

Evaluation of Anatomical and Biochemical Causes for Seed Deterioration in Rice Genotypes under Ageing

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ABSTRACT: Seed deterioration is an undesirable event occurs with ageing that decreases the ability of the seed to survive. Deterioration is generally fast in oil and pulses seeds than cereals. Rice is an important cereal food crop and consumed by more than 60 per cent of the people in the world. Though loss in viability of rice under ageing is not the major concern, some of the important rice genotypes lose their viability very quickly. In Tamilnadu, ADT 38, and ADT (R) 46 are important medium duration rice genotypes in seed supply chain and both the varieties loses their germinability very quickly as the storage time advances. In order to conserve the ADT (R) 46 seeds from loss, the causes for poor storability was studied under accelerated ageing in comparison with ADT 39, ADT (R) 49 and ADT (R) 50 genotypes. The results revealed that variety ADT (R) 46 lost its viability quickly as compared to other varieties. The histological study revealed that all the genotypes except ADT (R) 46 had undamaged aleurone layer with intact cell and distinct cytoplasm even after 10 days of accelerated ageing whereas, variety ADT (R) 46 had collapsed aleurone layer with poor distinct cytoplasm of cells which causes poor germination during ageing.

Keywords: Accelerated ageing, Anatomical, Biochemical, Rice, Seed deterioration, Seed quality

Rice (*Oryza sativa* L.) is the most important staple food and often known as the grain of life for Asians as it supplies majority of starch, protein and micronutrient requirements. The productivity and production of rice need to be improved for making India self-sufficient in food production. Among the various factors that influence agricultural production viz., irrigation water, fertilizer application, pest and disease management etc., sowing with quality seed registers a significant major contribution to the crop yield up to an extent of 10 to 20% [1]. Thus, the role of quality seed is prominent in boosting the agricultural productivity and production of the country. The quality of seeds is influenced by several factors of which loss of quality during storage is considered as one of the most important factors that results in poor crop establishment and yield. Hence, high attention is very much needed to minimize the loss and maintain the seed quality of important high yielding crop varieties/ hybrids during storage.

Since rice is cultivated in humid tropical and subtropical climates of the country associated with high temperature and relative humidity, rapid deterioration of seeds in storage is inevitable. Seed deterioration can be defined

as “deteriorative alterations occurring with time that increase the seed’s exposure to external challenges and decrease the ability of the seed to survive”. Seed deterioration is loss of seed quality, viability and vigour due to the effect of adverse environmental factors [2]. It is a natural process which involves cytological, physiological, biochemical and physical changes in seeds. These changes reduce the viability and ultimately cause the death of the seed. Seed deterioration is an undesirable attribute of agriculture. Annual losses due to seed deterioration can be as much as 25% of the harvested crop. It is one of the basic reasons for low productivity [3]. The rate of deterioration of seeds fluctuates critically from one species to another and also among the varieties of the same species [4]. The ageing of seed is manifested as a reduction in germination percentage, while those seeds that do germinate, produce weak seedlings [5]. Therefore, the present study was taken to understand the mechanism and find the causes of seed deterioration in rice seeds.

MATERIALS AND METHODS

The laboratory studies were conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural

University, Coimbatore during 2016-17. Freshly harvested seeds of rice varieties viz., ADT 39 (V1), ADT (R) 46 (V2), ADT (R) 49 (V3) and ADT (R) 50 (V4) were cleaned, graded and dried for safe moisture level then subjected for accelerated ageing by exposing the seeds at 40°C temperature and 100 per cent relative humidity for 0 day (D1), 2 days (D2), 4 days (D3), 6 days (D4), 8 days (D5) and 10 days (D6) as required to bring down the germination percentage. Observations on germination [6], root length (cm), shoot length (cm), dry matter production (mg per 10 seedlings), vigour index [7], Electrical conductivity [8], α -Amylase activity [9], DPPH [10], Peroxidase [11] and Ascorbate Peroxidase [12] were observed on respective day of accelerated ageing.

Histological studies on anatomical changes of 10 day accelerated aged seeds and control seeds were analyzed. The seeds were processed and sectioned by rotary microtome [13] and viewed under binocular microscope (Magnified up to 40x).

