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Seed Coating in Relation to Minimizing the Effects of Seed Ageing in Rice *(Oryza sativa L.)*

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Abstract

Rice is the second largest principal food crop in the world after wheat, which is one of the main staple food crops in India. Seed deterioration is a major issue in the developing countries, where proper control of humidity and temperature of stored seeds are not maintained. Seed coating of MTU-7029, PR-115 and Swarna Sub-1 rice varieties with royal flow, vitavax and imidacloprid along with polykote enhanced the seed quality parameters after exposing the seeds to adverse levels of temperature (40-45 ^{0}C) and 100% relative humidity for varying length of time (48 h, 96h, 144 h and 196 h). Among the seed coating treatments, the combination (polykote + royal flow+ imidacloprid + vitavax - T4) showed 50. 18% of germination at 48 h, seedling length of 36.97 cm and dry weight of 0.112 mg/seedling. Moreover, enhanced nitrate, nitrite and amylase enzyme activities under seed ageing signify the efficiency of seed coating in maintaining the seed quality. Thus, seed coating process substantiates that it is cost effective to enhance the seed quality parameters under seed ageing regime.

Key words: Pesticides, Rice, ROS, Seed deterioration, Seed quality parameters

Introduction

Rice (*Oryza sativa* L.) is an important staple and cereal food for half of the world's population, particularly in tropical South and Southeast Asia, East and Latin America. It can be grown under broad range of environments consisting 11% (approx.) of world arable lands (Abdul-Baki and Anderson,1973). On the other hand, burgeoning population, shrinkage of land and water resources and aggravating climate changes jeopardizing the quality rice production. For increased crop production, quality rice seed is a *numero uno* input and needs to strengthen under vagaries of climatic change. However, most of the Indian farmers use saved seeds of traditional varieties (less seed replacement rate) that hampers the quality of seed. During storage, seed viability looses due to accumulation of DNA, lipid and protein damages caused by reactive oxygen species (ROS). This loss of DNA, protein and lipids integrity ultimately leads to poor performance and death of seed (Agarwal and Kumar, 2016; Kumar *et al.,* 2015; Kumar *et al.,* 2016).

Reactive oxygen species plays dual functions in seed germination and protection based on the concentration during the respective physiological process. Recent literature suggests that under low or limiting threshold values, these species acts as a secondary messenger and enhances the seed germination. In contrary, at higher concentrations these may damage the integrity of lipids, protein and DNA that are responsible for seed longevity. In addition, storage conditions such as temperature and water content further intensifies the seed aging process. Because at low temperatures, seed deterioration is mainly resulted from free radical damage induced by Amadori and Maillard reactions. Similarly, at higher temperatures, proteins inactivation occurs; as a result, it leads to seed deterioration (Akazawa and Hara-Hishimura, 1985).

To address the issue, several approaches have been suggested like storing seeds at lower temperature and humidity (water content). However, formation of Amadori and Maillard reactions could takes place even at very low moisture content (Beck E and Ziegler P, 1989; Chandusingh *et al.,* 2017). Hence, an alternative technique is essential to conserve the seed. Seed coating is one such technique, where seed is coated with different coating materials, to reduce the adverse effects of seed ageing and may enhance the germination capability under ambient conditions. Seed coating is used for protection against seed-borne fungi, which simultaneously enhanced the shelf life of paddy (Dadlani *et al.,* 1992; Delouche and Baskin, 1973). In another study, seed coating with polymer, fungicide and insecticide in cotton has improved seed quality parameters (Ferrari and Varner, 1969). Seed coating is generally done with plasticizer polymer, which forms thin films and adheres firmly to pesticide and insecticide. Coating facilitates precise application of insecticides and pesticides to protect from pathogens. In addition, the coating material is readily available either in the form of liquids or powder that enables to apply directly to the seed surface. This explains the possible mechanisms of insecticide or pesticide in protection or enhancement of seed quality parameters (Gomes and Garcia, 2013). However, an interesting question arises is whether these ingredients have any effect on nitrite reductase, nitrite reductase and α- amylase that are relevant to nitrogen assimilation in plants. Hence, in the present study, the effect of coating ingredients and their effect on antioxidant enzymes relevant to seed ageing have been explored.

