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# Tocopherols and Antioxidants Assay to Understand the Mechanism of Soybean Seed Longevity

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## ABSTRACT

**Background:** Soybean, an important oilseed crop of India, is rich in protein, oil, vitamins, isoflavones and minerals. Being an oilseed crop and the inherent physiological and biochemical properties, soybean seeds looses itsviability andvigour rapidly during storage, resultinginto poor stand establishment and productivity, upon subsequent planting. The current study aimed to understand the mechanism of antioxidants and antioxidant enzymes in soybean seed longevity.

**Methods:** Ten good storer and ten poor storer, identified after storing for 8 months in ambient laboratory condition, were phenotyped biochemically to understand the mechanism of antioxidants [tocopherols (4 isomers) and isoflavones (12 isomers)] and antioxidant enzymes [catalase (CAT), peroxidise (POX), superoxidedismutase (SOD) and glutathione reductase (GR)]in seed longevity.

**Result:** Good and poor storer genotypes recorded a mean initial germination of 99 and 96%, decreased to 91 and 67%, respectively upon eight months of laboratory ambient storage. Significant differences were observed in the activities of SOD, CAT, GR, alpha (α-T) and delta (δ-T) tocopherol between good and poor storer genotypes. Isomers of tocopherols *ie.* α-T and δ-T were found to beeither negatively (r= -0.55) or positively (r= 0.60) associated with seed longevity, indicated the role of individual tocopherol isomer than those to total tocopherols in seed longevity.

**Key words:** Antioxidants, Enzymes, Isomers, Seed longevity, Soybean, Tocopherols.

## INTRODUCTION

Soybean [*Glycine max* (L.) Merrill], a major oilseed crop in India, also rich in proteins, vitamins, isoflavones and minerals, is very common fulfilling the nutritional requirement of vegetarians. Owing to its high oil and protein contents, the seeds have poor storability. Majority of the soybean are grown in an area having higher temperature and relative humidity, congenial for acceleration of seed deterioration, resulting into poor field stand and productivity upon planting (Singh and Ram, 1986). Though soybean, in general, is among least storable group (Justice and Bass, 1978), existence of genetic variation in storability was reported (Hosamani, 2013). Gowda (2017) reported an association of seed coat colour and seed size (100-seed weight) with seed storability in soybean; black seeded genotypes were better storers. Similarly, association of seed coat colour with seed quality had been reported in chickpea and mungbean crops (Lamichaney *et al.,* 2016; Lamichaney and Katiyar, 2017; Lamichaney *et al.*, 2018). In soybean, free radical mediated or enzymatic peroxidation of PUFA were reported to play an important role in seed deterioration. Production and accumulation of free radicals or reactive oxygen species due to lipid peroxidation caused rapid loss of seed viability and vigour by disturbing cellular integrity (Corbineau *et al.*, 2002). By scavenging the free radicals cell protects itself and maintains cellular integrity. The most important mechanism of scavenging free radicals is via antioxidants (vitamin E or tocopherols, isoflavones) or antioxidant enzymes (SOD, CAT, POX, GR), which lowers the content of free radicals and maintain seed viability and vigour during storage; their role during storage is inconsistent (Hosamani *et al.*, 2013). Therefore, the present study wasconducted to understand the mechanism of antioxidants (isomer) and/or antioxidant enzyme(s) association with seed longevity on chosen soybean genotypes.

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## MATERIALS AND METHODS

### Experimental materials

From the previous investigations (Sooganna *et al.*, 2016), from 59 soybean genotype screened for seed storability [loss

of germination under laboratory ambient storage (average 25±2°C and 65±5% RH)], ten genotypes each from good and poor storers were chosen for further biochemical assays (Table 1). The study was carried out during the period of 2014-2015 in the Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi.

**Standard germination**

One hundred pure seeds placed in between moist germination paper, rolled and covered by blotter paper to avoid moisture loss, were incubated in dark for seven days at 25°C. On eighth day from incubation, individual seeds/ seedlings were examined and categorized into normal seedlings, abnormal seedlings; hard seeds and dead seeds following ISTA rules (Anonymous, 2015). Only the percentage of normal seedlings was considered to calculate germination values.

