Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. 3**(ISC-2013),** 15-18 **(2014)** *Res. J. Recent. Sci.*

Effect of Accelerated ageing on Seed Viability and Biochemical Components of the Edible Bamboo *Dendrocalamus brandisii* (Munro) Kurz

Lakshmi C.J.[1*], Seethalakshmi K.K.[1], Chandrasekhara Pillai P.K.[2] and Raveendran V.P.[3]
[1]Tree Physiology Department, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, INDIA
[2]Silviculture Department, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, INDIA
[3]Extension and Training Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, INDIA

Available online at: www.isca.in, www.isca.me
Received 27th November 2013, revised 3rd January 2014, accepted 3rd February 2014

Abstract

Dendrocalamus brandisii is a very large evergreen bamboo, which is commonly used for house building, for making baskets, handicrafts and furniture. Young shoots of D. brandisii are edible. Although, abundant seed production is observed during gregarious flowering, viability of seeds under natural conditions is very short. In the present study, accelerated ageing test was carried out to predict the storability of seeds. Seeds were subjected to accelerated ageing at 42±1°C with a relative humidity of 100% for 0, 1, 3, 5 and 8 days, in a covered water bath. Germination test and biochemical analyses were carried out for control and aged seeds. The initial germination percentage was 59.71% and after accelerated ageing germination declined to 15.39%. Total soluble proteins, sugars and starch content decreased during the ageing process. There was a gradual decrease in the activity of acid and alkaline phosphatase and peroxidase, while the activity of α-amylase and β-amylase increased during accelerated ageing. Hence the decrease in the viability of D. brandisii seeds may be due to the changes in the biochemical content and the activity of enzymes involved in the degradation of seed reserves.

Keywords: *Dendrocalamus brandisii*, accelerated ageing, seed viability, edible bamboo, amylases, peroxidase.

Introduction

Dendrocalamus brandisii is a large evergreen sympodial bamboo. It is widely cultivated in Karnataka and Kerala and the species is found growing in the tropical forests, chiefly on calcareous rocks up to an altitude of 1300 m[1]. *D. brandisii* is a highly preferred bamboo for cultivation in homesteads due to its non thorny erect nature. Moreover, young shoots are edible and is of good quality[2]. In this species gregarious flowering is observed with a long flowering cycle of 40-45 years. Large amounts of seeds are produced during flowering, but viability of seeds under natural conditions is very short. In order to ensure the availability of seeds for afforestation and plantation programmes, it is necessary to develop appropriate storage methods that would prolong the viability of seeds. Accelerated ageing is the commonly used test to predict the storability of seeds, during which seeds were exposed to high temperature and high relative humidity[3]. The main factors influencing seed storage are high temperature, relative humidity and moisture content of seeds[4]. The present work was undertaken to study the effect of accelerated ageing on seed viability and biochemical components of *D. brandisii*.

Material and Methods

The experiment was carried out in the Tree physiology laboratory of Kerala Forest Research Institute, Peechi during 2012-13. The species was in bloom during 2010-12 in Coorg areas of Karnataka. Seed samples were obtained from Ponnampet, Karnataka (12° 08' 32.5" N 75° 55' 16.0" E). Collected seeds were air dried to about 8% moisture level. Dried samples were divided into 5 lots. From each lot four replications of 100 seeds were used for the experiment. Seeds were subjected to accelerated ageing at 42 ± 1°C with a relative humidity of 100% for 1, 3, 5 and 8 days, in a covered water bath. Germination tests were carried out using polyurethane foam sheet[5]. The poly urethane foam sheets were kept moist by adding water daily. Germination percentage was calculated using the formula1:

