



Biochemical changes in naturally aged seeds of soybean genotypes with good and poor storability

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ABSTRACT

Seed deterioration during storage is associated with various metabolic and chemical alterations that vary among genotypes in soybean. In the present study, five genotypes with good storability viz., *kalitur*, MACS 1416, EC 18761, CO1 and DSB21 and five genotypes with poor storability viz., JS 71-05, DS 228, MAUS 61, NRC 93 and DSB 24 were selected and evaluated for biochemical changes to identify the best donors for storability. Among the genotypes, all the poor storer genotypes showed faster accumulation of free amino acids, free fatty acids and malondialdehyde content as a result of oxidative stress. Poor storer genotypes also showed the increased lipoxxygenase I & II enzymes activity. All the good storer genotypes showed higher anti-oxidative enzymes activity causing slower lipid peroxidation. Among the good storers, *kalitur* showed favourable biochemical features for storability while MAUS-61 was identified as poor storer exhibiting less favourable biochemical features.

Key words: Biochemical changes, Deterioration, Genotypes, Seed, Soybean, Storability.

INTRODUCTION

Seeds exhibit their maximum germination potential at physiological maturity and thereafter seed vigour declines and finally lose their viability. The rate of seed deterioration varies among crop species and cultivars (Shelar 2002; Jain *et al.*, 2006 and El-Abady *et al.*, 2012). The exact causes of seed viability loss are still unknown, but the viability is affected by quality of the seed at the time of storage, pre-storage history of seed (environmental factors during pre and post-harvest stages), moisture content of seed or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents (Shelar *et al.*, 2008; Bellaloui *et al.*, 2011).

Biochemical changes occurring in seed during ageing play an important role in seed storability. Membrane disruption is one of the primary reasons attributed to seed deterioration. As a result, seed cells are not capable to hold their normal physical condition and function. Causes of membrane disruption are enhanced free fatty acid level and formation of free radicals by lipid peroxidation (Ghassemi-Golezani *et al.*, 2010). There are also mechanisms by which the cell protects its integrity by mopping-up excessive free radicals by the activity of anti-oxidative enzymes viz., superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) (Sung, 1996). Activity

of such enzymes lowers active oxygen/hydroxyl radical formation and loss of seed viability is reported to be associated with a reduction in the activities of antioxidant enzymes (Hosamani *et al.*, 2013).

The mechanism of ageing that differ with genotypes is still an enigma. Therefore, it was thought imperative to investigate the biochemical changes during storage to understand the basis of seed deterioration, which would help in both identifying reasons for improved storage life of seeds and also provide information that would enable incorporation of trait for better storability in the genetic background of high yielding varieties. Hence, the present investigation was taken to evaluate biochemical changes in naturally aged seeds of genotypes with good and poor storability.

MATERIALS AND METHODS

Seed samples of 30 soybean genotypes were grown under isoclimatic conditions at Agricultural Research Station (Bhavanisagar) of Tamil Nadu Agricultural University, Coimbatore during *kharif* 2014. Seeds were shade dried after harvesting to about 8.5% moisture content. These 30 genotypes were screened for storability through accelerated ageing and selected five genotypes with good storability viz., *kalitur*, MACS 1416, EC 18761, CO1 and DSB21 and five genotypes with poor storability viz., JS 71-05, DS 228, MAUS 61, NRC 93 and DSB 24 (Vijayakumar and Vijaya

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kumar, 2016). Seeds of these 10 genotypes were stored in cloth bags under the ambient conditions of Coimbatore for a period of 10 months and they were evaluated for biochemical changes at bi monthly intervals.