**Biochemical assay from seeds**

For the assay of antioxidant enzymes, one gram of soybean seeds were homogenized in 100 mM potassium phosphate buffer (pH 7.8) with a pinch of poly vinyl pyrrolidone and centrifuged at 15000 rpm for 10 min at 4°C. The supernatant was collected and used for enzyme assays *viz*. peroxidase (POX), glutathione reductase (GR), superoxidedismutase (SOD) and catalase (CAT). The SOD activity was quantified following Beauchamp and Fridovich (1971). The CAT activity was measured following Aebi (1984). The (POX) activity was assayed (as increase in optical density due to oxidation of guaiacol to tetra-guaiacol) following Castillo *et al*. (1994). The GR activity was determined at 25°C and 340 nm (the rate of NADPH oxidation) following Smith *et al*. (1988). Estimation of tocopherols was done using HPLC (Saha *et al.,* 2013). C30 column was used as the stationary phase. Separation was accomplished by using 100% methanol at a flow rate of 1.0 ml/min for 20 min. Column oven temperature was maintained at 40°C. Resolution of tocopherols was detected at 295 nm using a PDA detector. One gram of finely ground soybean flour was added in 20 ml of acetone and subjected to sonication for 10 min and left overnight for extraction. The sample was filtered using Whatman filter paper and 10 ml acetone was added and again subjected to sonication. The sample was filtered with Whatman filter paper and filtrate was subjected to rotary evaporator. 20 µl of the filtrate was injected into HPLC and allowed to run for 20 min. The amount of different isomers of tocopherols (α, β, γ and δ) present in the sample was estimated by putting the value of peak area of sample into the equation derived from the standard graph and expressed in µg/g sample. Isoflavones were also estimated using HPLC. C18 column was used as a non-polar stationary phase. A binary gradient of 0.1% formic acid in acetonitrile (13%: solvent A) and 0.1% formic acid in water (87%: solvent B) was used as mobile phase, run using the following time programme (% solvent A/solvent B): 0 min (0/100), 5 min (10/90), 20 min (0/100) and 50 min (0/100). The flow rate of mobile phase was 0.75 ml/min for 50 min. Column oven temperature was maintained at 40°C. The resolution of isoflavones was detected at 260 nm using a PDA detector. One gram of finely ground soybean flour was extracted with 20 ml of 80% methanol and subjected to ultra-sonication for 35 min and centrifuged at

**Table 1:** List of soybean seed genotypes and their characteristics.

| Genotypes | Seed colour | Hilum colour | 100-seed weight (g) | Germination (%) | | Storability |
|---|---|---|---|---|---|---|
| | | | | Fresh seeds | Eight months stored seeds | |
| AMSS-34 | Black | Grey | 6.5 | 100 | 95 | Good storer |
| DS-74 | Black | Grey | 6.7 | 99 | 93 | Good storer |
| EC-13969 | Yellow | Grey | 6.9 | 98 | 92 | Good storer |
| G-2253 | Black | Grey | 6.5 | 99 | 91 | Good storer |
| G-2603 | Black | Grey | 7.2 | 97 | 92 | Good storer |
| G-2614 | Black | Brown | 6.5 | 99 | 90 | Good storer |
| G-2651 | Black | Brown | 6.7 | 97 | 91 | Good storer |
| M-253 | Black | Grey | 6.5 | 98 | 90 | Good storer |
| M-11913 | Black | Grey | 7.0 | 100 | 90 | Good storer |
| MACS-1311 | Yellow | Brown | 6.8 | 98 | 89 | Good storer |
| P-250 | Yellow | Brown | 9.2 | 96 | 70 | Poor storer |
| P-876 | Yellow | Brown | 7.6 | 97 | 68 | Poor storer |
| PS-1480 | Yellow | Brown | 10.0 | 98 | 66 | Poor storer |
| JS(SH)-93-37 | Black | Brown | 11.2 | 95 | 70 | Poor storer |
| DSB-19 | Yellow | Brown | 14.0 | 97 | 65 | Poor storer |
| VLS-81 | Yellow | Brown | 14.0 | 95 | 64 | Poor storer |
| AMS-56 | Yellow | Brown | 12.4 | 95 | 65 | Poor storer |
| MAUS 608 | Yellow | Brown | 14.2 | 98 | 66 | Poor storer |
| KBS-2011 | Yellow | Grey | 12.7 | 95 | 68 | Poor storer |
| DS-2708 | Yellow | Brown | 12.5 | 98 | 69 | Poor storer |

