Mysore J. Agric. Sci., 57 (2) : 301-309 (2023)

Seed Morphometric Changes Influenced by Accelerated Ageing in Contrast-coloured Maize (*Zea mays* L.) Genotypes

V. Satya Srii and Nethra Nagarajappa
All India Coordinated Research Project on Seed (Crops), Seed Technology Research Unit,
UAS, GKVK, Bengaluru - 560 065
e-Mail : nethraharsha@gmail.com

Authors Contribution

V. Satya Srii : Conceptualization, formal analysis, investigation and original draft preparation; Nethra Nagarajappa : Conceptualization, project administration, provided resources, review and editing manuscript

Corresponding Author :
Nethra Nagarajappa
All India Coordinated Research Project on Seed (Crops), Seed Technology Research Unit,
UAS, GKVK, Bengaluru

Received : August 2022
Accepted : February 2023

Abstract

Accelerated ageing induces many deteriorative changes to seeds which ultimately lead to reduced germination and loss of viability. Taking advantage of the image analysis, the seed morphometric changes after accelerated ageing was investigated. Three contrasting colour maize genotypes *viz.,* African tall (white), MAH 14-5 (orange) and local land race (red) selected were artificially aged for 96 and 120 hours. The aged seeds showed 100 per cent viability but reduced germination per cent due to the increased fraction of abnormal seedlings. The parameters like area, diameter, perimeter, roundness increased, elongation decreased while roughness and compactness remained constant in artificially aged seeds. Area increase in 96 and 120 hours of ageing recorded 18 and 23 per cent in African Tall, 11 and 14 per cent in MAH 14-5 and 9 and 10 per cent in local landrace respectively while the diameter and perimeter showed similar per cent increase. There was a significant difference in morphometric changes between genotypes with white genotype showing a higher increase in size parameters compared to coloured genotypes which may be attributed to impermeability resulting out of oxidation of phenols in coloured genotypes. Lesser the change in morphometric characters after ageing higher was the germination indicating that change in seed morphometric characters during ageing as a deteriorative sign.

Keywords : Imbibition, Deterioration, Perimeter, Area

Seed ageing is an irreversible and inexorable process of a progressive decrease in vigour ultimately leading to the loss of seed viability (Stewart & Bewley, 1980 and Lehner *et al*., 2008). The rate of seed ageing depends upon the genotype/ species, the conditions prevailing during storage like moisture content, temperature, humidity and seed composition (Roberts, 1973). It has been reported that high moisture content and high temperature usually accelerate seed deterioration (Ellis and Hong, 1991; Goel *et al.*, 2003) based on which seeds are accelerated to artificially age by exposing them to high humidity and temperature of about 40 - 45p °C and 100 per cent RH (Delouche and Baskin, 1973). Accelerated ageing is shown to induce many deteriorative changes to seeds during storage like genetic damage, protein degradation, enzyme inactivation and loss of membrane integrity (Bailly *et al*., 1996; Merritt *et al*., 2003; Bailly., 2004; Ratajczak & Pukacka, 2005; Wang *et al*., 2011 and Ratajczak *et al*., 2015) which ultimately lead to reduced germination (Walters, 1998) and loss of viability. Though there are numerous studies on physiology, ROS and its mechanisms of membrane degradation during ageing, there is less knowledge about the seed morphometric changes brought about by accelerated ageing.

Ellis *et al*. (1992) reported that the rate of seed deterioration depends upon the moisture content and temperature of storage conditions. Among these two factors, the sensitivity of seeds to high temperatures is strongly dependent on their water content, loss of

viability being faster with increasing moisture content indicating the crucial role of moisture in seed deterioration (McDonald, 1999 and Roberts & Ellis, 1989). In accelerated ageing conditions, the increased humidity (~100% RH) leads to moisture absorption by seeds (ISTA, 2010 and Kapoor *et al.*, 2011). This moisture absorption during ageing would lead to swelling of seeds altering the seed size parameters. So, the morphometric changes like seed perimeter, area, diameter, roundness, roughness, elongation and compactness after ageing can be effectively captured by image analysis which in turn would indicate the rate of seed deterioration and help in varietal identification (Nethra *et al*., 2005). A strong relation between water absorption and change in seed perimeter measured by an image analyser was established by Satya Srii *et al*. (2020). Previous studies (Renuka *et al.*, 2022) have reported the use of morphological differences between parental lines in hybrid seed production to help characterization, however use of seed morphometric characters to study imbibition during ageig is unexplored.