Formula-1

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Biochemical assays were carried out for control and on the first, third, fifth and eighth days of accelerated ageing test. Protein was estimated using the bovine serum albumin as standard. Extraction of protein from the sample was carried out using tris HCl buffer (*p*H 7.8) and the supernatant was used for the estimation and the absorbance was measured at 660 nm[6]. Estimation of starch was carried out by treating the sample with 80% ethanol to remove sugars and then starch was extracted using perchloric acid and anthrone reagent. Glucose was used as standard and absorbance measured at 630 nm[6]. For the extraction of total soluble sugars, sample was

ground with 10 ml of 80% methanol and centrifuged at 5000 rpm and the supernatant thus obtained was used for the estimation. The concentration of soluble sugars was calculated using glucose as standard and the absorbance was read in a UV visible spectrophotometer at 490 nm[7]. Peroxidase activity was estimated by extracting the sample in 3 ml of 0.1 M phosphate buffer (*p*H 7) by grinding with a pre-cooled mortar and pestle and centrifuged at 18000 g at 5^0C for 15 min and the supernatant thus obtained was used for the enzyme estimation[6]. Acid and Alkaline phosphatases were estimated using p-nitrophenol as standard. Sample was homogenized in 10 ml of ice-cold buffer solution (Citrate buffer (*p*H 5.3) for acid phosphatase and glycine NaOH buffer (*p*H 10.4) for alkaline phosphatase) in a pre-chilled pestle and mortar. The filtrate obtained was subjected to centrifugation at 10,000g for 10 min and supernatant was used for enzyme estimation. The absorbance was read at 405nm[6]. The activities of α and β-amylases were estimated according to Peter Bernfield[8]. Sample was extracted using 0.02 M Sodium phosphate buffer (*p*H 6.9) for α-amylase and 0.016 M Sodium acetate buffer (*p*H 4.8) for β-amylase. To the supernatant 1% starch solution was added and kept at 25^0C for 3 min. Dinitrosalicylic acid was used as the color reagent and maltose as standard. The absorbance was measured at 540 nm.

Results and Discussion

Initial germination percentage of *Dendrocalamus brandisii* seeds was 59.71±1.85 %. The germination decreased drastically with ageing and on the very first day of ageing germination decreased to 55.60±3.29%. Thereafter it decreased to 44.28±4.64%, 30.19±4.93% and 15.39±3.01% on the third, fifth and eighth day of ageing. A significant difference was also observed in the time taken for germination among the control and aged seeds. For control, first and third day of ageing, seeds started germinating from the third day of sowing. As the ageing progressed, the time taken for germination also increased and on the final day of ageing, the seed germination was observed on the tenth day of sowing. The number of seeds germinating on each day was also more for control when compared with aged seeds.

One way analysis of variance revealed significant differences in biochemical parameters due to ageing at one per cent level. Total soluble proteins, sugars and starch content decreased during the ageing process. There was a gradual decrease in the activity of acid and alkaline phosphatase and peroxidase, while the activity of α-amylase and β-amylase increased during accelerated ageing. The results are presented in figures 1, 2, 3 and 4.

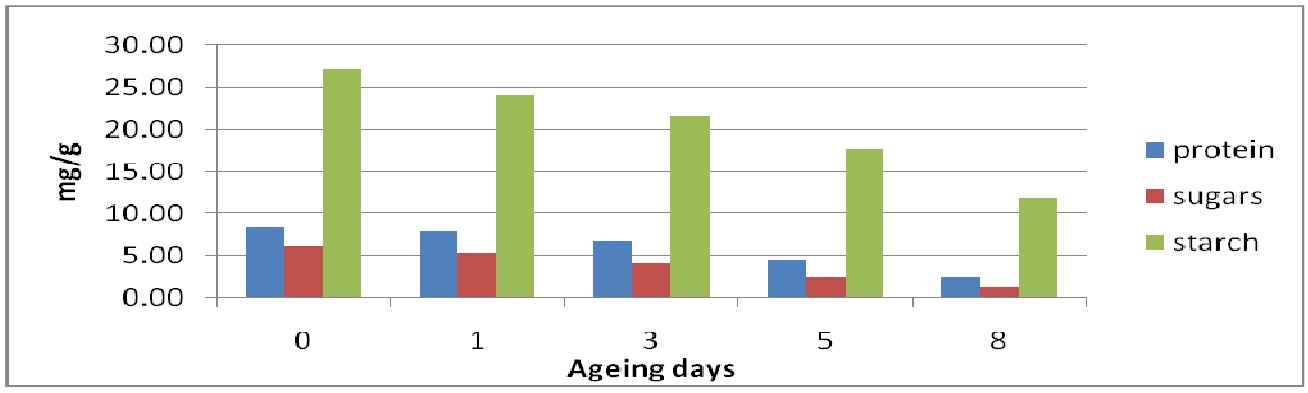

Figure-1
Variation in protein, sugars and starch content induced by accelerated ageing in *D. brandisii* seeds

