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Research Article

PHYSIOLOGICAL EFFECTS ASSOCIATED WITH FRESHLY HARVESTED BAMBOO SEEDS OF THREE SPECIES

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ABSTRACT

Bamboos are fascinating plants (tree-grasses) with a wide range of values and uses. They are indicators of high biodiversity, play a significant role in soil conservation and extensively used for soil and water management. The flowering of a bamboo is usually quite an extraordinary event - for the simple reason that it very rarely happens .Seeds of bamboos cannot be obtained every year and after seeding the bamboos die. Seeds can be used for seedling production only for short duration of maximum six months. Our aim was to observe the physiological studies include seed viability studies, membrane integrity and enzyme analysis in three species of bamboos *viz*. *Dendrocalamus hamiltonii*, *Dendrocalamus strictus* and *Bambusa bambos* in freshly harvested seeds. Maximum germination was found to be in case of *Dendrocalamus hamiltonii* (81.2%) out of all the three species and *D. strictus* (76%) showed minimum germination which was in correlation with physiological study showing vigour index, electrical conductivity, electrolyte leakage, TTC activity, α amylase, β amylase and catalase activity of all the three species.

INTRODUCTION

Bamboo is a tribe of flowering perennial evergreen plants in the grass family Poaceae, Subfamily Bambusoideae, tribe Bambuseae. The importance of bamboo as an ecofriendly raw material capable of meeting multifarious needs of the people at large is gaining global acceptance. From a raw material known as poor's man timber. Bamboo is currently being elevated to the status of "the timber of the 21st century". They are distinguished from other member of the family by having woody culms, complex branching and generally robust rhizome system and infrequent flowering. The flowering of a bamboo is usually quite an extraordinary event - for the simple reason that it very rarely happens. Bamboo flowering in this way spends an enormous amount of energy producing the flowers and seeds which usually stresses the plant to such an extent that it will die. A particular species can flower and die all over the world at the same time which happens because all plants originating from a particular source are clones of the mother plant (since bamboos are usually multiplied via cuttings or clump divisions) though it also happens in naturally regenerated clumps through seed. Seeds of bamboos cannot be obtained every year and after seeding the bamboos die. Seeds can be used for seedling production only for short duration of maximum six months. There are bamboos which have not produced seeds yet. So, production of planting stock in bamboo is difficult due to absence of regular seeding and short viability of seeds. In the year following the flowering of the bamboo or years in the case of gregarious flowering, blooms tend to be concentrated in the months between November and April. Seeds are generally available from March to April onwards. Bamboo seeds need to be collected immediately before rains set in as seeds lose viability rapidly on exposure to excess moisture. The biochemical processes occurring in grain are directly influenced by moisture content, air temperature, contact with air and condition of grain (degree of damage) (McDonald, 1999). The deterioration of seed quality depends on two environmental factors-relative humidity that regulates seed moisture content and temperature and both influence by affecting the metabolic rate of seeds. Our present study was aimed at studying the physiology of three species of bamboo seeds so that their physiology change with ageing could be understood well as enough literature is not available.

MATERIAL AND METHODS

Seeds of three species i.e. *Dendrocalamus hamiltonii, Dendrocalamus strictus and Bambusa bambus* were procured from KFRI, Peechi and Royal Forest Department, Bangkok, Govt of Thailand. Various physiological studies were carried

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out on freshly harvested seeds. All the following experiments were done in triplicates. Prior to germination studies the seeds were surface sterilized by soaking them in 0.5% Mercuric Chloride ($HgCl_2$) for two minutes followed by thorough washing in running water. They were later rinsed with distilled water 2-3 times. Ten randomly selected surface sterilized seeds were placed equidistantly in pre-sterilized petridishes (Ø 9.0 cm) lined with filter paper. One set of seeds were placed in petridishes, with filter paper soaked in distilled water as control. The entire experiment was conducted in laboratory condition in seed germinator where temperature was maintained at 28°C ± 2°C. The seeds were observed daily and the number of seeds germinated and their respective root and shoot length were recorded for 14 days

Germination percentage (G%)

Emergence of radicle was considered as an indicator of germination. Number of seeds germinated was noted after every 24 hours for 14 days.

