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Evaluating different artificial ageing techniques and variability in tolerance to ageing of seeds of *Brassica* spp. and *Eruca sativa*

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Abstract

The seeds of *B. juncea, B. napus, B. rapa* and *Eruca sativa*, a related genus of *Brassica* sp., were subjected to different ageing treatments including rapid ageing through immersion into hot water at 58±2°C, controlled deterioration by raising the seed moisture content to 15% at 40±2°C, and subjecting the seeds to higher relative humidity of 95% using saturated solution of Potassium nitrate at 40±2°C. Each of the accession was studied for physiological factors like germination percentage, mean germination time, and vigour index which declined with all the ageing treatments. Maximum decline was shown by accessions of *B. napus* and *E. sativa*. The accessions of *B. juncea* and *B. rapa* were found to be better performers as compared to the accessions of *B. napus* and *E. sativa*. The biochemical assays like electrical conductivity and lipid peroxidation, increased with the progress of deterioration. Decline in enzyme activity of peroxidase and catalases were observed, and these enzymes become non-functional when the seeds were dead. All the accessions showed a coherent response to ageing treatments irrespective of the species under study. Hot water ageing was a rapid method of ageing but controlled deterioration by equilibrating the seed moisture to a higher level (15%) and subjecting to elevated temperature of 40±2°C (controlled deterioration) was more reliable method of accelerated ageing technique for predicting the storability of seeds of *Brassica* and related genera.

***Key words**: Artificial ageing, Brassica spp., seed deterioration, Viability, Vigour index*

Introduction

Availability of genetic diversity in the gene pool of cultivated species is key for a planned genetic improvement and variety development programme. In *Brassica*, availability of genetic diversity has been one of the major constraints. This is because the sizable collection available in the country does not represent the total spectrum of variability, and that despite recognition of the importance of genetic diversity, it is being eroded because of poor conservation efforts. In case of cultivated plant species, where most of the genetic diversity is confined to the farmer's fields, *ex situ* conservation of plant genetic resources is the most common method for conservation. Appropriate conditions have to be identified to restrict the deterioration of seed during processing and to prolong the life of the seed in storage. Frequent fluctuation in temperature and relative humidity make the processing and storage of seeds difficult to minimize the loss of viability and change in genetic integrity of the seed, thereby conservation of genetic resources.

Exposure of seeds to high temperature and moisture conditions had been the commonly used method for ageing seeds in the laboratory. Such treatments to seeds, prior to germination may yield useful information concerning the loss of seed viability, vigour and longevity of viability (Delouche and Baskin, 1973; Powell and Matthews, 1977 and Tian *et al*., 2008) and may be used as a tool for predicting the relative seed-longevity. Artificially aged seeds are known to germinate and grow into seedlings in a normal manner comparable with

naturally aged seeds, making it possible to arrive at safe conclusions regarding the loss of seed viability and mechanism of seed deterioration under storage. Fabrizius *et al.* (1999) confirmed the possibility of predicting the actual germination of soybean seeds during natural ageing by applying accelerated ageing test since one of the main impediment for natural ageing being the time needed for natural ageing and degree of seed deterioration. In the present investigation, an effort was made to identify quick artificial ageing method for measuring and predicting loss of seed viability and vigour by assessing the physiological and biochemical parameters during ageing of seeds and to analyze the variability in seed storage capacity of various species of *Brassica* spp. and *Eruca sativa*.

Materials and Methods

Three accessions each of *B. juncea*, *B. napus* and *B. rapa*, and two accessions of *Eruca sativa* (a related genus of *Brassica*) were used in the study. The seeds were collected and procured from NBPGR, New Delhi, India. The accessions were *B. juncea* – IC (Indigenous Collection) number - 355331(V1), IC-241634(V2), IC-241632(V3), *B. napus* - IC-241650(V4), IC-355318(V5), IC-355321(V6), *B. rapa*.var.yellowsarson - IC-342764(V7), IC-241661(V8), IC-355302(V9), and *Eruca sativa*- RTM-314(V10) and TMLC-3(V11).

The seeds were subjected to various ageing treatments viz. hot water ageing, accelerated ageing using saturated solution of Potassium nitrate, and controlled deterioration. In hot water ageing, (Bhattacharya *et al*., 1985) the samples were subjected to hot water treatment at 58±2°C for different lengths of time (10, 20, 30, 40 and 50 minutes). At the end of each treatment, seeds were quickly removed from the bags and spread on blotting paper. After drying at room temperature, seeds were tested for laboratory vigour parameters and other biochemical assays. In accelerated ageing using potassium nitrate, the seed samples were kept over saturated solution of potassium nitrate (95% RH) in sealed containers at 40±2°C in an incubator. The seeds were removed after 8 days and assessed for various physiological and biochemical parameters. In Controlled deterioration (CD), the moisture content of all seed lots was raised to 15 percent by determining the amount of water to be added to the sample by using the formula given by Powell and Mathews (1981) as mentioned below:

$$WF = \frac{WI \times 100\text{-}IM}{100\text{-}FM}$$

Where,

WF	=	final weight of sample
WI	=	Initial weight of sample
IM	=	initial moisture content of sample
FM	=	final moisture content of sample

The seed lots were sealed in Aluminium foil packets and kept at 10^0C for 48 hours for moisture equilibration and were incubated at 40±2°C. The initial moisture content of the various seed lots was estimated by the low constant oven method (103±2°C for 17 hours). Every 24 hours a packet was drawn up to 5 days and various parameters were studied.

