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Enzyme changes during seed storage in groundnut (*Arachis hypogaea* L.)

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Abstract: A change in enzyme activity in seeds due to ageing is a topic of scientific importance. Vigour is essentially a physiological phenomenon influenced by the reserved metabolites, enzyme activities and growth regulators. The exact cause of loss of seed vigour and viability is still unknown as deterioration of seed is a complex process. In the presence of oxygen, ageing of seed can lead to peroxidative changes in polyunsaturated fatty acids. The free radical -induced non-enzymatic peroxidation, which has the potential to damage membrane, is likely to be a primary cause of deterioration of stored seeds. Certain anabolic enzymes help in maintaining viability while some catabolic enzymes decrease viability. The seed catalase and peroxidase activity seem to be decreased during storage. The results revealed that the peroxidase enzyme activity decreased from 0.236 to 0.444 OD 10 min^{-1} when storage period increased. A decrease in catalase activity from 0.454 to 0.444 µg H_2O_2 mg^{-1} min^{-1} followed by a small increase from 0.434 to 0.452 µg H_2O_2 mg^{-1} min^{-1} was observed during storage. But the activity of lipase enzyme increased from 0.236 to 0.231 meq $min^{-1}g^{-1}$ of sample when the storage period was increased. The study would help to know the deterioration pattern of stored groundnut seeds.

Keywords: Enzyme changes, Groundnut, Seed, Storage

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, earthnut, monkey-nut and goobers is the thirteenth most important food crop and fourth most important oilseed crop of the world (Reddy *et al.*, 2011). It is grown in more than 100 countries in the world. India, China, Nigeria, USA and Indonesia alone contribute 74% of the total world production (Mehrotra, 2011). India contributes 19% of world production. It occupies an area of 6.41 million ha with a production of 9.824 million tonnes and possesses an average yield of 1.6 tonnes. Groundnut is usually harvested and stored dry in different storage facilities, traditional and modern. Being an oil seed, it losses its viability within a short period due to the irreversible phenomena of ageing. Intensive crop improvement programme has resulted in the development of large number of high yielding varieties in groundnut. Many of the varieties are in seed production chain in the organized sector. Thus, production and distribution of quality seeds to the farmers become increasingly important.

In a seed production programme, storage of seeds till the distribution during next season assumes paramount importance. A change in enzyme activity due to ageing of seeds was reported by many researchers. Cakmak *et al.* (2009) observed a decrease in the activities of catalase and peroxidase enzymes, but an increase in activity of superoxide dismutase in both the old dry seeds of legumes during storage for 40 years. Scialabba *et al.* (2002) and Pallavi *et al.* (2003) reported a sharp decline in peroxidase emzyme activity in aged seeds of radish and sunflower, respectively. Goel *et al.* (2003) stated that the decrease in germinability was well correlated with increased accumulation of total peroxide and malondialdehyde content and decreased activities of antioxidant enzymes peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase. Rao *et al.* (2006) opined that activity of peroxidase catalase and super oxide dis mutase were decreased in onion seeds under prolonged storage. Loycrajjou *et al.* (2008) reported that ageing induced deterioration and increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes. vigour is essentially a physiological phenomenon influenced by the reserved metabolites, enzyme activities and growth regulators. The exact cause of loss of seed vigour and viability is still unknown as deterioration of seed is a complex process. In the presence of oxygen, ageing of seed can lead to peroxidative changes in polyunsaturated fatty acids. The free radical-induced non-enzymatic peroxidation, which has the potential to damage membrane, is likely to be a primary cause of deterioration of stored seeds (Sung and Chiub, 1995).

Certain anabolic enzymes help in maintaining viability while some catabolic enzymes decrease viability. The seed catalase and peroxidase activity seem to be decreased during storage. Therefore, the present investigation was conducted to study the enzyme changes during seed storage in groundnut (*Arachis hypogaea* L.)

MATERIALS AND METHODS

The seed samples of groundnut var. VRI 2 collected from three different locations of Tamil Nadu *viz.*, Vridhachalam, Tindivanam and Villupuram were used as base material for this study. The collected samples were hand sorted, cleaned thoroughly and tested for their initial enzyme activity and mean values were considered. The data pertaining to the observations recorded in the laboratory were analyzed using Completely Randomized Design adopting the procedure as described by Panse and Sukhatme (1967).

Peroxidase activity: Two replicates of 500 mg pre-germinated seed samples were homogenized in five ml 0.25 M Tris buffer (pH 6.0) and centrifuged at 10,000 rpm for 10 min. at 5^{o}C to extract the enzymes. To a 0.4 ml of enzyme extract, 0.5ml of one % H_2O_2 and 0.5ml of 0.5% aqueous solution of pyrogallol were added and incubated for 10 min. at 25^{o}C. The reaction was stopped by adding 0.5 ml of 5 % (v/v) H_2SO_4. The OD at zero time and expiry of 10 min. was measured at 420 nm in spectrophotometer. The peroxidase activity was expressed as difference in OD 10 min^{-1} (Malik and Singh, 1980).