Seed image analysis works based on the extraction of numerical data from a captured image of seeds and seedlings and their subsequent data processing with the help of suitable computer software (Hemender *et al*., 2018). The major advantage of image analysis over other conventional methods is the easy determination of dimensional changes in time without any manipulation of the seeds (Tanabata *et al*., 2012) and its capacity to detect even the smallest changes in seed dimensions (Dell'Aquila *et al*., 2000; Dell'Aquila, 2005). Taking advantage of the image analysis procedure, in this study we investigated the changes in seeds morphometric parameters like area, diameter, perimeter, roughness, elongation, roundness and compactness after subjecting seeds to accelerated ageing. As the rate of moisture absorption during ageing depends upon the initial moisture content of seeds and the genotype, we studied morphometric changes in three contrast coloured maize genotypes (white, orange and red) in response to accelerated ageing conditions *i.e.,* genotypic difference in moisture absorption ie morphometric changes and ultimately in deterioration.

Material and Methods

Seed Material

The three contrasting colour maize genotypes African tall fodder maize (white), MAH 14-5 (Orange) and local landrace from Tamil Nadu, India (red) were selected for the study and the fresh seeds of African fodder maize and MAH 14-5 were received from Seed Stores, National Seed Project, University of Agricultural Sciences, Bangalore, India and local red landrace was collected from Maize Research Station, Vagarai, Tamil Nadu, India. The fresh seeds were checked for optimum moisture content and stored at -20p °C until further use.

Accelerated Ageing

Artificial ageing was performed according to ISTA guidelines (ISTA, 2010). The moisture content of the samples was determined and those with optimal moisture content between 10-14 per cent moisture was kept for accelerated ageing. For artificial ageing (AA), the plastic AA boxes were first sterilized with 5 per cent sodium hypochlorite and dried then, each AA box was filled with 40 ± 1.0 ml of distilled water. Seeds were placed on the screen one layer deep to ensure an even uptake of moisture from the humid environment and the lid was placed on each plastic AA box. These AA boxes were placed on the shelves of the ageing chamber (Manufacturer: Thermo Scientific, Model: IGS 60/100/180) allowing air space of 2.5 cm between plastic AA boxes to assure temperature uniformity. The temperature was set to 41 ± 0.3p °C with 100 per cent RH and was monitored at regular intervals. After 96 hours and 120 hours of ageing, the AA boxes were removed from the chamber. The control seeds were stored in sealed, optimum storage conditions for the same duration as of ageing.

Germination Per cent and Viability

Seed germination per cent and viability of the aged seeds along with control (fresh, non- aged seeds) were measured using standard ISTA protocol (ISTA, 2010) to confirm the process of ageing. Seed germination test was performed for 100 seeds in 4 replicates for each ageing treatment and control by between paper

method. The viability testing of seeds was performed for 50 seeds in 2 replicates for each ageing treatment using Tetrazolium (Tz) staining. Tz staining was performed by soaking the seeds in water for 12 hours followed by cutting the seeds longitudinally through the embryo and ¾ of the endosperm and then soaking the seeds in 1 per cent Tz solution for 2 hours and evaluating the uptake of stains.

Image Analysis for Seed Morphometric Characters

Seed morphometric studies were performed using the image analysis approach. Two replicates of 25 seeds in each ageing treatment and control were taken for the study. Seed morphometric parameters like area, diameter, perimeter, roundness, compactness, elongation and roughness of the individually labelled seeds in Petri dishes was measured using an image analyser (Manufacturer: Expert Vision Labs Private Limited, Model L-2000 in conjunction with biovis software) and recorded as seed morphometric characters before ageing, then the seeds were subjected to accelerated ageing and storage in case of control for different periods after which the seeds were again analysed through image analysis. The parameters like roughness, compactness, elongation and roughness of seeds were measured by specifically developed macros based on image analysis library and formulas (Varma *et al.*, 2013).

Statistical Analysis

Descriptive statistics were performed using Microsoft Excel 2010. Significance of values of different parameters before and after ageing within a genotype was performed using Paired t-Test (paired two samples for mean) in Microsoft Excel 2010. The significance of the difference in values between genotypes and also between two ageing periods was analysed individually using SPSS software (ANOVA with single factor). Correlation between various parameters was confirmed using the r^2 value calculated using Microsoft Excel 2010 in which most parameters showed strong correlation ($r^2 > 0.7$) with few having moderate correlation ($0.5 < r^2 < 0.7$). Per cent increase/ decrease in values of each parameter before and after ageing was calculated which was used to plot a radar plot using Microsoft Excel 2010.