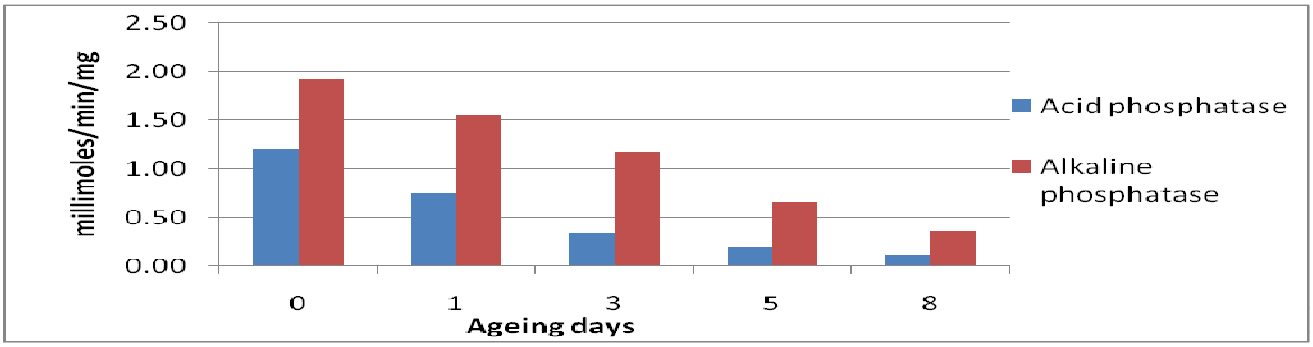

Figure-2
Variation in activity of acid and alkaline phosphatase induced by accelerated ageing in *D. brandisii* seeds

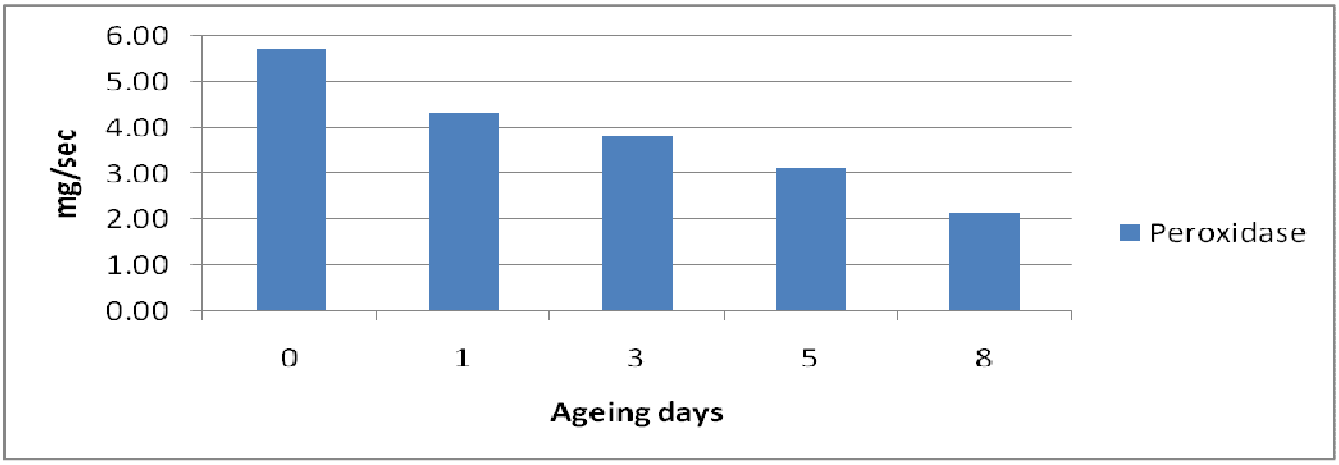

Figure-3
Variation in the activity of peroxidase induced by accelerated ageing in *D. brandisii* seeds