The seed viability was tested by plating 25 seeds in petriplates in two replications with wet filter paper and kept in germinator maintaining adequate humidity and temperature of 20±2^{0}C (ISTA, 1993). Seeds were considered to be germinated if 1 mm radicle had emerged. Vigour index was computed adopting method of Abdul-Baki and Anderson (1973) by using formula:

Vigour index = Root length + Shoot length (in cm) x Germination (%)

Speed of germination was estimated using formula

$$\text{Mean germination time (MGT)} = \frac{\sum nd}{\sum n}$$

n = number of seeds which germinate on day 'd'
d = number of days counted from the beginning of germination test

The seedling vigour was computed adopting method of Perry (1977). For this, the seeds were plated on germination paper and kept vertically on the template placed over water in a tray and covered with another tray to prevent entry of light.

Table1. Effect of different ageing techniques on germination percentage of various accessions of *Brassica* spp and *Eruca sativa*

Treatment Variety	Control T_0	HW_{10} T_1	HW_{20} T_2	HW_{30} T_3	HW_{40} T_4	HW_{50} T_5	KNO_3 T_6	CD_{24} T_7	CD_{48} T_8	CD_{72} T_9	CD_{96} T_{10}	CD_{120} T_{11}	Mean (Variety)
V_1	99.5	98.3	98.3	95.8	94.5	90.7	97.2	98.3	97.2	93.3	00	00	80.3
	(90.0)	(86.4)	(86.4)	(81.1)	(79.4)	(72.8)	(82.8)	(86.4)	(82.8)	(75.8)	(4.1)	(4.1)	(69.3)
V_2	99.5	98.3	95.8	93.3	90.7	85.3	84.0	97.2	82.7	82.7	00	00	75.8
	(90.0)	(86.4)	(81.1)	(75.8)	(72.8)	(68.4)	(66.9)	(82.8)	(66.0)	(65.8)	(4.1)	(4.1)	(63.4)
V_3	97.2	97.2	95.8	93.3	90.7	85.3	95.8	88.0	78.7	76.0	00	00	74.8
	(82.8)	(82.8)	(81.1)	(75.8)	(72.8)	(68.2)	(81.1)	(70.4)	(63.2)	(61.1)	(4.1)	(4.1)	(62.3)
V_4	94.7	93.3	85.3	86.7	79.3	77.3	65.3	95.8	92.0	49.3	00	00	68.3
	(77.5)	(75.8)	(68.2)	(69.1)	(63.4)	(62.1)	(54.2)	(81.1)	(74.5)	(44.9)	(4.1)	(4.1)	(56.6)
V_5	96.0	96.0	94.7	89.3	80.7	69.3	68.0	86.7	72.0	00	00	00	62.7
	(79.2)	(79.2)	(75.5)	(72.5)	(64.5)	(56.7)	(55.9)	(69.2)	(58.4)	(4.1)	(4.1)	(4.1)	(52.1)
V_6	93.3	90.6	88.0	90.7	90.7	69.3	80.0	94.7	81.0	61.3	00	00	70.0
	(76.2)	(72.8)	(70.4)	(72.8)	(72.8)	(56.7)	(64.1)	(77.5)	(65.4)	(51.9)	(4.1)	(4.1)	(57.4)
V_7	98.3	97.7	97.2	92.0	93.3	44.0	89.3	98.3	98.3	74.7	00	00	73.6
	(86.4)	(82.8)	(82.8)	(74.5)	(75.8)	(41.8)	(72.4)	(86.4)	(86.4)	(60.1)	(4.1)	(4.1)	(63.1)
V_8	99.5	97.2	97.2	97.2	85.3	57.3	92.0	98.3	98.3	62.7	00	00	75.8
	(90.0)	(82.8)	(82.8)	(82.8)	(68.4)	(49.5)	(74.5)	(86.4)	(86.4)	(52.7)	(4.1)	(4.1)	(63.7)
V_9	98.3	98.3	98.3	92.0	82.7	78.7	94.7	98.3	88.0	64.0	00	00	74.4
	(86.4)	(86.4)	(86.4)	(74.5)	(66.2)	(63.2)	(77.5)	(86.4)	(70.4)	(53.4)	(4.1)	(4.1)	(63.2)
V_{10}	92.0	88.0	83.3	83.3	77.3	52.0	74.7	78.7	72.0	28.3	00	00	60.8
	(74.5)	(70.4)	(66.5)	(66.5)	(62.1)	(46.4)	(60.2)	(62.9)	(58.5)	(32.1)	(4.1)	(4.1)	(50.7)
V_{11}	94.7	88.0	88.0	80.0	82.7	42.7	63.3	90.5	85.3	70.7	00	00	65.8
	(77.5)	(70.4)	(70.4)	(63.9)	(66.0)	(41.0)	(53.1)	(75.7)	(67.9)	(57.5)	(4.1)	(4.1)	(54.3)
Mean	96.6	94.8	92.9	90.3	86.2	68.4	82.2	93.2	86.0	60.3	00	00	
(Treatment)	(82.8)	(79.7)	(75.6)	(73.5)	(69.5)	(57.0)	(67.5)	(78.7)	(70.9)	(50.9)	(4.1)	(4.1)	
CD (P = 0.01)		Treatment		2.6									
		Variety		2.5									