Seed moisture content was estimated as per ISTA (2007). For the estimation of free amino acid content, four replications of fifty seeds from each genotype were drawn, prewashed well with distilled water and soaked in 100ml distilled water for 9 hrs to obtain the seed leachates. Modified method of Moore and Stein (1948) and Misra *et al.* (1975) was followed for estimation of free amino acid content. The oil content was estimated by using the Soxhlet extraction apparatus (Sadasivam and Manickam, 1995). For estimation of free fatty acid content, a known quantity of oil extracted from seeds was dissolved in 50ml neutral solvent in a 250ml conical flask (Neutral solvent was prepared by mixing 25ml ether, 25ml of 95% ethanol and 1 ml of 1 % phenolphthalein and neutralized with 0.1N NaOH). Then few drops of phenolphthalein was added and titrated against 0.1N potassium hydroxide solution to obtain the pink colour end point which persist for at least 15 seconds. The free fatty acid content was calculated based on their oil content using the following formula and expressed in per cent oleic acid (Christiansen and Moore, 1961).

Free fatty acid (%) =

$$\frac{\text{Titre value (ml)} \times \text{Normality of KOH}}{\text{Weight of sample (g)}} \times 100$$

The MDA level was estimated spectro-photo metrically according to Heath and Parker (1968). Free radical scavenging activity of the aqueous acetone seed extracts was assessed based on their ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, using a modified DPPH assay of Koleckar *et al.* (2007). Lipoxxygenase enzyme activity was assayed as per the procedure described by Grossman and Zakut (1979).

Anti-oxidative enzymes assay

Preparation of enzyme extract: Enzyme extract for superoxide dismutase, peroxidase, catalase and glutathione reductase was prepared by first freezing the weighed amount of seed samples (0.2g) in liquid nitrogen to prevent proteolytic activity followed by grinding of seed samples with 2ml extraction buffer (0.1M phosphate buffer, pH 7.5, containing 0.5mM EDTA). Then, the extract was centrifuged for 20 min at 15000 rpm and the supernatant was used as enzyme.

Assay: The assay of SOD is based on the formation of blue coloured formazone by nitro-blue tetrazolium and O_2^- radical, which absorbs at 560 nm and the enzyme (SOD) decreases this absorbance due to reduction in the formation of O_2^- radical by the enzyme (Dhindsa *et al.*, 1981). Peroxidase activity was assayed as increase in absorbance due to the oxidation of guaiacol to tetra-guaiacol (Castillo *et al.*, 1984). The catalase assay was based on the reduction

of hydrogen peroxide to water and molecular oxygen by the activity of catalase (Aebi, 1984). The glutathione reductase assay is based on the formation of a red coloured complex by reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), which absorbs at 412nm (Smith *et al.*, 1988). All the absorbance readings were taken by UV-Vis spectrophotometer (Systronics 2205).

RESULTS AND DISCUSSION

Seed moisture content was significantly affected by storage period irrespective of genotypes and it significantly increased only after two months of storage period (Table 1). Moisture content increased due to absorption of moisture and the attainment of equilibrium with atmospheric moisture as the seeds are hygroscopic in nature (Harrington, 1972; Shelar, 2008). However, there was no significant difference among genotypes with respect to seed moisture content.

Free amino acid (FAA) content in the seed leachate was almost doubled (increased to the tune of 107%) after 10 months of storage in good storer genotypes, whereas, it was increased by 4 times (324%) in genotypes classified under poor storers. Among the genotypes, kalitur maintained lowest FAA (93µg) and was followed by MACS 1416 (163.2µg), while highest FAA was recorded in poor storer genotype MAUS 61 (980.7µg) followed by NRC 93 (359.3µg) (Table 1). Similar genotypic differences in FAA content during seed storage was also reported by Salam *et al.*, 2018. Loss of membrane integrity due to higher lipid peroxidation might be reason behind higher free amino acid content in seed leachate of poor storers.

