



Effect of natural ageing on biochemical changes in relation to seed viability in okra (*Abelmoschus esculentus*)

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ABSTRACT

Effect of natural ageing on various storage enzymes, viz. peroxidase, catalase, superoxide dismutase (SOD), malondialdehyde (MDA) content and dehydrogenase activity (DHA) were studied during storage of seed in relation to seed viability and germination on three cultivars of okra, viz. ArkaAnamika, HisarUnnat and VarshaUphar. The present investigation revealed that the level of various enzymes have been studied so as to find the exact cause of seed deterioration. The activities of peroxidase, catalase, superoxide dismutase (SOD) and dehydrogenase activity (DHA) decreased whereas, malondialdehyde (MDA) content increased as the ageing period progressed in all the varieties. The cultivar VarshaUphar recorded higher activities of peroxidase, catalase, SOD and DHA and lower in MDA content among all the cultivars at the end of the storage period. In natural aged seed lot catalase and peroxidase activities decreased as the ageing progressed in all the three varieties, the rate of decreasing of both the enzymes activity was higher after 14 months of storage. Dehydrogenase activity was maximum up to 14 months of storage and after that it declined in terms of absorbance among all the varieties. It decreased at faster rate after 18 months of storage. During natural ageing no major changes in protein spectrum were recorded. No significant differences were observed in banding pattern of soluble proteins during ambient storage in all the three varieties. The germinability/viability of the seeds decreased as the ageing period progressed as a result of decreased in peroxidase, catalase, SOD and DHA activity and increased in MDA content in all the three varieties of okra.

Key words : Accelerated ageing, Catalase, Dehydrogenase, Natural ageing, Peroxidase, Seed quality, Okra

Okra [*Abelmoschus esculentus* (L.) Moench] is an important fibrous vegetable crop cultivated in tropical, subtropical and low altitudes regions of Asia, Africa and America. It is grown during summer and rainy season and hence classified as warm season crop (Yadav *et al.* 2012). The most important factor affecting crop production is the quality of seed. All seeds undergo aging process during long-term storage which leads to deterioration in seed quality, especially in the humid tropical regions. However, the rate of seed deterioration can vary among various plant species (Merritt *et al.* 2003). As the seeds aged, they come to germinate more slowly than fresh seeds, respire slower and become more susceptible to diseases, chromosomal

abnormalities and increased proportion of abnormal seedlings are produced. Rapid deterioration of stored seed is a serious problem in the tropical and subtropical countries like India. Retention of seed viability during storage has always been of utmost concern to seedsman. Thus it will be highly relevant to develop an insight into the basic phenomena of seed ageing and longevity. One of the approaches adopted in this direction is to identify the physiological and biochemical changes accompanying seed deterioration during seed storage.

Peroxidase and catalase are compounds of protoplasm with specific proteins. Catalase converts H₂O₂ to water and molecular oxygen. The enzyme is closely related to peroxidases in structure and function and both the enzymes are sometimes considered together as hydroperoxides. Catalases have extremely high maximum catalytic rates but low substrate affinities, since the reaction requires the simultaneous access of two H₂O₂ destruction is via peroxidases. These are found throughout the cell (Jimenez *et al.* 1997) and these have a high molecular affinity for H₂O₂ than catalase. However, peroxidase requires a reductant, since they reduce H₂O₂ to H₂O (Haschkel and Friedhoff

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1978). Superoxide dismutase is generally considered as a key enzyme in the regulation of intracellular concentrations of superoxide radical and peroxides from hydroxyl, which can react in the Haber-Weiss reaction to form hydroxyl radicals (Gutteridge and Halliwell 1990).

