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Physiological and Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza sativa* L.)

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ABSTRACT

The present study was carried out in five rice varieties *viz*. Pusa-1, Sugandha-4, Sugandha-5, Saket-4 and Saket-370, for analyzing the varietals differences during seed deterioration by subjected them to Accelerated Ageing (AA) at 45°C and 100% relative humidity (RH) for 24, 48, 72 and 96 h. Various physiological parameters such as moisture content, germination percentage, seedling vigour, vigour index and biochemical parameters such as reducing sugar based on DNS assay, soluble protein by Lowry's method were explored. Different levels of seed vigor were obtained with different varieties and duration of accelerated aging treatments. Prolongation of ageing led to deterioration of both germinability and seed viability. The physiological results were coinciding with the biochemical parameters. Ageing conditions resulted in increased moisture content in all the varieties. The study concludes that accelerated ageing showed effect on seed quality of all the varieties of rice. All the test experiments performed concluded that Sugandha-4 is the best variety, followed by Saket-370 among all five varieties and Sugandha-5 and Saket-4 are highly sensitive varieties with respect to accelerated ageing.

Key words: Accelerated ageing, poaceae, pusa, saket, sugandha, vigour index

INTRODUCTION

Rice (*Oryza sativa* L.) a member of family Poaceae is a cereal foodstuff which forms an important part of the diet of many people worldwide. It is a staple food for more than 60% of the world population, especially in tropical Latin America and East, South and Southeast Asia, making it the second-most consumed cereal grain. It provides 21% of the human per capita energy and 15% of protein globally (FAO, 2003). India is the second chief producer of rice (116.58 mt), in India the total area under rice is 42.17 million hectares which accounts for 33.3% of the total food crop area (Venkataramani, 2002).

Indian rice is highly appreciated in world market for its taste and suitability. Rice is not only a rich source of carbohydrate and proteins but also provides vitamins, minerals and fibers. Rice cultivation in the country is undertaken in the humid tropical and sub-tropical climate characterized by high temperature and high relative humidity, resulting in changes in genetic integrity leading to more rapid deterioration of seeds.

Seed deterioration, a natural process is expressed as the loss of quality, viability and vigour during ageing or adverse environmental conditions. It is an irreversible degenerative process that occurs during storage. The rate of deterioration is however, influenced by the seed moisture content and the temperature of the storage, an increase in either leading to more rapid deterioration (Ellis *et al.*, 1992). Many physiological and biochemical manifestations of seed deterioration have been extensively reported (Kalpana and Rao, 1995; Maeda and Wutke, 1996; McDonald, 1999; Kruse, 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 vigor have been developed based on the physiological effect of ageing (ISTA, 1993; Tekrony, 1993; Woltz and Tekrony, 2001; 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 moisture content. It provides a simple and good method for studying sequence and relationship of process of deterioration over short periods.

Accelerated ageing test procedures have been standardized for several crop species such as soyabean (Tekrony, 1993; Sung, 1996; Krishnan *et al.*, 2003) corn (Woltz and Tekrony, 2001) pea (Jatoi *et al.*, 2004) chickpea (Maeda and Wutke, 1996; Kapoor *et al.*, 2010), pigeon pea (Kalpana and Rao, 1995) radish (Jain *et al.*, 2006) and rice (Krishnaswamy and Sheshu, 1990; Ray *et al.*, 1990; Ellis *et al.*, 1992; Zhou *et al.*, 2002; Bam *et al.*, 2006).

The changes associated with ageing are many and depending on ageing conditions, differences among cultivars (Kalpana and Rao, 1995; Jain *et al.*, 2006) or harvesting conditions (Shepherd *et al.*, 1995). A broad review of this aspects related to rice seed quality with regards to accelerated ageing was also published by several researchers (Ray *et al.*, 1990; Fashui, 2002; Gowda *et al.*, 2002; Bailly, 2004). Varietals differences in the longevity of rice seeds stored under ambient conditions have also been reported by Krishnaswamy and Sheshu (1990) and Ray *et al.* (1990). 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 that would enable incorporation of trait for better storability in the genetic background of the high yielding varieties. Thus, present investigation was aimed to investigate various physiological and biochemical changes during seed deterioration as a result of accelerated ageing as well as to find out superior variety among five experimental varieties of rice (*Oryza sativa* L.).

MATERIALS AND METHODS

Experimental materials: Seed deterioration process was examined by using five rice varieties named Pusa-1, Sugandha-4, Sugandha-5, Saket-4 and Saket-370. The varieties were procured from the National Seed Cooperation, Pusa Campus, New Delhi and seed gene bank, SVBPU, Meerut, India in October 2008. This work was carried out at Department of Biotechnology, MIET, Meerut during 2008-2009.

