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# ASSESSMENT OF SEED VIGOUR DETERIORATION PATTERN BASED ON PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES IN PADDY (*Oryza sativa* L.) DURING STORAGE

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## ABSTRACT

**Seven paddy cultivars *viz.*, 'ADT 38', 'ADT(R) 46', 'CO 43', 'CO(R) 50', 'CR 1009', 'IW Ponni' and 'Swarna' were assessed for deterioration pattern during natural and artificial ageing. Various physiological and biochemical attributes associated with seed deterioration were studied in relation to seed vigour. Decline in dehydrogenase, α-amylase, catalase, peroxidase, superoxide dismutase activities and increase in electrical conductivity of seed leachate were observed during natural ageing. The deterioration during artificial ageing ($40^0$C temperature and 100% relative humidity) followed the same pattern but with accelerated rate. The disintegration of embryo and aleurone layer was also conspicuous in accelerated aged seeds. These biochemical changes occurring during ageing are quite well reflected in terms of physiological attributes like loss of viability and vigour. Among the evaluated paddy cultivars, 'CR 1009' proved superior over all the other cultivars in maintaining higher viability and vigour potential while cv. 'ADT 38' and 'ADT(R) 46' showed poor storability.**

**Keywords:** Ageing, antioxidants, germination, seed vigour, storability

## INTRODUCTION

Rice (*Oryza sativa* L.), a member of family *Poaceae*, is a staple cereal food of more than 60% world population, especially in tropical Latin America and East, South and Southeast Asia. In India, rice is a predominant food crop in terms of area, production and consumer preference. In world India ranks as the 2$^{nd}$ largest producer and consumer of rice followed by China. The total area under rice cultivation in India is 43.8 million ha with annual production of 116.4 million tones and productivity of 2659 kg ha$^{-1}$ (DAC Annual Report, 2018-19). Tamil Nadu state in India has an area of 2.04 million ha under rice with annual production of 7.98 million tones and productivity of 3912 kg ha$^{-1}$ (DAC, 2018).

Seed deterioration, an irreversible degenerative natural process, is expressed as the loss of quality, viability and vigour during ageing due to adverse climatic conditions. However, the rate of deterioration is influenced by seed moisture content and the temperature of storage environment; and an increase in either factor leads to rapid deterioration (Ellis *et al*., 1992). Many physiological and biochemical manifestations of seed deterioration have extensively been reported (Mc Donald, 1999; Jatoi *et al*., 2004). The most widely accepted single criterion of seed deterioration is reduced germinability. However, many tests for measuring the loss in seed vigour have been developed based on physiological effect on ageing (Jatoi *et al*., 2004; Khan *et al*., 2007; Malik and Shamet, 2009). Among them the most important method is accelerated ageing which is done by subjecting seeds to elevated temperature and high relative humidity. It provides a simple and reliable method for studying sequence and relationship of process of deterioration over short periods. It was, therefore, thought worthwhile to study the physiological and biochemical changes in seed so as to understand the basis of its deterioration. This would help not only in identifying the traits for improving storage life of seeds, but also provide information that would enable incorporation of these traits for better storability in the genetic background of high yielding varieties for improving storage life of poor storers. Thus, the present study was aimed to assess various physiological and biochemical changes during seed deterioration under accelerated ageing and natural ageing so as to screen the good storers.

## MATERIALS AND METHODS

The ambiently stored seeds (for eight months) and freshly harvested seeds of various paddy varieties were obtained from the Department of Rice, TNAU, Coimbatore (India). The freshly harvested seeds were cleaned and dried to bring the moisture content down to 11%. The seeds were then graded using the recommended sieve size with aperture 1.80s (in mm). The fresh and old seeds were invariably treated with carbendazim (Bavistin) @ 2.5 g $kg^{-1}$ and imidacloprid @ 1 mL $kg^{-1}$ to avoid the infestation of storage fungi and insects, respectively. The study conducted in the Department of Seed Science & Technology, TNAU, Coimbatore.

