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BRIEF COMMUNICATION

Biochemical changes induced by accelerated ageing in *Bambusa bambos* seeds

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Abstract

Decrease in seed viability and germination rate may be caused by biochemical changes associated with seed ageing. Different biochemical assays were conducted to investigate the changes occurring at the ageing of *Bambusa bambos* seeds. A reduction in the total content of food reserves such as sugars, proteins and lipids were recorded. Decreased activity of peroxidase, acid phosphatase, alkaline phosphatase were also noticed during accelerated ageing. A substantial increase in total free amino acids and the activity of amylases confirms the degradation of stored biomolecules in seeds during ageing.

Additional key words: bamboo, α and β-amylase, peroxidase, acid and alkaline phosphatase.

Accelerated ageing was initially developed as a test to estimate longevity of seeds in warehouse storage. Subsequent studies have substantiated the accuracy of this test in predicting the life span of a number of different species under a range of storage conditions. Delouche and Baskin (1973) and Baskin (1977) proposed that by using the accelerated ageing test one can predict stand establishment of peanuts. Seeds deteriorate and lose their germinability during periods of prolonged storage. The early deteriorative changes have been attributed to denaturation of biomolecules, accumulation of toxic substances, and to loss of membrane integrity (Woodstock and Grabe 1967, Abdul Baki and Anderson 1972, Roberts 1972, Basavarajappa *et al.* 1991).

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Bamboo is one of the most economically important forest species propagated by seeds. Although, huge quantity of seeds are available during gregarious flowering, we are not able to use them because of quick loss of viability. Till-date there is no information about biochemical changes involved in bamboo seed deterioration. Therefore the present investigation has been undertaken to unravel the various biochemical changes associated with ageing in *Bambusa bambos* seeds with a view to evolve seed storage strategies.

Bambusa bambos Willd. seeds provided by the Kerala Forest Research Institute, Peechi were subjected to accelerated ageing at 42 ± 1 °C and 100 % relative humidity for 96 h in a covered water bath placed in a regulated incubator. Seeds were kept in such a way that they never got direct contact with water bath, during the experimental period. 100 seeds were used for each experiment. Seeds subjected to various ageing conditions were disinfected as previously described by Mocquet *et al.* (1977). Later, the seeds were allowed to germinate in paper rolls according to Internatinal Rules for Seed Testing (given by International Seed Testing Association in 1985) and germination percentage was determined after 12 d.

Three replicates of seeds, each weighing about 1 g were used for biochemical assays using standard methods. Total soluble proteins were estimated according to Lowry *et al.* (1951), bovine serum albumin was used as the standard. For estimation of sugars, amino acids and starch 1 g of the seed sample was homogenised in 10 cm^3 of 80 % methanol, centrifuged at 8 000 *g* for 10 min at 5 °C and the supernatant was used for the analyses of sugars, amino acids and starch. Total soluble sugars were estimated by the methods of Dubois *et al.* (1956). The absorbance was measured at 490 nm. Glucose was used as the standard. Total free amino acids were estimated by the methods of Troll and Canon (1953). To 1 cm^3 of the sample, 0.1 cm^3 of 80 % phenol was added, kept in boiling water for 10 min, then 0.2 cm^3 of 0.5 % ninhydrin was then added and kept in a boiling water for further 10 min. The mixture was then cooled and the absorbance read at 575 nm. L-glycine was used as the standard. Starch was estimated by the following the methods of McCready *et al.* (1950). To the pellet after methanol extraction, 6.5 cm^3 of perchloric acid and 5.0 cm^3 of distilled water, were added and centrifuged at 6 400 *g* for 5 min. To 0.5 cm^3 of the supernatant, 4.5 cm^3 of distilled water and 10.0 cm^3 of 0.2 % anthrone were added. The absorbance was measured at 630 nm. Glucose was used as the standard. Lipids were estimated by the method of Bragdon (1951). One g of seed powder was soaked in chloroform for 48 h, centrifuged and the chloroform extract was evaporated to dryness. To this 10 cm^3 of $K_2Cr_2O_7$-H_2SO_4 reagent was added and diluted with equal volume of distilled water. The absorbance was read at 580 nm. Stearic acid was used as the standard. Peroxidase activity was estimated by the method of Malik and Singh (1980). One g of seed was homogenised in 0.1 M phosphate buffer of pH 6.5 and centrifuged. To 0.5 cm^3 of the sample, 0.1 cm^3 of orthodianisidine solution and 0.2 cm^3 of H_2O_2 were added. The increase in the absorbance for every 30 s was recorded upto 3 min at 430 nm. Acid and alkaline phosphatase activity were estimated by following the methods of Ikawa *et al.* (1964) and Torriani (1967). One g of seed was homogenised in 0.1 M acetate buffer of pH 5.0 for acid phosphatase and 0.1 M tris HCl buffer of pH 8.2 for alkaline phosphatase and centrifuged. To 1 cm^3 of the

supernatant 1 cm^3 of 6.6 mM nitrophenyl phosphate substrate was added and incubated at 30 °C for 30 min. The reaction was terminated by the addition of 2 M NaOH for acid phosphatase and 0.2 M Na_2HPO_4 for alkaline phosphatase. The absorbance was measured at 410 nm. The activities of α- and β-amylase were estimated according to Dure (1960). One g of seed was ground in 0.1 M citrate buffer (of pH 5.5 for α-amylase and 3.4 for β-amylase) and centrifuged. To 1 cm^3 of supernatant, 2 % soluble starch was added and kept at 30 °C for 30 min. Dinitrosalicylic acid was used as the colour reagent and the absorbance was read at 540 nm. Maltose was used as the standard.

