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Bhaskaran M
Tamil Nadu Open University, Chennai, Tamil Nadu, India

R Jerlin
Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

T Venupriya
Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Correspondence
Bhaskaran M
Tamil Nadu Open University, Chennai, Tamil Nadu, India

Analysing pattern of tissue viability loss in seeds of ground nut genotypes differing in dormancy, pre-storage treatment and storage conditions

Bhaskaran M, R Jerlin and T Venupriya

Abstract
Deterioration of quality in two contrasting groundnut genotypes differing in dormancy (VRI 7, a dormant genotype and CO 7, a non-dormant genotype), seed treatment (halo polymer and iodine impregnation treatment) and storage. Scanning electron microscopic analysis of anatomical changes showed that cells were intact and stable in seeds of dormant VRI 7 whereas cells started smudging showed structural irregularities in non-dormant CO 7. Seeds of dormant genotype VRI 7 subjected to halo polymer and cold storage treatment were found to possess significantly higher viability percentage.

Keywords: Groundnut, kernel, dormancy, storage, morphological and anatomical structure, viability

Introduction
Among the oilseeds, groundnut is very sensitive to deterioration during storage. Wide array of physical, physiological, biochemical, molecular and morphological changes taking place during storage which affects seed viability. The above changes are closely associated with seed dormancy which exhibits significant impact over seed viability during storage (Jenifer Sandhya, 2015) [6]. Suitable pre-storage seed treatments combined with optimum storage conditions can enhance the storage potential of groundnut seeds. Colour coating of groundnut kernel with halogenated polymer enabled maintenance of higher viability even after six months of storage under ambient conditions (Jenifer Sandhya, 2015) [6]. The present study was carried out to analyse the pattern of anatomical and morphological changes in kernels of dormant and non-dormant groundnut genotypes through periodical assessment of viability using tetrazolium staining pattern and high resolution imaging of kernels at initial and end of storage period.

Materials and Methods
Seeds of groundnut varieties (CO 7, non-dormant variety and VRI (Gn) 7, a dormant genotype) were obtained from Agricultural Research Station, Vaigaidam, Tamil Nadu, India. Seeds were cleaned, processed (graded based on size), sorted and subjected to seed treatments which included halogenations with iodine and seed coating with iodine based halo-polymer.

Seed treatment using halogen powder
Talc powder was used as a carrier for iodine gas. Talc powder was impregnated with iodine at the rate of 3 g kg^{-1} of powder and the mixture was incubated in a bottle for a week for diffusion of iodine vapours into the carrier. Other treatmental details are as follows:

Seed treatments
T_1 - Control (Kernel)
T_2 - Seed dressing with halogen impregnated powder @ 3 g kg^{-1} of seeds
T_3 - Halo polymer treatment @ 3 g kg^{-1} of seeds
T_4 - Control (pods)

Varieties
V_1 - Dormant - VRI (Gn) 7
V_2 - Non dormant CO 7

Storage

S_1 - Ambient (28 + 2^0C; 55 %)

S_2 - Cold (4 + 2^0 C; 30 %)

Seed samples were subjected to above treatments and stored under ambient (28 $\pm$ 2^0C) and cold (4 $\pm$ 2^0C) storage conditions for six months. Seed samples were drawn at monthly intervals and subjected to TTZ test. Histological studies *viz.*, topographical tetrazolium staining and anatomical changes were conducted on seed samples drawn at initial and end of the storage period.

Topographical tetrazolium staining (TTZ test) (Lakon, 1949) [8]

Seeds were subjected to tetrazolium staining for appropriate duration (2h) and washed with water and observed using a Digital Image Analyser and the pattern of staining was observed and recorded. Seeds were categorized as viable (Complete staining, staining in more than ½ the area with complete staining of embryonic axes) and non-viable (Complete non-staining, staining in less than ½ the area with complete staining of embryonic axes and embryonic axes fully unstained).

Scanning Electron Microscopic (SEM) analysis

Seed morphological traits were assessed using SEM FEI QUANTA 250. Embryonic axis was excised from the seed and cut laterally and transversely. For taking images, the cut section of the embryonic axis was placed facing upwards and to view the external surface the axis was placed as such on the double sided adhesive carbon conducting tape. The tape was then mounted on 8 mm diameter aluminium stub. Sample surface was observed at 5 KV, 1-9 Pa pressure, 500X and 4000X magnification and the images were recorded. The detector used was Everhart- Thornley detector.