***Seed material***

The fresh seeds of seven rice varieties i.e. 'ADT38' (V1), 'ADT(R)48' (V2), 'CO43' (V3), 'CO(R)50' (V4), 'CR1009' (V5), 'Improved White Ponni' (V6) and 'Swarna' (V7) were packed in cloth bags and stored under ambient conditions for 12 months. Seed samples were drawn from fresh and stored seeds (maintained under ambient conditions) after 8 (P1), 10 (P2) and 12 months (P3) of storage. The seed samples were also drawn for fresh seeds i.e. 0 days of accelerated ageing (D1) and 20 days of accelerated ageing (D2) and the observations were recorded. The experiment was conducted in a factorial completely randomized design and each treatment replicated four times.

***Moisture content***

Five grams of ground seed material were placed in a moisture weighing bottle and kept in a hot air oven maintained at 130 ± 2°C for 2 h for drying. The material was cooled in a desiccator containing silica gel for 30 min. The weight of seeds along with moisture bottle before and after drying was recorded. The moisture content of seeds was calculated using the below given formula and expressed as percentage (ISTA, 2011).

$$\text{Moisture content } (\%) = \frac{M_2 - M_3}{M_2 - M_1} \text{ X } 100$$

Where, $M_1$ = Weight of moisture weighing bottle alone, $M_2$ = Weight of moisture weighing bottle + seed sample before drying, $M_3$ = Weight of moisture weighing bottle + seed sample after drying.

***Electrical conductivity test***

Three replicates of 25 seeds from each variety were drawn, washed with distilled water to remove any adhering chemicals and then soaked in 25 mL distilled water for 16 h at 30 ± 1°C. After soaking, the seed steep water was decanted to obtain seed leachate. The electrical conductivity of seed leachate was measured in a digital conductivity meter with a cell constant of one and expressed as $\mu Sm^{-1}$ (Presley, 1958).

***Dehydrogenase activity test***

For estimating dehydrogenase activity and using the test 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 replications of ten seeds from each variety were taken and pre-conditioned 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 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 colour intensity measured in an ELICO UV-VIS spectrophotometer (Model SL-159) using blue filter (470 nm) with methyl cellosolve as the blank. The optical density (OD) value obtained was reported as dehydrogenase activity (Kittock and Law, 1968).

***Assay of α-amylase activity***

Three replicates of 500 mg of each pre-germinated seed sample were homogenized in 1.8 mL of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min to extract enzymes. To 0.1 mL of enzyme extract, 1.0 mL of 0.067% starch solution was added. The reaction was stopped after 10 min of incubation at 25°C by the addition of 1 mL of iodine HCl solution (60 mg KI and 6 mg $I_2$ in 100 mL of 0.05 N HCl). The change in colour was measured at 620 nm using a spectrophotometer (model SL-159). The activity was calculated and expressed as mg maltose min$^{-1}$ (Paul *et al.,* 1970).

***Assay of superoxide dismutase activity***

Superoxide dismutase activity was estimated by recording the decrease in OD of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme (Dhindsa *et al.*, 1981). Superoxide dismutase enzyme extract was prepared by first preconditioning the weighed amount of seed (0.2 g) in between the moist germination paper disc, followed by grinding with 2 mL extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA). The extract was centrifuged for 20 min at 15,000 rpm and the supernatant used as enzyme. The enzyme assay mixture was taken in a test tube and reaction started by adding 60 M riboflavin (0.1 mL) and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal colour, served as control. Switching off the light and putting the tubes into dark stopped the reaction. A non-irradiated complete reaction mixture served as blank. The absorbency was recorded at 560 nm in a spectrophotometer (model SL-159) and one unit of enzyme activity was taken as the amount of enzyme, which reduced the absorbency reading to 50% in comparison with tubes lacking enzyme.

***Assay of peroxidase activity***

Peroxidase activity was assayed as increase in OD due to the oxidation of guaiacol to tetra-guaiacol (Castillo *et al.*, 1984). The enzyme extract for peroxidase was prepared by first preconditioning the weighed amount of seed samples (0.2 g) in between the moist germination paper disc followed by grinding with 2 mL extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA). Then, the extract was centrifuged for 20 min at 15,000 rpm and the supernatant was used as enzyme. The absorbance due to the formation of tetra-guaiacol was recorded at 470 nm in a spectrophotometer (model SL-159) and the enzyme activity calculated as per extinction coefficient of its oxidation product, tetra-guaiacol (= 26.6 mM$^{-1}$ cm$^{-1}$). The enzyme activity was expressed as mmol tetra-guaiacol formed min$^{-1}$ g$^{-1}$ seed.