The germinability of *Bambusa bambos* seeds were decreased during accelerated ageing. Initial germination percentage was 67.2 and after 96 h it was only 38.6 (Fig. 1). Although content of total soluble sugars and starch were decreased during

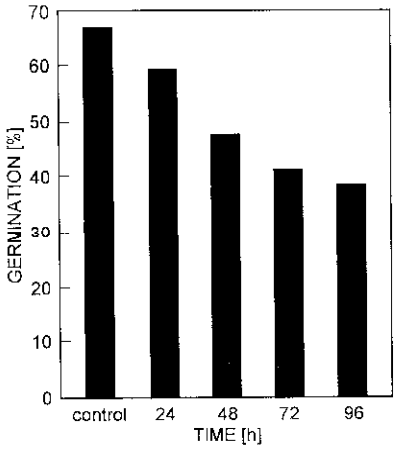

Fig. 1. Effect of accelerated ageing on germination of bamboo seeds.

accelerated ageing of seeds, the rate of sugar decrease was very slow (Table 1). The findings of the present study corroborates with that of the earlier observations of Basavarajappa *et al.* (1991) and Bernal-Lugo and Leopold (1992). They noticed the

Table 1. Changes in contents of proteins, amino acids, sugars, starch and lipids [mg g^{-1}(d.m.)] induced by accelerated ageing of bamboo seeds (mean ± SD, $n = 3$).

Time	Proteins	Amino acids	Sugars	Starch	Lipids
Control	0.826 ± 0.058	0.216 ± 0.0027	0.472 ± 0.0041	7.000 ± 0.259	0.247 ± 0.0028
24 h	0.811 ± 0.051	0.224 ± 0.0029	0.461 ± 0.0040	6.131 ± 0.245	0.231 ± 0.0026
48 h	0.621 ± 0.042	0.231 ± 0.0030	0.441 ± 0.0037	5.261 ± 0.229	0.223 ± 0.0022
72 h	0.567 ± 0.033	0.256 ± 0.0032	0.421 ± 0.0032	4.571 ± 0.129	0.200 ± 0.0022
96 h	0.449 ± 0.027	0.273 ± 0.0035	0.382 ± 0.0029	4.169 ± 0.124	0.182 ± 0.0018

decreasing trend in the content of carbohydrates during accelerated ageing. The decrease could be due to utilization in respiration or due to an increase in amylase activity (Table 2). Depletion of essential metabolites, including loss of food reserves, is one of the important factors responsible for loss in seed viability (Roberts 1972). Moreover, prolonged moist storage may lead to the fungal infection, which may partly contribute to the loss of viability (Harrington 1972, King and Roberts 1979).

The content of proteins was decreased to half of the initial value during accelerated ageing (Table 1). This could be due to their degradation because a sharp increase in total free amino acids (Table 1) was observed. Similar findings were recorded by Coolbear *et al.* (1984), Nautiyal *et al.* (1985) and Basavarajappa *et al.* (1991). Decrease in lipid content has been observed previously in cucumber (Koostra and Harrington 1969), pea (Harman and Mattick 1976, Powell and Mattews 1981, Pearce and Abedel Samad 1980), soybean (Stewart and Bewley 1980), sunflower Gidrol *et al.* (1989) and tomato (Francis and Coolbear 1984) seeds. Our findings also confirm such a decrease in total lipid content (Table 1).

Table 2. Changes in α- and β-amylase [pmol(maltose released) mg^{-1}(protein) h^{-1}], acid and alkaline phosphatase [pmol(nitrophenol released) mg^{-1}(protein) h^{-1}], and peroxidase [U mg^{-1}(protein) h^{-1}] induced by accelerated ageing of bamboo seeds.

Time	α-amylase	β-amylase	Acid phosphatase	Alkaline phosphatase	Peroxidase
Control	138.30 ± 1.23	71.16 ± 1.24	22.19 ± 0.20	47.25 ± 0.35	159 ± 2.52
24 h	143.36 ± 1.43	78.11 ± 1.48	19.76 ± 0.18	41.62 ± 0.28	143 ± 2.28
48 h	155.02 ± 1.46	80.30 ± 1.49	14.91 ± 0.17	36.21 ± 0.16	127 ± 1.98
72 h	165.91 ± 1.47	86.44 ± 1.89	12.12 ± 0.12	31.12 ± 0.13	112 ± 1.74
96 h	174.47 ± 1.51	93.69 ± 1.91	10.64 ± 0.11	28.79 ± 0.11	70 ± 1.36

Our findings indicate a decrease in the peroxidase activity during ageing (Table 2) similar to the reports of NKang (1988) and Basavarajappa *et al.* (1991). Basavarajappa *et al.* (1991) showed that activity of acid phosphatase and phosphomonoesterase decreased after ageing treatment in maize seeds. In the present study, the activities of acid phosphatase and alkaline phosphatase were found to be decreased during the treatment (Table 2). Since acid phosphatase is involved in the maintenance of constant cellular pool of phosphate, this condition might affect the phosphate metabolism in seeds during ageing. In the light of the aforesaid facts, it is concluded that seed deterioration may be due to marked changes in the activities of enzymes involved in degradation of stored reserves.

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