Figures in parenthesis indicate transformed values

CD_{24} - Controlled deterioration for 24 hours
CD_{48} - Controlled deterioration for 48 hours
CD_{72} - Controlled deterioration for 72 hours
CD_{96} - Controlled deterioration for 96 hours
CD_{120} - Controlled deterioration for 120 hours

HW_{10}- Hot water ageing for 10 minutes
HW_{20}- Hot water ageing for 20 minutes
HW_{30}- Hot water ageing for 30 minutes
HW_{40}- Hot water ageing for 40 minutes
HW_{50}- Hot water ageing for 50 minutes

V1,V2,V3 =*Brassica juncea*
V4,V5,V6=*Brassica napus*
V7,V8,V9=*Brassica rapa* var.yellow sarson
V10,V11=*Eruca sativa*

Observations were taken on the seventh day of sowing. Three replications of 10 seeds each were sown. The length of roots and shoots were measured in centimeter and calculated on the basis of total number of seeds plated.

The biochemical parameters viz., electrical conductivity was measured by using conductivity meter, expressed as µS/cm/g and lipid peroxidation (Heath and Parker, 1968), and enzyme activity viz., catalase (Kato and Shimizu, 1987) and peroxidase (Shannon *et al*., 1966) were assessed at regular intervals. Analysis of variance was done by factorial CRD and difference among means was tested for significance using least significant difference tests, at 1% probability level.

Results and Discussion

The effects of ageing treatments on germination were more drastic for seeds subjected to controlled deterioration than those subjected to hot water ageing (Table 1). Even after 50 minutes of hot water treatment, the final mean germination percentage of most of the accessions was maintained above 50%, except for *B. napus* (V_7 and V_8) and *E. sativa* (V_{10} and V_{11}) accessions. On the other hand, controlled deterioration over 72 hours was lethal and seeds of all the accessions of various species were dead in this treatment. One of *B. napus* accession (V_4) lost its viability very quickly even in CD for 72 hours duration. *Brassica juncea* accession maintained 66-82% viability and *B. rapa* maintained 72-77% viability at the end of this test.

Seeds of all the accessions subjected to accelerated ageing at 95% RH maintained viability between 53-82.8% in comparison to other two treatments. The reduction in mean germination was more drastic for *B. napus* and *E. sativa* accessions. Similar results of declined germination percentage consequent to accelerated ageing treatment in chick pea were reported by Kapoor *et al*. (2010) and have attributed this decline in germination to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for early stages of germination. Comparison of means of various ageing treatments clearly indicates that *B. juncea* and *B. rapa* accessions maintained germinability between 70-80% after ageing treatments, whereas accessions of *B. napus* and *E. sativa* deteriorated significantly faster with a final germination range of 60-70%. This could be attributed to genotype differences between *E. sativa* and *Brassica* sp., as reported by Balesevic-Tubic *et al*. (2011) in soybean and sunflower seeds, as well as several other factors like seed size, 100 seed weight, and seed coat colour etc. Also, it was stated that the rate at which the seed ageing process takes place depends on the ability of the seeds to resist degradation changes and protection mechanisms, which are specific for each plant species (Mohammadi *et al*., 2011).

The mean germination time (MGT) which is indicative of germination uniformity and seedling vigour (Hunter *et al*., 1984) increased with prolonged exposure to hot water ageing as well as increased duration of controlled ageing. Maximum MGT was observed for *E. sativa* (V_{10}) which was 3.69 after 72 hours of CD, followed by *B. napus* (3.58) for the same treatment. Moderately higher values for this parameter were observed when the seeds were subjected to accelerated ageing under saturated solution of Potassium nitrate (KNO_3) at 95% RH (Table 2). According to Bailly et al. (2000), the cause of increasing mean time to germination probably is due to delay in the process when germination started since, repairing the membrane damage and other part of cells and also restarting antioxidant system activity and preventing oxidative stress is time consuming process.

