DOI : <http://doi.org/10.22438/jeb/40/2/MRN-753>

Correlation among physiological and histological changes in soybean seeds during storage

Paper received: 18.09.2017

Revised received: 22.01.2018

Re-revised received: 07.06.2018

Accepted: 18.07.2018

Authors Info

H.P. Vijayakumar^{1*},
A. Vijayakumar², P. Srimathi²,
G. Somasundaram¹, S.R. Prasad¹,
S. Natarajan¹, K. Raja²,
R. Dhandapani¹ and
K. Vishwanath³

¹ICAR- Indian Institute of Seed
Science, Mau-275 103, India

²Tamil Nadu Agricultural University,
Coimbatore-641 003, India

³University of Agricultural Sciences,
GKVK, Bengaluru-560 065, India

*Corresponding Author Email :
vijayhpm@yahoo.com

Edited by

Professor Prakash Geetha

Reviewed by

Dr. Satheesh Naik
Dr. J. Alice R. P. Sujeetha

Abstract

Aim: The aim of the study was to correlate the histological changes in cotyledon and embryonic axis in aged seeds of soybean genotypes with the physiological changes and storability.

Methodology: Ten genotypes with differing storability as predicted by accelerated ageing test, were selected and various physiological parameters viz., germination per cent, seedling length and dry weight, seedling vigour, mobilization efficiency were studied over a period of ten months at bi-monthly interval. These were correlated with histological changes like cell arrangement and cell membrane integrity in cotyledon and embryonic axis of seed. The findings were further supported by electrical conductivity test.

Results: Good storer/performer (DSB 21) maintained comparatively better cell membrane integrity in both cotyledon and embryonic axis at the end of ten months of storage when compared to poor storer/performer (MAUS 61).

Interpretation: Maintenance of cell membrane integrity in good storer genotype, as revealed by scanning electron microscopy, as result of better tolerance mechanism for biochemical degradation, is the main reason for higher physiological performance of all the good storer/performers genotypes during storage.

Key words : Cell membrane, Embryonic axis, Histological changes, Soybean seeds, Storability

Selection of
genotypes
based on
prediction by
accelerated
ageing test

Initial seed
germination

Initial cell membrane
integrity in cotyledon
as seen in SEM
image

Germination
after 10 months
storage

Cell membrane
integrity in cotyledon
as seen in SEM
image after 10
months storage

Good Storer

Poor Storer

How to cite : Vijayakumar, H.P., A. Vijayakumar, P. Srimathi, G. Somasundaram, S.R. Prasad, S. Natarajan, K. Raja, R. Dhandapani and K. Vishwanath: Correlation among physiological and histological changes in soybean seeds during storage. *J. Environ. Biol.*, **40**, 217-225 (2019).

Introduction

In the tropical and subtropical countries, storage of soybean seed has become a big challenge to the seed industry as seed loses viability below minimum seed certification standard (70%) before the next growing season due to higher temperature. Deteriorated seeds in field produces uneven stands, spotty fields and fewer plants per hectare, there by, resulting in lower productivity (Kapoor *et al.*, 2010; Biabani *et al.*, 2011). Annual losses due to deterioration could be as much as 25 per cent of the harvested crop.

Storability of seeds is a genetically regulated character and is influenced by initial quality, pre-storage history, moisture content, relative humidity and temperature of storage environment; duration of storage and biotic agents (Khatun *et al.*, 2009; Biabani *et al.*, 2011). The storage life of seeds vary from species to species (Harrington, 1972) and even with in varieties of species (Basu *et al.*, 2004). The mechanism of ageing that differ with varieties of same species is still not clear. The changes associated with ageing are many but differ among genotypes (Jain *et al.*, 2006). Many processes associated with seed ageing during storage appears to be free-radical mediated, and lipid peroxidation is suggested to be a primary cause of deterioration in stored seeds (McDonald, 1999). The damage caused by lipid peroxidation can be visualized through scanning electronic microscope by studying the histological changes occurring during ageing and, thereby, help in identifying changes that are responsible for poor storability. In view of the above, a study was conducted to correlate physiological changes with histological changes in seeds of soybean genotypes during storage.