Total oil content of seeds is slightly affected by storage/seed deterioration (Mathur and Sinha 1978). Membrane and membrane lipids are damaged during storage of many seeds and this is thought to be one of the most important factors in loss of viability and vigour during storage. The peroxidation of seed lipids is one of the processes through which seed viability is lost under storage conditions (Harrington 1972, McDonald 1999). It has been reported that free fatty acid contents of seeds increase during seed deterioration (Basavarajappa *et al.* 1991). Malonaldehyde content is a product of lipid peroxidation. The determination of Malonaldehyde content is a convenient method for quantifying the extent of lipid peroxidation in oil rich seeds (Sung and Jeng 1994).

MATERIAL AND METHODS

The present research work was carried out in the laboratories of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar. The seed material was comprised of three varieties of okra, viz. ArkaAnamika, Hisar Unnat and Varsha Uphar having germination above minimum seed certification standards (76.00 - 89.69%). All the three varieties are ruling currently in market of Haryana, Punjab, Uttar Pradesh, Rajasthan region. The seed material (one year old) was collected from the Department of Vegetable Science and was stored in paper bags into glass desiccators with calcium chloride, sealed with vacuum silicon grease maintaining temperature at 25°C ± 2°C and relative humidity <10 percent. Natural ageing, observations were recorded every two months on the stored okra seed in cotton bags in ambient conditions up to one year till germination fall below as compared to fresh seed lot.

For standard germination test hundred seeds of each variety in four replicates placed in between sufficient moistened rolled towel papers and kept at 25°C in seed germinator. The first count was taken on 4th day and final

count on 10th day and only normal seedlings were considered for percent germination according to the rules of International Seed Testing Association (ISTA 2003). Tetrazolium viability test (Moore, 1973) based on three replications of 50 seeds each were soaked in 50 ml water for 16 hr at 25°C to activate dehydrogenase enzymes. After removal of seed coat, the seeds were stained in 0.5 percent tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) for 4 hr at 38°C, in petri plates. After that solution was poured off and seeds were rinsed briefly in water and examined under magnifications. The number of seeds stained entirely red were considered as viable seeds and expressed in percentage.

Peroxidase activity (mg/protein/min) was determined by the method of Shannon *et al.* (1960), following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H₂O₂).

The catalase activity (mg/protein/min) was assayed by the method as described by Aebi (1983) based on the reduction of potassium dichromate to chromic acetate by hydrogen peroxide (H₂O₂).

The SOD enzyme activity was assayed by the method of Giannopolitis and Ries (1977). One unit of SOD was defined as the enzyme activity which inhibits the photo reduction of NBT to blue formazan by 50% and expressed as units SOD mg/protein/min.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in the seeds. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction by the method of Heath and Packer (1968). The concentration of MDA was calculated using its estimation coefficient of 155 m/M/cm.

Tetrazolium reduction ability by the enzyme dehydrogenase was determined by the method of Kittock and Law (1968). The observations were expressed as change in O D/g/ml.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for different enzymes activities during natural ageing are presented in Table 1. The mean sum of squares due to variety and ageing for all the enzymes were found significant revealed sufficient level of

Table 1 Analysis of variance for various enzymes activity during natural ageing in okra

Parameters ↓	Source →			
	Variety (A)	Ageing (B)	A × B	Error
Standard germination	250.92**	673.43**	15.79	14.70
Tetrazolium test	344.44**	216.40**	31.48	52.38
Peroxidase activity	98424.51**	522845.89**	10878.68**	3073.10
Catalase activity	5201.03**	24358.95**	254.19**	62.08
Superoxide dismutase activity	947.42**	16996.79**	80.07	138.98
Lipid peroxidation	1024.64*	22298.57**	304.40	246.38
Dehydrogenase activity	0.01*	0.30**	0	0

** P=0.01 *P=0.05

variability present in the material studied. The interactions were found significant in peroxidase and catalase activities only.

Standard germination and viability

Standard germination slightly increased up to 14 months of storage and after that decreased significantly (Table 2). After 20 months of storage the germination decreased at a faster rate in all the three varieties of okra. It was below minimum seed certification standard after 24 months of storage in ArkaAnamika (61.00) and HisarUnnat (64.33) whereas varsha Uphar recorded higher germination percentage among all the varieties.