***Assay of catalase activity***

The assay of catalase activity 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). Enzyme extract for catalase was prepared by first preconditioning the weighed amount of seed samples (0.2 g) in between the moist germination paper disc followed by grinding with 2 mL extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA). Then, the extract was centrifuged for 20 min at 15,000 rpm and the supernatant was used as enzyme. The enzyme reaction was initiated by adding $H_2O_2$, and decrease in absorbance at 240 nm was recorded for 1 min in an UV-VIS spectrophotometer (ELICO, model SL-159). The 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. The enzyme activity was calculated as the concentration of hydrogen peroxide reduced (initial reading - final reading = quantity of hydrogen peroxide reduced in µmol) $min^{-1}$ $g^{-1}$ fresh seed weight.

***Measurement of seed deterioration pattern under artificial ageing conditions***

Freshly harvested seeds of paddy varieties were subjected for accelerated ageing by exposing them to 40°C temperature and 100% relative humidity for as many number of days as required to bring down the germination percentage by 50% of Indian minimum seed certification standard i.e. 20 days. All the observations were recorded for the aged seeds at D1= 0 days and D2 = 20 days.

***Vigour index I***

Vigour index I was computed using following formula as per Abdul-Baki and Anderson (1973) and the mean values expressed as whole number.

Vigour index I = Germination (%) x Seedling length (cm)

***Statistical analysis***

The data was analyzed statistically as per Rangaswamy (2002). Wherever necessary the values were transformed to angular (arcsine) values. The critical differences (CD) were calculated at 5% probability level. The F test expressed non-significant of variant was indicated by the letter. 'ns'

## RESULTS AND DISCUSSION

In present study, various physiological and biochemical degenerative changes occurring during seed deterioration and the loss of vigour and viability were studied in paddy and the data generated was analyzed to assess the pattern of seed deterioration in naturally and artificially aged seeds.

***Seed moisture content***

In natural ageing a significant difference in moisture content was observed in the varieties evaluated. The interaction between the varieties and period of storage was non-significant (Fig. 1). Among the varieties, cv 'ADT38' [V1] (10.9) recorded minimum moisture content as compared to the other varieties. The variety 'ADT38' was at par with cv. 'CO(R)50' [V4] (11.0), 'Improved White Ponni' [V6] (11.0) and 'Swarna' [V7] (11.2). The maximum moisture content was recorded in cv. 'CR1009' [V5] (11.6). The moisture content significantly increased from P1 (11.0) to P3 (11.4). Deterioration of seed quality was found associated with moisture content during storage

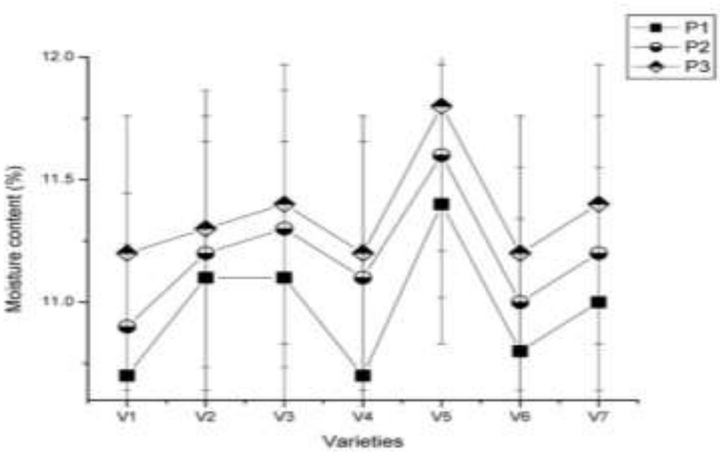


**Fig. 1: Pattern of change in moisture content in different varieties of paddy seeds during natural ageing:** P1 = 8 months storage, P2 = 10 months storage, P3 = 12 months storage (the data are the mean ± SEM. S = Significance at P ≤ 0.05 and P ≤ 0.01, using Origin 6.1

