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Viability and enzyme activity of ageing seeds of bamboo (*Dendrocalamus strictus* (Roxb.) Nees) in relation to exogenous plant growth regulators

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The effect of plant growth regulators (PGRs) on enzyme activity and germinability was studied at six monthly ageing intervals in bamboo (*Dendrocalamus strictus* (Roxb.) Nees) seeds. Enzyme activity of α-amylase and β-amylases was assayed at 48, 72 and 96 h of germination and peroxidase activity at 96, 120 and 144 h of germination after the application of PGRs, viz. GA_3 (40 ppm), IBA (20 ppm), 1,2,4-acid (1-amino-4-sulphonate-β-naphthol, 20 ppm) and resorcinol (1,3-dihydroxybenzene; 20 ppm). All the treatments increased the activities of α- and β-amylase enzyme over the control. IBA (20 ppm) and resorcinol (20 ppm) proved to be the best treatments in increasing enzymes activity after 96 h of germination and in turn the germination percentage. After six months ageing interval, the effectiveness of these two treatments increased significantly in increasing the germinability and enzyme activity and effectiveness of the treatment was highly concentration dependent. After six months of ageing, effectiveness of all treatments except IBA (20 ppm) declined. Peroxidase activity declined drastically during six months of ageing and the PGRs used in the study were not effective on membrane system repair mechanisms as the electrolyte leakage increases drastically with ageing, suggesting that ageing in bamboo seed leads to two major changes in the metabolism, viz. (i) the hormonal imbalance causing rapid loss in germinability which can be recovered only till certain stage of ageing by exogenous PGRs and (ii) the slow deterioration of membrane integrity which is irreversible, leading to permanent loss of viability.

Keywords: Enzymes, exogenous, germination, plant growth regulators, viability.

BAMBOO seeds have no dormancy and lose their viability very fast[1,2] which may be attributed to metabolic changes occurring during ageing. Changes in activity of various enzymes have been shown to accompany the seed deterioration, which precedes loss of seed viability. Seed ageing is occasionally accompanied by increase in the activity of α,β-amylase and protease[3] but more commonly there is a loss of enzyme activity during ageing[4,5]. Plant growth regulators (PGRs) are known to regulate various metabolic processes, which in turn promote or inhibit growth. Endogenous hormone levels change with

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ageing, resulting in decrease in viability of bamboo seeds[6]. Exogenous application of growth regulators like gibberellins and kinetins often cause enzymatic changes in seeds and in turn induce germination[7–9].

The present study was aimed at improving the viability of ageing bamboo seeds by using some PGRs and studying the role of various enzymes, which are active during germination, in promoting the germination percentage in relation to exogenous PGRs.

Dendrocalamus strictus (Roxb.) Nees seeds (five months old) were procured from the Bamboo Society of India, Bangalore, in May 2000 and stored in polythene bags in a desiccator at 4°C. The seeds were surface sterilized with 0.5% mercuric chloride ($HgCl_2$) for 2 min and were washed thoroughly in running water, and finally rinsed with sterilized water. Ten seeds were placed equidistantly in pre-sterilized petri dishes (15 cm dia) lined with moistened Whatman filter paper. Four types of PGRs were used, viz. gibberellic acid (GA_3) (40 ppm), indole-3-butyric acid (IBA) (20 ppm), resorcinol (20 ppm) and 1,2,4-acid (20 ppm). These solutions were prepared afresh for each experiment. The filter paper was moistened with different PGR solutions in treated seeds. Three replicates were kept for each treatment. The petri dishes were placed in an incubator at 28 ± 2°C and the percentage germination was studied for 14 days.

The seeds were dehusked, weighed and homogenized with 6 ml phosphate buffer and a pinch of acid-washed sand in cold pestle and mortar from the above mentioned treatments and control. These were centrifuged at 3000 rpm for 10 min and the supernatant was used as enzyme extract. The latter was deep freezed and enzyme assaying was done within 24 h. All the stages in the preparation of enzyme extracts were carried out at 1–4°C. The estimations were done for α-amylase, β-amylase and peroxidase. The activity of α-amylase and β-amylase was estimated after 48, 72 and 96 h of germination and was calculated by the method of Bernfeld[10]. Peroxidase activity was assayed after 96, 120 and 144 h of germination, according to the method of Malik and Singh[11]. Each experiment was carried out in triplicates and repeated twice.

Electrolyte leakage was also studied in 5 and 11-month-old seeds at 48, 72 and 96 h of germination to assess the membrane integrity. At five months of ageing, electrolyte leakage was 3.2, 13.4 and 15.8 m.mhos/cm (at 24, 72, 96 h respectively) which increased to 20.4, 27.2 and 31.6 m.mhos/cm at 24, 72, 96 h respectively.

