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BRIEF COMMUNICATION

Seed viability and biochemical changes associated with accelerated ageing in *Dendrocalamus strictus* seeds

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Abstract

Accelerated ageing of *Dendrocalamus strictus* Ness seeds at 42 ± 1 °C and 100 % relative humidity for 1 to 8 d was conducted. Seeds lost viability and changed their biochemical constituents. Reductions in the contents of sugars, starch, proteins and lipids were found. Decrease in the activity of the peroxidase as well as acid and alkaline phosphatase were also observed. Increase in total free amino acids content and the activity of amylase confirmed the degradation of seed reserves.

Additional key words: amino acids, amylase, *Dendrocalamus strictus*, lipids, peroxidase, phosphatase, proteins, starch, sugars.

Bamboo species, giant woody perennials belonging to the family *Poaceae*, are propagated by seeds. Even though huge quantities of seeds are produced, most of them cannot be utilised because of rapid loss of viability. Accelerated ageing was developed as a test to estimate the longevity of seed under a range of storage conditions. Helmer (1962) suggested that accelerated ageing might have additional utility as a test for predicting seed performance other than storability. Delouche and Baskin (1973) and Bishnoi and Delouche (1975) proposed that by using an accelerated ageing test one can predict stand establishment of peanuts and cotton respectively. Ravikumar *et al.* (1998) reported biochemical changes induced by accelerated ageing in *Bambusa bambos* seeds. The present investigation was undertaken to find out the various biochemical changes associated with ageing in *Dendrocalamus strictus* seeds with a view to evolve seed storage strategies.

Fresh seeds of *Dendrocalamus strictus* Ness were generously provided by Dr. K.K. Seethalakshmi, of the Kerala Forest Research Institute, Peechi, Kerala, India. The seeds were subjected to accelerated ageing at 42 ± 1 °C and 100 % relative humidity for 8 d in a covered water bath placed in a regulated incubator. Seeds were kept in such a way that they never had direct contact with water during the experimental period. The seeds were subjected to biochemical analyses on the 1st, 3rd, 5th, and 8th days. One hundred seeds were used for each experiment. Seed viability was tested using polyurethane foam sheet (Chacko 1983). The number of seeds germinated within the first week of sowing were counted in each treatment separately. The germination percentage for control and aged seeds were determined.

Total soluble proteins were estimated according to Lowry *et al.* (1951), bovine serum albumin was used as a 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 using *Systronics* spectrophotometer. Glucose was used as a standard. Total free amino acids were estimated by the methods of Troll and Cannan

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(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 5 % ninhydrin was added and kept in boiling water for further 10 min. The mixture was then cooled and the absorbance read at 575 nm. L-glycine was used as a standard. Starch was estimated by 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 cm^3 of 0.2 % anthrone were added. The absorbance was measured at 630 nm. Glucose was used as the standard. Lipid contents 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 at 2 000 *g* for 10 min. To 0.5 cm^3 of the sample, 0.1 cm^3 of orthodianisidine solution (1 mg in 1 cm^3 of methanol) and 0.2 cm^3 of 0.2 M H_2O_2 were added. The increase in the absorbance was recorded every 30 s up to 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 seeds 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 at 2 000 *g* for 10 min. To 1 cm^3 of the supernatant 1 cm^3 of 6.6 mM nitrophenyl phosphate was added as substrate 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 seeds was ground in 0.1 M citrate buffer (of pH 5.0 for α-amylase and 3.4 for β-amylase) and centrifuged at 2 000 *g* for 10 min. To 1 cm^3 of supernatant, 2 % soluble starch was added and kept at 30 °C for 3 min. Dinitrosalicylic acid was used as the colour reagent and the absorbance was read at 540 nm. Maltose was used as the standard.

Initial germinability of *Dendrocalamus strictus* seeds was 73.8 % and declined during accelerated ageing (Table 1). Total soluble sugars and starch content decreased during accelerated ageing of seeds, the rate of sugar decrease was very slow (Table 1). The reduction of sugar content may possibly be due to hydrolysis, followed by Amadori and Maillard reactions (Feeney and Whitaker 1982). 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 found a decreasing trend of the same content during accelerated ageing. The decrease in starch content could be due to an increase in amylase activity (Table 1). Roberts (1972) concluded that depletion of essential metabolites, including loss of food reserves, is one of the important factors responsible for loss in seed viability. Prolonged moist storage may lead to fungal infection, which may be responsible for further loss of viability (King and Roberts 1979).