Oil content significantly decreased over a period of storage irrespective of genotypes. However, good storers showed minimum reduction (16%) over the 10 months of storage, while genotypes identified for poor storability shown more reduction in oil content (23%). NRC 93 shown highest reduction in oil content (27.1%) among the poor storers (Table 2). Koutroubas *et al.* (2000) also reported genotypic variability in rate of reduction of oil content during seed storage. The decrease in oil content of sunflower, soybean and maize seeds during storage was also reported by Simic *et al.* (2007). On the other hand, good storer genotypes also maintained lower free fatty acid (FFA) content over the storage period with 98 per cent increase in free fatty acid content when compared poor storer genotypes which recorded 168 per cent increase. The increase in FFA during storage was also documented by Abreu *et al.* (2013) and it was mainly due to lipid peroxidation.

The genotypes with large lipid reserves like soybean, are subject to a slow and consistent oxygen attack, forming hydrogen peroxides and free radical. The polyunsaturated fatty acids such as linoleic and linolenic acids are susceptible to oxidative degradation by enzymatic and non-enzymatic reactions as opined by Anderson and Baker (1983). The extent of increase in malondialdehyde content

Table 1: Influence of genotypes and natural ageing period on seed moisture content and free amino acid content in soybean genotypes.

| Genotypes (G) | Seed moisture content (%) | | | | | Free amino acid content ($\mu\text{g}/50$ seeds/100 ml leachate) | | | | | | |
|----------------------------|-----------------------------|-------------|-------------|--------------------|-------------|---|---------------------|--------------------|--------------|--------------------|--------------|--------------|
| | Storage Period (Months) (P) | | | | | Storage Period (Months) (P) | | | | | | |
| | Initial | 2 | 4 | 6 | 8 | 10 | Initial | 2 | 4 | 6 | 8 | 10 |
| MACS 1416 | 8.49 | 8.42 | 8.9 | 8.71 | 8.75 | 9.1 | Good Storers | | | | | |
| CO 1 | 8.46 | 8.38 | 9.04 | 8.89 | 8.92 | 9.15 | 8.73 | 8.81 | 8.83 | 8.85 | 8.77 | 8.73 |
| EC 18761 | 8.5 | 8.45 | 9.04 | 8.92 | 8.93 | 9.17 | 8.73 | 8.81 | 8.83 | 8.85 | 8.77 | 8.73 |
| DSB 21 | 8.57 | 8.5 | 9.05 | 8.89 | 8.9 | 9.18 | 8.73 | 8.81 | 8.83 | 8.85 | 8.77 | 8.73 |
| Kalitir | 8.52 | 8.49 | 8.93 | 8.78 | 8.85 | 9.09 | 8.73 | 8.81 | 8.83 | 8.85 | 8.77 | 8.73 |
| MAUS 61 | 8.44 | 8.37 | 9.05 | 8.94 | 8.95 | 9.13 | Poor Storers | | | | | |
| DSB 24 | 8.55 | 8.51 | 8.82 | 8.73 | 8.75 | 8.98 | 8.81 | 8.72 | 8.73 | 8.77 | 8.73 | 8.73 |
| NRC 93 | 8.52 | 8.48 | 8.82 | 8.76 | 8.78 | 9.03 | 8.81 | 8.72 | 8.73 | 8.77 | 8.73 | 8.73 |
| DS 228 | 8.46 | 8.4 | 8.99 | 8.79 | 8.83 | 9.15 | 8.81 | 8.72 | 8.73 | 8.77 | 8.73 | 8.73 |
| JS 71-05 | 8.48 | 8.41 | 9.01 | 8.85 | 8.9 | 9.17 | 8.81 | 8.72 | 8.73 | 8.77 | 8.73 | 8.73 |
| Mean | 8.5 | 8.44 | 8.96 | 8.83 | 8.86 | 9.11 | 88.2 | 114.3 | 141.4 | 172.6 | 221.2 | 297.9 |
| 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 | 7.6 | 15.1 | | 20 | | |
| Storage period (P) | | 0.03 | | 0.07 | | 0.09 | 5.9 | 11.7 | | 15.5 | | |
| G x P | | 0.11 | | NS | | NS | 18.7 | 37 | | 49 | | |