13,000 rpm for 5 min. The supernatant was filtered first with mira cloth and then with 5 µ filter to get a clear extract and subjected to rotary evaporator for 3-5 min. Methanol was added on the extract to make up the volume to 5 ml. 20 µl of this sample extract was injected into the HPLC column inlet and allowed to run for 50 min. The amount of different isomers of isoflavones present in the sample was estimated by putting the values of peak area of sample into the equation derived from the standard graph and expressed in µg/g sample. The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content (Ohkawa *et al.,* 1979).

To normalize the data, the per cent germination values were subjected to arcsine transformation and statistical analysis was done using SPSS software.

## RESULTS AND DISCUSSION

All the studied twenty genotypes had an initial germination ≥95%; of these, only good storer, but eight months stored genotypes (seeds), maintained higher germination value >89% with a mean germination value of 91.3%. In contrast, the germination values among poor storer, also eight months stored genotypes (seeds), declined drastically (<70%) with a mean value of 67.1% (Table 1). In general, good storer genotypes were black with exceptions [EC-13969 and MACS-1311 (yellow)] and smaller in size with a mean 100-seed weight of 6.7 g, whereas the poor storer genotypes were yellow with an exception [JS(SH)-93-37 (black)] andlarge with amean 100-seed weight of 11.8 g (Table 1). Similar association of seed storability with seed colour, seed size and weight had been reported previously by Hosamani (2013) and Gowda (2017) in soybean. Small size seeds have thick and higher proportion of seed coat (Gowda, 2017; Lamichaney *et al.,* 2016; Lamichaney *et al.,* 2017); the interface between the embryo and the external environment helps in maintaining seed viability during storage. Further, pigmentation in seeds, imparted by tannins and phenols (Caldes and Blair, 2009; Gowda, 2017), known to have antimicrobial properties (Farahin *et al*., 2016); higher contents of such compounds in black genotypes protects the seeds from fungal and insect attacks during storage and helps in maintaining seed longevity.

In the present investigation, MDA contents, a product of lipid peroxidation, ranged between 141.9 to 238.7 (nM/g seed) with a mean value of 190.4 (nM/g seed) among good storers, whereas between 225.8 to 434.8 (nM/g seed) with a mean value of 328.1 (nM/g seed) among poor storers (Table 2). A significant, but negative correlation between MDA

**Table 2:** Antioxidant enzymes and volatile aldehydes among soybean genotypes.

| Genotypes | SOD (Units/min/ g seed) | CAT (µmoles $H_2O_2$/ min/g seed) | POX (µmoles/min/ g seed) | GR (µmoles NADPH/ min/g seed) | MDA contents (nM/ g seed) |
|---|---|---|---|---|---|
| **Good storer** | | | | | |
| AMSS-34 | 9.6 | 17.6 | 6.3 | 3.1 | 161.0 |
| DS-74 | 8.4 | 17.1 | 3.2 | 3.0 | 232.3 |
| EC-13969 | 7.5 | 12.3 | 3.3 | 2.5 | 187.1 |
| G-2253 | 9.3 | 14.2 | 4.9 | 2.4 | 171.3 |
| G-2603 | 9.8 | 13.2 | 3.2 | 2.6 | 235.5 |
| G-2614 | 8.4 | 14.3 | 1.5 | 2.0 | 206.8 |
| G-2651 | 9.1 | 18.1 | 4.3 | 2.8 | 164.5 |
| M-253 | 9.4 | 16.2 | 3.3 | 2.5 | 141.9 |
| M-11913 | 9.2 | 16.4 | 4.9 | 2.8 | 164.5 |
| MACS-1311 | 7.8 | 11.5 | 3.4 | 2.2 | 238.7 |
| **Poor storer** | | | | | |
| P-250(129) | 5.3 | 8.6 | 1.3 | 1.0 | 335.5 |
| P-876(146) | 4.8 | 8.2 | 4.0 | 1.6 | 312.9 |
| PS-1480 | 4.1 | 11.4 | 3.1 | 1.4 | 225.8 |
| JS(SH)-93-37 | 6.6 | 11.6 | 5.1 | 1.7 | 290.3 |
| Dsb-19 | 2.7 | 8.6 | 2.7 | 1.2 | 320.3 |
| VLS-81 | 5.3 | 10.3 | 3.9 | 0.8 | 358.1 |
| AMS-56 | 2.7 | 7.1 | 2.8 | 1.3 | 322.6 |
| MAUS 608 | 3.6 | 6.3 | 2.1 | 1.3 | 348.4 |
| KBS-2011 | 5.6 | 7.5 | 4.4 | 1.0 | 434.8 |
| DS-2708 | 3.8 | 8.6 | 2.0 | 1.2 | 332.3 |
| LSD *(P < 0.05)* | 1.4 | 3.4 | 1.3 | 1.0 | 54.0 |
| Mean (Good storer) | 8.8 | 15.1 | 3.8 | 2.6 | 190.4 |
| Mean (Poor storer) | 4.5 | 8.8 | 3.1 | 1.2 | 328.1 |
| **Grand mean** | **6.6** | **12.0** | **3.5** | **1.9** | **259.2** |