Viability percentage was found maximum after 12 months of storage in all the varieties (Table 3), after that it was decreased. Varsha and HisarUnnat recorded maximum viability whereas ArkaAnamika recorded low viability in the end of storage period.

Standard germination test is an excellent measure of seed viability. In the present study, loss of germinability and viability occurs during natural ageing after 14 months of ageing and loss of germination was very high after 20 months of storage. These observations are similar to those already reported by various workers in different crops such as okra (Narwal 1995), cotton (Meena *et al.* 1999), carrot (Maskri *et al.* 2003), onion (Promila Kumari 1994), turnip (Khan *et al.* 2005).

The results showed that tetrazolium test expressed higher viability percentage than standard germination after natural ageing. The viability of seeds decreased as the ageing period increased in ArkaAnamika and VarshaUphar. The variety VarshaUphar showed the maximum viability among all the varieties during natural ageing. The present result are also in corroborate with the findings of Agarwal and Sinha (1980) and Saxena *et al.* (1987) where loss of seed viability and vigour increased with increase in period of storage.

Table 2 Effect of natural ageing on seed germination in okra

Varieties	Ageing period (months)							Mean
	12	14	16	18	20	22	24	
ArkaAnamika	76.00 (60.69)	83.00 (65.75)	81.00 (64.18)	71.33 (57.61)	70.00 (56.78)	67.00 (54.93)	61.00 (51.34)	72.76 (58.75)
HisarUnnat	88.00 (69.99)	87.67 (69.48)	83.00 (65.84)	79.33 (62.96)	73.67 (59.15)	70.66 (57.20)	64.33 (53.33)	78.09 (62.56)
VarshaUphar	89.67 (71.81)	90.00 (72.07)	87.00 (68.85)	78.33 (62.26)	73.33 (58.95)	68.67 (55.95)	67.67 (55.34)	79.23 (63.60)
Mean	84.55 (67.50)	86.89 (69.10)	83.67 (66.29)	76.33 (60.95)	72.33 (58.29)	68.78 (56.03)	64.33 (53.34)	
CD for factor A (Var.)	= 2.38							
Factor B (Ageing)	= 3.64							
A × B	= NS							

Data in parenthesis are arcsine transformed value

Table 3 Effect of natural ageing on seed viability (%) by tetrazolium test in okra

Varieties	Ageing period (months)							Mean
	12	14	16	18	20	22	24	
ArkaAnamika	88.33 (69.98)	86.67 (63.20)	82.33 (58.98)	76.67 (61.20)	73.33 (58.98)	70.00 (56.77)	66.67 (54.76)	77.71 (60.55)
HisarUnnat	90.00 (71.90)	88.67 (68.83)	85.67 (63.20)	82.67 (61.20)	78.00 (56.98)	73.33 (58.98)	73.33 (58.98)	81.67 (62.87)
VarshaUphar	93.33 (74.37)	90.00 (71.90)	88.33 (69.61)	83.00 (63.93)	78.33 (58.98)	72.67 (50.20)	73.33 (58.98)	82.71 (62.26)
Mean	90.55 (72.08)	88.45 (67.98)	85.44 (63.93)	80.78 (62.11)	76.55 (58.31)	72.00 (55.31)	71.11 (57.57)	
CD for factor A (Var.)	= 4.50							
Factor B (Ageing)	= 6.88							
A × B	= NS							