**Table 1: Effect of natural and artificial ageing on electrical conductivity and seed vigour in different varieties of paddy seeds**

| Varieties | Electrical conductivity ($\mu Sm^{-1}$) | | | | | | | Vigour index | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Natural ageing | | | | Artificial ageing | | | Natural ageing | | | | Artificial ageing | | |
| | Period of storage (months) | | | | Duration (days) | | | Period of storage (months) | | | | Duration (days) | | |
| | P1 | P2 | P3 | Mean | D1 | D2 | Mean | P1 | P2 | P3 | Mean | D1 | D2 | Mean |
| V1 | 80 | 80 | 117 | 98 | 62 | 171 | 116 | 1912 | 1796 | 1675 | 1794 | 2272 | 0 | 1136 |
| V2 | 79 | 79 | 112 | 95 | 60 | 165 | 112 | 2073 | 2044 | 1933 | 2017 | 2234 | 0 | 1117 |
| V3 | 68 | 68 | 89 | 78 | 54 | 149 | 101 | 2274 | 2266 | 2240 | 2260 | 2389 | 489 | 1439 |
| V4 | 70 | 70 | 96 | 83 | 57 | 158 | 108 | 2138 | 2102 | 1982 | 2074 | 2324 | 267 | 1296 |
| V5 | 62 | 62 | 80 | 71 | 46 | 140 | 93 | 2610 | 2531 | 2453 | 2531 | 2837 | 772 | 1804 |
| V6 | 70 | 70 | 93 | 81 | 56 | 153 | 104 | 2176 | 2155 | 2093 | 2141 | 2307 | 285 | 1296 |
| V7 | 63 | 63 | 82 | 72 | 51 | 144 | 98 | 2292 | 2265 | 2197 | 2251 | 2430 | 615 | 1522 |
| Mean | 70 | 70 | 96 | 83 | 55 | 154 | 105 | 2211 | 2166 | 2082 | 2153 | 2399 | 347 | 1373 |
| | V | V | V x P | | V | D | V x D | V | P | V x P | | V | D | V x D |
| SE | 1.00 | 1.00 | 1.73 | | 2.12 | 1.13 | 2.99 | 22.16 | 14.51 | 38.38 | | 32.73 | 17.5 | 46.3 |
| $CD_{0.05}$ | 2.02 | 2.02 | 3.50 | | 4.34 | 2.32 | 6.13 | 44.28 | 28.99 | ns | | 66.07 | 35.3 | 93.4 |

V = paddy varieties; P = Storage period; D = Duration of aging; P1 = 8 months storage, P2 = 10 months storage, P3 = 12 months storage; D1 = accelerated aging for 0 day, D2 = accelerated aging for 20 days.

(Ramanadane and Ponnuswamy, 2004). Under natural ageing, the moisture content increased with increase in storage period, irrespective of the varieties. Although, cv. 'ADT 38' initially had less moisture content, the maximum increase (4.7%) was observed in it as compared to the cv. CR1009, which recorded increase by 3.5% over a period of 12 months storage (Fig. 1). The increase in moisture content might be due to the increase in relative humidity of storage environment and also the differential behaviour of genotypes in absorbing the moisture during natural ageing. The finding is in agreement with Bhaskaran (1995) in rice, Bharathi (1991) in maize and Sawant *et al*. (2012) in wheat, which too showed increasing trend of moisture content with respect to the storage period.

***Vigour index I***

The paddy variety 'CR1009' proved superior in vigour index I (2531) over other varieties, irrespective of the storage period (Table 1). The variety 'ADT 38' had minimum vigour index of 17940 over a period of 12 months under natural ageing. The reduction in vigour index I was less in cv. 'CR 1009' (6%) but high in cv. 'ADT 38' (12%). The same trend was observed in accelerated ageing wherein the paddy varieties 'ADT 38' and 'ADT (R) 46' registered no vigour index I. The reduction in vigour index was less in cv. 'CR1009' (73%) as compared to other varieties, and it was 100% in cv. 'ADT 38' and 'ADT (R) 46'. Deshpande and Mahadevappa (1994) observed that the seedling vigour in rice decreased as the ageing and storage period prolonged. Atici *et al.* (2007) reported decreased vigour aged seeds and weak seedlings that were unable to survive once reintroduced into a habitat.