Materials and Methods

Seeds of 30 soybean genotypes were procured from different sources UAS, Dharwad; IARI, New Delhi; ICAR-IISR, Indore and Department of Pulses, TNAU, Coimbatore and they were multiplied under isoclimatic conditions at ARS, Bhavanisagar of TNAU, Coimbatore. Seeds were shade dried after harvesting to about 8.5% moisture content. These 30 genotypes were screened through accelerated ageing test for physiological parameters and identified five genotypes viz., Kalituri, EC 18761, MACS 1416, CO1 and DSB21 as good storers/performers and five genotypes viz., JS 71-05, DS 228, MAUS 61, NRC 93 and DSB 24 as poor storers/performer, based on germination per cent after accelerated ageing. Seeds of selected ten genotypes were stored in cloth bags under ambient conditions of Coimbatore for a period of ten months and they were evaluated for following physiological parameters at bi-monthly intervals. Seed moisture content was estimated as per ISTA (2007). Seed germination test was carried out using four replicates of 100 seeds in paper medium (ISTA, 2007) under laboratory conditions of $25 \pm 2^\circ\text{C}$ temperature and 95 ± 3 per cent

relative humidity. The number of normal seedlings was counted at the end of 8th day and the means were expressed as germination per cent. On final count, ten normal seedlings were selected from the germination test at random in each replication. The seedling length was measured from base of primary leaf to tip of the primary root and the mean was calculated and expressed in centimeters. The seedlings used for growth measurement were taken from each replications and after removing their cotyledons and seed coat, they were dried in hot air oven maintained at $80 \pm 2^\circ\text{C}$ for 24 hrs. Later, they were cooled in desiccator filled with silica gel for 30 min. The dry matter of seedlings was weighed using an electronic balance and the mean was expressed as mg per seedling.

Seedling vigour index I and vigour index II values were computed using the formula as suggested by Abdul-Baki and Anderson (1973) and Reddy and Khan (2001). For estimating mobilization efficiency, three replicates of 25 seeds from each genotype were weighed individually and germinated in complete darkness. After seven days of germination, the cotyledons and seedling were separated, dried for 24 hrs at $80 \pm 2^\circ\text{C}$, cooled in a desiccator for half an hour and weighed. The reduction in dry weight of cotyledons and increase in dry weight of embryonic axes during germination period was estimated, and mobilization efficiency was calculated by the formula given by Srivastava and Sareen (1974).

For electrical conductivity test, four replicates of twenty five seeds were taken from each genotype and they were cleaned and weighed. The seeds were then soaked in pre-cooled 50 ml of distilled water for 9 hrs at 20°C temperature. After soaking duration, the beaker was swirled for 10 to 15 sec and electrical conductivity of seed leachates was measured by immersing dip-type cell of a digital conductivity meter having a cell constant of one (Hampton and TeKrony, 1995).

Scanning Electron Microscope FEI QUANTA 250 was used to study the histological changes in fresh and naturally aged seeds of one good storer (DSB 21) and one poor storer genotype (MAUS 61). Fresh seeds and 10 months stored seeds were preconditioned for 3 hrs in between moist germination paper. Then embryonic axis and cotyledon were separated and dehydrated slowly by soaking in 25, 50, 70-75, 90-95% ethanol for 20 min at each concentration followed by soaking in 100% ethanol for 30 min (repeated twice) at room conditions and observed for cell arrangement pattern of embryonic axis and cotyledon.

Data obtained from experiment was analyzed statistically by adopting techniques described by Panse and Sukhatme (1985). Wherever necessary, the percent values were transformed to arc-sine values before analysis. The critical differences (CD) were calculated at 5 and 1% probability level.

Results and Discussion

Seed moisture content was significantly influenced by storage period and increased to 9.11% by the end of ten months of storage from initial level of 8.5% (Table 1) due to absorption of moisture and attainment of equilibrium with atmospheric moisture as seeds are hygroscopic in nature (Shelar *et al.*, 2008). However, there was no significant difference among the genotypes with respect to seed moisture content.