content and seed germination [fresh (r= -0.71) and eight months stored seeds (r= -0.84) substantiated the previous statement of lipid peroxidation as a major cause of seed deterioration in soybean (Table 5).

Free radical scavenging by antioxidants *viz.* SOD, CAT, GR, tocopherols and isoflavones, etcalleviate the damage to lipid membranes caused by free radicals and leads to greater seed longevity (Lehner *et al*. 2008; Hameed *et al*., 2014; Gowda, 2017). The present investigation with chosen good and poor storer soybean genotypes validated the role of antioxidants. Marked differences were observed between good and poor storer genotypes in SOD activity, ranged between 7.5 Units/min/g seed (EC-13969) to 9.8 Units/min/g seed (G-2603) with a mean value of 8.8 Units/min/g seed among good storer genotypes, whereas 2.7 Units/min/g seed (Dsb 19 and AMS-56) to 6.6 Units/min/g seed [JS(SH)-93-37] with a mean value of 4.5 Units/min/g seed among poor storer genotypes (Table 2). These findings were further substantiated by a significant and strong positive association (r= 0.93) between SOD activity and germination from eight months stored seeds (Table 5). Likewise, the CAT activities among good storer genotypes were higher >11 µmoles $H_2O_2$/min/g seedwith a mean value of 15.1 µmoles $H_2O_2$/min/g seed, whereas among poor storer genotypes were lower <10 µmoles $H_2O_2$/min/g seed with exceptions [PS-1480, JS(SH)-93-97 and VLS-81] having a mean value of 8.8 µmoles $H_2O_2$/min/g seed (Table 2). Catalase also showed a positive association (r= 0.86) with germination from eight months stored seeds (Table 5). Peroxidase activities, among good storer genotypes, ranged between 1.5 to 6.3 µmoles/min/g seed with a mean value of 3.8 µmoles/min/g seed, whereas among poor storers ranged between 1.3 to 5.2 µmoles/min/g seed with a mean value of 3.1 µmoles/min/g seed (Table 2). The activities of GR ranged between 2.0 to 3.1 µmoles NADPH/min/g seed and 0.8 to 1.7µ moles NADPH/min/g seed among good and poor storer genotypes, respectively (Table 2). GR activity also showed a positive and strong association (r= 0.94) with seed germination from eight months stored seeds (Table 5).

Tocopherols are known to have a free radical scavenging potential owing to its chromanol ring (Liebler, 1993). The content of total tocopherols and its different homologues derived are given (Table 3). The dominant tocopherol in soybean genotypes, as recorded, were γ-T, accounted for 69% of the total tocopherols, followed by δ-T (29%), α-T (0.9%) and α-T (0.4%). Chen *et al*. (2015) had reported the relation of total tocopherols with soybean seed longevity, whereas the present findings had focused on the