Accelerated ageing: For accelerated ageing, the seeds were exposed to 45°C temperature and 100% Relative Humidity (RH) for 24, 48, 72 and 96 h (Deluche and Baskin, 1973). Seeds which were not exposed to the ageing treatments were referred as control (0 h).

Physiological parameters

Germination percentage: Three replicates of twenty seeds each were planted between moistened germination papers and incubated in a germinator maintained at a constant temperature of 30°C. Seeds with 0.5 cm radicle and plumule were considered as germinated (ISTA, 1993).

Moisture content: Moisture content was tested by gravimetric method (high constant temperature oven method) at 130°C for 1 h (ISTA, 1993).

Seedling vigour: Three replicates of ten seeds each were planted between paper (3×2) and kept in the vigour stands which were maintained at 30°C. After seven days total root length and shoot length of all the seedlings were studied.

Vigour index: Vigour Index was calculated as the product of seedling vigour (root and shoot length) and germination percentage (Abdul-Baki and Anderson, 1973).

Biochemical parameters

Determination of reducing sugars: Reducing sugars from seeds were extracted in 80% ethanol. The supernatant were dried in a water bath at 80°C and remain was dissolved in distilled water. Reducing sugars were determined by DNS method. Glucose was used as standard.

Determination of soluble proteins: Seeds were extracted in 0.1 M phosphate buffer (pH 7.5) precipitated in 10% TCA, redisolved in 0.1 N NaOH and estimated by Lowry's method (Lowry *et al.*, 1951). Bovine serum albumin (BSA) was used as a protein standard.

RESULTS AND DISCUSSION

The various observations were recorded for physiological and biochemical parameters are as follows:

Physiological parameters

Germination percentage: The results of seed viability (germination percentage) in unaged (control) seeds of all the varieties showed high germination percentage (more than 80%) (Fig. 1). Accelerated ageing treatment of *Oryza sativa* L. varieties resulted in significant declined in germination percentage with the passage of ageing time in all the five varieties as it declines by 9% to 38.0% in Pusa-1, 80 to 39.6% in Sugandha-4, 90 to 40.2% in Sugandha-5, 85 to 41.2% in Saket-4 and 80 to 43.8% in Saket-370. Thus maximum decrease (in germination percentage) occurred in Pusa-1, followed by Sugandha-5, Saket-4. Saket-370 is least effected by accelerated ageing on the basis of germination percentage. Similar decreases in the germination percentage after accelerated ageing in rice has also been reported earlier by several workers (Renard, 1988;

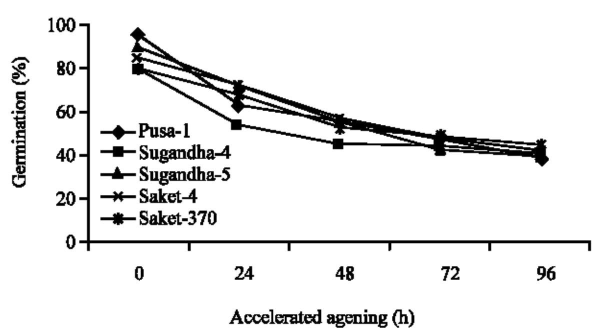

Fig. 1: Effect of accelerated ageing on germination percentage (%) of five *Oryza sativa* L. varieties

Ray *et al*., 1990; Kruse, 1999; Gowda *et al*., 2002; Lu, 2002; Vimala and Thiagarajan, 2002). Reduction in germination is due to degradation of mitochondrial membrane, leading to reduction in energy supply necessary for germination (Gidrol *et al*., 1998).

Moisture content: A significant increased were observed in moisture content after ageing in all the varieties (Fig. 2). With ageing moisture content increased by 9.46 to 16.67 in Pusa-1, 7.56 to 16.48 in Sugandha-4, 8.74 to 18.85 in Sugandha-5, 7.2 to 18.1 in Saket-4 and 9.17 to 15.55 in Saket-370. Under accelerated ageing the maximum increased was observed in Saket-4 followed by Sugandha-5. This increased could be explained by increased in imbibe water due to the disorganization of the cell membranes during ageing. Such differences in maintenance of seed moisture capacity have also been observed by Ellis *et al.* (1992) and Jain *et al.* (2006). This variation in moisture content implies greater sensitivity of longevity to the accelerated ageing time duration.