With respect to physiological seed quality, significant decrease in seed germination was observed in all the genotypes but the reduction was slow in Kalitur, which maintained 87% germination after ten months of storage and was on par with MACS 1416 (86%) (Table 1). In general, good performers screened from the accelerated ageing test maintained higher seed germination after 10 months of storage, where reduction was only 12% from initial germination. Genotypes classified as poor storers/performers showed drastic reduction of 55% from the initial which showed better reliability of accelerated ageing test in assessing seed storability. Similarly, Havstad *et al.* (2011) recommended accelerated ageing test for 24 hrs at 43-45°C as appropriate test to assess seed vigour and storage potential of red clover, while Eksi and Demir (2011) demonstrated that controlled deterioration test of 5 days could identify differences in seed lot vigour and longevity in onion.

Lima *et al.* (2010) reported that seed deterioration in soybean genotypes was more influenced by the linolenic acid content than presence of lipoxygenase. The seed quality of genotype containing lipoxygenase with normal linolenic acid content was found to be worst. Hosamani *et al.* (2013) reported that black-seeded landraces of soybean such as T-49, Kalitur and Birs-1 had better storability than yellow-seeded varieties. However, in the present study, yellow seeded genotypes also showed wider variability in seed quality characters, wherein EC 18761, MACS 1416, CO1 and DSB 21 maintained higher seed viability during storage as compared to other yellow seeded soybean genotypes such as MAUS 61, NRC 93, DS 228, JS 71-05 and DSB 24.

The genotypes which performed physiologically better in accelerated ageing test also maintained higher seedling length, dry matter production and seedling vigour over the storage period compared to poor performing genotypes. Mean seedling length significantly reduced after 4 months of seed storage and decreased to 29.6 cm after ten months of storage as compared to fresh seed (39.1 cm). Kalitur was found to produce longest seedlings (38.5 cm), while MAUS 61 produced shortest seedlings of 18 cm only (Table 2). The reduced seedling growth could be attributed to generation of free radicals during lipid peroxidation which damages cell organelles like mitochondria and leads to reduced mitochondria in cells and results in lower respiration and

Table 1 : Seed moisture content (%) and germination (%) as influenced by genotypes and natural ageing period in soybean

Genotypes (G)	Seed moisture content					Mean	Germination Per cent					Mean		
	Storage Period (Months) (P)						Storage Period (Months) (P)							
	Initial	2	4	6	8		10	Initial	2	4	6		8	10
Good performers in accelerated ageing test														
MACS 1416	8.49	8.42	8.9	8.71	8.75	9.1	8.73	98(84.2)	98(84.2)	96(80.0)	95(78.8)	94(77.9)	86(68.2)	95(78.9)
CO 1	8.46	8.38	9.04	8.89	8.92	9.15	8.81	93(76.9)	92(74.1)	88(70.0)	86(68.1)	83(65.6)	80(63.4)	87(69.7)
EC 18761	8.5	8.45	9.04	8.92	8.93	9.17	8.83	99(87.1)	96(80.4)	92(73.6)	89(70.7)	85(67.2)	83(65.6)	91(74.1)
DSB 21	8.57	8.5	9.05	8.89	8.9	9.18	8.85	89(70.7)	87(68.9)	84(66.4)	82(64.9)	79(62.7)	78(62.0)	83(65.9)
Kalitur	8.52	8.49	8.93	8.78	8.85	9.09	8.77	94(77.7)	94(77.9)	91(72.6)	90(71.6)	88(69.8)	87(69.1)	91(73.1)
Poor performers in accelerated ageing test														
MAUS 61	8.44	8.37	9.05	8.94	8.95	9.13	8.81	95(79.1)	90(74.1)	84(66.5)	75(60.8)	34(35.6)	5(8.7)	64(54.1)
DSB 24	8.55	8.51	8.82	8.73	8.75	8.98	8.72	89(71.2)	88(69.8)	82(64.9)	76(60.7)	71(57.4)	60(50.7)	78(62.5)
NRC 93	8.52	8.48	8.82	8.76	8.78	9.03	8.73	87(69.4)	85(67.2)	78(62.0)	69(56.1)	62(51.9)	25(29.9)	68(56.1)
DS 228	8.46	8.4	8.99	8.79	8.83	9.15	8.77	93(75.2)	87(69.0)	81(64.1)	75(60.0)	69(56.1)	58(49.6)	77(62.3)
JS 71-05	8.48	8.41	9.01	8.85	8.9	9.17	8.8	90(71.8)	85(67.2)	79(62.7)	72(58.0)	67(54.9)	54(47.2)	75(60.3)
Mean	8.5	8.44	8.96	8.83	8.86	9.11		93(76.3)	90(73.3)	86(68.3)	81(65.0)	73(59.9)	62(51.5)	
Source of variation		SEd		CD (P=0.05)		CD (P=0.01)			SEd	CD (P=0.05)		CD (P=0.01)		
Genotype (G)		0.04		NS		NS			1.4	2.76		3.64		
Storage period (P)		0.03		0.07		0.09			1.08	2.14		2.82		
G x P		0.11		NS		NS			3.43	6.76		8.93		

