



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 lose its viability and vigour rapidly during storage, resulting into 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), peroxidase (POX), superoxide dismutase (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 be either 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, Soybean, Seed longevity, 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

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et al., 2013). Therefore, the present study was conducted to understand the mechanism of antioxidants (isomer) and/or antioxidant enzyme(s) association with seed longevity on chosen soybean genotypes.

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 $25\pm 2^\circ\text{C}$ and $65\pm 5\%$ 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 (Anon, 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 100mM potassium phosphate buffer (pH 7.8) with a pinch of poly vinyl pyrrolidone and centrifuged at 15000rpm for 10min at 4°C . The supernatant was collected and used for enzyme assays viz. peroxidase (POX), glutathione reductase (GR), superoxide dismutase (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 340nm (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 20min. Column oven temperature was maintained at 40°C . Resolution of tocopherols was detected at 295nm using a PDA detector. One gram of finely ground soybean flour was added in 20ml of acetone and subjected to sonication for 10min and left overnight for extraction. The sample was filtered using Whatman filter paper and 10ml 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 20min. 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 $\mu\text{g/g}$ 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): 0min (0/100), 5min (10/90), 20min (0/100) and 50min (0/100). The flow rate of mobile phase was 0.75ml/min for 50min. Column oven temperature was maintained at 40°C . The resolution of isoflavones was detected at 260nm using a PDA detector. One gram of finely ground soybean flour was extracted with 20ml of 80% methanol and subjected to ultra-sonication for 35min 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

13,000rpm for 5min. The supernatant was filtered first with mira cloth and then with 5i filter to get a clear extract and subjected to rotary evaporator for 3-5min. Methanol was added on the extract to make up the volume to 5ml. 20il of this sample extract was injected into the HPLC column inlet and allowed to run for 50min. 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 ig/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 percent 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 $\geq 95\%$; 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 6.7g, whereas the poor storer genotypes were yellow with an exception [JS(SH)-93-37 (black)] and large with a mean 100-seed weight of 11.8g (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(μ moles H_2O_2 /min/g seed)	POX (μ moles/min/g seed)	GR(μ moles 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 (poorstorer)	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, etc alleviate 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 $>11 \mu\text{moles H}_2\text{O}_2/\text{min/g}$ seed with a mean value of $15.1 \mu\text{moles H}_2\text{O}_2/\text{min/g}$ seed, whereas among poor storer

genotypes were lower $<10 \mu\text{moles H}_2\text{O}_2/\text{min/g}$ seed with exceptions [PS-1480, JS(SH)-93-97 and VLS-81] having a mean value of $8.8 \mu\text{moles H}_2\text{O}_2/\text{min/g}$ 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 $6.3 \mu\text{moles/min/g}$ seed with a mean value of $3.8 \mu\text{moles/min/g}$ seed, whereas among poor storers ranged between 1.3 to $5.2 \mu\text{moles/min/g}$ seed with a mean value of $3.1 \mu\text{moles/min/g}$ seed (Table 2). The activities of GR ranged between 2.0 to $3.1 \mu\text{moles NADPH/min/g}$ seed and 0.8 to $1.7 \mu\text{moles NADPH/min/g}$ 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 ($\mu\text{g/g}$ seed)				Total tocopherols
	α -T	β -T	γ -T	δ -T	
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)											Total isoflavones										
	Daidzin	Glycitin	Genistin	Malony daidzin	Malony Iglycitin	Malony Igenistin	Acetyl diadzin	Acetyl glycitin	Acetyl genistin	Daidzein	Glycitein		Genistein									
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	0.9	0.5	0.9	0.5	0.9	0.9	0.5	0.9	0.5	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	0.5	0.3	0.5	0.3	0.5	0.5	0.3	0.5	0.3	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	0.5	0.5	0.5	0.5	0.5	0.5	0.5	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	0.7	0.2	0.7	0.2	0.7	0.7	0.2	0.7	0.2	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	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6	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	0.6	0.1	0.6	0.1	0.6	0.6	0.1	0.6	0.1	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	0.5	0.4	0.5	0.4	0.5	0.5	0.4	0.5	0.4	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	0.8	0.2	0.8	0.2	0.8	0.8	0.2	0.8	0.2	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	0.8	0.4	0.8	0.4	0.8	0.8	0.4	0.8	0.4	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	0.7	0.1	0.7	0.1	0.7	0.7	0.1	0.7	0.1	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	1.0	0.5	1.0	0.5	1.0	1.0	0.5	1.0	0.5	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	1.1	0.1	1.1	0.1	1.1	1.1	0.1	1.1	0.1	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	0.9	5.6	0.9	5.6	0.9	0.9	5.6	0.9	5.6	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	0.0	0.5	0.0	0.5	0.0	0.0	0.5	0.0	0.5	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	0.7	0.5	0.7	0.5	0.7	0.7	0.5	0.7	0.5	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	0.6	0.0	0.6	0.0	0.6	0.6	0.0	0.6	0.0	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	1.1	0.3	1.1	0.3	1.1	1.1	0.3	1.1	0.3	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	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.3	0.4	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	0.4	0.2	0.4	0.2	0.4	0.4	0.2	0.4	0.2	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	0.3	0.3	0.3	0.3	0.3	0.3	0.3	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	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	3.0
Mean (goodstorer)	20.3	4.7	41.3	30.3	4.6	60.4	4.5	0.7	1.6	0.1	0.3	0.7	0.7	0.3	0.7	0.3	0.7	0.7	0.3	0.7	0.3	169.6
Mean (poorstorer)	18.7	5.7	30.7	26.9	4.2	62.9	6.0	0.8	1.6	0.0	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.8	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	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6	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 85.9 $\mu\text{g/g}$ seed, whereas 1.8, 1.2, 248.4 and 126.4 $\mu\text{g/g}$ seed among good storers. Total tocopherols among good storers ranged between 174.0 (M-11913) to 523.3 $\mu\text{g/g}$ seed (G-2603) with a mean value of 377.7 $\mu\text{g/g}$ seed, and among poor storers between 120.4 [P-876(146)] to 468.2 $\mu\text{g/g}$ seed (Dsb-19) with a mean value of 345.9 $\mu\text{g/g}$ 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

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*
<i>Tocopherol</i>		
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 daidzin	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

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 -tocopherol to 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).

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 $\mu\text{g/g}$ seed with a mean value of 170.1 $\mu\text{g/g}$ seed, whereas among poor storer genotypes it ranged between 119.5 to 225.8 $\mu\text{g/g}$ seed with a mean value of 159.6 $\mu\text{g/g}$ 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, whereas δ -T was positively associated with seed longevity; inferred that the role of individual isomer of tocopherols rather than the total tocopherols in maintaining soybean seed longevity.

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