Results and Discussion

Viability of seeds was found to be decreased during storage. Adverse storage conditions can provoke significant variations in seed viability (Tatic *et al*., 2012) [11], and storage duration is negatively correlated with seed vigour (Simic *et al*., 2007) [10]. Seed ageing was a physiological phenomenon leading to an irreversible loss of viability during storage (Padma *et al.*, 2002) [9]. Loss of seed viability was also associated with decreased lipid content, lowering of protein and nucleic acid synthesis in addition to DNA damage (Devaiah *et al*., 2007) [4]. In the present study, the highest viability percentage was recorded in dormant variety treated with halo-polymer @ 3 g kg^{-1} of seed and stored under cold storage condition (Fig. 1). Up to 60 days of storage, the dormant variety had 100 % viability but germination was comparatively lower because of seed dormancy. It may be because of the period of dormancy that was extended up to 45 days. After 45 days it had reached 96 % of germination (Table 1). Cheema *et al*., (2010) [2] reported that low moisture content reduced respiration and deterioration can improve the stored seed quality. Seed is a hygroscopic material and the decline in seed viability will depend on high relative humidity and temperature of the environment in which the seeds are being stored. Whereas with course of time the decline in the viability of seeds, could be due to depletion of food reserve, increase in fat acidity, ultra structural changes, and reduced activity of enzymes and weakening of membrane integrity. These results are in line with the findings of Banovetz and Schiener (1994) [1] and Doijode (2004) [5].

Anatomical changes were observed in dormant and non-dormant seeds (Plate 1a and 1b). Process of cell degradation was found to be earlier in its onset and rapid in seeds of non-dormant genotype. This may be due to the maintenance of turgidity of cell wall. Whereas the untreated non-dormant seeds expressed shrunken parenchymatic cells in embryo and also exhibited significant difference in cell rigidity upon treatment and storage. This may be due to the fact that in dormant seeds the rate of cell membrane degradation is lesser than non-dormant seeds. The observations are in line with Yasseen Mohamed - Yasseen (1993) [13] who reported cell damage in onion to the present study seeds during ageing.

Results of this study clearly demonstrated that groundnut kernels deteriorate faster under ambient storage conditions and such processes are slower in dormant varieties compared to non-dormant varieties. Further, the seed quality can be maintained by treating the kernels with halo-polymer that quenches the reactive oxygen species which are generated during the seed deterioration. Initially, all the treated and untreated seeds had 100 % viability for both the varieties and storage conditions. At the end of the storage period, seeds of dormant genotype VRI 7 treated with halo-polymer and cold storage conditions retained 85 % of viability whereas the non-dormant had the least viability of 67 % (Table 1).

Cortelazzo *et al*., (2005) [3] reported that during seed ageing, cellular degradation began with loss of cell content. Based on morphological analysis of french bean seeds, they observed decreased size of starch grains in eight days accelerated aged seeds compared to freshly harvested seed. Cellular alterations and viability loss of the embryos during storage was studied by Jose *et al.,* (2006) [7]. They observed disappearance of starch granules, cell wall folding and cytoplasm fragmentation in stored seeds of *Inga vera.* Vasudevan *et al.,* (2012) [12] confirmed through ultra-structure examination that membranes undergo deteriorative changes with increasing seed age.

Scanning Electron Microscopic examination was undertaken in fresh and six months old dormant and non-dormant kernels of groundnut to determine the changes in seed coat, cotyledon and embryonic axis. In the present study, initially both dormant and non-dormant varieties had shown similar morphological features. After six months of storage, in non-dormant variety, cells in embryo was bulged compare to dormant variety (Fig. 2). At plumule region, non-dormant variety had enlargement of cells. At the end of storage period, the cells are damaged, parenchymatic cells are destruction (Fig. 3) and in cotyledon region shrinkage of cells were observed in non-dormant variety stored in ambient storage condition than dormant variety. The cell wall highly damaged in non-dormant variety than dormant at the end of storage period.

Results of this study revealed that dormant groundnut genotype retained better seed quality attributes during storage up to six months while same parameters showed decline in the non-dormant variety. Among the treatments, halo-polymer treatment enabled seeds to retain better seed quality attributes. Irrespective of the dormancy status and seed treatments, cold storage was found to be helpful in maintaining seed quality for a longer period.