Values in parenthesis are arc sine transformed values

energy metabolism (McDonald, 1999). All the genotypes showed significant decline in dry matter production, but MACS 1416 produced the highest dry matter (39.9 mg) even after ten months of seed storage, which was on par with Kalitur (39.6 mg), DSB 21 (39.5 mg), DSB 24 (38.4 mg) EC 18761 (35.9 mg) and DS 228 (34.9 mg). The lowest dry matter production was recorded in MAUS 61 (15.5 mg) (Table 2). Kapoor *et al.* (2010) observed significant decrease in all the physiological parameters in all the five chickpea varieties upon ageing and also found a differential response among varieties in tolerating ageing conditions.

There was a significant decrease of SVI I and SVI II in all the genotypes, however reduction was slow in Kalitur which maintained highest SVI I and SVI II (3351 and 3445) after a storage period of 10 months. MAUS 61 showed maximum decrease and recorded lowest SVI I and SVI II (90 and 78) (Table 3). There was only 27 and 26% reduction in SVI I and SVI II in good storers/performers after ten months of storage period but poor performing genotypes showed drastic reduction of 67 and 66% in SVI I and SVI II, respectively. Anuja and Aneja (2004) reported that soybean genotypes showed variability with respect to changes in the level of dehydrogenase, catalase, peroxidase, amylase, phosphatase and glutamic acid decarboxylase, which are associated with seed viability during storage. Such genotypic differences in capability to resist biochemical degradation resulted in variability in seedling vigour among genotypes.

Mobilization efficiency is the capacity of seed to mobilize the nutrients to the growing embryo. Mean mobilization efficiency

significantly declined after each storage period and finally reached 39.5% on 10th month of storage as compared to fresh seeds (68.9%). The reactive oxygen species generated during ageing cause damage to enzymes that are essential to convert reserve food in the embryo to usable form and finally production of normal seedling (Iqbal *et al.*, 2002). Although mobilization efficiency decreased over the storage period in all the genotypes, the extent of reduction at ten months of storage varied significantly among the genotypes. Among the genotypes, good storers/performers maintained higher mobilization efficiency recording a reduction percentage of 22 over 10 months' period, while the poor storers/performers showed 64% reduction. MACS 1416 maintained highest mobilization efficiency (58.8%) which was followed by Kalitur (57.3%), while MAUS 61 could maintain only 2.1% (Table 4).

The genotypic variation in seed viability and seedling vigour of aged seeds was also observed by Basu *et al.* (2004) in maize; Zhang *et al.* (2006) in rapeseed and mustard and Kannababu *et al.* (2016) in sweet sorghum. Good storers/performers have better tolerant mechanisms for biochemical degradation which further manifested in better mobilization efficiency and physiological seed quality during seed storage when compared to poor performing genotypes.