Seedling vigour: Besides germination percentage, shoot and root length also elicited a significant decline over the control seeds (Fig. 3, 4). Maximum shoot length was measured in Saket-4 and lowest shoot length was measured in Pusa-1 in both before and after accelerated ageing treatments. Among the five varieties the maximum decline were observed in Sugandha-4 followed by Saket-4 and Sugandha-5 and minimum decline were in Pusa-1 followed by Saket-370. Pusa-1 variety exhibited minimum differences in shoot as well as root length before and after accelerated ageing, showing least deterioration. Saket-4 and Saket-370 exhibited maximum influence of ageing on root

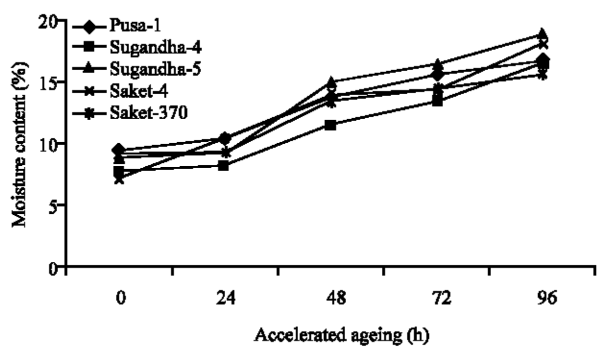

Fig. 2: Effect of accelerated ageing on moisture content (%) of five *Oryza sativa* L. varieties

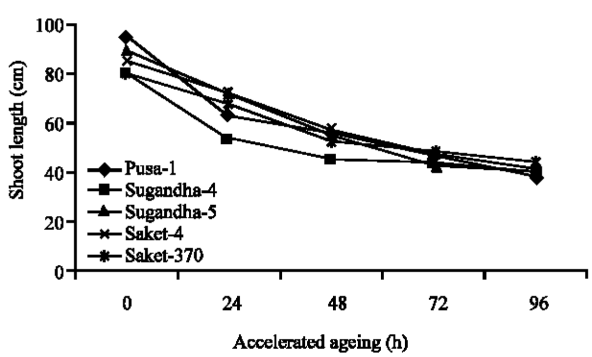

Fig. 3: Effect of accelerated ageing on shoot length (cm) of five *Oryza sativa* L. varieties

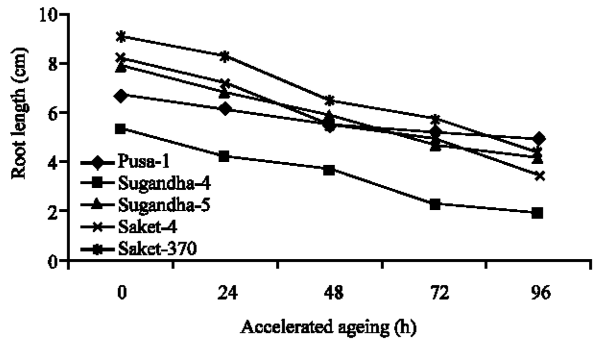

Fig. 4: Effect of accelerated ageing on root length (cm) of five *Oryza sativa* L. varieties

parameter. Present results are in harmony with those obtained by Jatoi *et al.* (2004), Jain *et al.* (2006) and Munnujan *et al.* (2007). Growth efficiency and other seedling characteristics like shoot and root length also decreased with increased accelerated time period.

Vigour index: With the decrease in germination percentage and seedling length in the entire five varieties vigour index also showed a decline pattern during accelerated ageing (Fig. 5). Vigour index decreased from 1491.5 to 395.2 in Pusa-1, 1384 to 332.64 in Sugandha-4, 1719 to 438.18 in Sugandha-5, 1729.75 to 449.08 in Saket-4 and 1528 to 464.28 in Saket-370. In the control the maximum vigour index were observed in Saket-4 followed by Sugandha-5, Saket-370 and then by Pusa-1 and the least value was observed in Sugandha-4, whereas, in the accelerated ageing treated seeds the maximum and minimum vigour index was observed in Saket-370 and Sugandha-4, respectively. Thus Sugandha-4 was found to be having least germination growth potential in natural as well as in ageing conditions. Deterioration may be indicative of an inability to reform functionally competent membranes during rehydration of seeds resulting in loss of vigour and lack of germination.

On the basis of differences in vigour index before and after accelerated ageing it was cleared that Sugandha-5 and Saket-4 are very sensitive varieties followed by Pusa-1, whereas Sugandha-4 and Saket-370 are least influenced varieties. Similar conclusion on growth efficiency and seedling vigour as a result of ageing effects with regard to varietal responses were also reported by several workers (Ellis *et al.*, 1985; Tekrony, 1993; Kalpana and Rao, 1995; Gowda *et al.*, 2002; Jatoi *et al.*, 2004; Munnujan *et al.*, 2007; Kapoor *et al.*, 2010).