RESULTS AND DISCUSSION

Physiological parameters

The changes associated with ageing are many and depending on ageing conditions and differences among cultivars [14]. Varietal differences in the longevity of rice seeds stored under ageing conditions have been reported [15]. It was therefore, thought imperative to investigate the physiological and biochemical changes to understand the basis of seed deterioration. This would help not only

in identifying reasons for improving storage life of seeds but also provide information to enable application of suitable treatment for better storability. Thus in the present investigation, various physiological and biochemical changes and main causes for seed deterioration were studied under accelerated ageing. Highly significant results were obtained in all the cases of physiological, biochemical and anatomical changes during accelerated ageing condition from zero to 10 days of accelerated ageing.

Among the above varieties, ADT 39 registered maximum germination (80 per cent) and ADT (R) 46 recorded the minimum (74 per cent). The reduction in germination over the period of 10 days of accelerated ageing was less in ADT 39 (20 per cent) as compared to ADT (R) 46 (30 per cent) (Table 1). The retention of higher germination potential of the paddy varieties viz., ADT 39, ADT (R) 49 and ADT (R) 50 might be due to genetic makeup as it was shown in the aleurone layer having intact cells with distinct cytoplasm even after 10 days of accelerated ageing (Plate 1). Whereas, in the variety ADT (R) 46, there was collapse of aleurone layer with poor distinct cytoplasm of cells in the same at 10 days of accelerated ageing. Disintegration of cells was observed in the aleurone layer in most of the area was also observed in 10 days of accelerated aged seeds. This might be the reason for poor storability of the cultivar ADT (R) 46 as compared to other varieties under accelerated ageing. The decline in germination over the period of storage

Table 1. Effect of accelerated ageing on germination (%) of different rice varieties

Varieties (V)	Germination (%)						
	Ageing duration (D)						
	0 day	2 days	4 days	6 days	8 days	10 days	Mean
V1	90 (71.5)	87 (68.8)	82 (64.8)	77 (61.3)	74 (59.3)	70 (56.7)	80 (63.4)
V ₂	88 (69.7)	85 (67.2)	80 (63.4)	72 (58.0)	66 (54.3)	58 (49.6)	74 (59.3)
V ₃	89 (70.6)	86 (68.0)	81 (64.4)	75 (60.0)	71 (57.4)	67 (54.9)	78 (62.0)
V ₄	88 (69.7)	85 (67.2)	82 (64.8)	76 (60.6)	70 (56.7)	66 (54.3)	77 (61.3)
Mean	89 (70.6)	85 (67.2)	81 (64.4)	74 (65.6)	69 (56.1)	64 (53.1)	77 (613)
SEd(±)	D			V			D×V
	0.940			0.858			2.102
CD (p= 0.05)	1.868			1.705			NS