Electrical conductivity (EC) of seed leachate *i.e.*, leaching of sugars and leaching of amino acids were employed for evaluating the membrane integrity and correlated with seed vigour and viability (Mavi *et al.*, 2014). In the present study, mean

Table 2 : Seedling length and dry matter production as influenced by genotypes and natural ageing period in soybean

Genotypes (G)	Seedling length (cm)						Mean	Dry matter production (mg per seedling)						Mean	
	Storage period (months)							Storage period (months)							
	Initial	2	4	6	8	10		Initial	2	4	6	8	10		
Good performers in accelerated ageing test															
MACS 1416	36.2	35.8	35.1	33.2	31.7	30.9	33.8	47	46.5	46	45.7	43.8	39.9	44.8	
CO 1	41.2	39.5	38.8	37.7	36.7	33.9	38	40	41	40.5	38.8	36.9	34.1	38.5	
EC 18761	39	39.4	38	36.9	34.8	32.4	36.8	43	43.4	42.1	41	39.8	35.9	40.9	
DSB 21	38.7	38.6	37.4	35.8	34.5	32	36.2	47.1	45.2	45	44.7	42.9	39.5	44.1	
Kalitur	45	44.7	43.8	42.6	40.8	38.5	42.6	46.9	47.5	45.6	43.1	42.6	39.6	44.2	
Poor performers in accelerated ageing test															
MAUS 61	42.2	41.8	39.3	36.9	24.9	18	33.8	47.2	47.5	47.5	43.5	34.4	15.5	39.1	
DSB 24	41.7	40.5	39.2	37.4	35.5	32.5	37.8	47.3	48.3	48.3	45.2	41.6	38.4	44.7	
NRC 93	39.6	38.5	37.5	36.1	32.2	27.3	35.2	49.7	46.2	46.2	44.9	37.8	30.3	42.4	
DS 228	34.5	33.7	32.3	30.5	28.8	25.9	31	45.6	43.8	43.8	38.4	35.5	34.9	40	
JS 71-05	33.2	32.2	31.4	29.7	28.3	24.7	29.9	42.6	43	43	37	33.3	32.3	38.3	
Mean	39.1	38.5	37.3	35.7	32.8	29.6		45.6	45.2	44.2	42.2	38.9	34.1		
Source of variation	SEd	CD (P=0.05)			CD (P=0.01)				SED	CD (P=0.05)			CD (P=0.01)		
Genotype (G)	0.67	1.32			1.75				1.21	2.38			3.15		
Storage period (P)	0.52	1.02			1.35				0.93	1.85			2.44		
G x P	1.64	3.24			4.28				2.96	5.85			7.71		

Table 3: Seedling vigour index I and II as influenced by genotypes and natural ageing period in soybean

Genotypes (G)	Seedling vigour index I							Seedling vigour index II							Mean
	Storage period (months)						Mean	Storage period (months)							
	Initial	2	4	6	8	10		Initial	2	4	6	8	10		
Good performers in accelerated ageing test															
MACS 1416	3552	3510	3368	3155	2978	2654	3203	4608	4556	4418	4337	4119	3435	4246	
CO 1	3827	3633	3416	3246	3042	2711	3313	3720	3772	3564	3339	3061	2727	3364	
EC 18761	3864	3783	3491	3287	2959	2692	3346	4257	4165	3872	3649	3380	2979	3717	
DSB 21	3443	3360	3141	2934	2727	2497	3017	4187	3935	3780	3664	3392	3083	3674	
Kalitur	4232	4201	3983	3833	3593	3351	3865	4413	4465	4150	3877	3753	3445	4017	
Poor performers in accelerated ageing test															
MAUS 61	4009	3759	3301	2769	847	90	2463	4479	4277	3901	3263	1168	78	2861	
DSB 24	3713	3564	3212	2842	2520	1947	2966	4207	4248	3879	3433	2951	2305	3504	
NRC 93	3447	3271	2922	2491	1998	681	2468	4322	3928	3568	3095	2345	757	3002	
DS 228	3209	2935	2619	2284	1989	1503	2423	4236	3807	3389	2878	2449	2026	3131	
JS 71-05	2991	2735	2478	2135	1899	1333	2262	3830	3657	3287	2665	2232	1747	2903	
Mean	3629	3475	3193	2898	2455	1946		4226	4081	3781	3420	2885	2258		
Source of variation	SED	CD (P=0.05)			CD (P=0.01)			SED	CD (P=0.05)			CD (P=0.01)			
Genotype (G)	59.3	117			154.4			101.3	199.9			263.8			
Storage period (P)	45.9	90.7			119.6			78.4	154.8			204.3			
G x P	145.3	286.8			378.4			248.2	489.8			646.2			