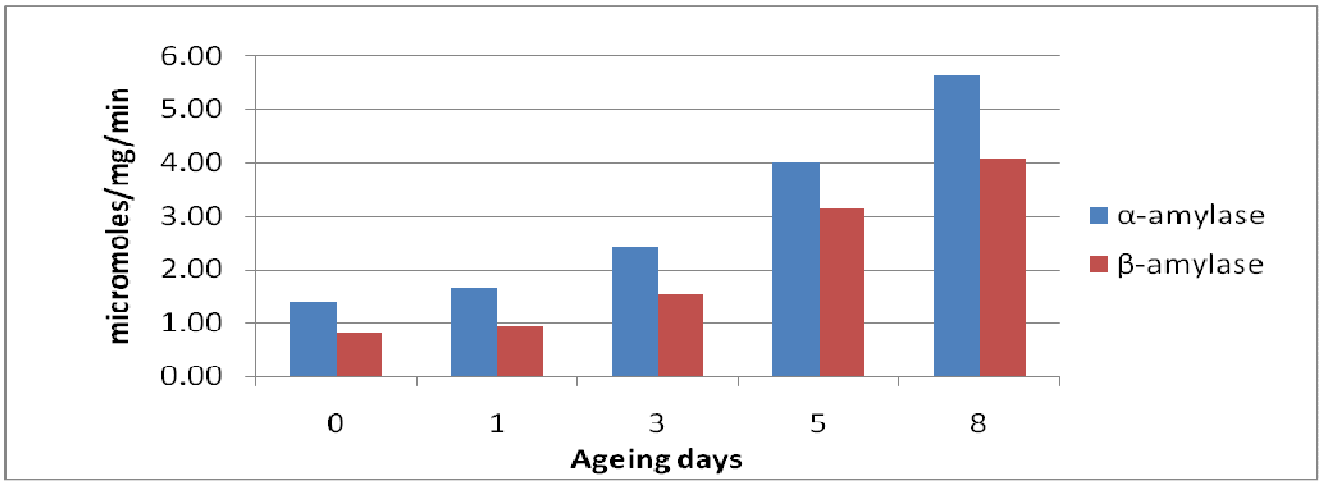

Figure- 4
Variation in the activity of α and β- amylase induced by accelerated ageing in *D. brandisii* seeds

The loss of seed germination capacity during ageing is in accordance with the findings in the seeds of *Bambusa bambos* and *Dendrocalamus strictus*[9, 10]. In the present study germination percentage of *D. brandisii* seeds decreased from 55.60% to 15.39%. Germination decreased from 73.8 to 51.5% and 67.2 to 38.6% in *D. strictus* and *B. bambos* seeds respectively. Variation in the biochemical components of *D. brandisii* seeds was in conformity with the findings of [9,10,11,12]. Previous studies in *B. bambos* and *D. strictus* seeds indicated that with accelerated ageing the total content of food reserves such as sugars, proteins and lipids, activity of peroxidase, acid phosphatase and alkaline phosphatase were reduced. Increase in total free aminoacids and the activity of amylases confirmed the degradation of seed reserves [9, 10]. The biochemical analysis of the *Bambusa bambos* seeds stored in different storage conditions showed qualitative and quantitative changes in food reserves specially sugars and proteins[13]. The decline in the protein content during ageing may be due to the degradation by proteinases. Similar results were reported in *Arachis hypogaea* and *Zea mays*[11, 14]. During ageing, the starch content in seeds were hydrolysed by the activity of starch degrading enzymes α and β- amylases, which results in increase in the activity of amylases and decrease of starch content. Seed deterioration may be due to the denaturation of biomolecules, accumulation of toxic substances and loss of membrane integrity[11, 15]. The decrease in the activity of phosphatases and peroxidases during ageing may adversely affect the metabolism of seeds which results in the deterioration of seeds and loss of viability.