Figures in parentheses indicate arc sine values

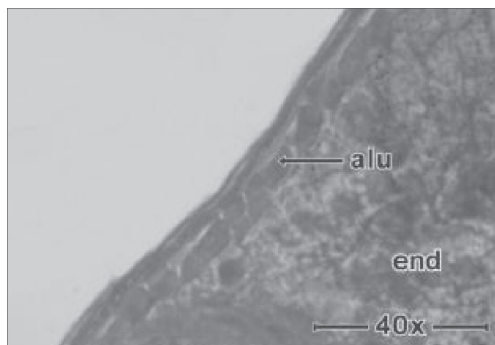
V₁ – ADT 39

V₂ – ADT (R) 46

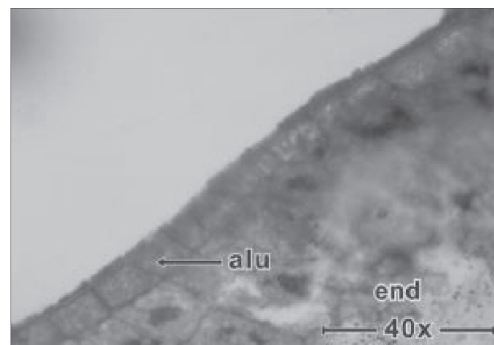
V₃ – ADT (R) 49

V₄ – ADT (R) 50

I. ADT 39

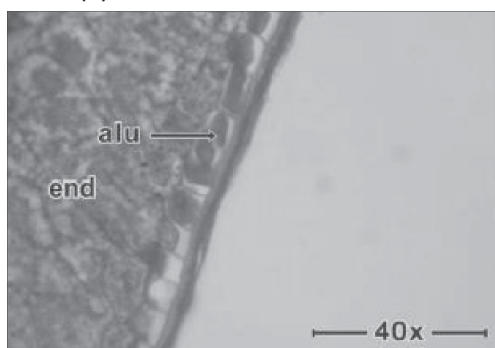


A) L.S. of fresh seed

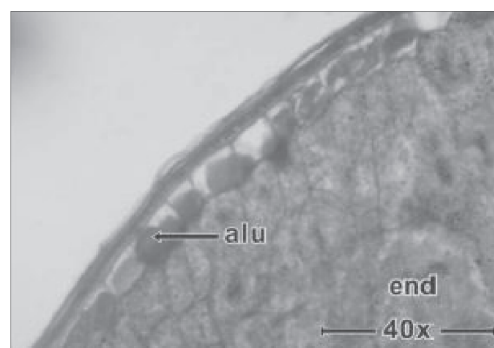


B) L.S. of 10 days accelerated aged

II. ADT (R) 46



A) L.S. of fresh seed



B) L.S. of 10 days accelerated aged

Plate 1. Histological study- Microtome sectioning of seed of rice varieties

might be due to depletion of food reserves coupled with decline in synthetic activity. In the present investigation, among the varieties, irrespective of the duration, maximum vigour value was recorded in ADT 39 (2184) and the minimum in ADT (R) 46 (2107) at 10 days of accelerated ageing (Table 2). The reduction in the vigour index from 0 to 10 days of accelerated

ageing was less in ADT 39 (38 per cent) whereas, it was high in ADT (R) 46 (51 per cent), thus revealing the poor storability of the rice genotype ADT (R) 46, compared to other tested varieties. This finding is corroborated with [9, 10] in rice. Use of aged seeds of rice caused rapid decline in germination, root and shoot length, seedling dry matter, vigour index [16].

Table 2. Effect of accelerated ageing on vigour index of different rice varieties

Varieties (V)	Vigour index						Mean
	Ageing duration (D)						
	0 day	2 days	4 days	6 days	8 days	10 days	
V ₁	2633	2405	2214	2140	1944	1907	2184
V ₂	2536	2262	2164	2039	1966	1677	2107
V ₃	2621	2293	2197	2076	1938	1859	2166
V ₄	2518	2351	2204	2124	1885	1764	2141
V ₅	2562	2337	2163	2137	1932	1782	2173
Mean	2574	2330	2188	2103	1933	1819	2158
SEd(±)		D		V			D×V
		27.41		26.02			61.29
CD (p= 0.05)		54.46		52.04			122.58
V ₁ – ADT 39	V ₂ – ADT (R) 46	V ₃ – ADT (R) 49	V ₄ – ADT (R) 50				

Biochemical parameters

The biochemical parameters *viz.*, alpha-amylase, electrical conductivity, peroxidase, DPPH and ascorbate peroxidase activity were measured quantitatively in all the tested genotypes from 0 to 10 days of accelerated ageing. The activity of all the above enzymes had decreased at subsequent storage interval under accelerated ageing. There is a sharp decline in the activity of enzymes during ageing of seeds. Changes in free radical scavenging enzymes, increased free radical production and increase in free amino acid were attributed to reduce the germination and seedling vigour during ageing of seeds. Electrical conductivity of seed leachates is a good index of seed deterioration. Intensity of membrane damage during storage was measured by electrical conductivity of the seed leachates. Among the varieties, ADT (R) 46 recorded the maximum value (0.250 dSm^{-1}) and ADT 39 which has a good storage potential recorded the minimum (0.226 dSm^{-1}) value. The rate of increases in electrical conductivity was less in ADT 39 (36 per cent) and the highest in ADT (R) 46 (48 per cent) (Figure 1). Alpha-amylase activity was decreased to $1.201 \text{ mg maltose min}^{-1}$ at initial to $1.128 \text{ mg maltose min}^{-1}$ at 10 days of accelerated ageing. The variety ADT 39 recorded the highest alpha-amylase ($1.200 \text{ mg maltose min}^{-1}$) activity and ADT (R) 46 recorded the lowest ($1.148 \text{ mg maltose min}^{-1}$). The rate of reduction over the period 0 to 10 days of accelerated ageing was maximum in ADT (R) 46 (12 per cent) and minimum in ADT 39 (7 per cent) (Figure 2). This might be due to varietal differences in exhibiting the hydrolytic enzymes and this

can be supported by the histological studies of the above varieties wherein the integrity of aleurone layer and embryo differed in the degree of intactness in various genotypes over a period of ageing.