Materials and Methods

Chemicals

All the chemicals were procured from Hi-media and Qualigens that are of analytical grade. The fungicides vitavax, imidacloprid, royal flow and polykote polymer have been procured from authorized dealer of the local market.

Seed germination tests

Seed germination tests were conducted using 'top of paper' method as per the standards of International Seed Testing Association (ISTA) protocol (Gupta and Singh, 1993; ISTA, 1990). The germination of seed would be considered, when the radicle and plumule attained 0.5 cm as per ISTA guidelines (ISTA, 1993). Mean seedling length and dry weight were also recorded (ISTA) to understand the seed quality parameters (ISTA, 2008). Assessment of seed vigour was based on seedling length, germination percentage, and seedling dry weight according to Abdul-Baki and Anderson (1994) and expressed as below.

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

Seedling Vigour index II = Germination (%) x Mean seedling dry weight (mg)

Accelerated ageing test (AAT)

Accelerated ageing test is carried out at adverse levels of temperature (40-45°C) and 100% relative humidity for varying length of time (48h, 96h, 144 h and 196 h); thereafter the vigour was assessed by regular germination tests. Basis of this test is that higher vigour tolerates the high temperature and humidity (Jarande *et al.,* 1994).

Field experiments

Field experiments were conducted during 2012-13 at the research farm of Directorate of Seed Research, Kushmaur, Mau. The seed material of three elite genotypes i.e. MTU-7029, PR-115 and Swarna Sub-1 of rice were obtained from the seed production and processing unit of Directorate of Seed Research. The collected seeds were initially surface sterilized with 0.2%(v/v) mercuric chloride and then coated with various treatments. The seed coating treatment designated as T1, T2, T3 and T4 and their combinations are given in Table-1.

Table 1: Seed coating treatments and their desired combinations

Designated symbol	Treatment combinations
T1	polykote (4mL/kg) + royal Flow 40 sec (2.4mL/kg)
T2	polykote (4mL/kg) + imidacloprid (6mL/kg)
T3	polykote (4mL/kg) + vitavax (2g/kg)
T4	polykote (4mL/kg) + royal Flow (2.4mL/kg) + imidacloprid (6mL/kg) + vitavax 200 (2g/kg)
Control	untreated seeds

Nitrate reductase and nitrite reductase assay

Nitrate assimilatory enzymes such as nitrate reductase and nitrite reductase activities were assayed as per the methods developed by Jaworski (1971) and Ferari and Varner (1969), respectively.

α –Amylase assay

Activity of α -amylase was assayed according to the method of Kunkur *et al.,* (2007).

Statistical Analysis

The data were statistically analyzed using analysis of variance appropriate for completely randomized design (CRD). Main and interaction effects were compared using LSD test at 0.05 level of probability, when the F-values were significant.

Results

Seed quality parameters under ageing regime

Seed quality parameters such as germination percentage, seedling length and seedling dry weight are important features to determine the quality of seed. Generally, seed upon storage several changes occurs at biochemical and molecular levels, as a result, the quality of seed deteriorates. The seed treated with different combinations have showed a general phenomenon that upon storage of seed germination percentage was decreased. This might be observed that upon inducing ageing treatment, the free radicals generated could facilitate a chain of reactions, as a result the damage could have envisaged. Treatments applied to minimize the adverse effects of seed ageing, including (T1, T2, T3 and T4) have significantly improved the seed quality parameters over the uncoated control. However, among the treatments applied, the combination of polykote + royal flow + imidacloprid + vitavax (T4) has resulted with higher germination percentage (50.18%; at 48 h) followed by polykote + vitavax, polykote + royal flow and polykote + imidacloprid. Similarly, seedling length of 36.97 cm and dry weight of 0.112 mg/seedling in T-4 have been observed. These results implies that the treatment have significant impact in enhancing the seed quality parameters under storage conditions (Table 2, 3 and 4).