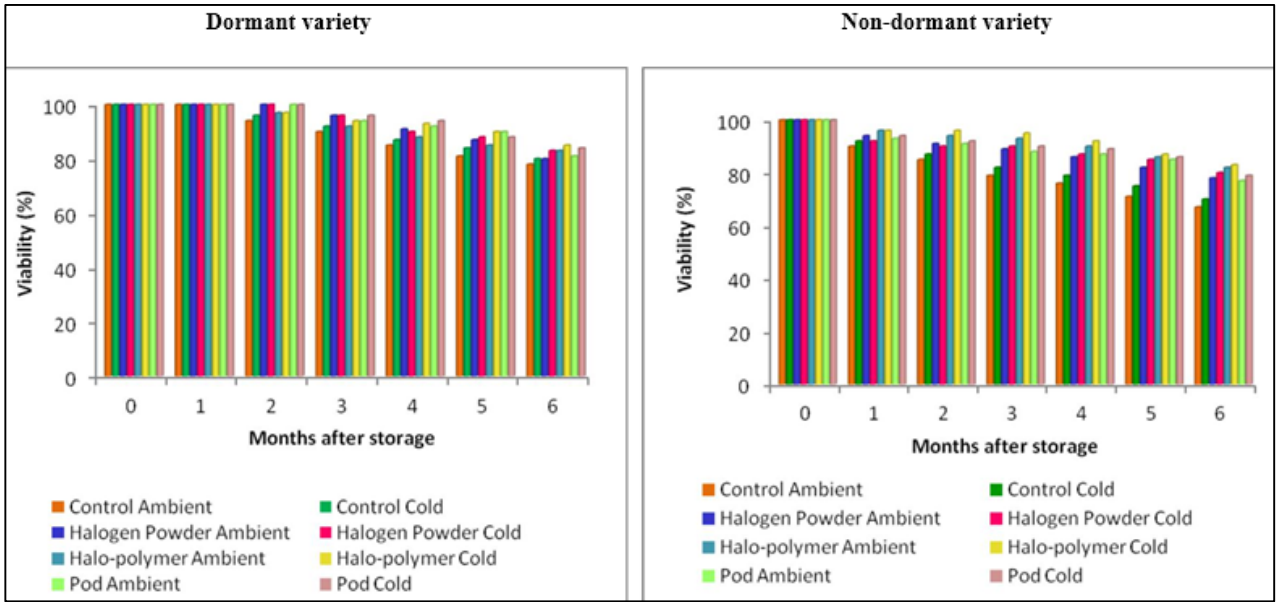

Fig 1: Effect of seed dormancy, halogenation and storage conditions on viability (%) of groundnut seeds

Table 1: Effect of seed dormancy, halogenation and storage condition on viability (%) of groundnut seeds

Varieties	Treatments	Ambient storage condition							Cold storage condition						
		Period of storage(in months)													
		P0	P1	P2	P3	P4	P5	P6	P0	P1	P2	P3	P4	P5	P6
Dormant	T1	100	100	94	90	85	81	78	100	100	96	92	87	84	80
	T2	100	100	100	96	91	87	80	100	100	100	96	90	88	83
	T3	100	100	97	92	88	85	83	100	100	97	94	93	90	85
	T4	100	100	100	94	92	90	81	100	100	100	96	94	88	84
Non-dormant (CO 7)	T1	100	90	85	79	76	71	67	100	92	87	82	79	75	70
	T2	100	94	91	89	86	82	78	100	92	90	90	87	85	80
	T3	100	96	94	93	90	86	82	100	96	96	95	92	87	83
	T4	100	93	91	88	87	85	77	100	94	92	90	89	86	79

Not statistically analyzed Legends

T1 - Control; T2 - Halogen (Iodine) impregnated powder at 3g kg^{-1}; T3 - Halopolymer at 3g kg^{-1}; T4 - Pod without treatment (control)