Results and Discussion

Seed Physiological Quality After Ageing

The germination per cent, viability per cent of three genotypes of seeds aged for different time intervals along with control evaluated as per ISTA protocol (ISTA, 2010) is given in Table 1. One way ANOVA was used to test the significance of data and there was a significant difference ($P<0.01$) in seed quality parameters between genotypes and between different ageing treatments.

Ageing treatments of 96 and 120 hours were selected after standardising viability at different periods of ageing, to have seeds that are still viable but less vigorous *i.e.,* at the initial stages of deterioration to study the initial changes brought about by ageing to morphometric characters of seeds which might, in turn, correlate to deteriorative changes. Results of viability and germination tests showed that all three genotypes remained viable after 96 and 120 hours of ageing yet there was a decrease in germination

Table 1

Seed quality parameters measured at different ageing periods for two Maize genotypes

Genotypes	Seed germination(per cent)			Viability (per cent)		
	Control	Ageing (96 h)	Ageing (120 h)	Control	Ageing (96 h)	Ageing (120 h)
African Tall (white)	100 ± 0.00 **	90 ± 0.83 **	84 ± 0.70 **	100 ± 0.00	100 ± 0.00	100 ± 0.00
MAH 14-5 (orange)	100 ± 0.00 **	93 ± 1.22 **	88 ± 0.44 **	100 ± 0.00	100 ± 0.00	100 ± 0.00
Local landrace (red)	100 ± 0.00 **	96 ± 0.54 **	93 ± 0.70 **	100 ± 0.00	100 ± 0.00	100 ± 0.00

Values are expressed as mean (±SD). ** indicates significant difference between ageing times at pd ≤ 0.01.

per cent as shown in Table 1. This contradiction in results of viability and ageing is due to the fraction of abnormal seedlings produced by aged seeds which would not be considered as germinated in germination tests as per ISTA test guidelines. Though aged seeds had 100 per cent viability even after ageing the deteriorative changes that occurred due to ageing reflected as abnormal seedlings. It was also reported in peas and soybean that accelerated ageing increased the proportion of abnormal seedlings which reduced the germination per cent of aged seeds (Veselova and Veselovsky, 2003 and Rastegar *et al*., 2011). But there was a significant difference in germination per cent between genotypes after ageing where African Tall showed the least germination per cent after ageing followed by MAH 14-5 and Local landrace which might be due to varying genetic potential between genotypes. An interesting point is that the darker the colour, the greater the resistance to ageing *i.e.,* coloured genotypes incurred lesser damage compared to colourless genotype due to ageing. The reason for the increased germination after ageing in coloured genotypes could be attributed to the presence of proanthocyanidins with free radical scavenging activity in the seedcoat (Takahata *et al.*, 2001) which would in turn help in cell repair mechanisms preventing membrane damage (Bailly, 2004).

Seed Morphometric Analysis

Seed morphometric parameters like area, diameter, perimeter, roundness, compactness, elongation and roughness measured for different seed groups revealed that the control (fresh, non-aged) seeds had no change in morphometric characters before and after the storage period while the seeds subjected to ageing showed a significant difference in values after ageing. The parameters like area, diameter, perimeter, roundness increased, elongation decreased while roughness and compactness remained constant when the seeds were artificially aged. Data recorded for various parameters before and after ageing along with percent change in parameter after ageing for three genotypes are presented in Table 2, 3 and 4. This data (Fig. 1) shows that there was a

TABLE 2

Seed morphometric parameters of white maize (African Tall- fodder maize) measured before and after ageing in T1 and T2 along with percent change in parameters

Parameters	Before ageing	After ageing	Per cent change
T1 (white maize)			
Area square (cm)	0.73831	0.86961	18%
Diameter (cm)	0.87641	0.959068	9%
Perimeter (cm)	2.98482	3.257892	9%
Roundness	1.03697	1.111904	7%
Roughness	1.03261	1.033192	0%
Elongation	1.25762	1.225448	-3%
Compactness	12.1804	12.35156	1%
T2 (white maize)			
Area square cm	0.78291	0.962952	23%
Diameter cm	0.875407	0.986072	13%
Perimeter cm	2.87533	3.245132	13%
Roundness	1.02521	1.150136	12%
Roughness	1.03253	1.033884	0%
Elongation	1.240747	1.17448	-5%
Compactness	12.29995	12.47616	1%