Seed quality parameters in addition to above characteristics, some other important features are vigour indices -1 and II. Decreasing trend in vigour indices have been observed with increasing ageing regime. However, T-4 showed 2557.15 of vigour index-I and 6.85 vigour index-II that is higher in comparison to control and other treatments (Figures 1 and 2). The combination of polykote + royal flow + imidacloprid + vitavax (T4) has shown significant response that might be accrued by synergistic effect of polymer and fungicides. For instance, Thobunluepop (2009) has reported enhancement of seed quality parameters up to 6 months using conventional captan; but treatment with captan coupled with eugenol incorporated chitosan-lignosulphonate polymer has resulted 9 months storage in rice (Kumar *et al.,* 2017a; 2017b; Matheus *et al.,* 2011). This observation may be explained with synergistic effects of polymer and bioactive agent of eugenol, which acts as fungicide.

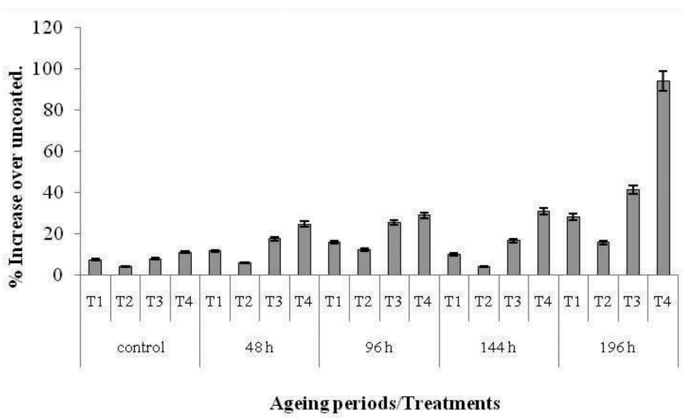

Figure 1: Effect of seed coating treatments on vigour index-I against the increasing seed ageing period in rice

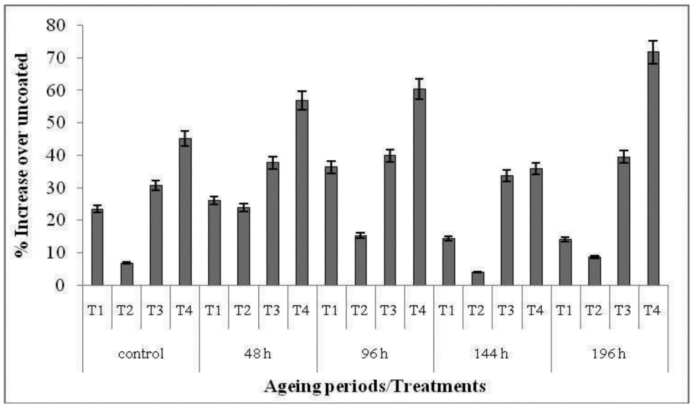

Figure 2: Effect of seed coating treatments on vigour index-II against the increasing seed ageing period in rice

Nitrate Assimilatory Enzymes

Nitrate reductase activity

Nitrate reductase activity (n mole g^{-1}f.w.h.$^{-1}$) clearly revealed that there was sharp decrease in nitrate reductase activity with the increasing ageing period in all varieties evaluated. Among the treatments applied, T4 combination has significantly improved the nitrate reductase activity over the other treatments and uncoated control. The combination of polykote + royal flow + imidacloprid + vitavax (T4) has showed maximum activity of 35.29, 32.27, 29.48 and 28.08 nmole g^{-1}f.w.h.$^{-1}$ at 48 h, 96 h, 144 h and 196 h, respectively (Figure 3).The trend of decreasing NR activity under ageing regime implies that the enzyme could have deteriorated by reactive oxygen species.