Table 4 : Mobilisation efficiency and electrical conductivity as influenced by genotypes and natural ageing period in soybean

Genotypes (G)	Mobilisation efficiency (%)							EC of seed leachate ($\mu\text{S cm}^{-1} \text{g}^{-1} \text{seed}$)							Mean
	Storage period (months)						Mean	Storage period (months)							
	Initial	2	4	6	8	10		Initial	2	4	6	8	10		
Good performers in accelerated ageing test															
MACS 1416	73	71.5	69.5	66.1	63.6	58.8	67.1	82	85.6	90.4	98.4	110.9	121.3	98.1	
CO 1	68.1	66.4	64.2	60	57.6	51	61.2	85.7	93	100	108.6	115.2	126.6	104.8	
EC 18761	71.5	69	65.7	60.4	58.1	53.2	62.9	84.7	91.9	101.4	109.8	118.6	127.4	105.6	
DSB 21	67.2	65.4	61.1	59	55.9	52.4	60.2	80.4	90	96.1	105.9	113.3	126.9	102.1	
Kalitur	70.2	67.8	65.3	62.4	59.9	57.3	63.8	61.7	63.9	65.9	68	70.1	75	67.4	
Poor performers in accelerated ageing test															
MAUS 61	69	66	59	50.9	23	2.1	45	95.5	108.8	120.4	132.8	179.3	231.6	144.7	
DSB 24	67.3	65	60.5	52	45.7	36.7	54.5	92.5	105.6	115.5	124	140.2	160.8	123.1	
NRC 93	66.8	63.9	57.7	46.9	38.9	17.8	48.7	93.4	107.7	118.8	129.9	151.1	190.3	131.9	
DS 228	68.6	65.6	60.6	50.6	42.5	34.5	53.7	89.6	102.8	110.3	125.9	142.9	161.4	122.1	
JS 71-05	67.1	63.6	59	48.9	40.2	31.4	51.7	87.2	105.3	112.5	127.2	145.5	164.1	123.6	
Mean	68.9	66.4	62.3	55.7	48.5	39.5		85.3	95.4	103.1	113.1	128.7	148.5		
Source of variation	SED	CD (P=0.05)			CD (P=0.01)			SEd	CD (P=0.05)			CD (P=0.01)			
Genotype (G)	1.18	2.34			3.1			1.81	3.6			4.76			
Storage period (P)	0.91	1.81			2.4			1.4	2.78			3.68			
G x P	2.9	5.75			7.6			4.45	8.82			11.66			

EC increased with every storage period and recorded maximum value ($148.5 \mu\text{S cm}^{-1}$) at the end of 10 months from $85.3 \mu\text{S cm}^{-1}$ (initial) (Table 4). Lipid peroxidation of membranes may lead to decline in membrane integrity that would increase the membrane

permeability and electrical conductivity (Ratajczak and Pukacka, 2005). Kalitur showed the least increase in electrical conductivity over the ten months storage period (22%), whereas MAUS 61 expressed drastic rise of 142%. All the good storer/performer

Fig. 1 : Histological changes in cotyledon of fresh and aged seeds of soybean genotypes: (A) Fresh seeds of DSB21; (B) Fresh seeds of MAUS61; (C) Aged seeds of DSB21 and (D) Aged seeds of MAUS61. Arrows indicate damage of cell membranes and lack of clear demarcation of cells in cotyledons of aged seeds of MAUS61.

genotypes maintained lower EC over the storage period compared to poor storer/performer genotypes.

Khan *et al.* (2004) reported that onion seeds exhibited very short life, and lost their germinability, vigour and viability due to loss of membrane integrity which leads to decreased vigour and ultimately viability of seeds. Demir *et al.* (2012) also reported a negative correlation of electrical conductivity with percentage of normal seedlings in radish seed lots. Similarly, Mavi *et al.* (2014) reported that electrical conductivity of seed soaked water after 17

and 24 hrs of nine commercially acceptable seed lots (79-100% standard germination) of salad radish cv. Findik were negatively correlated with field emergence.