TABLE 3

Seed morphometric parameters of orange maize (MAH 14-5) measured before and after ageing in T1 and T2 along with percent change in parameters

Parameters	Before ageing	After ageing	Per cent change
T1 (Orange maize)			
Area square cm	0.51764	0.575841	11%
Diameter cm	0.7081	0.759013	7%
Perimeter cm	2.36762	2.5442125	7%
Roundness	1.17417	1.218283	4%
Roughness	1.02265	1.023017	0%
Elongation	1.12385	1.113441667	-1%
Compactness	10.88918	10.90508	0%
T2 (Orange maize)			
Area square cm	0.55045	0.625274	14%
Diameter cm	0.739703	0.802678	9%
Perimeter cm	2.48067	2.708144	9%
Roundness	1.122687	1.196872	7%
Roughness	1.023893	1.023856	0%
Elongation	1.115287	1.079171722	-3%
Compactness	11.22109	11.16935	0%

TABLE 4

Seed morphometric parameters of red maize (local landrace) measured before and after ageing in T1 and T2 along with percent change in parameters

Parameters	Before ageing	After ageing	Per cent change
T1 (Red maize)			
Area square cm	0.501706	0.546908	9%
Diameter cm	0.707217	0.738156	4%
Perimeter cm	2.39521	2.490916	4%
Roundness	1.099223	1.100836	0%
Roughness	1.03078	1.02716	0%
Elongation	1.119803333	1.119312	0%
Compactness	11.49296	11.45215	0%
T2 (Red maize)			
Area square cm	0.514243	0.5644548	10%
Diameter cm	0.714353	0.759934	6%
Perimeter cm	2.41172	2.549356	6%
Roundness	1.105403	1.109316	0%
Roughness	1.02853	1.02934	0%
Elongation	1.11161	1.1124916	0%
Compactness	11.40051	11.40193	0%

difference in seed size of different genotypes even before subjecting to ageing where the African Tall (white) were larger followed by MAH 14-5 (Orange) while Local landrace (red) was the smallest among three. Among various parameters, roundness and elongation were seen to be negatively correlated with $r^2 > 0.5$. All genotypes recorded similar roughness values which were cross-verified by physical examination of seed surface.

To eliminate exaggeration of increase/decrease in parameters between genotypes, per cent increase was calculated taking into account the initial value and the change in value after ageing for all genotypes. These results of per cent change in morphometric parameters at different periods of ageing for three genotypes are presented in form of a radar chart in Fig. 2, which shows that the increase/ decrease per cent for all parameters was higher in African Tall (white genotype) followed by MAH 14-5 (orange genotype) and least in Local landrace (red). Between ageing periods, 120 hours of ageing recorded more increase/ decrease per cent than 96 hours of ageing in all three genotypes. Area increase in 96 and 120 hours