Table 2: Influence of genotypes and natural ageing period on oil content and free fatty acids content in soybean genotypes.

| Genotypes (G) | Oil content (%) | | | | | Free fatty acids content (%) | | | | | | |
|----------------------------|-----------------------------|--------------|--------------|--------------------|--------------|------------------------------|---------------------|--------------------|-------------|--------------------|-------------|-------------|
| | Storage Period (Months) (P) | | | | | Storage Period (Months) (P) | | | | | | |
| | Initial | 2 | 4 | 6 | 8 | 10 | Initial | 2 | 4 | 6 | 8 | 10 |
| MACS 1416 | 19.00 | 18.57 | 18.07 | 17.6 | 16.81 | 16.10 | Good storers | | | | | |
| CO 1 | 18.57 | 18.02 | 17.59 | 17.23 | 16.3 | 15.15 | 17.69 | 17.14 | 17.55 | 17.87 | 18.17 | 17.08 |
| EC 18761 | 19.20 | 18.47 | 18.00 | 17.49 | 16.87 | 16.03 | 17.69 | 17.14 | 17.55 | 17.87 | 18.17 | 17.08 |
| DSB 21 | 18.80 | 17.97 | 17.80 | 17.39 | 16.35 | 15.37 | 17.69 | 17.14 | 17.55 | 17.87 | 18.17 | 17.08 |
| Kalitir | 18.60 | 18.10 | 17.53 | 17.30 | 16.53 | 16.00 | 17.69 | 17.14 | 17.55 | 17.87 | 18.17 | 17.08 |
| MAUS 61 | 18.70 | 18 | 17.30 | 16.73 | 15.30 | 14.23 | Poor storers | | | | | |
| DSB 24 | 19.47 | 18.69 | 18.10 | 17.34 | 16.70 | 15.03 | 16.71 | 16.16 | 16.52 | 16.88 | 17.24 | 17.60 |
| NRC 93 | 20.39 | 19.54 | 18.90 | 18.22 | 17.10 | 14.87 | 16.71 | 16.16 | 16.52 | 16.88 | 17.24 | 17.60 |
| DS 228 | 19.02 | 18.4 | 17.49 | 16.62 | 16.00 | 14.97 | 16.71 | 16.16 | 16.52 | 16.88 | 17.24 | 17.60 |
| JS 71-05 | 19.00 | 18.3 | 17.20 | 16.53 | 15.81 | 14.78 | 16.71 | 16.16 | 16.52 | 16.88 | 17.24 | 17.60 |
| Mean | 19.07 | 18.41 | 17.80 | 17.25 | 16.38 | 15.25 | 0.38 | 0.46 | 0.55 | 0.65 | 0.73 | 0.89 |
| Source of variation | | SED | | CD (P=0.05) | | CD (P=0.01) | SED | CD (P=0.05) | | CD (P=0.01) | | |
| Genotype (G) | | 0.11 | | 0.22 | | 0.29 | 0.03 | 0.06 | | 0.08 | | |
| Storage period (P) | | 0.08 | | 0.17 | | 0.22 | 0.02 | 0.04 | | 0.06 | | |
| G x P | | 0.27 | | 0.54 | | 0.71 | 0.07 | 0.15 | | NS | | |

over the storage period varied among the genotypes with all the good storer genotypes showing lesser malondialdehyde content than poor storer genotypes. Kalitur maintained lowest MDA (60.2nmol) among the genotypes, followed by MACS 1416 (73.3nmol), CO1 (76.2nmol) and EC 18761 (78.7nmol), while MAUS 61 shown maximum increase (99.6nmol) which was on par with NRC 93 (97.5nmol) (Table 3). The genotypic differences in MDA during ageing of soybean was also noticed by Balešević-Tubić *et al.* (2011) and attributed such differences to biochemical characteristics of soybean genotypes to resist the negative consequences of aging (Kibinza *et al.*, 2006). The aldehydes produced as a result of lipid peroxidation further react with sulfhydryl groups leading to an inactivation of proteins and inhibit tubulin, the main protein of microtubules which is necessary for mitotic spindle formation and thereby affecting seed germination (Gabriel *et al.*, 1977). Hence, higher lipid peroxidation with increased MDA content might have resulted in reduced germination during storage in poor storers.