At five months of ageing, when germinability of the seeds was 30%, the α-amylase activity was enhanced over the control by all PGR treatments after 96 h of germination. As *de novo* synthesis of α-amylase starts after 3–4 days of germination, visible signs of germination in bamboo seeds also occur. Resorcinol (20 ppm) treatment increased the activity to the maximum, followed by GA_3 (40 ppm) and IBA (20 ppm). These treatments also increased the germination percentage except for GA_3 (40 ppm) treatment (Figure 1).

After 11 months of ageing, the response of these seeds to the same concentrations of PGRs changed. At this stage, when germination percentage was reduced to 20, IBA (20 ppm) was the most effective treatment in increasing germination percentage and the enzyme activity after 96 h of germination followed by 1,2,4-acid (20 ppm) and resorcinol (20 ppm) treatments. Age-related loss of amylases has been reported by several workers in rice and wheat seeds[5,12,13]. All PGR treatments increased the enzyme activity over the control at one or the other stage of germination. The enzyme activity after 96 h of germination is the indicator of response produced by that PGR treatment on metabolism of the germinating seed and in turn the germination percentage[14].

At five months of ageing, the β-amylase activity increased by all PGR treatments over the control after 48 h of germination. It was maximum in IBA (20 ppm)-treated seeds followed by 1,2,4-acid (20 ppm) and resorcinol (20 ppm) treatments. The germination percentage was also increased by IBA (20 ppm) treatment from 30% in control to 50%. After six months of ageing, the effectiveness of these PGR concentrations in increasing this enzyme activity was reduced. Similarly, the effectiveness of the same concentrations of these PGRs on germination percentage also reduced.

Again, IBA (20 ppm) was the best treatment for increasing the germination percentage as well as the enzyme activity after 96 h of germination (Figure 1). According to Das and Sen-Mandi[15], in wheat unlike α-amylase which is synthesized *de novo*, some of the long-lived β-amylase surviving through dry storage degrade starch to initiate germination and seedling growth. The pre-formed β-amylase activity initiates germination at 48 h and at a particular stage of ageing (i.e. at 5 months) but at 11 months, PGRs increased enzyme activity but not significantly. Concentrations of PGRs are very specific at this stage of ageing as sometimes if concentrations used are low they fail to produce desired response and if high, they are inhibitory due to supra-optimal levels[16]. Optimum concentration of PGRs would have given best results but the precise co-relation between the best treatment for germination and enzyme activity could not be made in these seeds as these are time-bound studies.

At five months of seed ageing, the peroxidase activity was maximum in control whereas all the treatments proved to be inhibitory for the enzyme activity. After 11 months of ageing, the peroxidase activity in control seeds was reduced tremendously (Figure 1). The enzyme activity was marginally enhanced by some of the PGRs like 1,2,4-acid (20 ppm), IBA (20 ppm) and GA_3 (40 ppm) after 144 h of germination. An increase in the levels of this enzyme with treatment of PGRs is reported by many workers[17–19].