$$\text{Germination Percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total No. of seeds sown}} \times 100$$

Vigor index (VI) (Abdul- Baki and Anderson, 1973)

Vigor index was calculated by the formula here under:-

VI=G % X Root Length (cms)

Emergence index (EI)

Emergence index was calculated by the formula:
$Ei = £n_1/dn_1 + n_2\,dn_2 + n_3/dn_3$------nx/dnx
Where
n = number of newly germinated seeds on each day
dn = number of days from the day of sowing
dnx = number of days to the final count

Electrolyte leakage measurements of seed leachates

10 seeds of each species were placed in 20 ml of distilled water for 12, 24 and 48 hours each, in conical flasks which were covered with aluminium foil to reduce evaporation and dust contamination and maintained at 20°C ±2. The solutions were filtered & final volume was made to 20ml. The medium was gently swirled for 10-15 seconds and the conductivity was measured using conductivity meter. Electrolyte leakage was calculated by the formula:

$Pi= [1-(1-T_1/T_2)/(1-C_1/C_2)X100$
T_1 = First conductivity measurement
T_2= Second conductivity measurement
C_1 = Control
C_2 = second conductivity measurement of control after damage.

TTC (2,3,5- triphenyltetrazolium chloride) Test (Viability Test) (Steponuks and Lanphear, 1967)

The samples used to perform the test were seeds. The seeds were taken in 3 replicates with 10 seeds. All the replicates were then placed in small glass tubes containing 3ml of 0.1M K_2HPO_4-KH_2PO_4 buffer (pH-7) with 0.5% (w/v) TTC. The ovules were incubated for 20 hours at 28°C in darkness. The TTC solution was drained. The seeds were washed twice and 2ml of ethanol was added. The tubes were kept in a water bath at 95°C until complete evaporation of the ethanol. 4ml of ethanol was added again and tubes were vigorously shaken. The absorbance due the formazon formation was recorded at 520nm against ethanol.

Enzyme analysis

The enzyme extracts were made after 12 hours, 24 hours and 48 hours of germination. For this the seed were deglumed, weighed and homogenized with 6 ml of buffer and a pinch of acid washed sand in cold pestle and mortar. The homogenate was centrifuged at 3000 rpm for 10 min. and the supernatant was retained and used for enzyme assaying. All the above mentioned steps were carried out at 1-4°C.To 1.0 ml of starch substrate added 0.5 ml of enzyme extract which forms the reaction mixture. At 0 time taken an aliquot of 0.2 ml of reaction mixture. Added 3 ml of Kl solution and the absorbance of resulting blue colored solution at 620 nm. After 30 min. taken another aliquot of 0.2 ml of reaction mixture. Added 3.0 ml of KI solution and read blue- colour solution for absorbance at 620 nm. The enzyme activity was estimated by measuring the decrease in starch concentration in the reaction mixture. In case of β- amylase (Shuster and Gifford, 1962) Reaction mixture containing 0.2 ml enzyme extract and 1 ml of freshly prepared starch solution was added and incubated the whole reaction mixture at 30°C for one hour. Reaction was terminated by adding 1 ml DNSA reagent All test tubes were kept in boiling water bath for 10 min, cooled the tubes to room temperature and added 2 ml of distilled water to each tube and recorded the absorbance at 560 nm. Control for every reaction mixture was read simultaneously. In Catalase (Teranishi 1974), The reaction mixture (3 ml) containing 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2, 0.1 ml of enzyme extract. The reaction was stopped by adding 2 ml of titanium reagent. It was centrifuged at 10,000 rpm to 10 min. The absorbance was read at 410 nm. The catalase activity was measured using the extinction coefficient 40 mM^{-1} cm^{-1} and expressed as µ Mol H_2O_2 reduced/sec/g FW.