***Electrical conductivity***

The electrical conductivity of seed leachate is a good index of seed deterioration. The intensity of membrane damage during storage was measured by electrical conductivity of seed leachate (Matthews and Bradnock, 1968). In present study, though the electrical conductivity of seed leachate increased with increase in storage period. It was minimum in cv. 'CR 1009' (V5) (71 $\mu Sm^{-1}$) as compared to the other paddy varieties tested, irrespective of the storage period (Table 1). The variety 'ADT 38' (V1) recorded maximum electrical conductivity of 98 $\mu Sm^{-1}$ over 12 months of storage under natural ageing. The increase in electrical conductivity was less in 'CR1009' (29%) than 'ADT 38' (46%) under natural ageing. Similarly, under accelerated ageing, cv. 'CR1009' performed better in terms of electrical conductivity and it was minimum (140 $\mu Sm^{-1}$) at 20 days of accelerated ageing as compared to other varieties while varieties 'ADT 38' (V1) and 'ADT (R) 46' (V2) registered electrical conductivity of 171 and 165 $\mu Sm^{-1}$, respectively. The pattern in increase of electrical conductivity under artificial ageing was similar to that of natural ageing. The results are in conformity with those of Manonmani (1990) and Jerlin (1990) in rice. This might be due to increase moisture content that might have promoted the metabolic process resulting in leaching of free sugars and free amino acids. Deshpande and Mahadevappa (1994) observed that the electrical conductivity of rice seed leachate increases with prolonged ageing and storage period. Ramanadane and Ponnuswamy (2004) observed that electrical conductivity of seed leachate showed similar changes in both natural and accelerated ageing conditions.

***Dehydrogenase activity***

The dehydrogenase activity (OD value 10 seeds$^{-1}$) under natural ageing declined from 0.163 at 8 months to 0.129 at 12 months storage, irrespective of the varieties (Table 2). The maximum dehydrogenase activity (0.206) was recorded in cv. 'CR 1009'. The cv. 'ADT 38' depicted minimum dehydrogenase activity (0.122) over 12 months storage under natural ageing. The reduction in dehydrogenase activity was less in cv. 'CR 1009' (12%) than cv. 'ADT 38' (40%) under natural ageing**.** Similar trend was observed in artificial ageing. These findings are in agreement with Rame Gowda (1992), Raja (2003), Ramanadane (2003) in rice and Basavarajappa *et al*. (1991) in maize.

***α-amylase activity***

Under natural ageing the α-amylase activity in paddy varieties differed significantly from one another. Comparatively higher α-amylase activity (2.34 mg maltose min$^{-1}$) was found in cv. 'CR 1009' (V5), irrespective of the storage period; while cv. 'ADT 38' (V1) recorded minimum α-amylase activity (1.20 mg maltose min$^{-1}$) [Table 3]. The reduction in α-amylase activity was less in cv. 'CR 1009' (11%) than cv. 'ADT 38' (17%) under natural ageing of 12 months period (Fig. 5). Similarly, cv. 'CR1009' recorded maximum α-amylase activity (0.99 mg maltose min$^{-1}$), whereas cv. 'ADT 38' showed minimum activity (0.21 mg maltose min$^{-1}$) under artificial ageing at 20 days. The reduction in α-amylase activity was less in cv. 'CR 1009' (60%) as compared to cv. 'ADT 38' (86%) during accelerated ageing of 20 days duration**.** This might be due to the varietal differences in hydrolytic enzymes which is supported by the anatomical results (results not presented) wherein integrity of aleurone layer and embryo differed in genotypes over a period of ageing. In paddy variety 'CR 1009', the integrity of aleurone layer and embryo was good than the poor storer, which might have triggered the synthesis of α-amylase activity. The α-amylase actually represents the predominant contribution of carbohydrate metabolism in the endosperm of rice seeds and represents a variable spectrum of germination potential (seed vigour) (Galani *et al*., 2011). The α-amylase activity is a biochemical