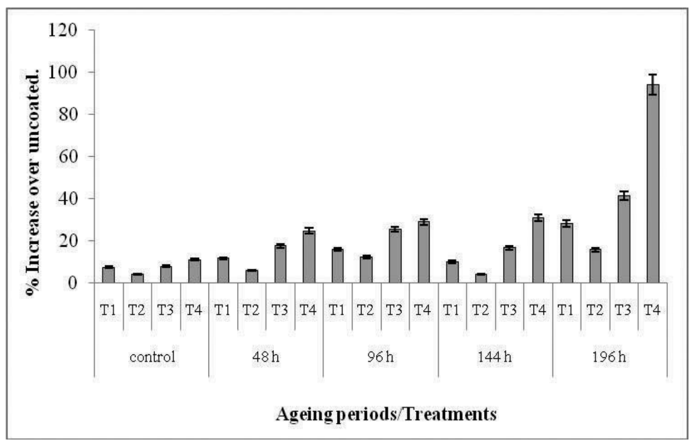

Figure 3: Nitrate reductase activity in treatments against the increasing seed ageing period in rice

Nitrite reductase activity

Nitrite reductase activity (n mole g^{-1}f.w.h.$^{-1}$) decreased with the increasing ageing period in all varieties evaluated. Treatments given to minimize the adverse effects of seed ageing, including (T1, T2, T3 and T4) significantly improved the nitrite reductase activity over the uncoated control. However, among the treatments the combination of polykote + royal flow + imidacloprid + vitavax (T4) has retained the activity of 80.31, 76.38, 62.29 and 68.07 at 48 h, 96 h, 144 h and 196 h, respectively (Fig. 4) than the other treatments and control. Nitrate reductase is a rate-limiting step in nitrogen assimilation in most of the crops. It has Mo element as a co-factor. Similarly, nitrite reductase catalyzes the reduction of nitrite to ammonium, which has iron and copper as the metal ions. The decrease in enzyme activity upon increase of ageing period has been observed in both the cases. It may be hypothesized that the reactive oxygen species synthesizes by Amadori and Maillard reactions. Further, it may be aggravated in presence of metal ions, since these ions enhance the lipid peroxidation by triggering the enzyme activity of lipoxygenase (Murthy *et al.,* 2003; Sano *et al.,* 2016). Due to above phenomenon, the activity could be decelerated upon increase of ageing treatments.

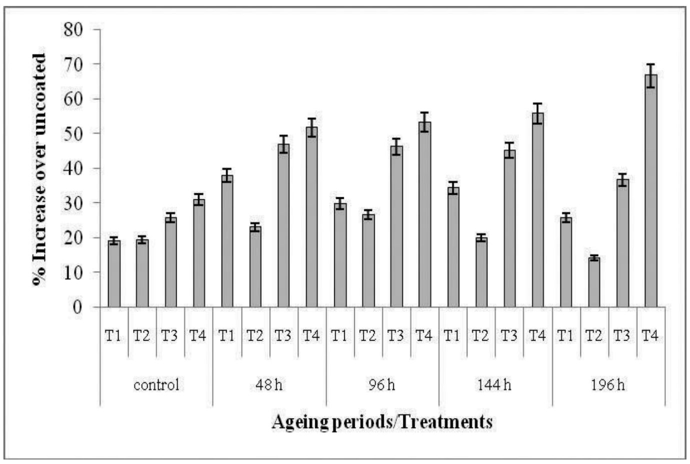

Figure 4: Nitrite reductase activity in treatments against the increasing seed ageing period in rice

α- Amylase activity (mg. of maltose released/mL)

The data recorded on α- amylase activity and the results obtained showed that the enzyme activity has been decreased with the increasing ageing period in all varieties evaluated. However, treatments applied to minimize the adverse effects of seed ageing, including (T1, T2, T3 and T4) have significantly improved the α-amylase activity over the uncoated control. Combinatorial treatment (T4) such as polykote + royal flow + imidacloprid + vitavax has responded with 0.97, 0.92, 0.88 and 0.84 mg of maltose released/mL, respectively. The behavior of amylase activity with increased ageing seed has been depicted in Figure 5. In the course of seed germination, α-amylase plays an important role in hydrolyzing the endosperm starch into soluble sugars in the aleuronic layer that provide the energy for the growth of roots and shoots (Rao *et al.,* 996). As seen from the Fig. 8, upon increase of ageing period increase in ROS threshold or owing to calcium metallo-enzyme it could have deteriorated by lipid peroxidation (Savitri *et al.,* 1998; Seck *et al.,* 2012; Singh *et al.,* 2017).

