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Effects of Seed Storage Conditions on Biochemical Changes of Freshly Harvested High Moisture Undried Rice Seeds cv. CO 51

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A B S T R A C T

Freshly harvested high moisture undried rice seeds will induce the deterioration rapidly, it affect the storability of the seed material. The present study was carried out to access the biochemical changes of high moisture and dried seeds taking place under cold and ambient storage condition. The freshly harvested high moisture rice seeds was harvested and stored under cold and ambient storage condition for 15 days in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. In order to know the effectiveness of the storage condition the seeds drawn at 5, 10 and 15 days after storage and it was dried to optimum moisture content, followed by the enzymatic changes was carried out *viz.*, dehydrogenase, α- amylase, catalase and peroxidase. The seeds under cold storage performed better than ambient storage condition. The enzymes which will helps the storability of seed was maximum in undried and dried seeds stored under cold storage condition *viz.*, Dehydrogenase (0.174 and 0.773 OD value), α- amylase (1.38 and 1.43 mg maltose $min.^{-1}$), due to lower respiratory activity in seeds, which was higher than the ambient storage condition. The rising of enzyme activity in undried and dried seeds stored under ambient condition such as Catalase (4.228 and 4.210 µg H_2O_2 mg^{-1} min^{-1}), Peroxidase activity (2.27 and 2.12 m moltetraguaicol g^{-1}), will shown signaling of seed deterioration.

Introduction

Changes in free radical scavenging enzymes, increased free radical production, degradation of protein and DNA and increase in free amino acid were attributed to reduce the germination and seedling vigour during ageing (McDonald, 2004). Decrease in enzymatic activity in stored seeds with increase in storage time resulted reduction in germination and vigour (Khan *et al.*, 2013).The biochemical parameters *viz.*, electrical conductivity, dehydrogenase, α-amylase, catalase, peroxidase and superoxide dismutase activity were measured quantitatively. The activity of all the enzymes had decreased at subsequent storage interval under natural ageing. Chauhan *et al.*, (2011) studied the level of various enzymes and found that reason might be cause of seed deterioration under natural ageing is decrease in enzyme activity in seeds lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the

germinating seed. Several changes in the enzyme macromolecular structure may contribute to their lower effectiveness. They may undergo compositional changes by losing or gaining certain functional groups, by oxidation of sulfhydral groups or by conversion of amino acids within the protein structure

Materials and Methods

Dehydrogenase activity (OD Value)

For estimating the dehydrogenase activity and utilizing it as a measure of seed vigour, 0.5% 2, 3, 5-triphenyl tetrazolium chloride solution dissolved in Sorenson's buffer solution as solvent was used. Three replication of ten seeds from each variety were taken and preconditioned by soaking in water for 7 h. Seeds were soaked in tetrazolium solution and kept in dark for 2 h at 40ºC for staining. After staining, the excess solution was drained and the seeds were washed thoroughly with distilled water and transferred to a test tube containing 5 ml of 2-methoxy ethanol (Methyl Cellosolve). The test tube was closed air tight and allowed to remain in the incubator in darkness overnight for extracting the red coloured formazon. The coloured solution was decanted and the colour intensity was measured in an ELICO UV-VIS Spectrophotometer (Model SL-159) using blue filter (470 nm) with Methyl Cellosolve as the blank. The OD value obtained was reported as dehydrogenase activity (Kittock and Law, 1968).

α-Amylase activity (mg maltose/min)

Three replicates of 500 mg of each pre-germinated seed samples were homogenised in 1.8 ml of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 20000 rpm for 20 min. to extract enzymes. To the 0.1 ml of enzyme extract, one ml of 0.067 per cent starch solution was added. The reaction was stopped after 10 min. of incubation at 25ºC by the addition of one ml of iodine HCl solution (60 mg KI and 6 mg I2 in 100 ml of 0.05 N HCl). Change in colour was measured at 620 nm Spectrophotometer (Model SL-159). The activity was calculated and expressed as mg maltose/min (Paul *et al.*, 1970).

$$\alpha - \text{amylase activity} = \frac{\text{OD Value}}{\text{Volume of sample pipetted out}} \times \frac{1000}{500}$$

Protease activity (OD value)

One ml of embryo extract was added to 0.5 ml of 1 per cent casein solution containing 0.1M Tris-HC1 buffer (pH 8.5). After incubation at 37°C for 30 min. the reaction was stopped by adding 1.25 ml of 5% of Tricarboxylic acetic acid (TCA). After 1h at room temperature the precipitate was filtered and the absorbance of the filtrate was read at 280 nm in an Optima UV-VIS spectrophotometer (Model SP-3000) against a blank prepared by adding casein solution to the incubation mixture after TCA. An increase in 0.01 absorbance unit at 280 nm under the conditions of assay was considered as one protease activity unit. The protease activity was measured as OD value (Naithani, 1987).

Catalase (µmol min.$^{-1}$ g^{-1} seed)

Catalase assay is based on the absorbance of H_2O_2 at 240 nm in UV-range. A decrease in the absorbance is recorded over a time period as described by Aebi (1984).