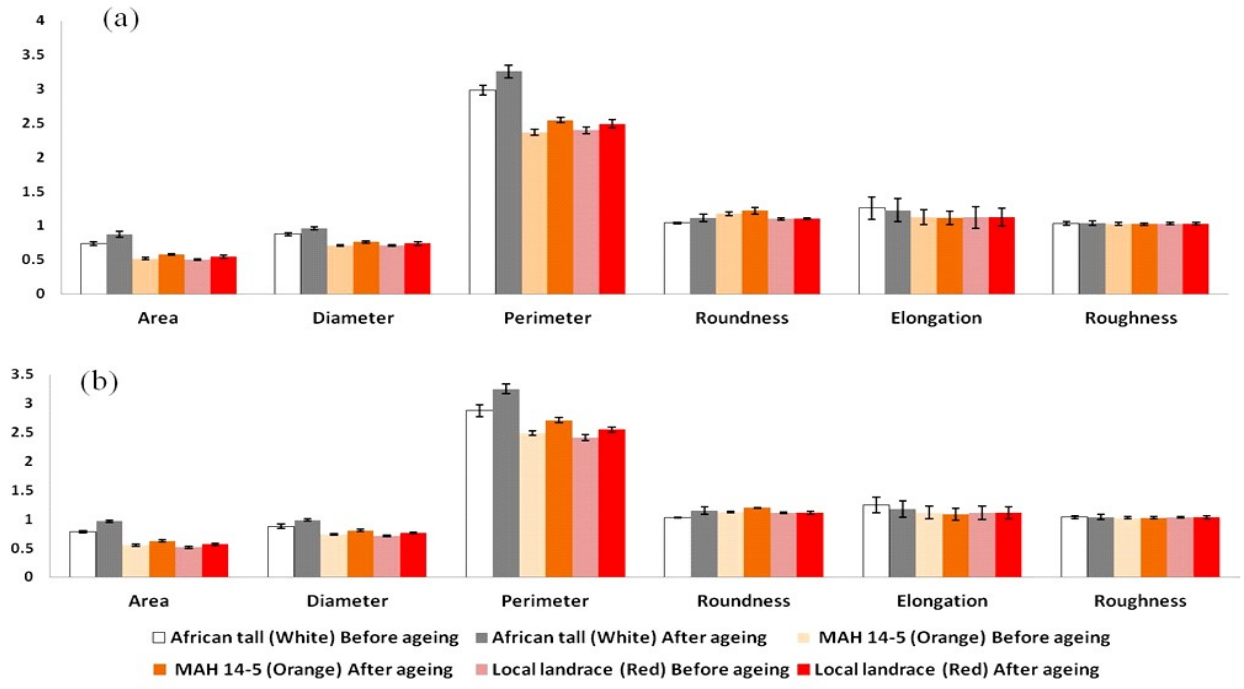

Fig. 1: Morphometric parameters of maize seeds of three genotypes after ageing for (a) 96 hours and (b) 120 hours. Black bar indicates standard error

The Mysore Journal of Agricultural Sciences

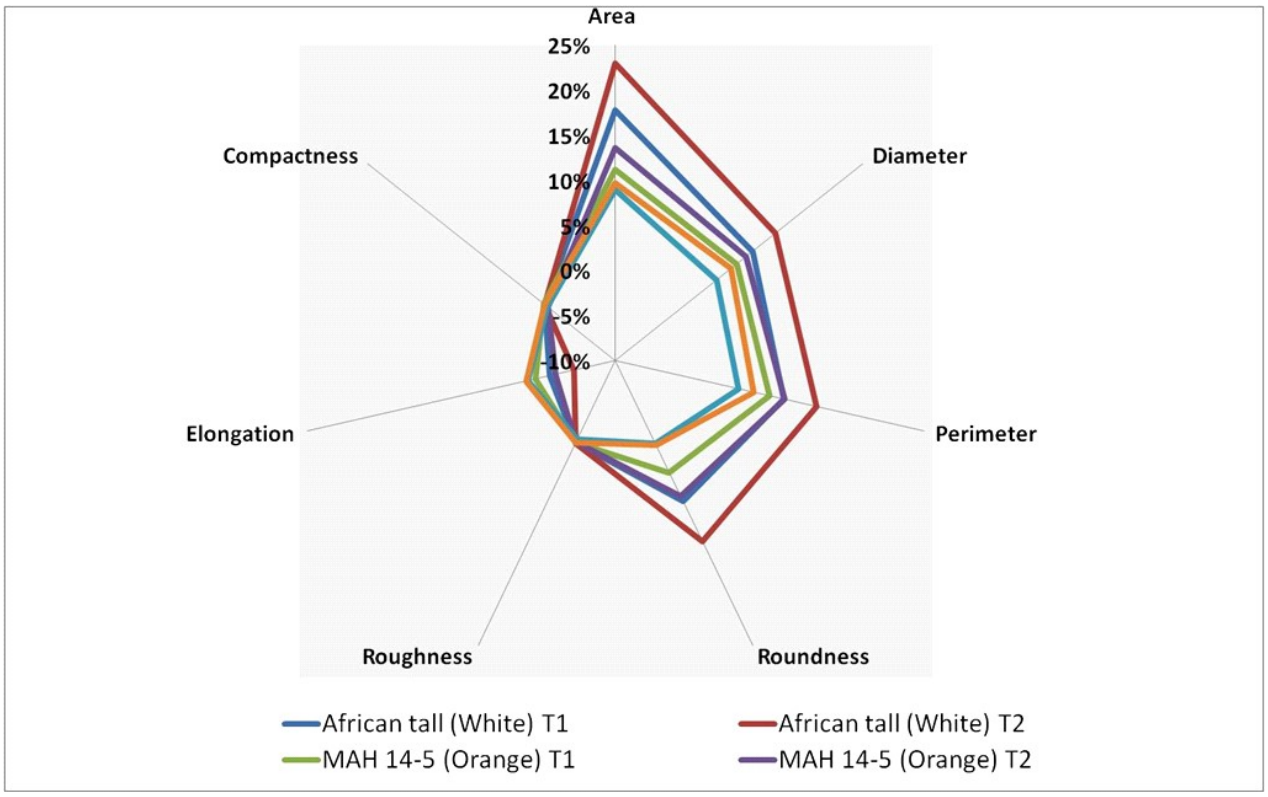

Fig. 2: Per cent change in seed morphometric characters brought about by different periods of ageing in three genotypes