Conclusion

The results clearly indicated that accelerated ageing test can be used to predict the storability of *D. brandisii* seeds. This caused biochemical changes in the seeds that are known to occur during degradation of seeds, such as decrease in total soluble proteins, sugars and starch content, decrease in activity of acid and

alkaline phosphatase and peroxidase and increase in the activity of α-amylase and β-amylases.

References

1. Seethalakshmi K.K. and Kumar M.S., Bamboos in India-a compendium. INBAR Technical Report No.17. Kerala Forest Research Institute, Peechi and International Network for Bamboo and Rattan, New Delhi **(1998)**

2. Rao A N., Rao R.V. and Williams J.T., Priority species of Bamboo and Rattan. International Plant Genetic Resources Institute, Malaysia and International Network for Bamboo and Rattan, China, 95 **(1998)**

3. Delouche J.C. and Baskin C.C., Accelerated ageing techniques for predicting the relative storability of seed lots, *Seed Sci. Technol*., **1**, 427-452 **(1973)**

4. Abdul-Baki A.A., Biochemical aspects of seed vigor, *Hort Sci.*, **15,** 765–71 **(1980)**

5. Chacko K.C., Polyurethane foam as substratum for germination tests, *Indian J. Forest*., **6,** 325 **(1983)**

6. Sadasivam and Manickam A., Biochemical Methods for Agricultural Sciences, New Age International (P) Ltd., New Delhi **(1996)**

7. Dubois M., Gilles K., Hamilton J.K., Roberts P.A. and Smith F., A colorimetric method for the determination of sugars, *Nature*, **168,** 167 **(1956)**

8. Peter Bernfield. In: Methods of Enzymology (*Eds* Colowick, S and Kaplan, N O)Academic press New York **1,** 149 **(1955)**

9. Ravikumar R., Ananthakrishnan G., Ganapathi A. and Appasamy T., Biochemical changes induced by accelerated ageing in *Bambusa bambos* seeds, *Biologia Plantarum*, **40(3),** 459-464 **(1998)**

10. Ravikumar R., Ananthakrishnan G., Girija S. and Ganapathi A., Seed viability and biochemical changes associated with accelerated ageing in *Dendrocalamus strictus* seeds, *Biologia Plantarum*, **45(1),** 152-156 **(2002)**

11. Basavarajappa B.S., Shetty H.S. and Prakash H.S., Membrane deterioration and other biochemical changes associated with accelerated ageing of maize seeds, *Seed sci. technol.,* **19,** 279-286 **(1991)**

12. Bernal-Lugo I. and Leopold A.C., Changes in soluble carbohydrates during seed storage, *Plant Physiol*., **98,** 1207-1210 **(1992)**

13. Appasamy T., Studies on bamboo seed-biology and its propagation, Ph.D. thesis, Bharathidasan University, Thiruchirappally, 72 **(1993)**

14. Rao M.R.K., Sreeramulu N. and Rao I.M., Respiratory rate and food reserves in TMV-2 groundnut seeds (*Arachis hypogaea* L.) in relation to loss of viability due to storage, *The Andhra Agricultural Journal*, **17,** 27-29 **(1970)**

15. Roberts E.H., Cytological, genetical and metabolic changes associated with loss of viability, In: Roberts, E.H. (ed.): Viability of seeds, Chapman and Hall, London 14-58 **(1972)**

16. Ahirwar J.R., Effect of seed size and weight on seed germination of Alangium lamarckii, Akola, India, *Res.J.Recent.Sci.,***1(ISC-2011)**, 320-322 **(2012)**

17. Islam M.A., Begum S., Nakaba S. and Funada R., Distribution and pattern of availability of storage starch and cell death of ray parenchyma cells of a conifer tree (Larix kaempferi), *Res.J.Recent Sci*., **1(5)**, 28-37 **(2012)**