Scanning electron microscopic observations revealed that in fresh seeds of good storer (DSB 21), the cells in cotyledon were large, turgid and there was a clear demarcation of cells, however, in poor storer (MAUS 61) the cells were comparatively small and less turgid. Even after ten months storage, the integrity of cell membrane was maintained in DSB 21 and the extent of cell

Fig. 2 : Histological changes in plumule of fresh and aged seeds of soybean genotypes: (A) Fresh Seeds of DSB21; (B) Fresh Seeds of MAUS61; (C) Aged Seeds of DSB21 and (D) Aged Seeds of MAUS61. Arrow symbol indicates collapse of cells and damage of cell membranes in plumule of aged seeds of MAUS61.

damage was less and turgidity of cells was maintained as that of fresh seed when compared to MAUS 61 wherein there was severe collapse of cells and the damage of cell membrane could be distinguishably visualised (Fig. 1). In plumule and radicle of fresh seeds of both the genotypes, the cells were compact and well arranged with clear demarcation and with intact membrane. After ten months of storage, the proportion of collapsed cells was more in MAUS 61 with membrane damage and also the cells were not expanded (Fig. 2, 3), which is the first sign of germination. It

clearly indicates that cell elongation stage was affected in MAUS 61 due to dead cells at the root tip, whereas in DSB 21 cells were triggered and elongated.

Higher lipid peroxidation in MAUS 61 might have led to more generation of reactive oxygen species and further damage of cell membrane resulting in collapse and death of cells in cotyledon (Fig. 1) and embryonic axis (Plate 2, 3). Whereas, in DSB 21, cells exhibited better tolerance mechanisms for deterioration which

Fig. 3 : Histological changes in radicle of fresh and aged seeds of soybean genotypes: (A) Fresh seeds of DSB21; (B) Fresh seeds of MAUS61; (C) Aged seeds of DSB21 and (D) Aged seeds of MAUS61. Arrows indicate collapsed cells and membrane damages and no cell elongation in radicle of aged seeds of MAUS61.

leads to higher physiological quality indicating good correlation among histological changes and physiological quality. Kavitha *et al.* (2017) reported that cells of embryonic axis were clearly visible and found intact in fresh seeds while it was collapsed in both accelerated and naturally aged seeds of black gram. The damage was severe when ageing period was extended.

The present study confirms that poor storer genotypes of soybean maintained lower physiological seed quality during

storage due to histological changes like disintegration of cell membranes of cotyledon and embryonic axis as they are less tolerant to biochemical changes.

Acknowledgments

Authors are thankful to the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore for providing SEM facility. We also thank Prof. Sundareswaran

(Seed Technology) for helping in seed multiplication of soybean genotypes at ARS, Bhavanisagar.