of ageing recorded 18 and 23 per cent in African Tall, 11 and 14 per cent in MAH 14-5 and 9 and 10 per cent in Local landrace respectively. Diameter and perimeter showed the same per cent increase confirming the accuracy of measurements made by image analysis where they increased by 9 and 13 per cent in African Tall, 7 and 9 per cent in MAH 14-5 and 4 and 6 per cent in Local landrace at 96 and 120 hours of ageing respectively.

The paired t-test showed a high level of significance for both single and two-tail tests ($P<0.001$) for all the parameters measured before and after ageing. Also, there was a significant difference ($P< 0.01$) for values between genotypes tested using ANOVA (single factor). Significant correlations between parameters ($r^2> 0.5$) showed the accuracy and relevance of values measured by the image analyser.

The increase in size parameters like area, diameter, perimeter in aged seeds is due to the absorption of moisture by seeds in the ageing chamber which is maintained at 100 per cent RH. There was an increase in area, perimeter and diameter, in turn, reflecting an increase in roundness while the decrease in elongation. This absorption of moisture leads to swelling/ increased size of seeds after ageing and it is seen that seeds absorb more moisture if they are exposed to the ageing chamber for a longer duration which is reflected as the increased per cent of the size increase in seeds that was aged for a longer duration. Absorption of moisture increases the moisture content of seeds which in turn leads to faster deterioration. It is reported that higher moisture content in seeds leads to faster deterioration (Ellis *et al*., 1992). Results from germination also correlate to the above fact that the genotype which showed a higher increase in size after ageing *i.e.,* more absorption of water had lesser germination per cent. African tall which showed 18 and 23 per cent increase in the area after 96 and 120 hours of ageing had only 90 and 84 per cent germination respectively, on the other hand, local landrace which showed only 9 and 10 per cent increase in the area after 96 and 120 hours

had 96 and 93 per cent germination while MAH 14-5 which had an intermediate increase of about 11 and 14 per cent showed 93 and 88 per cent germination after 95 and 120 hours respectively.

This differential ability of genotypes to absorb moisture may be due to its differential permeability properties. The primary line of protection to seeds against ageing conditions is offered by seed coats as they are the main interface between seed and external environment. It is reported the cracks, cleavages, fissures and scratches resulting either from ageing or genetics or handling procedures (*e.g.,* harvesting, drying, processing and sowing operations) in seed coats could lead to deteriorative changes affecting the embryo and ultimately decreasing the seed longevity. Thus the primary resistance of seeds against deterioration can be attributed to thick impermeable seed coats that could guard the embryo from adverse external conditions (Black & Halmer, 2006 and 205 Brooker *et al.,* 2007). The impermeability of seed coat is proposed to be created by oxidation of phenolic compounds by polyphenol oxidase or peroxidase which could, in turn, provide impermeability to seed coats (Pourcell *et al.*, 2005 and Rajjou & Isabelle, 2008). Also, studies show that phenolic compounds in seed coats contribute to seed longevity by limiting the permeability to oxygen and moisture (Pourcell *et al.*, 2007). This may be one of the reasons why the cultivars with dark seed coats in chickpea (Gvozdeva and Zhukove, 1971), soybean (Shahi and Pandey, 1982), snap bean (*Phaseolus vulgaris*) (Prasad and Weigle, 1976), french bean (Powell, 1986) were found to be less permeable than the light seeded cultivars. Studies show that coloured seed coats are impermeable compared to colourless ones in legumes with pigmentation negatively correlating to permeability (Souza and Marcos-Filho, 2001). The results were again proven in our study where the white genotype showed more moisture absorption compared to orange and red with red absorbing the least moisture. Thus the impermeability resulted from oxidation of phenolic compounds may be one of the reasons for reduced damage and deterioration reflected as higher germination per cent in local landrace (red) and MAH14-5 (Orange) compared to African Tall (white) after ageing.