**Table 3:** Tocopherol and its homologues among soybean genotypes.

| Genotypes | Tocopherol and its homologues (µg/g seed) | | | | |
|---|---|---|---|---|---|
| | α-T | β-T | γ-T | δ-T | Total tocopherols |
| **Good storer** | | | | | |
| AMSS-34 | 1.1 | 0.5 | 282.3 | 132.4 | 416.3 |
| DS-74 | 2.7 | 1.6 | 268.5 | 155.2 | 427.9 |
| EC-13969 | 1.8 | 0.6 | 266.6 | 129.5 | 398.6 |
| G-2253 | 1.7 | 0.7 | 260.5 | 117.4 | 380.3 |
| G-2603 | 2.4 | 0.6 | 332.4 | 188.0 | 523.3 |
| G-2614 | 2.7 | 0.9 | 270.6 | 146.8 | 421.0 |
| G-2651 | 2.8 | 0.5 | 278.6 | 130.6 | 412.4 |
| M-253 | 0.6 | 3.5 | 144.8 | 92.9 | 241.7 |
| M-11913 | 0.0 | 1.4 | 104.3 | 68.2 | 174.0 |
| MACS-1311 | 2.2 | 1.4 | 275.6 | 102.7 | 381.9 |
| **Poor storer** | | | | | |
| P-250(129) | 7.7 | 0.6 | 311.5 | 109.7 | 429.5 |
| P-876(146) | 0.4 | 0.2 | 70.2 | 49.6 | 120.4 |
| PS-1480 | 0.5 | 3.6 | 96.4 | 62.6 | 163.0 |
| JS(SH)-93-37 | 5.1 | 1.7 | 282.0 | 107.5 | 396.3 |
| Dsb-19 | 3.7 | 0.6 | 353.9 | 110.0 | 468.2 |
| VLS-81 | 7.0 | 1.7 | 295.9 | 93.3 | 397.9 |
| AMS-56 | 6.7 | 0.5 | 356.9 | 107.5 | 471.5 |
| MAUS 608 | 4.5 | 0.7 | 343.7 | 100.0 | 449.0 |
| KBS-2011 | 2.8 | 4.1 | 154.1 | 52.5 | 213.6 |
| DS-2708 | 10.0 | 1.1 | 272.6 | 65.9 | 349.6 |
| LSD *(P < 0.05)* | 2.3 | 1.2 | 6.2 | 4.6 | 5.9 |
| Mean (Good storer) | 1.8 | 1.2 | 248.4 | 126.4 | 377.7 |
| Mean (Poor storer) | 4.8 | 1.5 | 253.7 | 85.9 | 345.9 |
| **Grand mean** | **3.3** | **1.3** | **251.1** | **106.1** | **361.8** |