Figures in parenthesis are arcsine value

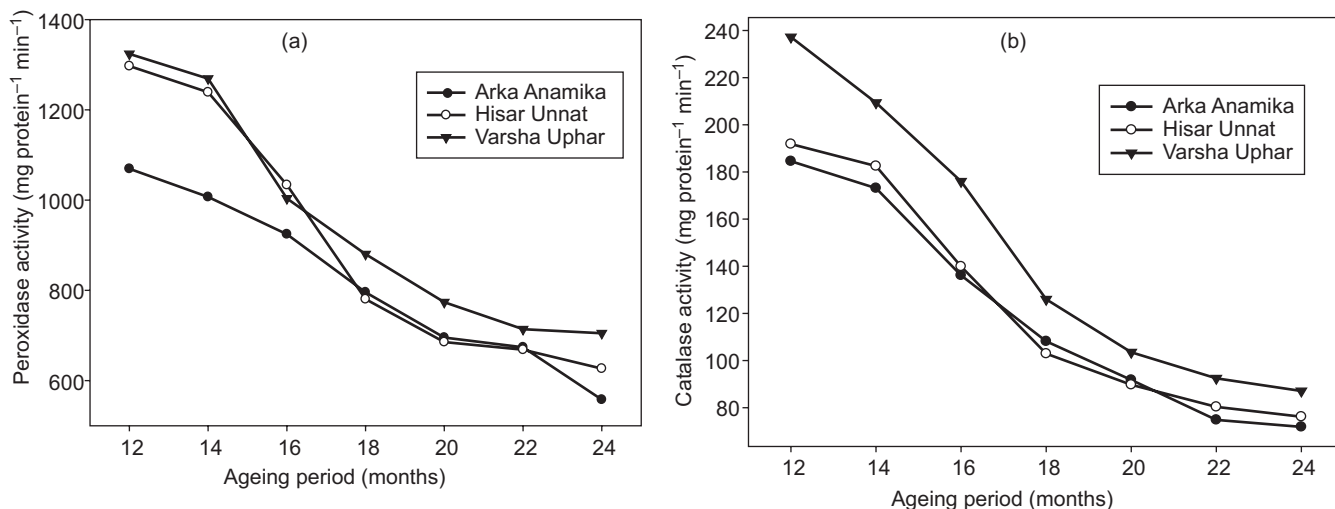


Fig 1 Effect of natural ageing on (a) peroxidase enzyme activity and (b) catalase enzyme activity in okra

Peroxidase enzyme activity

The activity of peroxidase decreased significantly with advancement of ageing period in all the three varieties, but decreased at a faster rate after 14 months of ageing. The maximum activity of this enzyme was recorded in VarshaUphar (705.04) which is significantly higher than other varieties whereas minimum activity was recorded in ArkaAnamika (557.94) (Fig 1a).

Catalase activity

The catalase activities decreased significantly as the ageing period progressed in all the three varieties. The rate of decline of catalase activity was higher after 14 months of storage among all the varieties. In the end of storage period the activity of catalase was found significantly higher in VarshaUphar whereas lower was found in ArkaAnamika (Fig 1b).

Catalase and peroxidase activities decreased which results into decrease in seed viability as the ageing progressed in all the three varieties. The rate of decreasing of both the enzymes activity was higher after 14 months of storage. In the present study, the level of various enzymes have been studied so as to find the exact cause of seed deterioration under natural ageing. In general, decrease in enzyme activity in seed lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the germinating seed. Several changes in the enzyme macromolecular structure may contribute to their lower effectiveness. They may undergo compositional changes by losing or gaining certain functional groups, by oxidation of sulf-hydral groups or by conversion of amino acids within the protein structure. The enzymes may undergo configurational changes such as partial folding or unfolding of ultrastructure, condensation to form polymers and degradation to sub units. However, the basic reason for reduced enzymatic activities in deteriorating okra seed is yet

to be established. The present results are also in accordance with the findings of sampietro (1931) and Ghosh *et al.* (1978) in rice, Sung and Chiu (1995) and Sung (1996) in soybean during natural ageing.