Accessions of *B. napus* and *E. sativa* that had comparatively lower vigour level, tended to deteriorate faster than the high vigour lots of *B. juncea* or *B. rapa* in hot water ageing and controlled deterioration tests. Considerable evidences are available for several crop species supporting the assumption that all seed lots within a given species deteriorate at the same rate when stored in the same environment (Parkes *et al*., 1990). Our finding that, the rate of *Brassica* seed deterioration did not differ significantly among the accessions of the same species, but did differ between accessions of different species as well as those of *E. sativa*, is in line with these results.

Table 2. Effect of different ageing techniques on Mean Germination Time of various accessions of *Brassica* spp and *Eruca sativa*

Treatment Variety	Control T_0	HW_{10} T_1	HW_{20} T_2	HW_{30} T_3	HW_{40} T_4	HW_{50} T_5	KNO_3 T_6	CD_{24} T_7	CD_{48} T_8	CD_{72} T_9	CD_{96} T_{10}	CD_{120} T_{11}	Mean Variety
V_1	1.0	1.1	1.1	1.7	1.9	2.4	1.1	1.7	2.0	2.2	0.0	0.0	1.3
V_2	1.1	1.3	1.3	1.5	1.9	3.7	2.0	1.6	2.5	3.2	0.0	0.0	1.7
V_3	1.2	1.1	1.2	1.5	1.8	2.8	2.0	2.1	2.7	3.1	0.0	0.0	1.6
V_4	1.1	1.2	1.5	2.2	2.5	2.8	2.6	2.0	2.3	3.6	0.0	0.0	1.8
V_5	1.0	1.2	1.8	2.2	2.9	3.3	2.1	2.1	2.6	0.0	0.0	0.0	1.6
V_6	1.1	1.2	1.9	2.1	2.4	3.2	2.3	2.1	2.3	2.9	0.0	0.0	1.8
V_7	1.0	1.2	1.2	1.4	1.8	4.0	2.2	1.3	2.2	2.6	0.0	0.0	1.6
V_8	1.0	1.2	1.5	1.7	2.2	3.1	2.2	1.4	2.1	2.8	0.0	0.0	1.6
V_9	1.0	1.3	1.5	1.8	2.1	2.9	2.1	1.6	2.3	2.5	0.0	0.0	1.6
V_{10}	1.1	1.4	1.6	2.8	2.5	3.0	2.8	2.2	2.8	3.7	0.0	0.0	2.0
V_{11}	1.1	1.3	1.4	2.4	2.8	3.8	2.9	1.5	1.9	2.1	0.0	0.0	1.8
Mean, Treatment	1.1	1.2	1.5	1.9	2.3	3.2	2.2	1.8	2.3	2.6	0.0	0.0	

CD, P = 0.01	Treatment	0.1
	Variety	0.1

CD_{24} - Controlled deterioration for 24 hours
CD_{48} - Controlled deterioration for 48 hours
CD_{72} - Controlled deterioration for 72 hours
CD_{96} - Controlled deterioration for 96 hours
CD_{120} - Controlled deterioration for 120 hours
V1,V2,V3 = *Brassica juncea*
V7,V8,V9= *Brassica rapa* var.yellow sarson

HW_{10}- Hot water ageing for 10 minutes
HW_{20}- Hot water ageing for 20 minutes
HW_{30}- Hot water ageing for 30 minutes
HW_{40}- Hot water ageing for 40 minutes
HW_{50}- Hot water ageing for 50 minutes
V4,V5,V6= *Brassica napus*
V10,V11= *Eruca sativa*

The mean vigour index gradually decreased with increased exposure to hot water ageing as well as controlled deterioration treatment in all the accessions of various species under study (Table 3). Controlled deterioration for 48 and 72 hours, as well as accelerated ageing for 8 days over KNO_3, could differentiate the species based on their rate of vigour loss than hot water ageing during any duration. Genetic factors and seed chemical composition influence the expression of seed deterioration and vigour decline (Balesevic-Tubic, 2001 and Malencic et al., 2003) and is indicated by the differential response of various accessions of species to different types of ageing. The accessions of *E. sativa* and *B. napus* deteriorated much faster than the accession of other species under all ageing treatments.

Table 3. Effect of different ageing techniques on vigor index of various accessions of *Brassica* spp and *Eruca sativa*