References

- Abdul-Baki, A.A. and J.D. Anderson: Vigour determination of soybean seed by multiple criteria. *Crop Sci.*, **13**, 630-633 (1973).
- Anonymous: International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland (2007).
- Anuja, G. and K.R. Aneja: Seed deterioration in soybean varieties during storage in physiological attributes. *Seed Res.*, **32**, 26-32 (2004).
- Basu, S., S.P. Sharma and M. Dadlani: Storability studies on maize (*Zea mays* L.) parental line seeds under natural and accelerated ageing conditions. *Seed Sci. Technol.*, **32**, 239-245 (2004).
- Biabani, A., L.C. Boggs, M. Katozi and H. Sabouri: Effects of seed deterioration and inoculation with *Mesorhizobium cicerion* on yield and plant performance of chickpea. *Aust. J. Crop Sci.*, **5**, 66-70 (2011).
- Demir, I., C. Cebeci and T. Guloksuz: Electrical conductivity measurement to predict germination of commercially available radish seed lots. *Seed Sci. Technol.*, **40**, 229-237 (2012).
- Eksi, C. and I. Demir: The use of a shortened controlled deterioration vigour test in predicting field emergence and longevity of onion seed lots. *Seed Sci. Technol.*, **39**, 190-198 (2011).
- Hampton, J.G. and D.M. TeKrony: Handbook of Vigour Test Methods. International Seed Testing Association, Switzerland (1995).
- Harrington, J.F.: Seed storage and longevity. In: Seed Biology (Ed.: T.T. Kozlowski). Academic Press, New York, pp. 145-245 (1972).
- Havstad, T., S. Aamlid and A. Lomholt: Evaluation of vigour tests for determination of seed storage potential in red clover (*Trifolium pratensis* L.) and timothy (*Phleum pratense* L.). *Seed Sci. Technol.*, **39**, 637-648 (2011).
- Hosamani, J., M. Dadlani, I.M. Santha, M.B. Arun Kumar and S.R. Jacob: Biochemical phenotyping of soybean [*Glycine max* (L.) Merrill] genotypes to establish the role of lipid peroxidation and antioxidant enzymes in seed longevity. *Agr. Res.*, **2**, 119-126 (2013).
- Iqbal, N., A. Shahzad, M. Basra and K.U. Rehman: Evaluation of vigor and oil quality in cotton seed during accelerated aging. *Int. J. Agri. Biol.*, **4**, 318-322 (2002).
- Jain, N., R. Kooper and S. Saxena: Effect of accelerated ageing on seeds of radish (*Raphanus sativus* L.). *Asian J. Plant Sci.*, **5**, 461-464 (2006).
- Kannababu, N., S.S. Rao, B. Prabhakar, G. Shyamprasad, K. Srinivasa Babu, A. Dhandapani and J.V. Patil: Genetic variability for seed ageing and longevity among the advanced sweet sorghum genotypes and cultivars. *Sugar Tech.*, **18**, 100-104 (2016).
- Kapoor, N., A. Arya, M.A. Siddiqui, A. Amir and H. Kumar: Seed deterioration in chickpea (*Cicer arietinum* L.) under accelerated ageing. *Asian J. Pl. Sci.*, **9**, 158-162 (2010).
- Kavitha, S., C. Menaka and M. Ananthi: Comparative study of physical, physiological, biochemical and histological changes due to accelerated and natural ageing of blackgram seeds. *Int. J. Chem. Stud.*, **5**, 1969-1973 (2017).
- Khan, M.M., M.J. Iqbal, M. Abbas, H. Raza, R. Waseem and A. Ali: Loss of vigour and viability in aged onion (*Allium cepa* L.) seeds. *Int. J. Agri. Biol.*, **6**, 708-711 (2004).
- Khatun, A., G. Kabir and M.A.H. Bhuiyan: Effect of harvesting stages on the seed quality of lentil (*Lens culinaris* L.) during storage. *Bangladesh J. Agri. Res.*, **34**, 565-576 (2009).
- Lima, W.A.A., A. Borém, D.C.F.S. Dias, M.A. Moreira and L.A.S. Dias: Lipoxigenase and physiological quality of soybean seeds during storage. *Seed Sci. Technol.*, **38**, 767-771 (2010).
- Mavi, K., F. Mavi, I. Demir and S. Matthews: Electrical conductivity of seed soak water predicts seedling emergence and seed storage potential in commercial seed lots of radish. *Seed Sci. Technol.*, **42**, 76-86 (2014).
- McDonald, M.B.: Seed deterioration: Physiology, repair and assessment. *Seed Sci. Technol.*, **27**, 177-237 (1999).
- Panse, V. G. and P. V. Sukhatme: Statistical Methods for Agricultural Workers. ICAR publication, New Delhi, p. 359 (1985).
- Ratajczak, E. and S. Pukacka: 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 (2005).
- Reddy, Y.T.N. and M.M. Khan: Effect of osmopriming on germination, seedling growth and vigour of khirni (*Mimosa hexandra*) seeds. *Seed Res.*, **29**, 24-27 (2001).
- Shelar, V.R., R.S. Shaikh and A.S. Nikam: Soybean seed quality during storage: A review. *Agricultural Reviews*, **29**, 125-131 (2008).
- Srivastava, A.K. and K. Sareen: Physiology and biochemistry of deterioration in soybean seeds during storage. I. Mobilization efficiency and nitrogen metabolism. *Seed Res.*, **2**, 26-32 (1974).
- Zhang, X.K., G.T. Yang, L. Chen, J.M. Yin, Z.L. Tang and J.N. Li: Physiological differences between yellow-seeded and black-seeded rapeseed (*Brassica napus* L.) with different testa characteristics during artificial ageing. *Seed Sci. Technol.*, **34**, 373-381 (2006).