**Table 2: Effect of natural and artificial ageing on dehydrogenase and superoxide dismutase activities in paddy seeds of different varieties**

| Varieties | Dehydrogenase activity (OD value $10^{-1}$ seeds) | | | | | | | Superoxide dismutase activity (OD value $10^{-1}$ seed | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Natural ageing<br>Period of storage (months) | | | | Artificial ageing<br>Duration (days) | | | Natural ageing<br>Period of storage (months) | | | | Artificial ageing<br>Duration (days) | | |
| | P1 | P2 | P3 | M | D1 | D2 | Mean | P1 | P2 | P3 | M | D1 | D2 | Mean |
| V1 | 0.135 | 0.109 | 0.081 | 0.122 | 0.183 | 0.012 | 0.097 | 36.7 | 30.5 | 20.3 | 29.2 | 27.3 | 0.7 | 14.0 |
| V2 | 0.146 | 0.129 | 0.110 | 0.128 | 0.181 | 0.022 | 0.101 | 38.3 | 32.8 | 27.3 | 32.8 | 32.8 | 1.6 | 17.2 |
| V3 | 0.149 | 0.133 | 0.113 | 0.131 | 0.183 | 0.049 | 0.116 | 55.5 | 53.1 | 49.2 | 52.6 | 53.1 | 7.0 | 30.1 |
| V4 | 0.160 | 0.145 | 0.128 | 0.144 | 0.189 | 0.038 | 0.113 | 47.6 | 42.2 | 36.7 | 42.2 | 42.1 | 6.2 | 24.2 |
| V5 | 0.218 | 0.207 | 0.193 | 0.206 | 0.216 | 0.065 | 0.141 | 65.6 | 63.3 | 60.2 | 63.0 | 63.3 | 10.9 | 37.1 |
| V6 | 0.154 | 0.139 | 0.121 | 0.138 | 0.185 | 0.042 | 0.113 | 50.0 | 46.9 | 42.2 | 46.4 | 46.9 | 7.0 | 26.9 |
| V7 | 0.182 | 0.160 | 0.154 | 0.165 | 0.221 | 0.054 | 0.137 | 60.9 | 57.8 | 53.9 | 57.6 | 57.8 | 8.6 | 33.2 |
| Mean | 0.163 | 0.146 | 0.129 | 0.146 | 0.200 | 0.040 | 0.120 | 50.7 | 46.6 | 41.4 | 46.2 | 46.2 | 6.0 | 26.1 |
| | V | P | V x P | | V | D | V x D | V | P | V x P | | V | D | V x D |
| SEd | 0.0023 | 0.0015 | 0.0039 | | 0.0025 | 0.0013 | 0.0035 | 0.55 | 0.36 | 0.95 | | 0.57 | 0.31 | 0.81 |
| $CD_{0.05}$ | 0.0046 | 0.0030 | 0.0080 | | 0.0050 | 0.0027 | 0.0071 | 1.10 | 0.72 | 1.91 | | 1.18 | 0.63 | 1.67 |

V = paddy varieties; P = Storage period; D = Duration of aging; P1 = 8 months storage, P2 = 10 months storage, P3 = 12 months storage; D1 = accelerated aging for 0 days, D2 = accelerated aging for 20 days

**Table 3: Effect of natural and artificial ageing on α-amylase and catalase and peroxidase activities in seeds of some paddy varieties**