Treatment Variety	Control	HW_{10}	HW_{20}	HW_{30}	HW_{40}	HW_{50}	KNO_3	CD_{24}	CD_{48}	CD_{72}	CD_{96}	CD_{120}	Mean Variety
V_1	1795.3	1492.1	1283.5	1094.8	1085.8	978.9	1490.4	1584.0	1442.6	1012.0	0.0	0.0	1079.4
V_2	1742.7	1683.7	1557.0	1006.2	901.6	700.1	1106.6	1548.7	1422.4	820.1	0.0	0.0	1040.8
V_3	1640.8	1483.7	1289.3	1321.7	1123.6	1002.8	1504.9	1463.6	1227.5	558.1	0.0	0.0	1051.4
V_4	1150.4	1136.3	977.5	784.1	702.3	397.2	798.4	1168.8	874.3	541.0	0.0	0.0	710.9
V_5	1354.8	1258.4	1077.8	580.3	545.2	279.5	404.9	732.1	221.4	0.0	0.0	0.0	537.9
V_6	1291.6	1121.3	902.4	842.5	599.2	297.2	686.1	1095.5	826.8	0.0	0.0	0.0	682.4
V_7	1490.9	1319.6	1020.8	793.4	543.0	286.0	869.1	1072.0	926.7	0.0	0.0	0.0	736.1
V_8	1523.3	1288.1	1225.5	791.7	547.5	428.2	1297.0	1073.3	779.6	563.0	0.0	0.0	793.1
V_9	1557.3	1123.5	783.7	685.7	475.1	298.5	1203.2	952.2	784.1	478.7	0.0	0.0	695.2
V_{10}	959.4	984.9	826.5	612.3	487.2	240.3	497.7	680.1	545.0	109.0	0.0	0.0	495.2
V_{11}	1240.0	1084.5	950.4	489.5	493.5	249.4	380.4	904.3	547.3	547.3	0.0	0.0	573.9
Mean , Treatment	1431..5	1270.6	1081.3	790.5	682.2	468.9	930.8	1115.9	872.5	515.1	0.0	0.0	

CD, P = 0.01)	Treatment	77.8
	Variety	74.5

CD_{24} - Controlled deterioration for 24 hours
CD_{48} - Controlled deterioration for 48 hours
CD_{72} - Controlled deterioration for 72 hours
CD_{96} - Controlled deterioration for 96 hours
CD_{120} - Controlled deterioration for 120 hours
V1,V2,V3 =*Brassica juncea*
V7,V8,V9=*Brassica rapa* var.yellow sarson

HW_{10}- Hot water ageing for 10 minutes
HW_{20}- Hot water ageing for 20 minutes
HW_{30}- Hot water ageing for 30 minutes
HW_{40}- Hot water ageing for 40 minutes
HW_{50}- Hot water ageing for 50 minutes
V4,V5,V6=*Brassica napus*
V10,V11=*Eruca sativa*

Many authors have observed changes in biochemical parameters of artificially aged seeds, as well as certain relations to natural ageing (Walters *et al.*, 2001; Bailly *et al*., 2002 and Zilic *et al*., 2006). Enhanced solute leakage from imbibed seed is associated with the loss in seed vigour and viability (Maristela and Vieira, 2007 and Kumar *et al.*, 2011). The electrical conductivity (EC) measured for the various accessions showed increase for all the ageing treatments except for hot water treatment at 58°C, for various durations (Table 4).

A peculiar trend in the pattern of electrical conductivity of seed leachate was observed during hot water ageing except in *Eruca* sp. where a gradual decline in conductance with duration of ageing was observed. In all accessions of the *Brassica* spp., there was decline in EC following

Table 4. Effect of different ageing techniques on electrical conductivity of various accessions of *Brassica* spp and *Eruca sativa*

Treatment Variety	Control T_0	HW_{10} T_1	HW_{20} T_2	HW_{30} T_3	HW_{40} T_4	HW_{50} T_5	KNO_3 T_6	CD_{24} T_7	CD_{48} T_8	CD_{72} T_9	CD_{96} T_{10}	CD_{120} T_{11}	Mean, Variety
V_1	127.7	258.8	193.5	133.3	88.2	61.8	341.5	222.5	296.3	480.6	698.0	1316.4	351.5
V_2	210.0	303.4	205.0	155.2	83.2	59.8	549.0	409.1	499.8	665.4	1118.8	1350.2	467.4
V_3	256.8	304.1	244.3	171.5	111.4	83.2	560.7	345.7	437.5	537.3	1092.1	1283.5	452.3
V_4	174.8	328.3	228.4	164.5	131.4	112.1	440.8	465.2	647.8	572.0	965.3	1320.6	477.6
V_5	217.6	387.5	218.2	194.0	154.6	124.1	642.3	429.7	524.9	600.4	1398.6	1387.2	523.3
V_6	160.0	371.6	220.1	197.2	176.7	135.6	477.4	334.8	528.4	730.0	1006.2	1222.1	463.1
V_7	312.6	432.2	326.2	237.4	186.0	153.7	635.7	374.1	504.6	618.2	1232.6	1408.5	534.1
V_8	307.4	396.8	355.0	211.3	171.8	153.4	681.9	445.9	779.1	885.3	1263.0	1385.8	586.4
V_9	288.3	412.4	380.9	200.0	175.4	139.8	720.2	391.1	492.3	878.7	1234.3	1381.2	555.4
V_{10}	393.9	446.5	514.2	608.2	758.6	855.3	1423.5	632.3	802.3	886.1	1167.5	1626.0	842.9
V_{11}	309.8	357.6	401.2	497.5	664.4	820.9	1476.9	448.6	647.4	822.5	1522.0	1652.4	801.8
Mean Treatment	250.8	363.6	296.1	251.8	245.4	245.4	722.7	409.0	560.0	714.2	1154.4	1394.0	

