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Study of free radical and peroxide scavenging enzymes and content in different vigour lots of soybean (*Glycine max*)

T.N. Usha*[1] and Malavika Dadlani

Indian Agriculture Research Institute,
New Delhi-110 012, India.

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ABSTRACT

Free radical and lipid peroxidation are widely considered to be major contributor to seed deterioration in soybean. Three lots of soybean high, medium and low vigour were stored under ambient storage conditions. Free radical scavenging enzymes such as superoxide dismutase, catalase, glutathione reductase activities and ascorbic acid contents were measured for a year with three months interval. There was gradual increase in the activities of glutathione reductase and catalase reaching a peak earliest in the low vigour seed lots than in medium and high vigour lots during storage, followed by a steady decline. Similar trend was noticed incase of ascorbic acid content. This is the first such report (of rise in the enzyme activity during seed aging) which needs detailed examination for validation.

Key words: Ascorbic acid content, Free radical scavenging enzymes, Soybean, Storage.

INTRODUCTION

Loss of seed vigour and viability is highly dependent on temperature and seed moisture content (Roberts 1972, Priestley 1986), and may be associated with various biochemical and metabolic alterations that could result in loss of membrane integrity, and impaired RNA and protein synthesis and ATP production (Priestley 1986). Free radicals and lipid peroxidation are widely considered to be major contributors to seed deterioration in soybean (Priestley 1986, Wilson and McDonald 1986a). They generate changes in unsaturated fatty acids that affect the structure and functional properties of cell membranes, such as the inactivation of membrane bound proteins, an increase in membrane permeability there by loss of vigour and viability (Simon 1974). Kang and Saltveit, (2002) reported that high vigour cucumber radical had high catalse and ascorbic acid peroxidase activity in contrast to low vigour radical had super oxide dismutase and glutathione reductase.

Cellular damage caused by lipid peroxidation might be reduced or prevented by protective mechanisms involving free radical and peroxide scavenging enzymes Such as superoxide dismutase (SOD), catalase and glutathione reductase (GR). SOD is generally considered as a key enzyme in the regulation of intracellular concentrations of superoxide radical (O_2^{-}) and peroxides, which can react in the Haber-Weiss reaction to form hydroxyl radicals leading to lipid peroxidation. Catalase is implicated in removal of hydrogen peroxide (Fridovich, 1986). Similar to catalase, GR could also play a part in the control of endogenous hydrogen peroxide through an oxido-reduction cycle involving glutathione and ascorbate (Smith *et al*., 1989). Its role in H_2O_2 detoxication has been demonstrated in plants (Foyer and Halliwell, 1976). Moreover, it has been shown to protect membranes from peroxidation damage by trapping oxygen radicals (Barclay, 1988).

Soybean contains about 30-40% proteins, 30-40% carbohydrates and 20% oil of which approximately 60% are polyunsaturated fatty acids (PUFA) content, it is liable to rapid degradation making it a poor storer. Peroxidation of PUFA is considered to be one of the major causes of the rapid loss of vigour and viability (Wilson and McDonald, 1986a; Vijay and Dadlani, 2003). This loss of seed vigour and viability are more rapid in subtropical countries as compared to temperate environment, the conditions of high temperature and humidity in subtropical countries such as India make it difficult to produce quality seeds and maintain their viability during storage. Hence, the present study was undertaken to know activities of free radical and peroxide scavenging enzymes in three different vigour lots of soybean seed.

MATERIALS AND METHODS

This investigation was carried out at Division of Seed Science and Technology, IARI, New Delhi during 2008-2009. About 1000 g of GP 2601 soybean seeds of each low, medium and high vigour lots were packed in cloth bags stored under ambient condition in the laboratory. Different vigour lots of soybean seeds were obtained by accelerated ageing test (Anonymous 2004) Samples were drawn at every three months interval up to twelve months and the following enzyme activities were monitored.

*Corresponding author's e-mail and present address: drushatn@gmail.com; [1]Seed Unit, University of Agrilcultural and Horticultural Science, Shivamogga-577 225, Karnataka, India.