**Table 4:** Isoflavone and its isomers among soybean genotypes.

| Genotypes | Isoflavone and its isomers (µg/g seed) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Daidzin | Glycitin | Genistin | Malony ldaidzin | Malony lglycitin | Malony lgenistin | Acetyl diadzin | Acetyl glycitin | Acetyl genistin | Daidzein | Glycitein | Genistein | Total isoflavones |
| **Good storer** | | | | | | | | | | | | | |
| AMSS-34 | 16.8 | 8.1 | 26.9 | 28.7 | 8.7 | 59.7 | 5.8 | 1.0 | 2.1 | 0.9 | 0.5 | 0.9 | 160.1 |
| DS-74 | 35.9 | 3.8 | 53.4 | 24.8 | 2.3 | 43.4 | 3.9 | 0.2 | 1.4 | 0.0 | 0.3 | 0.5 | 169.8 |
| EC-13969 | 12.4 | 4.8 | 1.3 | 24.5 | 4.9 | 47.4 | 3.3 | 0.6 | 1.6 | 0.1 | 0.5 | 0.5 | 101.7 |
| G-2253 | 18.7 | 7.4 | 42.3 | 11.8 | 3.5 | 31.4 | 2.2 | 0.2 | 1.5 | 0.1 | 0.2 | 0.7 | 119.8 |
| G-2603 | 26.2 | 4.5 | 32.6 | 50.2 | 4.6 | 74.9 | 5.1 | 1.4 | 1.8 | 0.0 | 0.6 | 0.7 | 202.7 |
| G-2614 | 19.9 | 1.6 | 30.0 | 32.4 | 1.9 | 64.1 | 4.8 | 0.5 | 1.8 | 0.0 | 0.1 | 0.6 | 157.7 |
| G-2651 | 13.0 | 6.2 | 125.4 | 24.1 | 7.0 | 52.2 | 3.5 | 1.1 | 1.8 | 0.2 | 0.4 | 0.5 | 235.2 |
| M-253 | 19.8 | 1.8 | 22.8 | 35.3 | 2.3 | 65.3 | 4.2 | 0.4 | 1.1 | 0.0 | 0.2 | 0.8 | 154.1 |
| M-11913 | 19.6 | 5.6 | 29.7 | 39.9 | 6.6 | 76.5 | 6.6 | 0.4 | 2.1 | 0.2 | 0.4 | 0.8 | 188.4 |
| MACS-1311 | 21.1 | 3.8 | 48.2 | 31.7 | 4.3 | 88.7 | 5.8 | 0.9 | 1.2 | 0.0 | 0.1 | 0.7 | 206.3 |
| **Poor storer** | | | | | | | | | | | | | |
| P-250(129) | 19.2 | 2.5 | 39.9 | 27.2 | 3.2 | 68.0 | 5.9 | 1.6 | 1.7 | 0.1 | 0.5 | 1.0 | 170.7 |
| P-876(146) | 27.7 | 4.2 | 44.1 | 40.2 | 4.7 | 94.1 | 6.3 | 1.0 | 1.6 | 0.0 | 0.1 | 1.1 | 225.1 |
| PS-1480 | 25.4 | 2.5 | 33.9 | 42.5 | 2.1 | 75.4 | 0.9 | 0.0 | 1.4 | 0.0 | 5.6 | 0.9 | 190.5 |
| JS(SH)-93-37 | 29.9 | 3.2 | 35.0 | 41.6 | 5.9 | 51.9 | 0.8 | 0.0 | 2.2 | 0.0 | 0.5 | 0.0 | 170.9 |
| Dsb-19 | 12.2 | 6.1 | 29.7 | 14.8 | 5.4 | 47.6 | 4.1 | 1.4 | 1.5 | 0.0 | 0.5 | 0.7 | 124.1 |
| VLS-81 | 13.6 | 6.4 | 34.2 | 23.1 | 4.8 | 75.4 | 4.9 | 0.0 | 2.0 | 0.0 | 0.0 | 0.6 | 165.0 |
| AMS-56 | 11.8 | 5.0 | 32.1 | 15.2 | 4.8 | 59.8 | 4.8 | 1.9 | 1.7 | 0.1 | 0.3 | 1.1 | 138.6 |
| MAUS 608 | 15.1 | 20.3 | 11.8 | 16.1 | 4.0 | 27.5 | 24.0 | 1.0 | 0.9 | 0.1 | 0.4 | 0.3 | 121.5 |
| KBS-2011 | 19.2 | 2.5 | 23.9 | 27.1 | 2.9 | 81.7 | 5.5 | 0.6 | 1.6 | 0.0 | 0.2 | 0.4 | 165.6 |
| DS-2708 | 13.1 | 4.4 | 22.7 | 21.5 | 4.2 | 47.8 | 3.2 | 0.6 | 1.0 | 0.0 | 0.3 | 0.3 | 119.1 |
| LSD *(P<0.05)* | 3.2 | 2.5 | 6.3 | 4.2 | 2.3 | 4.3 | 2.2 | 0.9 | 0.6 | 0.1 | 0.2 | 0.2 | 3.0 |
| Mean (Good storer) | 20.3 | 4.7 | 41.3 | 30.3 | 4.6 | 60.4 | 4.5 | 0.7 | 1.6 | 0.1 | 0.3 | 0.7 | 169.6 |
| Mean (Poor storer) | 18.7 | 5.7 | 30.7 | 26.9 | 4.2 | 62.9 | 6.0 | 0.8 | 1.6 | 0.0 | 0.8 | 0.7 | 159.1 |
| **Grand mean** | **19.5** | **5.2** | **36.0** | **28.6** | **4.4** | **61.6** | **5.3** | **0.8** | **1.6** | **0.1** | **0.6** | **0.7** | **164.3** |