The soybean genotypes significantly varied with respect to activity of both lipoxygenase I (LOX I) and Lipoxygenase II (LOX- II) during storage. DSB 21 had lowest lipoxygenase I activity (841.1 units) at the end of storage period which was on par with kalitur (857.7 units). Whereas, highest activity recorded in MAUS 61 (1072.6 units) followed by NRC 93 (1028 units) (Table 4). All the good storer genotypes maintained lower lipoxygenase II activity over the storage period with kalitur having least

activity (274.2 units). While, poor storer MAUS 61 registered highest lipoxygenase II activity (480.7 units) followed by NRC 93 (427.2 units). (Table 4). Genotypic variability in lipoxygenase activity during seed storage was also reported by Hosamani *et al.* (2013) and Rahul *et al.* (2016). However, in the present study, genotypes shown more increase in lipoxygenase II activity over the storage period indicating that LOX-II might be directly involved in the deterioration of soybean seeds. The high activity of Lox II in poor storers could attribute to the loss of seed viability and storability through accumulation of their catalyzed hydroperoxide products.

Total antioxidant activity is measured by free radical scavenging activity. Antioxidant molecules include vitamin E, β -carotene, vitamin C and glutathione. Antioxidants can inhibit lipid peroxidation by reducing the level of active oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radical by blocking the propagation of free radical chain reactions. In the present study, Kalitur shown highest initial (66.6%) as well as mean free radical scavenging activity (71.2%) over the storage period, which was followed by MACS 1416 (45.1%) while, MAUS 61 recorded lowest (30.7%) which was on par with DSB 24 (32.5%) (Table 3). In poor storers, there was imbalance between antioxidants and reactive oxygen species due to reduced supply of the antioxidant molecules during oxidative stress which has resulted in cellular damage and loss of seed viability as observed in poor storer MAUS 61 (Kaewnaree *et al.*, 2011).

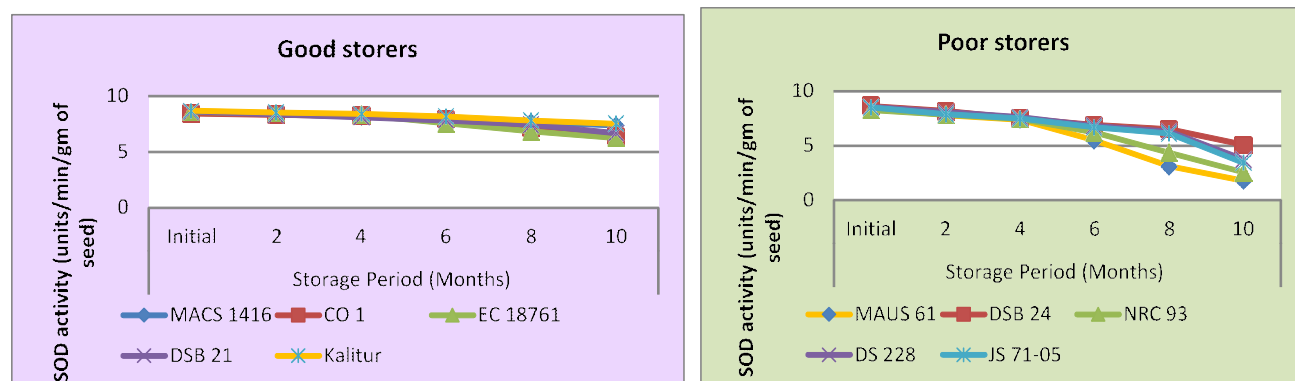


Fig 1: SOD activity in soybean genotypes during 10 months of storage.