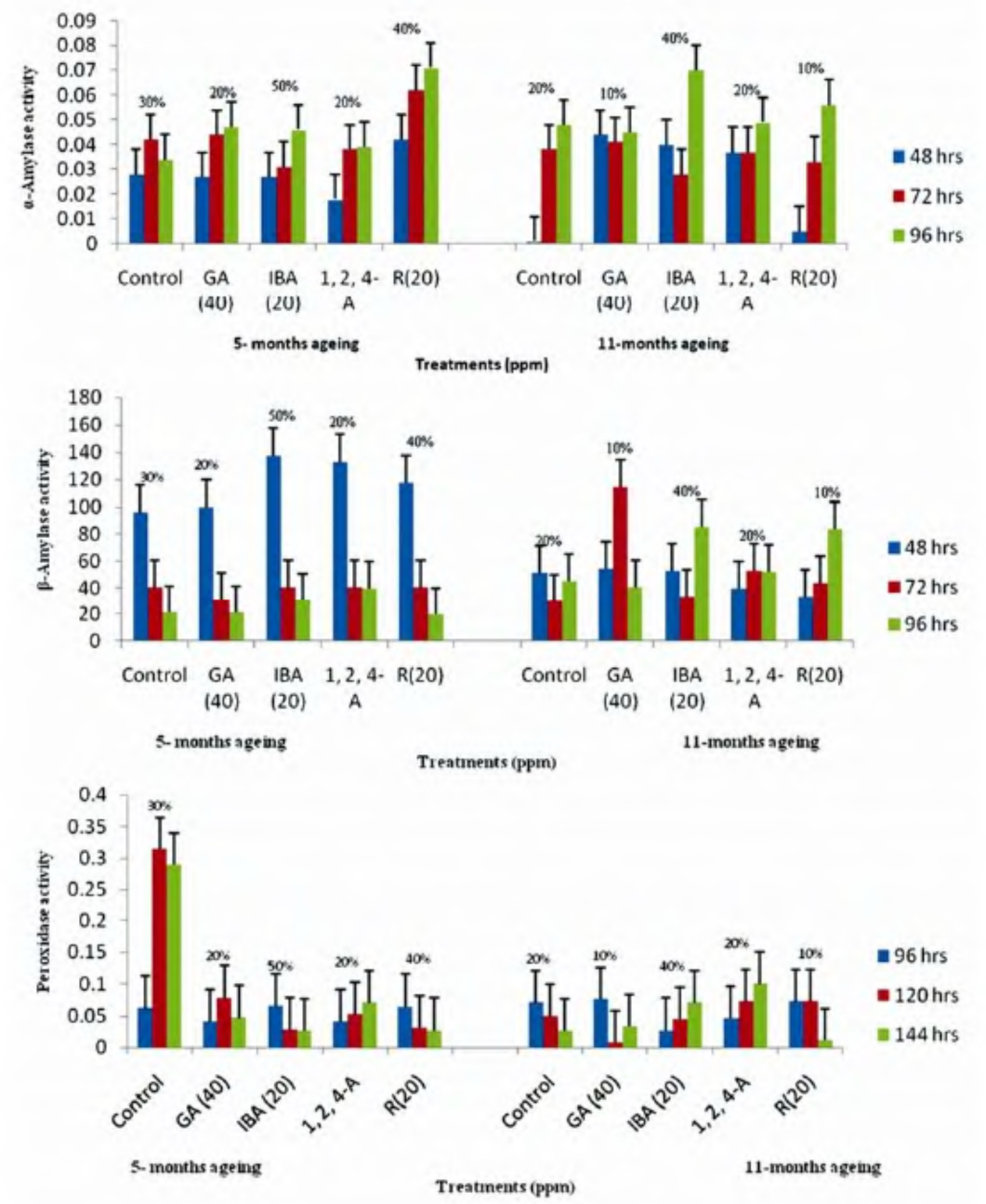

Figure 1. Changes in α,β-amylase activity (µg reducing sugars formed/g fr wt) after 48, 72, 96 h and peroxidase activity (µM/mg protein) after 96, 120 and 144 h of germination at six monthly ageing interval in *D. strictus* seeds with exogenous applications of plant growth regulators. (Bars represent SD at 0.05%.)

It appears that during seed ageing, the rate of seed deterioration depends upon the seed efficiency to maintain sufficient peroxidase enzymatic system as protection against the oxidative stress. The activity of peroxidase, which is involved in mitigation of oxidative damage to membranes, has been considered as an indicator of possible recovery of damaged cellular system and vigour of seeds[20,21].

The results of present study on the effect of PGRs on the peroxidase activity indicate that PGRs did not help in membrane system repair since peroxidase activity decreased with ageing leading to membrane damage and slow deterioration of seeds[22,23]. This slow degradation of seeds is irreversible which makes PGR treatments ineffective after certain stage of ageing. The bamboo seeds showed deterioration in germinability after six months of ageing not only due to depletions of hormones but also because of lack of presence of an efficient antioxidant enzymatic system to protect membranes from oxidative stress during ageing.

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A protocol for multiplication and restoration of *Ceropegia fantastica* Sedgw.: a critically endangered plant species

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***Ceropegia fantastica* Sedgw. (Asclepidaceae) is a critically endangered, endemic species in Western Ghats of India. The fruit and seed setting are very low and *in vitro* propagation is the only viable alternative for its sapling raising and restoration of this plant's population in the wild natural environment through reintroduction. Attempts have been made here for regeneration of this species through *in vitro* technique using nodal segments as explants and up to 13 multiple shoots were initiated on Murashige and Skoog's (MS) basal medium supplemented with 6-benzyl aminopurine (1.5 mg l^{-1}). Shoots were multiplied by routine periodic subcultures. The shoots of 3–4 cm length were isolated and rooted on MS basal medium (without $CaCl_2$) containing indole-3-buteric acid (1 mg l^{-1}). The rooted plantlets were hardened and successfully established in pots. More than 250 hardened plantlets in two successive years were transferred to their natural habitats of Western Ghats.**

Keywords: *Ceropegia fantastica*, nodal explants, restoration, Western Ghats.

THE genus *Ceropegia* includes more than 200 species distributed in the Old World ranging from South-East Asia, India, Madagascar, Tropical Arabia, Canary Islands, Africa except Mediterranean region, New Guinea and Northern Australia. In India, about 50 species are present and most of them are endemic to Western Ghats[1,2], which is one of the centres of diversity of *Ceropegia*[3,4]. The tubers of many *Ceropegia* species contain starch, sugar, gum, albuminoids, fats and crude fibre which are useful as a nutritive tonic[5]. The tuberous roots of many species of *Ceropegia* are edible and are eaten by local inhabitants and animals[6], causing threat to their survival in the nature. The bitter principle of the root is due to the presence of an alkaloid called Ceropegine[7]. Additionally, propagation either by seed or by vegetative techniques is rather difficult and cumbersome (unpublished work). Habitat modification is one of the major causes for reduction of natural population of this species. Because of these constraints, their distribution is strictly confined mainly to the highly protected areas. The majority of endemic species are grown in limited areas and some of them are

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