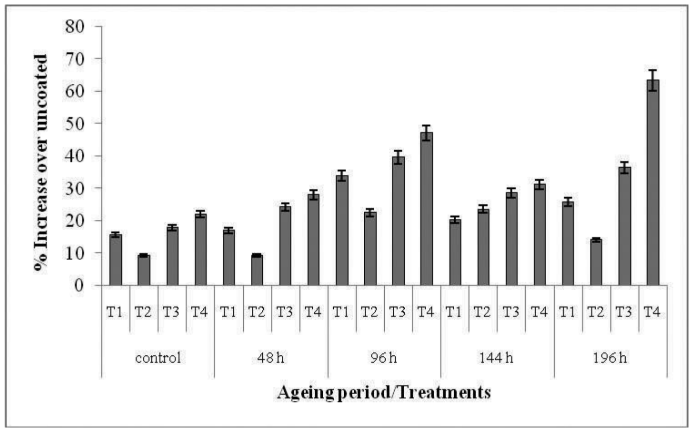

Figure 5: α- Amylase activity (mg. of maltose released/mL) in treatments against the increasing seed ageing period in rice

Discussion

Seed aging is a great concern, where seeds are stored in places that have no proper control of humidity and temperature. Moreover, fungus infection (biotic stress) and consequent structural and functional changes occur due to reactive oxygen species generation that may damage the lipids, DNA or proteins. In addition, any biotic stress such as fungus infection and Amadori and Maillard reactions synthesize reactive oxygen species. These ROS reduces its life span and ultimately leads to seed death (Sinha *et al.,* 2016; Sun and Leopold, 1995). ROS has to be maintained in controlled condition for prolonged period. In this study, seed coating with polymer and fungicides have been tested and found that the treatment (T-4) not only controlled the moisture content but also restricted the oxygen diffusion to the embryo along with reduction in leaching of inhibitors from the seed covering (Sun *et al.,* 1998; Sundaram and Thirupathihalli, 2014). On the one hand, fungicides like royal flow and vitavax protects the seeds against fungal invasion and safeguard from seed deterioration by reducing the physiological ageing. As a result, the seed germination, vigour indices-I and II, viability was maintained for comparatively longer period of time (Thobunluepop *et al.,* 2008; 2009). On the other hand, imidacloprid exhibit phytotonic effect that have resulted the enhanced seed quality parameters (Vamadevvappa, 1998;). These results are in congruent with the reports of several researchers in various crops (Vinutha *et al.,* 2015). Interestingly, in this study it has been found that the ageing affects nitrate reductase and nitrite reductase, which are key enzymes in nitrogen assimilation. Moreover, α-amylase also affected due to ageing that has profound role in mobilization of reserves (Vinutha *et al.,* 2014). Generally, seed longevity is declined in aged seeds due to oxidation of lipids, nucleic acids and proteins (Zarei *et al.,* 2012). To combat these conditions strategies like protection and repair mechanisms have been deployed. For instance, to restrict the mobility of ROS content, the cytoplasm attains glassy state coupled with antioxidant system is a good example of seed protection. On the other hand, repair system ponders the damage in the seed and removes with the help of methionine sulfoxide reductase and DNA glycosylase in proteins, RNA and DNA upon imbibition (Murthy *et al.,* 2003). Based on this, it is explained that ageing effected the enzyme activities; however, seed coating has enhanced the enzyme activities to maintain the seed longevity (Figure 6).

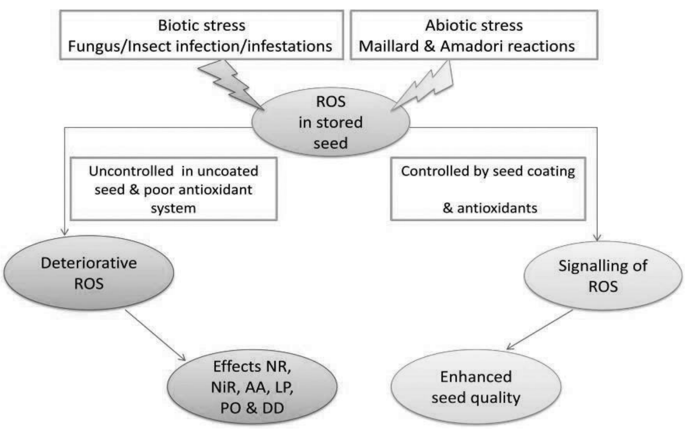

Figure 6: Role of seed coating in controlling the ROS to enhance seed quality parameters

NR: Nirate Reductase; NiR: Nitrite Reductase; AA: α-Amylase; LP: Lipid Peroxidation; PO:Protein Oxidation: DD; DNA damage.