Superoxide dismutase activity (SOD)

The activity of superoxide dismutase decreased significantly with the advancement of storage period in ArkaAnamika and HisarUnnat but in VarshaUphar it decreased significantly only after 16 months of storage. SOD activity decreased at very fast rate after 16 months of storage in all the three varieties. At the end of storage period VarshaUphar recorded higher activity whereas in ArkaAnamika lower activity of SOD was recorded (Fig. 2a).

In the present study, the SOD activity decreased with ageing. It declined very rapidly after 16 months of natural ageing in all the three varieties of okra. Similar decrease in SOD activity was reported by Sung and Chiu (1995) and Sung (1996) in soybean, Swaraj *et al.* (1995) in *Cajanus cajan*, Goel and Sheoran (2003) in cotton.

Lipid peroxidation (Malondialdehyde)

Malondialdehyde content of seed increased progressively and significantly with the advancement of ageing period in all the three varieties. The rate of increase in accumulation of MDA content was very high after 18 months of seed storage. The accumulation of MDA content was maximum in ArkaAnamika whereas minimum in VarshaUphar at the end of storage (Fig 2b).

All plant membranes are made up of lipid bilayers and any change in the lipid would result in the loss of membrane permeability and lipid peroxidation is considered as the major cause of loss in permeability. Lipid peroxidation may be attributed directly to the altered nature of phospholipids, which affect their packaging in the bilayer to damage incurred

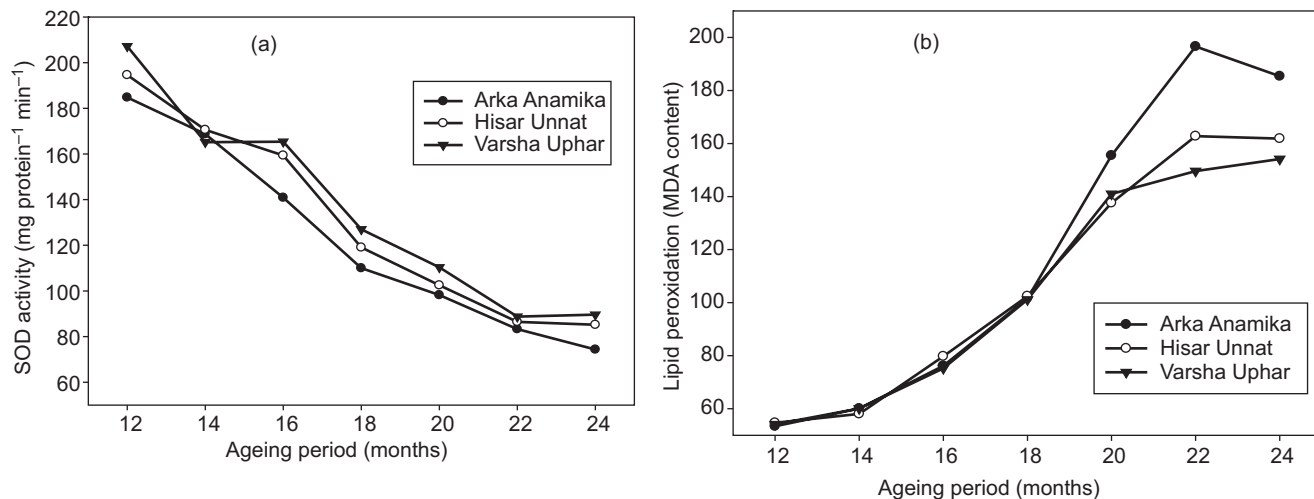


Fig 2 Effect of natural ageing on (a) Superoxide dismutase activity (SOD) and (b) Lipid peroxidation (Malondialdehyde) in okra

by membrane proteins in the course of the peroxidation reaction (Ponquett *et al.* 1992). Lipid peroxidation occurs in all the seeds but the mechanism of lipid peroxidation may be different under long term natural ageing as compared to accelerated ageing conditions (Wilson and McDonald 1986).