Peroxidase activity = (Difference in OD value/10 min.) X (1000/500) X 60

Catalase activity: Two replicates of 500 mg of pre germinated seed samples were homogenised in 0.066 M sodium phosphate buffer (pH 6.8) and centrifuged at 2000 rpm for 20 min. at 5^{o}C to extract enzymes. To a 0.2 ml of enzyme extract, five ml of phosphate buffer (pH 6.8) and four ml of 0.3N hydrogen peroxide (substrate) were added. The reaction was stopped after 15 min. of incubation by addition of ten ml of 2N H_2SO_4. The blank was maintained for each set which contained 0.2 ml enzyme extract with 2N H_2SO_4. The contents were titrated against 0.1N $KMnO_4$ and the titre values were noted down. Differences between the titre values will give the volume of permanganate equivalent to enzyme activity. The activity was expressed as µg H_2O_2 mg^{-1} min^{-1} (Povolotskaya and Sedenka, 1956).

Catalase activity= (Difference in titre value / Volume of the sample pipetted out) X (1 X 15 min.)

Lipase activity

Enzyme extract: Two gram of kernel was ground using pestle and mortar and homogenized with twice the volume of ice-cold acetone. Then the powder was washed with acetone, acetone:ether (1:1) and ether; dried and stored in refrigerator till used. Extract was obtained by centrifuging one gram of powder in 20 ml of ice-cold water at 15,000 rpm for 10 min.

Substrate: To two ml of clear vegetable oil (pH 7), 25 ml of water and 100 mg of bile salt (sodium taurocholate) were added and stirred well till an emulsion was formed. Then two gram of gum arabic was added to hasten the emulsification.

Five ml of phosphate buffer was added to 20 ml of substrate taken in a 500 ml beaker and the content was stirred slowly using magnetic stirrer cum hot plate maintained at the temperature of 35^{0}C and then the pH was adjusted to 7 using pH meter. 0.5 ml of enzyme extract was added to the mixture and the pH was recorded immediately and the timer was set on. When pH dropped by 0.2 unit, 0.1 N NAOH was added to bring the pH to the initial value and the titration was continued for 30 min.; enzyme activity was calculated from the amount of alkali consumed using the following formula and expressed in meq $min^{-1}g^{-1}$ of sample (Jayaraman, 1981).

Lipase activity = (Volume of alkali consumed / Weight of sample in g) X (Strength of alkali X Time in min.)

RESULTS AND DISCUSSION

During the course of investigation, a decrease in catalase activity followed by a small increase was observed during storage (Table 1). Increase in catalase activity is attributed to the secretion of catalase enzyme during biological stress condition and later on decreased as the storage period increased. The results were in conformity with the findings of Basavarajappa *et al.* (1991). Gidrol *et al.* (1989) reported that the decreased activity of peroxidase and catalase was due to accumulation of H_2O_2 as seeds neutralized free radicals and this accumulation of hydrogen peroxide

Table 1. Enzyme activity in groundnut seeds during storage**.**

Parameter	Periods of storage (months)				
	P0	P2	P4	P6	Mean
Catalase activity (µg H_2O_2 mg^{-1} min^{-1})	0.434	0.452	0.454	0.444	0.446
Peroxidase activity (difference in OD 10 min^{-1})	0.236	0.233	0.232	0.231	0.233
Lipase activity (meq $min^{-1}g^{-1}$ of sample)	0.231	0.232	0.233	0.236	0.233

	Catalase activity	Peroxidase activity	Lipase activity
SEd	0.0060	0.0026	0.0047
CD P=0.05	0.0126	0.0054	0.0097

itself was detrimental to seeds.

In the present study, the peroxidase activity decreased from 0.236 to 0.231 OD 10 min^{-1} when the storage period was increased. Cakmak *et al.* (2009) observed a decrease in the activities of catalase and peroxidase enzymes, but an increase in activity of superoxide dismutase in both the old dry seeds of legumes during storage for 40 years. Similar results were reported by Bhanuprakash *et al.* (2010) in bell pepper, Demirkaya *et al.* (2010) in onion seeds, Scialabba *et al.* (2002) in radish and Pallavi *et al.* (2003) sunflower.

Lipase is the enzyme which is produced abundantly in oil seeds during storage which breaks down the lipid into free fatty acid. In the present study, the activity of lipase enzyme increased during storage from 0.231 to 0.236 (meq $min^{-1}g^{-1}$ of sample). It might be due to the secretion of fungal lipase of the storage fungi which increased the rate of lipid degradation. Such type of increase in lipase enzyme activity was noticed in stored groundnut seeds by Kakde and Chavan (2011).

Conclusion

The present study concluded that the peroxidase enzyme activity decreased when storage period increased. A decrease in catalase activity was witnessed followed by a small increase during storage. But the activity of lipase enzyme increased the storage period was increased. Altering the enzymatic activity could help in maintaining viability for long storage of groundnut seeds.

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