The loss of seed viability was associated with the decrease in DPPH antioxidant activity in the seeds during storage. The total antioxidant activity of different varieties determined by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect revealed that the DPPH activity was found to be reduced from 49.95 per cent at initial to 39.78 per cent at 10 days of accelerated ageing. Among the varieties, the DPPH activity was maximum in ADT 39 (46.66 per cent) and minimum DPPH activity in ADT (R) 46 (44.59 per cent). Among the tested varieties, the rate of reduction in DPPH activity in ADT (R) 46 was 35 per cent and in ADT 39 was 20 per cent (Figure 3).

Reactive oxygen species and hydrogen peroxides are produced from several metabolic reactions and could be destroyed by the activity of scavenger enzymes like catalase and peroxidase. Peroxidase activity decreases substantially with ageing. In the present investigation, irrespective of duration of accelerated ageing, ADT 39 recorded the maximum peroxidase activity $0.203 \text{ m mol tetra-guaiacol min}^{-1} \text{ g}^{-1}$ seed and ADT (R) 46 recorded the minimum $0.168 \text{ m mol tetra-guaiacol min}^{-1} \text{ g}^{-1}$ seed. Among different varieties, the ADT (R) 46 recorded 67 per cent reduction in peroxidase activity ADT 39 registered 52 per cent reduction. Within the 10 days of accelerated ageing, the peroxidase activity reduced from $0.232 \text{ m mol tetra-guaiacol min}^{-1} \text{ g}^{-1}$ seed (0 days) to 0.154

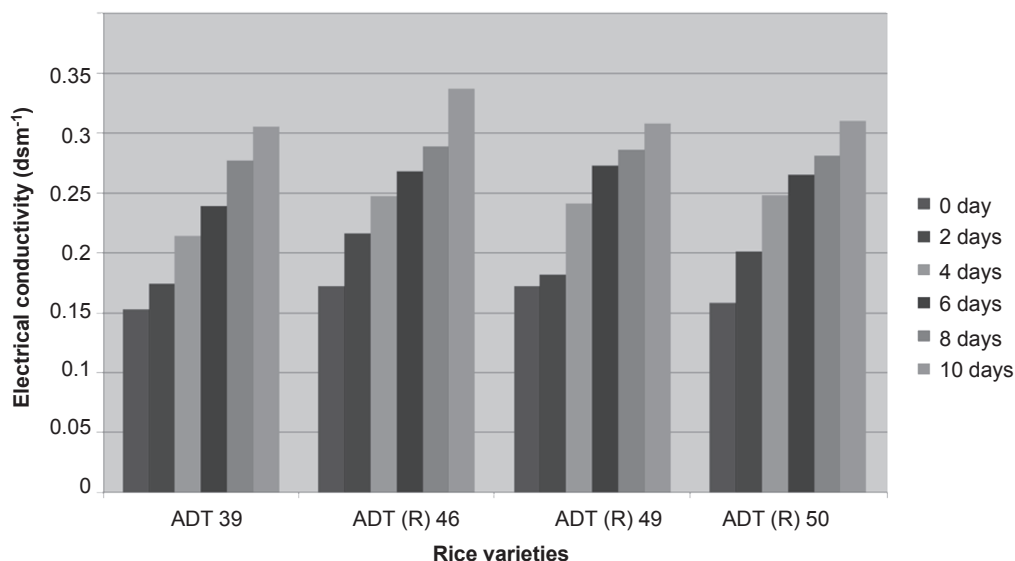


Figure 1. Effect of accelerated ageing on electrical conductivity (dSm^{-1}) of different rice varieties