| Varieties | α- amylase activity (mg maltose min.$^{-1}$) | | | | | | | Catalase activity (μmol $H_2O_2$ reduced min.$^{-1}$ g$^{-1}$ seed) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Natural ageing | | | | Artificial ageing | | | Natural ageing | | | | Artificial ageing | | |
| | Period of storage (months) | | | | Duration (days) | | | Period of storage (months) | | | | Duration (days) | | |
| | P1 | P2 | P3 | M | D1 | D2 | Mean | P1 | P2 | P3 | M | D1 | D2 | Mean |
| V1 | 1.31 | 1.20 | 1.09 | 1.20 | 1.53 | 0.21 | 0.87 | 10.1 | 7.6 | 5.1 | 7.6 | 12.6 | 0.0 | 6.3 |
| V2 | 1.70 | 1.62 | 1.52 | 1.57 | 1.89 | 0.40 | 1.14 | 12.6 | 7.6 | 5.0 | 8.4 | 14.3 | 0.0 | 7.2 |
| V3 | 2.12 | 2.06 | 1.98 | 2.05 | 2.31 | 0.89 | 1.60 | 12.7 | 10.1 | 7.6 | 10.1 | 15.8 | 6.3 | 11.1 |
| V4 | 1.99 | 1.87 | 1.76 | 1.87 | 2.09 | 0.74 | 1.42 | 10.1 | 7.6 | 5.1 | 7.6 | 15.2 | 4.8 | 10.0 |
| V5 | 2.48 | 2.33 | 2.20 | 2.34 | 2.51 | 0.99 | 1.75 | 35.5 | 35.5 | 33.0 | 34.7 | 38.3 | 16.2 | 27.2 |
| V6 | 2.07 | 1.98 | 1.89 | 1.98 | 2.19 | 0.87 | 1.53 | 20.3 | 20.3 | 20.2 | 20.3 | 21.6 | 8.6 | 15.1 |
| V7 | 2.36 | 2.16 | 2.03 | 2.18 | 2.41 | 0.92 | 1.66 | 30.4 | 30.4 | 27.9 | 29.6 | 33.2 | 14.4 | 23.8 |
| Mean | 2.00 | 1.89 | 1.78 | 1.89 | 2.13 | 0.72 | 1.42 | 18.8 | 17.0 | 14.8 | 16.9 | 21.6 | 7.2 | 14.4 |
| | V | P | | V x P | V | D | V x D | V | P | | V x P | V | D | V x D |
| SEd | 0.025 | 0.016 | | 0.043 | 0.026 | 0.014 | 0.037 | 0.26 | 0.17 | | 0.45 | 0.28 | 0.16 | 0.42 |
| $CD_{0.05}$ | 0.050 | 0.033 | | ns | 0.053 | 0.029 | 0.075 | 0.53 | 0.35 | | 0.92 | 0.56 | 0.32 | 0.84 |

V = paddy varieties; P = Storage period; D = Duration of aging; P1 = 8 months storage, P2 = 10 months storage, P3 = 12 months storage; D1 = artificial aging for … days, D2 = artificial aging for … days, ns = non-significant

indicator showing variable germination abilities of rice varieties thus leading to different seed vigour. This finding is in agreement with Ramanadane and Ponnuswamy (2004). Decrease in α-amylase and dehydrogenase activities during storage has also been reported by Rame Gowda (1992) in rice.

***Catalase, peroxidase and superoxide dismutase activities in seeds***

The enzyme activities assayed in rice varieties aged under natural and artificial ageing revealed that the catalase, peroxidase and superoxide dismutase activities decreased with increase in ageing period. Catalase activity was higher (35 µmol $H_2O_2$ min$^{-1}$ g$^{-1}$ seed) in cv. 'CR1009' as compared to the other paddy varieties, whereas cv. 'ADT 38' recorded less catalase activity (8 µmol $H_2O_2$ min$^{-1}$ g$^{-1}$ seed) under natural ageing of 12 months. The pattern of reduction showed that cv. 'CR1009' was superior with less reduction in catalase activity (7%); while cv. 'ADT 38' was poor with more reduction in catalase activity (58%) over 12 months storage period. Similarly, under accelerated ageing cv. 'CR1009' had maximum catalase activity (16 µmol $H_2O_2$ min$^{-1}$ g$^{-1}$ seed) than cv. 'ADT 38' and 'ADT (R) 46', which registered no catalase activity at 20 days. The reduction in catalase activity was less in cv. 'CR1009' (58%) than cv. 'ADT 38' and 'ADT (R) 46' wherein reduction percentage was 100%. These findings are in line with Zhou *et al*. (2002) and Abbade *et al*. (2014).