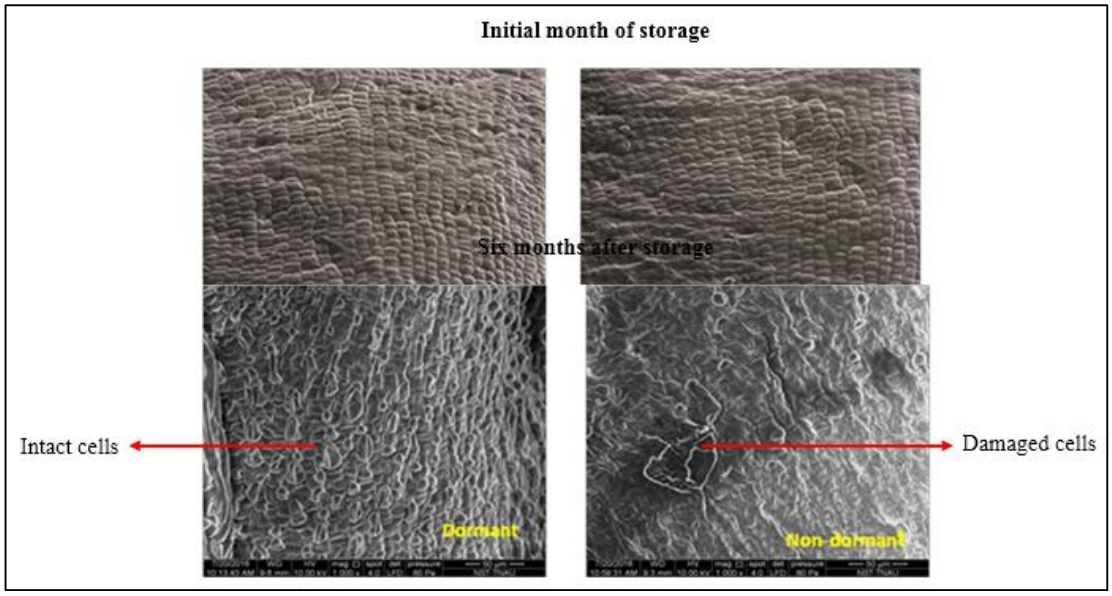

Fig 2: Scanning Electron Microscopic analysis of embryonic axis in dormant (VRI 7) and non-dormant

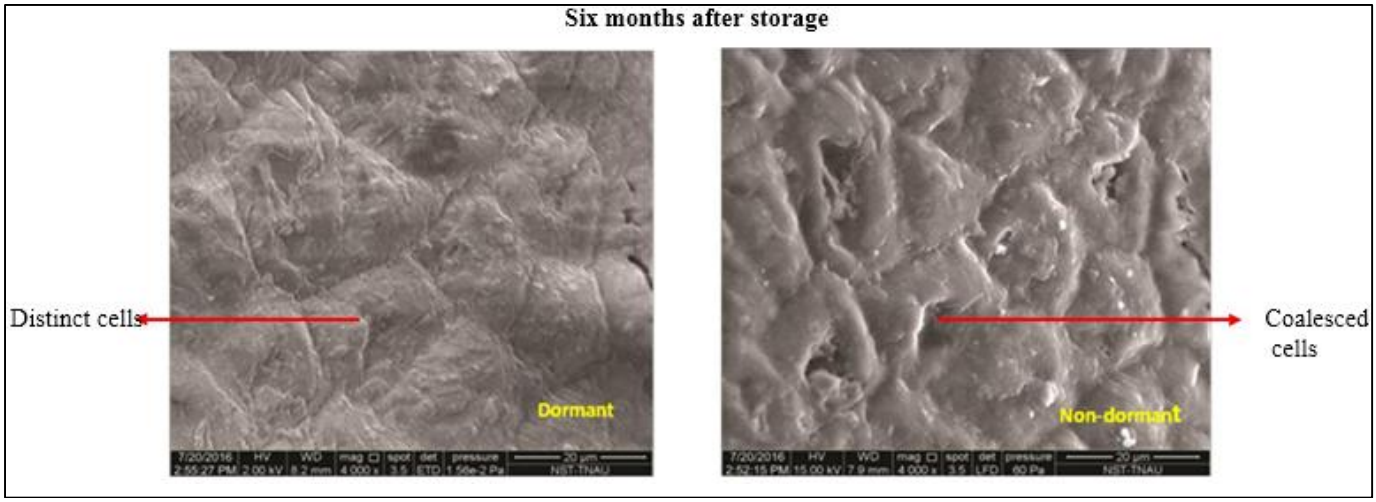

Fig 3: Scanning Electron Microscope imaging of parenchyma tic cells in dormant (VRI 7) and non-dormant (CO 7) groundnut kernels

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