proportions of individual tocopherol homologues among different soybean genotypes having contrasting longevity. Poor storers recorded more tocopherol contents of α-T, β-T and γ-T than those to good storers, which recorded higher δ-T and total tocopherols. α-T, β-T, γ-T and δ-T among poor storers recorded a mean values of 4.8, 1.5, 253.7 and 85.9µg/g seed, whereas 1.8, 1.2, 248.4 and 126.4 µg/g seed among good storers. Total tocopherols among good storers ranged between 174.0 (M-11913) to 523.3 µg/g seed (G-2603) with a mean value of 377.7 µg/g seed and among poor storers between 120.4 [P-876(146)] to 468.2 µg/g seed (Dsb-19) with a mean value of 345.9 µg/g seed (Table 3). None of the tocopherol homologues could establish a significant correlation with germination in fresh seeds, whereas the germination from eight months stored seeds recorded a negative association (r = -0.55) with α-T and positive association (r= 0.60) with δ-T (Table 5), implying its role in maintaining seed longevity. A similar negative association of α-T with seed longevity was reported by Lee *et al*. (2017) in rice. Mutants with faulty tocopherol cycle in *Arabidopsis* failed to maintain seed viability for longer period due to its inability to scavenge the free radicals produced as a result of lipid peroxidation (Sattler *et al*. 2004). Kamal-Eldin and Appelqvist (1996) demonstrated the role of α-tocopherol to have a strong antioxidant capacity,whereas, Jiang (2014) and Kim (2014) reported γ- and δ-tocopherolsto have better antioxidant activities than those to α-tocopherol. In the present study, the association of tocopherols with seed longevity was found to be inconsistent,as some reports showed correlation of tocopherol with seed longevity and some did not (Priestley *et al.* 1980; Simontacchi *et al*. 1993; Sattler *et al*., 2004).

**Table 5:** Correlation coefficients between seed longevity (germination%) and various biochemical parameters.

| Parameter | Germination (%) | |
|---|---|---|
| | Fresh seeds | Eight months stored seeds |
| **Antioxidant enzyme** | | |
| SOD | 0.52 | **0.93*** |
| Catalase | 0.61 | **0.86*** |
| Peroxidase | 0.13 | 0.31 |
| GR | 0.69 | **0.94*** |
| **Volatile aldehyde** | | |
| MDA | -0.71 | **-0.84*** |
| *Tocopherol* | | |
| α-T | -0.52 | **-0.55*** |
| β-T | -0.17 | -0.17 |
| γ-T | -0.22 | -0.03 |
| δ-T | 0.19 | **0.60*** |
| Total tocopherols | -0.12 | 0.15 |
| **Isoflavone** | | |
| Daidzin | 0.11 | 0.18 |
| Glycitin | 0.17 | -0.14 |
| Genistin | -0.07 | 0.22 |
| Malonyldaidzin | 0.05 | 0.20 |
| Malonylglycitin | 0.06 | 0.15 |
| Malonylgenistin | -0.22 | -0.09 |
| Acetyl diadzin | 0.10 | -0.19 |
| Acetyl glycitin | -0.21 | -0.12 |
| Acetyl genistin | -0.17 | 0.14 |
| Daidzein | 0.43 | 0.37 |
| Glycitein | 0.08 | -0.24 |
| Genistein | 0.13 | 0.01 |
| Total isoflavones | -0.09 | 0.15 |

Isoflavones are reported to have various physiological properties like antioxidants, anti-inflammatory, anti-tumour, and anti-aging (Wang *et al*., 2013). Twelve different isomers of Isoflavones were found in soybean namely,daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin and malonylglycitin. Genetic variation with isoflavones was reported in soybean, influenced by environment and storage period (Lee *et al*., 2003). In the present investigation, Isoflavones among good storer genotypes ranged between 102.1 to 236 µg/g seedwith a mean value of 170.1 µg/g seed, whereas among poor storer genotypes it ranged between 119.5 to 225.8 µg/g seed with a mean value of 159.6 µg/g seed (Table 4). None of the derived isoflavone isomers showed any significant association with seed longevity.

## CONCLUSION

The antioxidant enzymes *viz*. SOD, CAT and GR play the pivotal role in maintaining seed longevity. α-T, the most bio-active isomer of tocopherols, was negatively associated, where as δ-T was positively associated with seed longevity; inferred that the role of individual isomerof tocopherols rather than the total tocopherols in maintaining soybean seed longevity.

### Conflict of interest

All authors declared that there is no conflict of interest.

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