The peroxidase and superoxide dismutase activities followed similar trend as that of catalase activity under both natural and artificial ageing conditions (Table 3 & 4**).** After undergoing accelerated ageing for 20 days, cv. 'CR1009' lost 57% activity while cv. 'ADT 38' and 'ADT (R) 46'

**Table 4: Effect of natural and artificial ageing on peroxidase activity in seeds of some paddy varieties**

| Varieties | Peroxidase activity (m mol tetraguaicol g$^{-1}$seed) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Natural ageing | | | | Artificial ageing | | |
| | Period of storage (months) | | | | Duration (days) | | |
| | P1 | P2 | P3 | Mean | D1 | D2 | Mean |
| V1 | 0.759 | 0.579 | 0.368 | 0.568 | 1.071 | 0.022 | 0.546 |
| V2 | 1.073 | 0.759 | 0.394 | 0.742 | 1.315 | 0.030 | 0.673 |
| V3 | 1.244 | 1.075 | 0.883 | 1.067 | 1.507 | 0.300 | 0.904 |
| V4 | 1.139 | 0.943 | 0.706 | 0.930 | 1.436 | 0.120 | 0.778 |
| V5 | 1.515 | 1.364 | 1.202 | 1.360 | 1.546 | 0.518 | 1.032 |
| V6 | 1.210 | 1.067 | 0.872 | 1.050 | 1.466 | 0.255 | 0.860 |
| V7 | 1.274 | 1.128 | 0.962 | 1.121 | 1.514 | 0.451 | 0.982 |
| Mean | 1.173 | 0.988 | 0.770 | 0.977 | 1.408 | 0.242 | 0.825 |
| | V | P | V x P | | V | D | V x D |
| SEd | 0.011 | 0.007 | 0.020 | | 0.017 | 0.009 | 0.024 |
| $CD_{0.05}$ | 0.023 | 0.015 | 0.040 | | 0.035 | 0.019 | 0.05 |

V = paddy varieties; P = Storage period; D = Duration of aging; P1 = 8 months storage, P2 = 10 months storage, P3 = 12 months storage; D1 = accelerated aging for 0 day, D2 = accelerated aging for 20 days.

lost the activity completely. The complete loss of enzyme activities may be due to the rapid oxidative stress caused due to high temperature and relative humidity. Certain toxic metabolites like hydrogen peroxide might have formed as a consequence of oxidative stress. Accelerated ageing in wheat was found in association with accumulation of hydrogen peroxide (Lehner *et al*., 2008). Catalase activity decreased in *Melanoxylon brauna* seeds under storage (Corte *et al*., 2010) in maize and beans. Muniz *et al*. (2007) observed different banding patterns for catalase which indicated that the enzyme is associated with the decomposition of hydrogen peroxide in cells. In *Ginkgo biloba* seeds stored at 4°C, the catalase activity remained stable in embryo and endosperm, and its activity was significantly reduced only at 12 months. However, when stored at 25°C the catalase activity decreased at 6 months storage (Tommasi *et al*., 2006). Reduction in catalase activity has also been observed in onions during accelerated aging (Demirkaya *et al*., 2010).

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 (Vieira *et al*., 2008). The decline in peroxidase activity was also observed in *Copaifera langsdorffi* (Carvalho *et al*., 2006). Non-viable seeds showed reduced activities of dehydrogenase, α-amylase and catalase, which might have significantly contributed to the reduction of respiratory activity (Desai *et al*., 1997). Peroxidase activity was a reduced during storage of rice seeds (Zhou *et al*., 2002).

**Conclusions:** The present study revealed that among the paddy varieties tested, cv. 'ADT 38' and 'ADT(R) 46' were poor storers as they showed minimum physiological and biochemical seed quality attributes over a period of 12 months storage under natural ageing condition and also in 20 days of artificial ageing condition. The paddy variety 'CR 1009' was good storer as it recorded comparatively favourable features for above parameters. Biochemical studies showed a drastic increase in electrical conductivity over a period of natural and artificial ageing. The antioxidant enzyme activities, *viz*., catalase, peroxidase and superoxide dismutase, were reduced with increase in storage period. The paddy varieties with higher initial vigour continued to maintain higher viability as compared to the genotypes of lower vigour. In present study, cv. 'CR 1009' had maximum antioxidant enzyme activity, which resulted in higher vigour. The pattern of deterioration during natural and artificial ageing revealed cv. 'CR1009' to be superior with minimum reduction in catalase activity; while cv. 'ADT 38' proved inferior with maximum reduction in catalase activity. The peroxidase and superoxide dismutase activities followed the similar result as that of catalase.

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