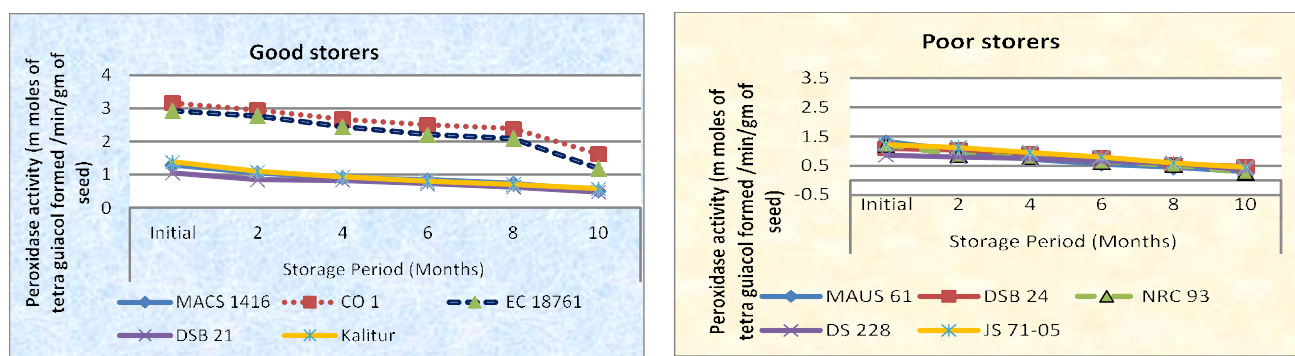


Fig 2: Peroxidase activity in soybean genotypes during 10 months of storage.

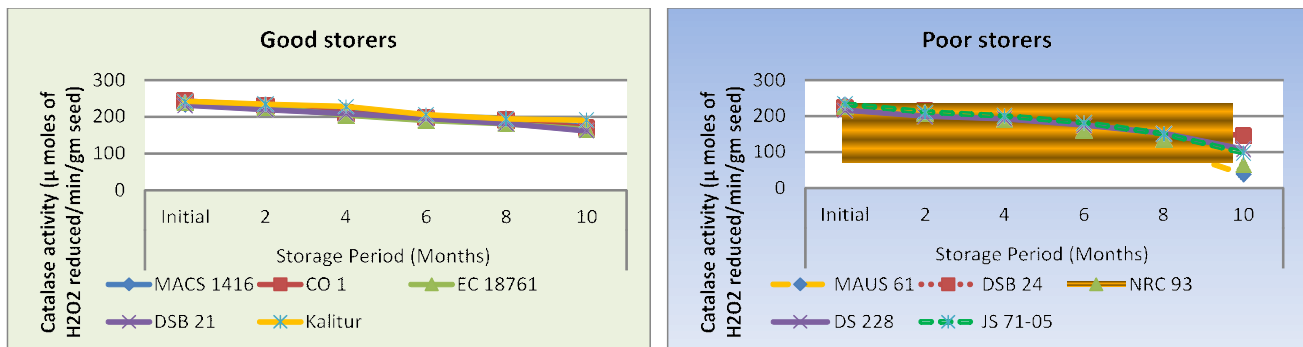


Fig 3: Catalase activity in soybean genotypes during 10 months of storage.