CD, P = 0.01)	Treatment	9.4
	Variety	9.0

CD_{24} - Controlled deterioration for 24 hours
CD_{48} - Controlled deterioration for 48 hours
CD_{72} - Controlled deterioration for 72 hours
CD_{96} - Controlled deterioration for 96 hours
CD_{120} - Controlled deterioration for 120 hours
V1,V2,V3 =*Brassica juncea*
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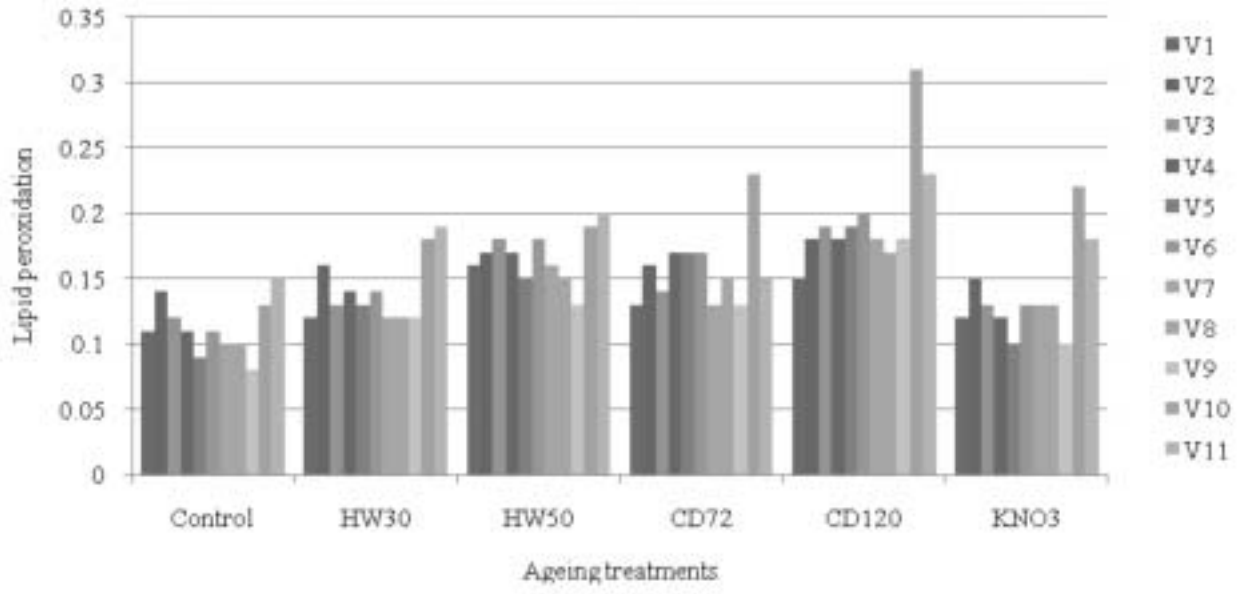
HW_{10}- Hot water ageing for 10 minutes
HW_{20}- Hot water ageing for 20 minutes
HW_{30}- Hot water ageing for 30 minutes
HW_{40}- Hot water ageing for 40 minutes
HW_{50}- Hot water ageing for 50 minutes
V4,V5,V6=*Brassica napus*
V10,V11=*Eruca sativa*

10 minutes of hot water ageing. This could be explained by the genotype difference between *Brassica* and *Eruca* sp. The reverse trend could have been due to simultaneous deterioration and exudation of electrolyte, into the immersion medium which increased with increased length of time. Since the electrolytes have already leaked out into the immersion medium, it did not increase significantly when re-immersed in distilled water for measuring the conductance. This has been reported by the results of Pandey (1989) in french bean seeds and Bailly *et al*. (1996) in sunflower seeds, where significant increase in EC with decrease in germination percentage was absent. On the other hand, gradual increase in EC with every step of CD was evident. The EC values reached the maximum in dead seeds for all the accessions and were highest for accessions of *Eruca* spp. Similar trend was observed during accelerated ageing over KNO_3 for 8 days. Increase in EC with every step of CD was reported earlier in Canola cultivars (*B. napus* L.) by Janmohammadi *et al.*, (2008) and have attributed to their capacity for sugar accumulation that occurs during seed filling period. The soluble sugars which are accumulated during the seed development in angiosperms (Amuti and Pollard, 1977) contribute to dessication tolerance and to longevity itself. (Bernal-Lugo and Leopold, 1995). These polyols maintain the structural integrity of membranes and provide stability for macromolecules such as proteins (Crowe and Crowe, 1986). In addition, as water is withdrawn from a solution of sugars, the sugars form a glassy state, and this can serve as a physical stabilizer (Franks *et al*., 1991). Because of the extremely high viscosity that can be obtained in the glassy state, deteriorative reactions could be suppressed. The EC values reached the maximum in dead seeds for all the accessions and were highest for accessions of *Eruca* spp. In another attempt to explain the increased leakage of solutes and electrolytes with seed ageing, Koostra and Harrington (1969) proposed that anti-oxidation of membrane lipids may lead to increased permeability.

