Amir A. Physiological & Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza sativa* L.). American Journal of Plant Physiology. 2011; 6(1):28-35.
12. Lehner A, Mamadou N, Come D, Bailly C, Corbineau F. Changes in soluble carbohydartes, lipid peroxidation and antioxidant enzyme activities in the embryo during agening in wheat grains. J. Cereal Science. 2008; 47:555-565.
13. Loycrajjou LY, Steven PC, Groot Belghazi M, Job C, Job D. Proteome wide characterization of seed ageing in arabidopsis. A comparison between artificial and natural ageing. Prot. Pl. Phy. 2000; 148:620-641.
14. Mathews S, Bradnock WT. Relationship between exudation and field emergence in peas and French beans. Horticulture Research. 1968; 8:89-93.
15. Natarajan N. Seed vigour, viability and productionstudies in blackgram (*Vigna mungo* (L) Hepper) cv CO3. PhD thesis. Tamil Nadu Agricultural University, Coimbatore, TN, India.1998.
19. Norastehnia RH, Sajedi M, Nojavan-Asghari. Inhibitory effects of methyl jasmonate on seed germination in maize (*Zea mays* L.) Effect on alpha-amylase activity and ethylene production. Gen. Appl. Plant Physiology. 2007; 33(1-2):13-23.
20. Pal P, Basu RN. Treatment of rice seed with iodine and chloride for the maintenance of vigour, viability and productivity. Indian agriculturist, 1988; 32(1):71-76.
21. Ramanadane T, Ponnuswamy AS. Ageing and anatomical influence on seed storability in rice (*Oryza sativa* L.) hybrids and parental lines. Tropical Agriculture Research. 2004; 16:37-50.
22. Rame Gowda. Studies on seed senescence and seed vigour in some genotypes of rice (*Oryza sativa* L.). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. 1992.
23. Rangaswamy R. A text book of agricultural statistics. New Age International Pvt., Ltd. New Delhi. 2002.
24. Ravichandran R. Studies on certain aspects of post harvest factors affecting quality of paddy Compaction effect of stacking, Impact of transportation and handling and 3) Provenance influence on storability. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. 1994; 2(1).
25. Roberts EH. Storage environment and the control of viability. In: Viability of seeds, Chapman and Hall, London, UK. 1972; p.14-58.
26. Singh RKP, Satapathy KK. The zero energy cool chamber: a low cost storage structure. Journal of the North Eastern Council, 2003; 23(3):27-30.
27. Sundaralingam K, Srimathi P, Vanagamudi K. Presowing seed management. In: Recent trendsand participatory approaches on quality seedproduction (K Vanangamudi, A Bharathi, P Natesan, R Jerlin, TM Thiyagarajan and SKannaiyan eds). Department of Seed Scienceand Technology, Tamil Nadu Agricultural University, Coimbatore, TN, India. 2001.
35. Thant KH, Duangpatra J, Romkaew K. Approppriate temperature and time for an accelerated agingvigor test in sesame (*Sesamum indicum* L.) Seed Journal of Nature Science. 2010; 44:10-16.
38. Varner JE, Ho THD. Hormones. In. J. Bonner, J.E. Varner, eds, Plant Biochemistry. Academic Press, New York, 1976; p.714-765.
39. Warham EJ. The effect of different packing materials on moisture uptake by dry wheat in stimulated humid tropical conditions. Seed Science and Technology. 1986; 14:641-655.