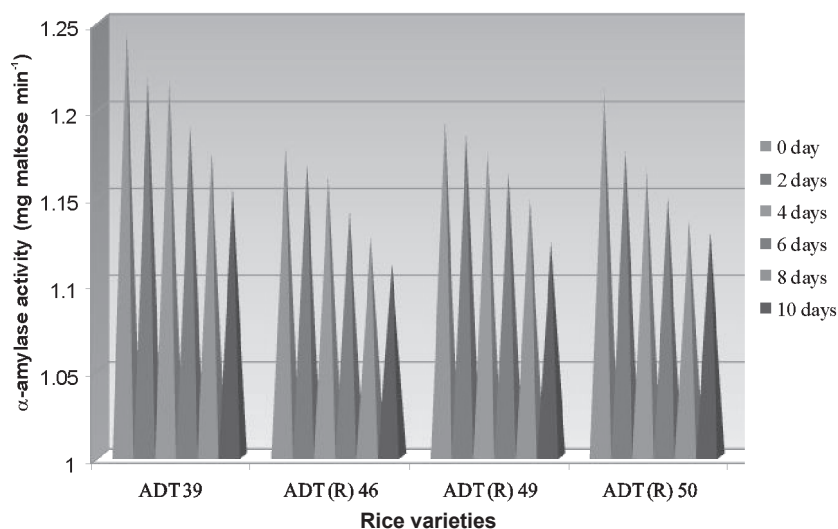


Figure 2. Effect of accelerated ageing on α -amylase activity ($\text{mg maltose min}^{-1}$) of different rice varieties

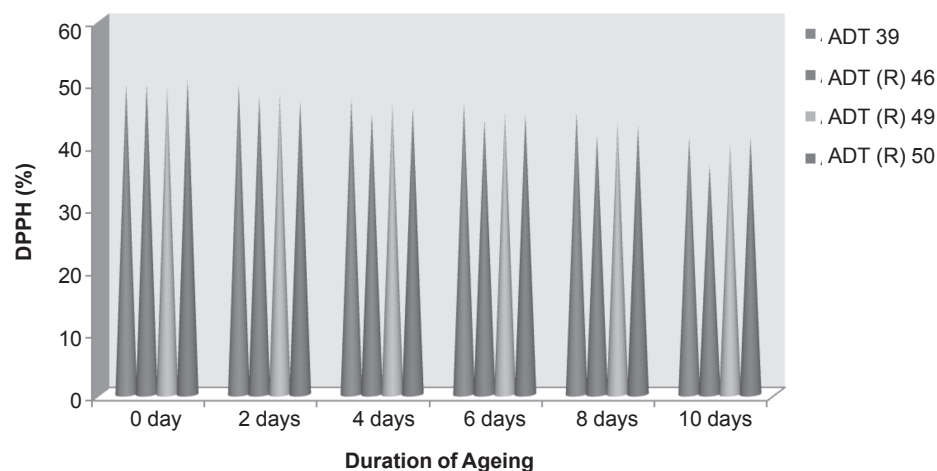


Figure 3. Effect of accelerated ageing on DPPH (%) (1, 1-diphenyl-2 picryl hydrazyl) of different rice varieties

mol tetra-guaiacol $\text{min}^{-1} \text{g}^{-1}$ seed (10 days) (Figure 4). The loss of seed viability was associated with the decreased, ascorbate peroxidase (APX) and antioxidant activity in the seeds. The present study revealed that ascorbate peroxidase activity was decreased over the period of accelerated ageing. Among the varieties, ADT 39 recorded the highest activity ($2.024 \text{m moles min}^{-1} \text{g}^{-1}$ seed) and the lowest activity was recorded by ADT (R) 46 ($1.775 \text{m moles min}^{-1} \text{g}^{-1}$ seed). The rate of reduction was maximum in ADT (R) 46 (83 per cent) and minimum in ADT 39 (57 per cent) (Table 3). Certain toxic metabolites like hydrogen peroxide might have formed as a consequence of oxidative stress. The complete loss of activity of enzymes may be due to the rapid oxidative stress caused due to high temperature and relative

humidity. Changes in free radical scavenging enzymes, increased free radical production and increase in free amino acid were attributed to reduce the germination and seedling vigour during ageing of seeds [17]. The above results obtained were supported with following findings by several authors [16 & 18].