Free radical induced lipid peroxidation (LP), which has the potential to damage membrane is likely to be the primary cause of deterioration of seeds (Stewart and Bewley, 1980 and Sung and Chiu, 1995). There was a concomitant increase in the lipid peroxidation expressed as increased absorbency values at a wavelength of 532 nm with artificial ageing of the seeds by various methods (Fig 1).

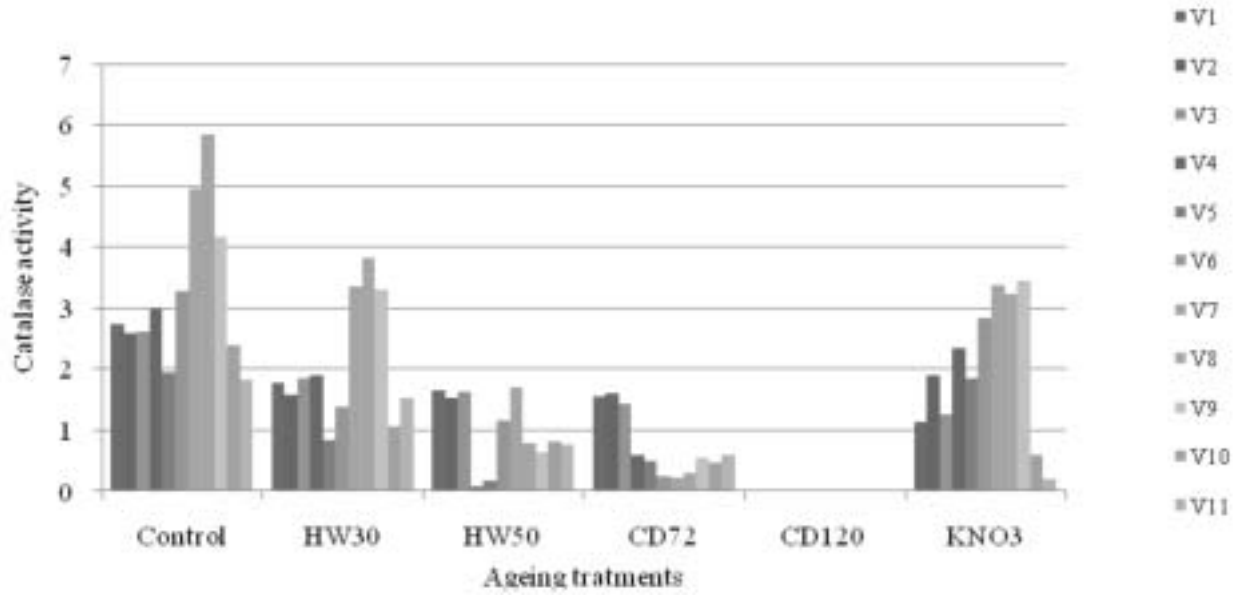
Fig 1. Effect of different ageing experiments on Lipid Peroxidation of various accessions of *Brassica* spp and *Eruca sativa*



Maximum accumulation of lipid peroxides was observed in *E. sativa* accessions (0.23) when subjected to controlled deterioration for 120 hours. The seeds were however non-viable after this treatment. Also, similar trend was observed for all the accessions of *Brassica* spp. However, Priestley and Leopold (1979) and Kalpana and Madhava Rao (1994) did not find any correlation between seed ageing and lipid peroxidation during ageing of seeds suggesting that this biochemical event is not the only mechanism involved in deterioration of seeds.

The activity of two main enzymes of hydroperoxide metabolism namely peroxidase and catalase, was determined which provide the protective mechanisms that could scavenge the peroxidatively produced free radicals and peroxides and which have evolved within the seeds to keep these deleterious compounds to a minimum level. A concomitant decrease in activity of both the enzymes occurred with increased duration of ageing. The initial levels of peroxidase as well as catalase were many times higher in all the species of *Brassica* examined. Fig 2. summarizes the results obtained regarding the changes in catalase activity during ageing of *Brassica* spp. and *E. sativa* seeds.