Conclusions

From the study, emerging evidence implies that upon ageing seed quality deteriorates. The seed quality parameters are however, enhanced with seed coating in terms of seed germination percentage, seedling length, weight and vigour indices I and II. Moreover, enzyme activities were decreased with increase in ageing regime. Nevertheless, the activities were tolerated with increase in ageing period in treatment (T4) and suggesting that the polymer and fungicides could implying that the seed coating could reduce the ROS and maintain to remain in controlled condition so that the seeds longevity can be improved.

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References

Abdul-Baki AA, Anderson JD. 1973. Vigour determination in soybean by multiple criteria. Crop Science 13: 630-63.

Agarwal DK, Kumar SPJ. 2016. IISS Annual report 2015-16. ICAR-Indian Institute of Seed Science. P. 25.

Akazawa T, Hara-Hishimura. 1985. Topographic aspects of biosynthesis, extracellular secretion, and intracellular storage of proteins in plant cells. Annual Review of Plant Biology 36: 441-472.

Beck E and Ziegler P. 1989. Biosynthesis and degradation of starch in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 40: 95–117.

Chandu Singh, Kumar SPJ, Sripathy KV, Somasundaram G, Udaya Bhaskar K, Ramesh KV, Madan Kumar, S. Rajendra Prasad. 2017. Characterization and identification of rice germplasm accessions using chemical tests. Seed Research 45 (1):75-83.

Dadlani M, Shenoy VV, Seshu V. 1992. Seed coating to improve stand establishment in rice. Seed Science Technology 20: 307 – 313.

Delouche JC and Baskin CC.1973. Accelerated ageing techniques for predicting the relative storability of seed lots. Seed Science Technology 1: 427–452.

Ferrari TE and Varner JE.1969. Substrate induction of nitrate reductase in barley aleurone layers. Plant Physiology 44: 85-88.

Gomes MP and Garcia QS .2013. Reactive oxygen species and seed germination. Biologia 68: 351–357.

Gupta A and Singh D.1993. Effect of fungicidal treatment on seed viability and mycoflora of stored vegetable seeds. Seed Research 20: 270-275.

ISTA. 1990. Amendments to have International Rules for Seed Testing, ISTA, Zurich.

ISTA. 1993. International Rules for Seed Testing. Seed Science & Technology 21, 1 288(suppl.).

ISTA. 2008. International rules for seed testing. Zurich, 31p.

Jarande NT and Dethe MD. 1994. Effective control of brinjal sucking pests by imidacloprid. Plant Protection Bulletin 46: 43-44.

Jaworski EG .1971. Nitrate reductase in intact plant tissues. Biochemical Biophysical Research Communications 43(6): 1274-1279.

Kumar SPJ, Rajendra Prasad S, Banerjee R, Thammineni C. 2015. Seed birth to death: dual functions of reactive oxygen species in seed physiology. Annals of Botany 116(4): 663–668.

Kumar SPJ, Rajendra Prasad S, Madan Kumar, Chandu Singh, Sinha AK, Avinash Pathak. 2016. Seed Quality Markers: A Review. Research & Reviews: Journal of Botanical Sciences. S3, 13-17.

Jeong YO and Cho JL.1995. Effect of coating materials and priming on seed germination of tomato and pepper. *Korean Journal of Horticulture Science Technology* 36 (2): 185-191.

Kumar SPJ, Kumar VG, Dash A, Scholz P, Banerjee R. 2017a. Sustainable green solvents and techniques for lipid extraction from microalgae: A review, Algal Research 21: 138–147.