Though numerous studies on the effect of artificial ageing on seed physiology, ROS and its mechanisms of membrane degradation, there are no reports of difference in morphometric characters of seeds brought about by artificial ageing. The study reveals that besides physiological, chemical and physical changes, seed morphometric characters also change due to ageing. The increase in size parameters of seeds shows that an increase in moisture content under imposed ageing conditions *i.e.,* 41 ± 0.3 °C with 100 per cent RH is the crucial deteriorating agent in artificial ageing while under natural conditions, there may be many other factors playing role in deterioration. Further studies on the comparison of changes in morphometric characters of natural and artificial ageing would provide deep insight into the mechanisms of the two process.

This study indicates the potential of image analysis to estimate moisture increase in aged seeds by capturing minute changes in seed dimensions. Thus, image analysis can be a better alternative for measuring moisture changes in seeds during storage as conventional moisture estimation methods are either destructive or time taking and are practically impossible for larger lots. Further study and standardisation of the utility of image analysis in moisture estimation would help in the efficient storage and handling of seeds.

References

Bailly, C., 2004, Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.,* **14** : 93 - 107.

Bailly, C., Benamar, A., Corbineau, F. and Come, D., 1996, Changes in malondialdehyde content and in super oxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plant,* **97** : 104 - 110.

Black, M. H. and Halmer, P., 2006, The Encyclopedia of Seeds: Science, Technology and Uses. Wallingford: CABI.

Brooker, N. L., Lagalle, C. D., Zlatanic, A., Javni, I. and Petrovic, Z., 2007, Soy polyol formulations as novel seed treatments for the management of soil-borne diseases of soybean. *Communications Agri. Appl. Biological Sci.,* **72** : 35 - 43.

Dell'aquila, A., 2005, The use of image analysis to monitor the germination of seeds of broccoli (*Brassica oleracea*) and radish (*Raphanus sativus*). *Ann. Appl. Bio.*, **146** : 545 - 550.

Dell'aquila, A., Van Eck, J. W. and Van Der Heijden, G. W. A. M., 2000, The application of image analysis in monitoring the imbibition process of white cabbage (*Brassica oleracea* L.) seeds. *Seed Sci. Res.*, **10** : 163 - 169.

Delouche, J. and Baskin, C. C., 1973, Accelerated Aging Techniques for Predicting the Relative Storability of Seed Lots. *Seed Sci. Tech.*, **1** : 427 - 452.

Ellis, R. and Hong, T., 1991, Seed storage behaviour in *Elaeis guineensis. Seed Sci. Res.,* **1** : 99 - 104.

Ellis, R. H., Hong, T. D. and Roberts, E. H., 1992, The Low-moisture-content Limit to the Negative Logarithmic Relation Between Seed Longevity and Moisture Content in Three Subspecies of Rice. *Ann. Bot.,* **69** (1) : 53 - 58.

Goel, A., Goel, A. K. and Sheoran, I. S., 2003, Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Plant Physiol.,* **160** : 1093 - 1100.

Gvozdeva, Z. V. And Zhukova, N. V., 1971, Influence of storage conditions on longevity of seeds of bean, chickpea and soybean. *Trudy Prikl. Bot.*, **45** : 161 - 168.

Hemender, T., Sushma, S., Mor, V. S., Jitender and Bhuker, A., 2018, Image analysis: a modern approach to seed quality. *Current J. Appl. Sci. Tech.*, **27** : 1 - 11.

Ista (International Seed Testing Association) (2010), International rules for seed testing: rules 1996. Seed Science and Technology.

Kapoor, N., Arya, Arvind, Siddiqui, D. R., Kumar, Amir and Asad., 2011, Physiological and Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza sativa* L.). *Amer. J. Plant Physiol.,* **6** (1) : 28 - 35.

Lehner, A., Mamadou, N., Poels, P., Come, D. and Bailly, C., Corbineau, F., 2008, Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. *J. Cereal Sci.,* **47** : 555 - 565.

Mcdonald, M. B., 1999, Seed deterioration: physiology, repair and assessment. *Seed Sci. Tech.*, **27** : 177 - 237.

Merritt, D. J., Senaratna, T., Touchell, D. H., Dixon, K. W. and Sivasithamparam, K., 2003, Seed ageing of four Western Australian species in relation to storage. *Seed Sci. Res.*, **13** : 155 - 165.

Nethra, N., Rajendra Prasad. S. and Rame Gowda., 2005, Varietal characterization based on seed, seedling and plant morphological traits in rice (*Oryza sativa* L.) genotypes. *Mysore J. Agric. Sci*, **39** (3) : 362 - 367.

Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L. and Debeaujon, I., 2007, Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends Plant Sci.,* **12** : 29 - 36.

Pourcel, L., Routaboul, J. M., Kerhoas, L., Caboche, M., Lepiniec, L. and Debeaujon, I., 2005, Transparent testa10 encodes a laccase-like enzyme involved in oxidative polymerization of flavonoids in Arabidopsis seed coat. *Plant cell*, **17** (11) : 2966 - 2980.

Powell, A. A., Oliveria, M. D. A. and Matthews, S., 1986, The role of imbibition damage in determining the vigor of white and colored seed lots of dwarf Frech bean (*Phaseolus vulgaris*). *J. Exper. Bot.*, **37** : 716 - 722.

Prasad, K. and Weigle, J. L., 1976, Association of seed coat factors with resistance to Rhizoctonia solani in *Phaseolus vulgaris*. *Phytopathology,* **6** : 342 - 345.

Rajjou, L. and Debeaujon, I., 2008, Seed longevity: Survival and maintenance of high germination ability of dry seeds. *Comptes rendus biologies*. **331** : 796 - 805.

RASTEGAR, Z., SEDGHI, MOHAMMAD, KHOMARI AND SAEID., 2011, Effects of Accelerated Aging on Soybean Seed Germination Indexes at Laboratory Conditions. *Notulae Sci. Biol*., **3** (3) : 126 - 129.

RATAJCZAK, E. AND PUKACKA, S., 2005, Decrease in beech (*Fagus sylvatica*) seed viability caused by temperature and humidity conditions as related to membrane damage and lipid composition. *Acta Physiol Plant*, **27** : 3 - 12.

RATAJCZAK, E., MALECKA, A., BAGNIEWSKA-ZADWORNA, A. AND KALEMBA, E. M., 2015, The production, localization and spreading of reactive oxygen species contributes to the low vitality of long-term stored common beech (*Fagus sylvatica* L.) seeds. *J. Plant Physiol.,* **174** : 147 - 156.

RENUKA, R., VASUDEVAN, S. N. AND SHIVAKUMAR, N., 2022, Morphological Characterization of New Parental Lines (AxB) of Rice for their Production Potential (*Oryza sativa* L.). *Mysore J. Agric. Sci*., **56** (1) : 292 - 298.

ROBERTS, E. H. AND ELLIS, R. H., 1989, Water and seed survival. *Ann. Bot.,* **63** : 39 - 52

Roberts, E. H., 1973, Loss of viability: Ultrastructural and physiology aspects. *Seed Sci. Tech.,* **1** : 529 - 545.

SATYA SRII, V., NETHRA, N., UMARANI, K., SOWMYA, H. AND DEVARAJU, P. J., 2020, Use of an image analyser to study the imbibition process of *Melia dubia* seeds. *Seed Sci. Tech.*, **48** (3) : 325 - 332.

SHAHI, J. P. AND PANDEY, M. P., 1982, Inheritance of seed permeability in soybean. *Ind. J. Genetics Plant Breed.*, **42** : 196 - 199.

SOUZA FRANCISCO, H. D. AND MARCOS-FILHO, J., 2001, The seed coat as a modulator of seed-environment relationships in Fabaceae. *Brazilian J. Bot.*, **24** (4) : 365 - 375.

STEWART, R. R. AND BEWLEY, J. D., 1980, Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiology,* **65** : 245 - 248.

TAKAHATA, Y., OHNISHI-KAMEYAMA, M. AND FURUTA, S., 2001, Highly polymerized procyanidins in brown soybean seed coat with a high radical-scavenging activity. *J. Agri. food chemis.*, **49** : 5843 - 5347.

TANABATA, T., SHIBAYA, T., HORI, K., EBANA, K. AND YANO, M., 2012, SmartGrain: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiology*, **160** : 1871 - 1880.

VARMA, S. V., KANAKA, D. K. AND KESHAVULU. K., 2013, Seed image analysis: its applications in seed science research. *Inter. Res. J. Agri. Sci.*, **1** (2) : 30 - 36.

VESELOVA, T. V. AND VESELOVSKY, V. A., 2003, Investigation of atypical changes during accelerated ageing of pea seeds. *Seed Sci. Tech.*, **31** : 517 – 530.

WALTERS, C., 1998, Understanding the mechanisms and kinetics of seed aging. *Seed Sci. Res.*, **8** (2) : 223 - 244.