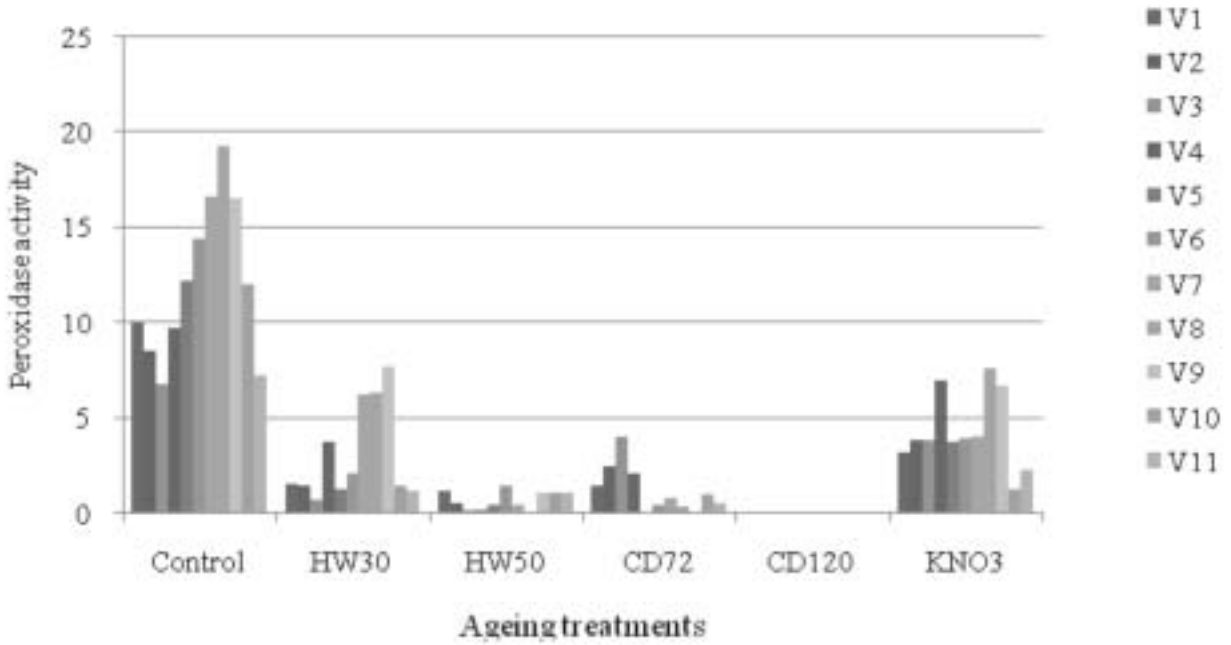
Fig 2. Effect of different ageing experiments on Catalase activity of various accessions of *Brassica* spp and *Eruca sativa*



The initial activity was maximum in *B.rapa* accessions and minimum in one of the accessions of *E. sativa* (V_{11}). Hot water treatments for 50 minutes drastically reduced the enzyme activity in all the accessions of both the genera. Accessions of *B. juncea*, however, retained more than 50% of activity in this treatment. For all other accessions of *Brassica* spp. and *Eruca* spp., there was remarkable decrease in the activity. Similar trend was obtained for seeds subjected to controlled deterioration for 72 hours where *B. juncea* accessions retained good activity, and in all others, there was a sharp decline. No enzyme activity was found in the seeds subjected to CD for duration over 72 hours. Accelerated ageing at 95% RH for 8 days also resulted in a sharp decline in catalase activity for all accessions considered for the study. Activity of peroxidase decreased markedly when aged in hot water for 30 minutes (Fig 3). The decrease was significantly more in *B. juncea* and *E. sativa* as opposed to catalase activity. However, after 50 minutes of ageing in hot water, the difference

between accession and species was minimized. On the other hand, accession of *B. juncea* subjected to CD for 72 hours maintained better activity than those subjected to hot water ageing for 50 minutes, indicating the extremely variable responses shown by the accessions/species for various ageing treatments.

Fig 3. Effect of different ageing experiments on Peroxidase activity of various accessions of *Brassica* spp and *Eruca sativa*



Accelerated ageing at 95% RH resulted in decline in peroxidase activity to a lesser extent as compared to the other ageing treatments. Significantly higher activities were observed for this treatment in all the accessions of all the species including *E. sativa*. Research evidence has shown that the longevity of seeds is shortened if the activities of these peroxides and radical scavenging enzymes are inhibited (Harrington, 1973; Bowler et al., 1992; Balesevic et al., 2005 and Cakmack et al., 2010). The decline in activity of these enzymes might have led to the accumulation of Malondealdehyde (the product of lipid peroxidation) in the tissues, which in turn might have caused an irreparable damage to the membrane system there by leading to deterioration of seeds.

It was observed that all the accessions showed a linear response to ageing treatments. Though hot water ageing was a rapid method, controlled deterioration by raising the moisture content of the seeds to 15% and subjecting to elevated temperature of 40 ± 2^0C was found to be a better ageing method than other treatments. Subjecting the seeds to higher relative humidity (95%) did not cause much deterioration even after 8 days. The physiological parameters like germination percentage, seedling vigour and vigour index declined with the ageing treatments. Maximum decline was shown by accessions of *B. napus* and *E. sativa*, and *B.juncea* and *B.rapa* were found to be the better performers. The biochemical parameters like electrical conductivity and lipid peroxidation increased with the progress of deterioration. Decline in activities of enzymes like peroxidase and catalase were observed and these enzymes become non-functional when the seeds were dead. These physiological and biochemical parameters can be used as safe indices for detecting early deterioration of seeds of various *Brassica* spp. and related genera.

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