Super oxide dismutase activity was assayed according to Beauchamp and Fridovich (1971) with some minor modifications. Enzyme extraction carried out as following One gram of seed was ground removing the seed coat and homogenized in 15 ml of 100 mM Potassium phosphate buffer (pH 7.8) with a pinch of PVP. The extract was centrifuged at 15000 g for 10 min at 4^0C. The supernatant was collected and used as enzyme extract. Reaction mixture contained 1 ml of 100 mM potassium phosphate buffer (pH 7.8); 100 µl of 2.25 mM NBT; 100 µl of 3 mM EDTA; 200 µl of 200 mM L-methionine and 1.75 ml distilled water; 200 µl enzyme extract and 150 µl of 0.075 mM riboflavin were mixed in test tubes and swirled well. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. The glass tube containing the mixture were immersed in a thermostat bath at 25° C and illuminated with a fluorescent lamp. Identical tubes which were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of SOD was defined as the enzyme activity which inhibited the photoreduction of NBT to blue farmazan by 50%, and SOD activity of the extracts was expressed as units SOD/ mg seed.

Glutathione reductase activity was assayed according to the procedure described by Mavis and Stellwagen (1968), by following the rate of NADPH oxidation at 340 nm. The assay mixture contained 3.4 mM EDTA in 100mM Potassium Phosphate buffer, 30 mM Glutathion , 0.8 mM b Nicotinamide adenine dinucleotide phosphate, 1% Bovine Serum Albumine and 100 ml of enzyme extract in total volume of 3 ml. GR activity was expressed as nmol NADPH oxidised /mg seed/min.

Catalase activity was assayed following the method of Bailly *et al*., (1996) with minor modifications. Reaction mixture contained 3 ml reaction mixture contained 200 µl enzyme extract and 2.8 ml hydrogen peroxide-phosphate buffer. Catalse activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm and was expressed as nmol H_2O_2 decomposed/mg seed/min.

Ascorbic acid content was assayed according to the procedure described by Sadasivam and Balasubraminan (1987). A 10-100 ìg standard dehydroascorbic solution was pipetted out into a series of tubes. Similarly, different aliquot (0.1 ml- 2 ml) of brominated sample extract was pipetted out. The volume was made up in each tube to 3 ml by adding distilled water. One ml of DNPH reagent was added, followed by 1-2 drops of thiourea to each tube. A blank was set as above but with water in place of ascorbic acid solution. The contents of the tubes were mixed thoroughly and incubated at 37^0 C for 3 hr. After incubation, the orange red osazone crystals formed after dissolved by adding 7 ml of 80% sulphuric acid. The absorbance was recorded at 540 nm. A standard curve of ascorbic acid concentrations versus absorbance was plotted and the ascorbic acid content in the sample was determined in the test solutions.

RESULTS AND DISCUSSIONS

SOD dismutates (mollifies) the superoxide radicals which were produced during electron transport processes into H_2O_2 and oxygen. SOD act as antioxidant against the free radicals. High vigour seed lot in soybean recorded the highest SOD activity (i.e., 0.190 enzyme units/ mg seed) as compared to medium (i.e., 0.184 enzyme units/ mg seed) and low (i.e., 0.140 enzyme units/ mg seed) vigour seed lots (Table 1). As the storage period progressed the SOD activity increased initially and then reduced significantly in all vigour lots.

Catalase activity is essential for the removal of the potentially toxic H_2O_2 produced under various stress conditions and then for the avoidance of oxidativestress-related damage. Catalase dismutates H_2O_2 into water and oxygen. In mature seeds, it is located mainly in glyoxysomes. A peak catalase activity was noticed (36.88 nmol H_2O_2/ mg seed /min) initially in soybean (Table 1). Later its activity declined to 28.53 (nmol H_2O_2/ mg seed /min). High vigour soybean seed lot recorded significantly higher activity i.e., 48.50 (nmol H_2O_2/ mg seed /min) than the medium and low vigour seed lots.