Kumar SPJ, Prasad SR, Banerjee R, Agarwal DK, Kulkarni KS, Ramesh KV. 2017b. Green solvents and technologies for oil extraction from oilseeds. Chemistry Central Journal 11:1-9.

Kunkur VK, Hunje R, Patil NKB, Vyakarnhal BS. 2007. Effect of seed coating with polymer, fungicide and insecticide on seed quality in cotton during storage. *Karnataka Journal of Agricultural Sciences* 20(1): 137 - 139.

Matheus MT, Lopes JC, Corrêa NB .2011. Maturação fisiológica de sementes de *Erythrina variegata* L. Ciência Florestal 21: 619-627.

Murthy UMN, Kumar PP, Sunday WQ .2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiate* (L.) Wilczek: Lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. *Journal of Experimental Botany* 54: 1057–1067.

Rao RVV, Sugunakar M, Rao C .1996. Effect of fungicidal treatment on the viability of groundnut (*Arachis hypogaea* L.) seed in storage. Seed Research 24 (1): 66–68.

Sano N, Rajjou L, Helen MN, Isabelle D, Annie M, Seo M. 2016. Staying alive: molecular aspects of seed longevity. Plant Cell Physiology 57 (4): 660-674.

Savitri H, Reddy SM, Reddy M .1998. Effect of seed treatment with fungicides and insecticides on seed borne fungi, storage pest, seed viability and seedling vigour in groundnut. Seed Research 26: 62 – 72.

Seck PA, Diagne A, Mohanty S, Wopereis MC .2012. Crops that feed the world 7: Rice. *Food Security* 4:7–24.

Singh RP, Agarwal DK, Sripathy KV, S Rajendra Prasad, Kumar SPJ. 2017. Varietal and seed replacement in the era of climate change. *ICAR-Indian Institute of Seed Science*, P. 42.

Sinha AK, Agarwal DK, Kumar SPJ, Tiwari TN, Chaturvedi AK. 2016. Novel technique for precluding hybrid necrosis in bread wheat. *International Journal of Tropical Agriculture* 34 (3); 761-765.

Sun WQ and Leopold AC .1995. The Maillard reaction and oxidative stress during ageing of soybean seeds. *Plant Physiology* 94: 94-105.

Sun WQ, Davidson P, Chan HSO.1998. Protein stability in the amorphous carbohydrate matrix: relevance to anhydrobiosis. Biochimie Biophysic Acta 1425: 245-254.

Sundarram A and Thirupathihalli PKM .2014. α-Amylase Production and Applications: A Review. *Applied Environment Microbiology* 2 (4): 166-175.

Thobunluepop P, Pawelzik E, Vearasilp S .2008. Possibility of biological seed coating application on direct-seed rice production: Emphasis on plant productivity and environment awareness. P.101 in Proceedings of the 6th National Conference for Post Harvest Technology, August 25-28, Khon Kaen, Thailand.

Thobunluepop P, Pan-in W, Pawelzik E, Vearasilp S .2009. The perspective 8 effects of various seed coating substances on rice seed variety Khao Dawk Mali 105 storability II: the case study of chemical and biochemical properties. *Pak J Biol Sci* 12(7): 574-81.

Vamadevappa H.1998. Studies on seed viability and vigour in relation to storage in soybean (*Glycine max* (L.) Merrill). M.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Vinutha KS, S Rajendra Prasad, Parashiva Murthy, Kumar SPJ, Rame Gowda, Ravishankar P. 2014. Influence of staggered sowing, planting ratio and subtending cob leaf clipping on seed quality parameters of maize. Seed Research 42 (1): 91-97.

Vinutha KS, S Rajendra Prasad, Parashiva Murthy, Kumar SPJ, Rame Gowda, Ravishankar P. 2014. Optimization of seed production techniques in a single cross maize hybrid. Seed Research 42 (1): 210-216.

Zarei I, Sohrabi Y, Heidari GR, Jalilian A, Mohammadi K. 2012. Effects of biofertilizers on grain yield and protein content of two soybean (*Glycine max* L.) cultivars. *African Journal of Biotechnology* 11(27): 7028-7037.