Biochemical parameters

Reducing sugar estimation: There was a significant ageing time duration x varieties interaction for reducing sugar contents (mg g^{-1} fw) as estimated by DNS method (Fig. 6). It decreased from 6.7 to 5.4 mg in Pusa-1, 6 to 5 mg in Sugandha-4, 7.1 to 5.5 mg in Sugandha-5, 6.5 to 5 mg in saket-4 and 6.2 to 5.5 mg in Saket-370. The maximum reducing sugar content was achieved in Sugandha-5 and least reducing sugar content in Sugandha-4. Similar to present findings Ray *et al.* (1990) also reported differences in soluble carbohydrate content in two cultivars of rice subjected to accelerated ageing conditions. This reduction in reducing sugar content is due to the inhibition of photosynthesis which is associated with decline in pigment contents resulted from the reduction in leaf area or due to decrease in leaf organic acid or due to less stomatal openings in leaf due to accelerated ageing.

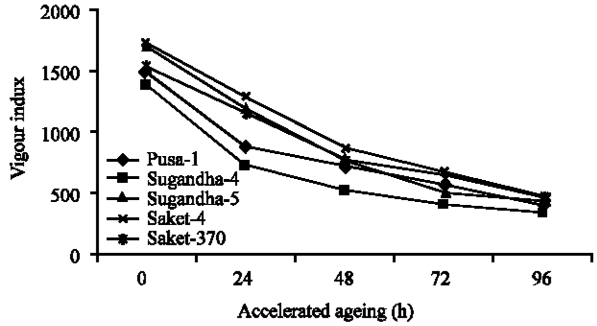

Fig. 5: Effect of accelerated ageing on vigour index of five *Oryza sativa* L. varieties

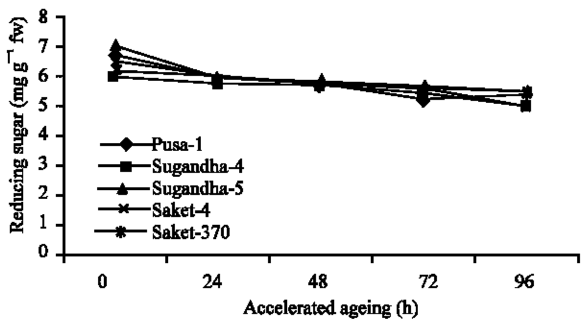

Fig. 6: Effect of accelerated ageing on reducing sugar (mg g^{-1} fw) of five *Oryza sativa* L. varieties

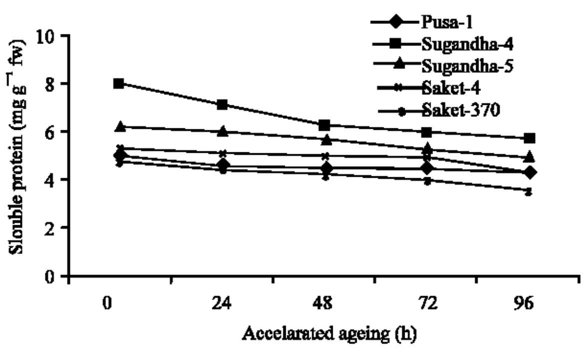

Fig. 7: Effect of accelerated ageing on soluble protein (mg g^{-1} fw) of five *Oryza sativa* L. varieties

Protein estimation: Another important parameter which is influenced by ageing was protein content (mg g^{-1} fw) as any changes in status of seeds eventually changed protein content (Fig. 7). Result showed that the protein content as estimated by Lowry's method decreased from 4.9 to 4.3 in Pusa-1, 7.9 to 5.7 in Sugandha-4, 6.2 to 5 in Sugandha-5, 5.3 to 4.3 in Saket-4 and 4.8 to 3.6 in Saket-370, although this decrease were not significant. In the control maximum value were observed in Sugandha-4 followed by Sugandha-5, whereas, Saket-370 had least protein content. After accelerated ageing maximum and minimum reduction in protein content were observed in Sugandha-4 and Pusa-1, respectively. Zhou *et al.* (2002) also reported no changes in protein content of rice during ageing. The decrease in soluble proteins content following accelerated ageing has been shown to be accompanied by denaturation of protein, elevated superoxide dismutase activity (Kalpana and Rao, 1995) and lack of ATP (Gidrol *et al.*, 1998).

CONCLUSION

The study was conducted in five different varieties of rice (*Oryza sativa*) namely Pusa-1, Sugandha-4, Sugandha-5, Saket-4, Saket-370, in order to investigate physiological and biochemical changes during seed deterioration. To understand basis of seed deterioration during storage all the varieties were subjected to accelerated ageing by keeping at 45°C and 100% RH for 96 h. It was clear from the study that no single criteria are sufficient to be used as an index of deterioration of seed due to accelerated ageing. The resultant plants from aged seeds recorded lower values for all the yield attributing parameters The cumulative study based on results of all the parameters concluded that among all experimental varieties Sugandha-5 and Saket-4 are highly sensitive varieties and Sugandha-4 and Saket-370 are least sensitive varieties.

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