TABLE 1: Activity of SOD and Catalse in different vigour lots of soybean during ambient storage

Storage period	SOD (Enzyme Units/ mg seed)				Catalase (nmol H_2O_2/ mg seed / min)			
	High vigour	Medium vigour	Low vigour	Mean	High vigour	Medium vigour	Low vigour	Mean
Initial	0.090	0.100	0.120	0.103	33.00	31.63	30.50	31.71
3 months	0.130	0.140	0.160	0.143	39.25	36.38	35.00	36.88
6 months	0.133	0.270	0.240	0.214	54.62	49.00	48.25	50.63
9 months	0.365	0.320	0.130	0.272	67.00	55.35	31.00	51.12
12 months	0.230	0.090	0.050	0.123	48.63	21.48	15.50	28.53
Mean	0.190	0.184	0.140		48.50	38.77	32.05	
			CD (P=0.05)					
Storage period (SP)	0.0518				0.36			
Lots (L)	0.0401				1.05			
SP x L	0.090				2.35			

TABLE 2: Activity of glutathione reductase and ascorbic acid content in different vigour lots of soybean upon ambient storage

Storage period	Glutathione reductase (nmol NADPH/ mg seed/min)				Ascorbic acid content (µg/g seed)			
	High vigour	Medium vigour	Low vigour	Mean	High vigour	Medium vigour	Low vigour	Mean
Initial	23.25	21.50	22.00	22.25	18.50	19.13	19.38	19.00
3 months	27.35	25.50	26.63	26.49	23.00	24.50	25.00	24.17
6 months	40.50	39.00	38.00	39.17	27.50	28.00	28.00	27.83
9 months	49.00	32.38	20.75	34.04	33.38	31.38	20.50	28.42
12 months	35.38	12.00	8.25	18.54	28.00	17.00	11.50	18.83
Mean	35.10	26.08	23.13		26.08	24.00	20.88	
			CD (P=0.05)					
Storage period (SP)	1.13				0.83			
Lots (L)	0.88				0.64			
SP x L	1.96				1.43			

GR take part in the control of endogenous hydrogen peroxide through an oxido-reduction cycle involving glutathione and ascorbate. A peak glutathione reductase activity (39.17 nmol NADPH/ mg seed /min) was recorded during six month of ambient storage. After six months of storage there was a gradual reduction in GR activity in medium and low vigour lots (Table 2). High vigour seed lot recorded significantly higher GR activity i.e., 35.10 (nmol NADPH/ mg seed /min) than the medium and low vigour seed lots.

Ascorbic acid is a non enzymatic antioxidant, which is used by a number of enzyme complexes for their catalytic activity. ASC peroxidase is probably the best known, due to its ability to use ascorbic acid specifically to remove hydrogen peroxide. Ascorbic acid content was significantly higher in high vigour seed lot as compared to the medium and low vigour seed lots (Table 2). In the beginning, ascorbic acid content was low in unaged seed, but it increased upto nine months and reduced subsequently by 12 months. Initially the ascorbic acid content was similar in all seed lots irrespective of vigour, the pattern remain same up to six months. A peak ascorbic acid content (28 µg/ g seed) was noted in low vigour seed lot at 6 months of storage, whereas in high and medium vigour lots peak were noted at nine months with ascorbic acid contents of 33.38 µg/ g of seed and 31.38 µg/ g seed, respectively.

There was a gradual increase in the activities of GR and catalase enzymes, reaching a peak earliest (6 months) in the low and medium vigour seed lots, than in high vigour lot (9 months) during storage. This was then followed by a steady decline. A similar trend was also recorded for ascorbic acid contents of high, medium and low vigour seed lots. Function of ascorbic acid in seeds is likely to be related to its action as a specific co-substrate for the activity of dioxygenases, in addition to its role in antioxidation defence. De'Tullio and Arrigoni, (2003) commented that the relationship between mitochondrial activity and ASC biosynthesis and the role of ASC in seed desiccation and oligosaccharide content (which has direct implications in seed longevity) need to be studied further. On the other hand, Bailly *et a*l., (1998) reported significant reductions in SOD, CAT and GR activities in oil-rich sunflower seeds during accelerated ageing, and restoration of activities by priming. However, it is becoming more and more evident (Priestley *et al*., 1985; Powell and Harman, 1985; Vijay *et al*., 2009) that the processes leading to the loss of viability may be different in artificially and naturally aged seeds.

This is the first such report (of rise in the enzyme activity during seed ageing), which needs detailed examination for validation. Whether the rise in antioxidative enzymes and antioxidant is some kind of defence signal for protecting the seed from loss of viability, needs to be studied.

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