The major cause of membrane lipid peroxidation under stress condition has been attributed due to the production of H₂O₂ and free radicals (Hendry 1993). The damage caused by free radicals to membrane lipids results in the production of large number of volatile aldehydes (Esashi *et al.* 1997). The presence of these volatile aldehydes indirectly indicates the occurrence of lipid peroxidation. Sung and Jeng (1994) reported that the determination of malondialdehyde content was a convenient way to quantify the extent of lipid peroxidation in peanut.

In the present study the MDA content increased with natural ageing. It increased very rapidly after 18 months of storage (Fig 2b). Similar increased MDA content was reported during natural ageing in pea (Harman and Mattick 1976), peanut (Jeng and Sung 1994), sunflower (Bailey *et al.* 1996), mung bean (Murthy and Sun 2000 and Murthy *et al.* 2003), carrot (Maskri *et al.* 2003) and cotton (Goel and Sheoran 2003).

Dehydrogenase activity

The activity of dehydrogenase was increased up to 14 months of storage and after that it decreased significantly with advancement of ageing period. It was decreased rapidly after 18 months of ageing in all the three varieties. Variety HisarUnnat showed more reduction as compared to ArkaAnamika and VarshaUphar.

Dehydrogenase activity was maximum up to 14 months of storage and after that it declined in terms of absorbance among all the varieties. It decreased at faster rate after 18 months of storage. Ray and Gupta (1980) also noted reduced DHA activity in terms of formazan formation in rice seeds

undergoing deterioration. Similar findings were given by Halder and Gupta (1980), Plallavi *et al.* (2003) in sunflower seeds, Narwal (1995) in okra, Verma *et al.* (2003) in *Brassica* spp.

Seed storage protein profile and similarity index

No significant differences were observed in banding pattern of soluble proteins during ambient storage in all the three varieties. However, neither any additional band appeared nor any band disappeared due to ageing. ArkaAnamika and VarshaUphar are grouped in one cluster whereas hisarUnnat remains separate after natural ageing. Similarity index based on the relative mobility of protein band and molecular weight indicated a close association in ArkaAnamika and VarshaUphar (90%) after natural ageing. The similarity index value varied from 0.400 to 0.900 (Fig 3).

The proteins are product of structural gene that encodes them. So analysis of protein profile can be used as an ideal mean of varietal characterization (Herrmann and Zanetti 2002 and Cooke *et al.* 1983).

It was thought that protein changes were another reason for the seed deterioration. Surprisingly, during natural ageing

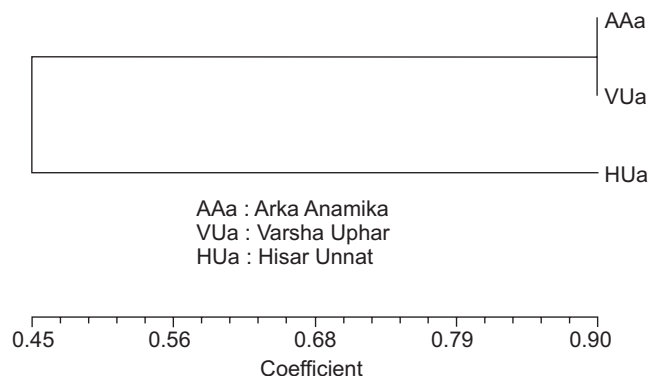


Fig 3 Dendrogram of natural aged (after 24 months) seed lots of okra (protein)

no major changes in protein spectrum were recorded. Similarly, no marked changes were reported in soluble protein profiles between naturally aged and fresh seed of cotton (Dadlani *et al.* 1992, Anuradha 2000), wheat (Yogeshia *et al.* 1998) and *P. vulgaris* (Choer *et al.* 1998) except for faint staining of protein bands in naturally aged seeds.

In conclusion, with the increased ageing periods the seeds were unable to sustain the scavenging enzymes like peroxidase, catalase, SOD & DHA activity. This study allowed important progress towards the understanding of seed aging mechanisms.

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