Adding H_2O_2 started reaction and decrease in absorbance at 240 nm was recorded for one minute in an ELICO UV-VIS spectrophotometer (Model SL-159). Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. The initial and final contents of hydrogen peroxide are

calculated by comparing with a standard curve drawn with known concentrations of hydrogen peroxide. Enzyme activity is calculated as concentration of hydrogen peroxide reduced (initial reading – final reading = quantity of hydrogen peroxide reduced in µmol) per min. per gram fresh weight of seed (µmol $min.^{-1}$ g^{-1} seed).

Peroxidase (m moltetraguaicol g^{-1})

Peroxidase activity was assayed as increase in optical density due to the oxidation of guaiacol to tetraguaiacol (Castillo *et al.,* 1984). Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm in Spectrophotometer (Model SL-159) and enzyme activity was calculated as per extinction coefficient of its oxidation product, tetraguaiacol ($\in$ = 26.6 $mM^{-1}cm^{-1}$). Enzyme activity is expressed as m moltetraguaiacol formed $min.^{-1}$ g^{-1} seed.

Results and Discussion

There was significant difference observed in biochemical properties of freshly harvested high moisture rice seeds stored under cold and ambient storage condition. The rice seeds were harvested and stored with high moisture content without drying for 15 days and the seeds taken back after 5, 10 and 15 days after storage evident that, storability of rice seeds with high moisture was not spoiling when its adopt with cold storage. Narayana Murthy *et al.,* (2003) reported that causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage. The enzymes which will help the storability of seed were maximum in undried and dried seeds stored under cold storage condition *viz.*, Dehydrogenase (0.174 and 0.773 OD value) and it was (0.1677 and 0.163 OD value) under ambient condition. 5, 10 and 15 DAS the dehydrogenase activity was increased in undried and dried seeds. Under the cold storage condition the dehydrogenase activity (0.174) was 4.2% higher than the ambient storage (0.167) condition (Table 1). This result is in agreement with the findings of Raja (2003), Ramanadane (2003) in rice and Basavarajappa *et al.*, (1991) in maize.

The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al.,* 1987) and have positive association with vigour and viability of seeds (Rudrapal and Basu, 1979; Halder and Gupta, 1982). Irrespective of undried and dried seeds α-amylase (activity was 1.38 and 1.43 mg maltose $min.^{-1}$) was 2% higher in cold storage condition than the ambient storage condition (Table 2).

Seeds stored and dried after 5, 10 and 15 days after drying has retain higher level of α-amylase activity in cold storage condition which was maintain 1.4% higher activity with 15 days of storage period. α-amylase activity actually represents the predominant contribution of the carbohydrate metabolism in endosperm of rice seeds and represents the variable spectrum of germination potential (seed vigour) of sindh rice cultivars (Galani *et al.,* 2011) due to lower respiratory activity in seeds, which was higher than the ambient storage condition.

The rate of respiration was minimum in the cold storage conditions which will helps to reduce the enzymatic reaction which will induce the deterioration process. Ramegowda (1992) reported a decrease in the activity of enzymes *viz*., α-amylase, catalase and peroxidase, coupled with progressive ageing of rice seeds. He further authenticated that α-amylase and peroxidase enzymes were more directly involved in the maintenance of better germination of differentially aged seeds.

Table.1 Effect of cold and ambient storage conditions on dehydrogenase (OD Value 10^{-1} seeds) activity of freshly harvested high moisture rice seeds cv. CO 51

Storage conditions (C)	Dehydrogenase (OD Value 10^{-1} seeds)			
	Days after storage (D)	Undried seeds	Dried seeds	Mean
Cold storage	**5**	0.168	0.721	**0.426**
	10	0.173	0.814	**0.494**
	15	0.181	0.823	**0.502**
	Mean	**0.174**	**0.773**	**0.474**
Ambient storage	**5**	0.155	0.603	**0.379**
	10	0.170	0.695	**0.433**
	15	0.175	0.720	**0.448**
	Mean	**0.167**	**0.673**	**0.420**

	C	D	M	CD	DM	CM	CDM
SEd	0.00414	0.00507	0.00414	0.00717	0.00717	0.00586	0.01015
CD (P=0.05)	0.01165	0.01427	0.01165	NS	0.02018	0.01648	NS

Table.2 Effect of cold and ambient storage conditions on α- amylase (mg maltose $min.^{-1}$) activity of freshly harvested high moisture rice seeds cv. CO 51

Storage conditions (C)	α- amylase activity (mg maltose $min.^{-1}$)			
	Days after storage (D)	Undried seeds	Dried seeds	Mean
Cold storage	**5**	1.37	1.46	**1.391**
	10	1.37	1.44	**1.398**
	15	1.39	1.40	**1.41**
	Mean	**1.38**	**1.43**	**1.399**
Ambient storage	**5**	1.35	1.40	**1.380**
	10	1.36	1.41	**1.393**
	15	1.37	1.42	**1.397**
	Mean	**1.36**	**1.41**	**1.390**