Table 1. Biochemical changes induced by accelerated ageing in *Dendrocalamus strictus* seeds (mean ± SD, $n = 6$).

	0 d (control)	1st day	3rd day	5th day	8th day
Germination [%]	73.8	68.0	62.3	57.0	51.5
Proteins [mg g^{-1}(d.m.)]	9.36 ± 1.41	8.51 ± 1.22	7.25 ± 1.12	6.27 ± 1.08	5.11 ± 0.99
Amino acids [mg g^{-1}(d.m.)]	2.80 ± 0.81	2.81 ± 0.83	2.86 ± 0.79	2.92 ± 0.84	2.97 ± 0.81
Sugars [mg g^{-1}(d.m.)]	6.15 ± 0.72	6.10 ± 0.70	6.00 ± 0.71	5.89 ± 0.68	5.72 ± 0.62
Starch [mg g^{-1}(d.m.)]	73.49 ± 1.59	67.61 ± 1.48	63.21 ± 1.21	60.10 ± 1.30	57.31 ± 1.29
Lipids [mg g^{-1}(d.m.)]	3.12 ± 0.72	3.10 ± 0.69	3.00 ± 0.68	2.89 ± 0.54	2.81 ± 0.52
α-Amylase [mg(maltose) g^{-1}(protein) h^{-1}]	61.21 ± 1.34	64.27 ± 1.28	69.27 ± 1.17	73.86 ± 1.19	77.65 ± 1.12
β-Amylase [mg(maltose) g^{-1}(protein) h^{-1}]	31.12 ± 0.98	31.63 ± 0.87	33.12 ± 0.89	34.71 ± 0.91	36.72 ± 0.98
Acid phosphatase [mmol(nitrophenol) g^{-1}(protein) h^{-1}]	26.27 ± 0.79	22.12 ± 0.72	19.79 ± 0.62	18.16 ± 0.61	16.76 ± 0.59
Alkaline phosphatase [mmol(nitrophenol) g^{-1}(protein) h^{-1}]	56.13 ± 1.21	48.72 ± 1.18	40.12 ± 1.17	36.71 ± 1.10	35.04 ± 1.11
Peroxidase [units mg^{-1}(protein) min^{-1}]	5.07 ± 0.89	4.91 ± 0.71	4.62 ± 0.71	4.31 ± 0.69	4.06 ± 0.62

The content of proteins decreased to half of the initial protein content during accelerated ageing (Table 1). This findings are supported by those of Nautiyal *et al.* (1985) and Basavarajappa *et al.* (1991). Ghosh *et al.* (1981) demonstrated leaching of amino acids into the imbibing medium during ageing of rice seeds and suggested that

proteins were hydrolysed during ageing. The protein loss depended on the severity of ageing conditions. We found an increase in total content of free amino acids during accelerated ageing (Table 1). This is similar to Coolbear *et al.* (1984) and Basavarajappa *et al.* (1991) who reported substantial increases in total free amino acids following ageing treatment. Decrease in lipid content has been observed previously by many authors in cucumber (Koostra and Harrington 1969), pea (Powell and Matthews 1981), soyabean (Stewart and Bewley 1980), sunflower (Gidrol *et al.* 1989) and tomato seeds (Francis and Coolbear 1984). Our findings also confirm such a decrease in total lipid content (Table 1).

Our findings also indicate decrease in the peroxidase activity during accelerated ageing (Table 1). Decline of peroxidase activity with increasing storage time was reported by Nkang (1988) and Basavarajappa *et al.* (1991). Basavarajappa *et al.* (1991) also showed that acid phosphatase and phosphomonoesterase decreased after ageing treatment in maize seeds. Ram and Wiesner (1988) showed a decrease in respiratory rate in aged wheat seeds. In the present study, the activities of acid phosphatase and alkaline phosphatase decreased during accelerated ageing (Table 1), which might affect the maintenance of a cellular pool of phosphate as well as phosphate metabolism in seeds. The activity of α- and β-amylases were increased due to accelerated ageing (Table 1). This was supported by the findings of Nkang (1988) who observed an increased amylolytic activity in *Guilfoylia monostylis* seeds.

In the light of the aforesaid facts, it is concluded that seed deterioration and loss of viability may be due to marked changes in the biochemical content and activity of enzymes involved in degradation of stored reserves in two different bamboo species (*Dendrocalamus strictus* and *Bambusa bambos*).

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