Histological study- Microtome sectioning of seed of rice varieties

Histological basis for seed deterioration during accelerated ageing was studied with microtome sectioning viewed under optical microscope (Euromex) at 40X magnification. Based on the result, it is revealed that paddy varieties viz., ADT 39, ADT (R) 49 and ADT (R) 50 exhibited the aleurone layer having intact cells

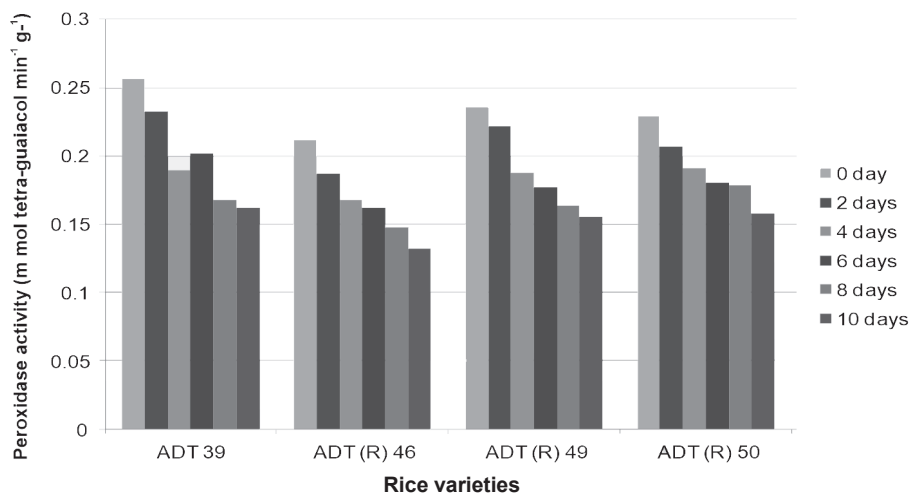


Figure 4. Effect of accelerated ageing on peroxidase activity (m mol tetra-guaiacol min⁻¹ g⁻¹) of different rice varieties

Table 3. Accelerated ageing on ascorbate peroxidase activity (mmol min⁻¹g⁻¹) of different rice varieties

Varieties (V)	Ascorbate peroxidase activity (m mol min ⁻¹ g ⁻¹)						Mean
	Ageing duration (D)						
	0 day	2 days	4 days	6 days	8 days	10 days	
V ₁	2.523	2.333	2.000	1.964	1.845	1.571	2.024
V ₂	2.231	2.071	1.785	1.666	1.555	1.333	1.775
V ₃	2.428	2.321	1.940	1.783	1.690	1.537	1.966
V ₄	2.511	2.321	2.140	1.903	1.714	1.429	2.003
Mean	2.379	2.244	1.968	1.837	1.708	1.443	1.930
SEd(±)		D		V			D×V
		0.082		0.075			0.185
CD (p= 0.05)		0.165		0.151			NS

with distinct cytoplasm after 10 days of accelerated ageing (Plate 1). Whereas, in the variety ADT (R) 46, there was collapse of aleurone layer with poor distinct cytoplasm of cells in the same at 10 days of accelerated ageing. Disintegration of cells was observed in the aleurone layer in most of the area was also observed in 10 days of accelerated aged seeds. This might be the reason for poor storability of the cultivar ADT (R) 46 as compared to other varieties under accelerated ageing. This present results support the 'classical' conception of [19] concerning the role of the aleurone layer in the production of α -amylase in barley. These results are in line with the findings of [10] in rice.

CONCLUSION

The study concluded that the seed quality in terms of physiological and biochemical characters drastically reduced with advancement of ageing period. The reduction in germination over the period of 10 days of

accelerated ageing was less in ADT 39 (20%) as compared to ADT (R) 46 (30%). The histological study for the above five varieties revealed that in all the varieties, except ADT (R) 46, the aleurone layer was having intact cell with distinct cytoplasm even after 10 days of accelerated ageing whereas, the variety ADT (R) 46 exhibited collapsed aleurone layer with poor distinct cytoplasm of cells which might be the cause for poor storability of ADT (R) 46. These biochemical and anatomical changes happening are well reflected in terms of loss of viability and vigour in this variety.

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