	C	D	M	CD	DM	CM	CDM
SEd	0.00973	0.01192	0.00973	0.01686	0.01686	0.01377	0.02384
CD (P=0.05)	0.02738	NS	0.02738	NS	NS	NS	NS

Table.3 Effect of cold and ambient storage conditions on catalase (µmol H_2O_2 reduced $min.^{-1}$ g^{-1} seed) activity of freshly harvested high moisture rice seeds cv. CO 51

Storage conditions (C)	Catalase (µmol H_2O_2 reduced $min.^{-1}$ g^{-1} seed)							
	Days after storage (D)	Undried seeds		Dried seeds		Mean		
Cold storage	**5**	4.198		4.186		**4.192**		
	10	4.202		4.188		**4.195**		
	15	4.207		4.192		**4.200**		
	Mean	**4.202**		**4.189**		**4.196**		
Ambient storage	**5**	4.213		4.201		**4.211**		
	10	4.223		4.209		**4.219**		
	15	4.247		4.221		**4.231**		
	Mean	**4.228**		**4.210**		**4.219**		
		C	D	M	CD	DM	CM	CDM
	SEd	0.01762	0.02158	0.01762	0.03052	0.03052	0.02492	0.04316
	CD (P=0.05)	0.04956	0.06070	0.04956	NS	NS	NS	NS

Table.4 Effect of cold and ambient storage conditions on peroxidase activity (m moltetraguaicol g^{-1}) of freshly harvested high moisture rice seeds cv. CO 51

Storage conditions (C)	Peroxidase activity (m moltetraguaicol g^{-1})							
	Days after storage(D)	Undried seeds		Dried seeds		Mean		
Cold storage	**5**	1.98		1.78		**1.88**		
	10	2.02		1.86		**1.94**		
	15	2.16		1.94		**2.05**		
	Mean	**2.05**		**1.86**		**1.96**		
Ambient storage	**5**	2.19		2.02		**2.10**		
	10	2.23		2.11		**2.17**		
	15	2.39		2.23		**2.31**		
	Mean	**2.27**		**2.12**		**2.20**		
		C	D	M	CD	DM	CM	CDM
	SEd	0.01687	0.02066	0.01687	0.02922	0.02922	0.02386	0.04132
	CD (P=0.05)	0.04744	0.05811	0.04744	NS	NS	NS	NS

The seed coat and the internal seed properties in the freshly harvested high moisture undried seeds was not closely integrated, due to this reason the releasing of seed leachates will occur. Increasing heat induction and biochemical and enzymatic activities also found higher in ambient storage condition. The rising of catalase activity in undried and dried seeds was reported in the seeds stored under ambient condition (4.228 and 4.210 µg H_2O_2 mg^{-1} min^{-1}) and the minimum catalase activity was reported in the seeds stored under cold condition (4.202 and 4.189 µg H_2O_2 mg^{-1} min^{-1}). After 5 days of storage the catalase

activity was noticed minimum in cold condition and the maximum catalase activity was observed in ambient storage condition (Table 3). Catalase activity decreased in *Melanoxylon brauna* seeds under storage in maize and beans. Muniz *et al.,* (2007) found different banding patterns for catalase, which was indicative that the enzyme is associated with the decomposition of hydrogen peroxide in cells. Reduction of catalase activity was also observed in onions during accelerated aging (Demirkaya *et al.,* 2010). In ambient storage condition the complete loss of activity of enzymes may be due to the rapid oxidative stress caused due to high temperature and relative humidity.

The present study revealed that higher peroxidase activity (2.27 and 2.12 m moltetraguaicol g^{-1}) was reported in seeds stored under ambient storage condition. The minimum peroxidase activity was observed in the undried and dried seeds stored under cold storage condition (1.98 and 1.78 m moltetraguaicol g^{-1}) (Table 4). Peroxidase plays a critical role in seed metabolism by using hydrogen peroxide as an acceptor, which may increase defense mechanisms and prevent loss of quality (Ushimaru *et al.*, 2001). In stored cotton seeds, peroxidase activity decreased after storage. The decline in peroxidase activity was also observed in *Copaifera langsdorffi*. In non-viable seeds, there is reduced activity of enzymes such as dehydrogenase, α- amylase and catalase, which can contribute significantly to the reduction of respiratory activity (Desai *et al.*, 1997). Ageing in wheat was in association with accumulation of hydrogen peroxide (Lehner *et al.,* 2008). Peroxidase activity was also reduced during storage of rice seeds (Zhou *et al.,* 2002). Failure of aged seeds to germinate might be due to peroxidation, mitochondrial disfunction and less ATP production changes in membrane lipids therefore could account for the increase in solute leakage. Deterioration of cells in the rice embryonic axes depends on the balance between free radical accumulation and the activity of oxygen scavenging enzymes which constituted the active oxygen scavenging system during early imbibitions. They also recorded the loss of enzyme activity during prolonged storage of seeds under hot and humid conditions.

The present study was concluded that storing the high moisture undried rice seeds under cold storage is found best for slowdown the enzymatic activity of high moisture undried rice seeds when compared to the ambient storage condition.

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