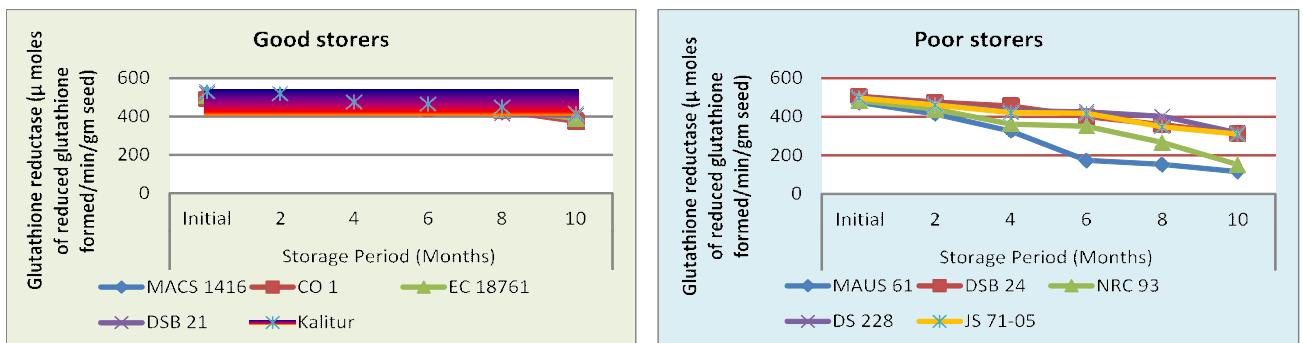


Fig 4: Glutathione reductase activity in soybean genotypes during 10 months of storage.

The protective mechanism within the seeds also involves several free radical scavenging enzymes such as superoxide dismutase (SOD), catalase, peroxidase and glutathione reductase (GR) (Sung, 1996). Superoxide dismutase (SOD) is first in the series of antioxidant system, which mollifies the superoxide radicals (O°), produced during electron transport process, into H_2O_2 and oxygen. It plays an important role in the process of free radical reaction and in seed ageing. Catalase enzyme is essential for the removal of the potentially toxic H_2O_2 produced under various stress conditions and hence, for the avoidance of oxidative-stress-related damage, by breaking hydrogen peroxide molecule to oxygen and water. In the present study, SOD, peroxidase, catalase, GR activity decreased over the storage period and reached 5.08 units, 0.62mmoles, 131.7 μ moles and 320.6 μ moles, respectively irrespective of genotypes from 8.53 units, 1.55mmoles, 232.1 μ moles and 502.9 μ moles, respectively (initial). The decrease in activity of anti-oxidative enzymes was also reported by Scialabba *et al.* (2002); Goel *et al.* (2003), Demirkaya *et al.* (2010) and Abreu *et al.* (2013). The decrease of antioxidative enzymes is linked to increase in peroxidation of lipids as well as to accelerated aging process, with a positive correlation between antioxidant capacity of the enzyme and the vigor of seeds (Bailly *et al.*, 2002).

Kalitur maintained highest SOD (7.51units), catalase (190.6 μ moles) and GR activity (418.7 μ moles) at

the end of storage period, while lowest was recorded in MAUS 61 (1.78 units, 38.8 μ moles and 115.2 μ moles, respectively) (Fig 1, 3 & 4). There was genotypic variation of peroxidase activity in initial as well as at the end of 10 months of storage wherein CO1 and EC 18761 recorded highest activity of 1.63 and 1.19mmoles, respectively. Whereas, all other genotypes shown significantly lesser activity (Fig 2). Loss of seed viability has been reported to be associated with a reduction in the activities of anti-oxidative enzymes *viz.*, SOD, CAT and GR which play a significant role in providing protection against highly reactive free radicals both under accelerated ageing and storage conditions (Kuchlan, 2006; Hosamani *et al.*, 2013).From the present study, it was evident that good storers were characterized by more efficient system of these radical scavenging enzymes activity which might have helped in reducing the level of ROS and thereby slow down the lipid peroxidation process and contributed for good seed storability.

CONCLUSION

Higher activity of anti-oxidative enzymes and lesser activity of lipoxygenase II in good storers might have contributed for lesser lipid peroxidation and which further helped in maintaining viability during storage. Suppression of LOX-II gene expression and enhancement of antioxidant molecules in seeds may be exploited for improving the seed storability in soybean.

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