RESULTS AND DISCUSSION

The germination studies include the study of various parameters of germination viz. Germination percentage (G%), Vigour index (VI), Emergence index. Seed deterioration can be defined as the loss of quality, viability and vigour either due to ageing or effect of adverse environmental factors. The rate of deterioration rapidly increases in either seed moisture content or temperature of storage. Seeds characteristics decrease under long storage condition due to ageing. Changes that occur in seed during aging are significantin terms of seed quality, the feature that among other things, also implies seed longevity (McDonald, 1999). The purpose of proper storage is to inhibit biological processes to the highest possible extent and to eliminate unfavorable environmental factors, which limit duration of the safe storage. Indeed, the ability of many orthodox seed to remain viable for tens or hundred of year in dry storage (Walters *et al.*, 2005; Daws *et al*., 2007), indicates that they can be used for the long term conservation of plant germplasm.

Table 1 Germination Parameters in seeds of three species of bamboos

Seed Stage	Species	*Dendrocalamus hamiltonii*	*Dendrocalamus strictus*	*Bambusa bambos*
Fresh Seeds	G %	81.2	76	80.2
	Vigour index	1236.4	1120	1214
	Emergence index	3.5	2.6	2.8

The longevity of seeds during dry storage is mainly determined by seed moisture content and storage temperature, with longevity increasing with decreasing temperature and moisture content (Ellis and Roberts, 1980). There is evidence that shows that some species produce seeds with much shorter longevity in dry storage. For example, seeds (with high initial viability) of *Anemone nemorosa* are predicated to survive for less than one year under seed bank storage conditions (Ali *et al*., 2007). Maximum germination can be found in case of *Dendrocalamus hamiltonii* and minimum in case of *Dendrocalamus strictus* as can be seen in Table 1

Table 2 Membrane stability index (MSI) in seeds of three species of bamboos

Species	Fresh
Dendrocalamus hamiltonii	39.78
Dendrocalamus strictus	40.667
Bambusa bambos	54.523

Table 3 Electrical conductivity (m.mhos/cm) at 12 hrs, 24 and 48 hrs of imbibitions in seeds of three species of bamboos

	Fresh		
Species	**12 hrs**	**24 hrs**	**48 hrs**
Dendrocalamus hamiltonii	10.23	15.567	20.170
Dendrocalamus strictus	8.13	11.03	18.310
Bambusa bambos	8.911	11.320	19.080

Table 4 Electrolyte leakage (m/ mhos) in seeds of three species of bamboos

Species	Fresh
Dendrocalamus hamiltonii	65.9
Dendrocalamus strictus	59.3
Bambusa bambos	62.4

Biological membranes with a normal composition and organization regulate the transport of material into and out of the cell. They play a key role in maintaining seed viability and vigour. Solute leakage include seed imbibitions during the process of membrane reorganization following rehydration. The rate of leakage depends on the degree of cell membranes damage and repair in response to ageing (Simon, 1978). Damage to the organization of cell membranes during seed ageing may constitute an important factor in explaining seed deterioration .Free fatty acid can damage lipid bilayer particularly of mitochondria leading to reduce energy production and free radicals have potential to damage membrane, DNA, enzymes, protein and ultimately cellular repair mechanism (Ghassemi-Golezani *et al,* 2010). In seed ageing, damage to cellular membranes, decrease in mitochondrial dehydrogenases activities, chromosomal aberration and DNA degradation increase. Loss of seed viability with ageing is usually linked with the loss of membrane integrity (Priestley, 1986; Bewley and Black, 1994) i.e Table 2,3 and 4.

Electrical conductivity measurements of seed leacheates are routinely used to determine seed vigour in a number of species. Leakage of sugars is considered as a less reliable index of membrane integrity than the leakage of electrolytes (Simon, 1974). The damage caused to membrane through deterioration that provides lower selectivity and hence increase in the leakage of solutes to the environment is one of the main cause of the decline in the physiological quality of seeds. One of the major changes during seed storage is membrane deterioration, which leads to the loss of seed viability. It suggested that the damage to cellular membranes during ageing could be manifested as an increase of solute and electrolyte leakage from seeds during imbibitions. Minimum leakage was in *D. strictus* as seen in Table 4. Membrane damage that occurs during seed storage contributes to a loss of viability and vigour. An oxidative change in the membrane polyunsaturated fatty acids is one of the reasons for deterioration of the seeds (Wilson and McDonald, 1986). Seed ageing and seed loss of viability is closely related to cellular damage and decrease in nucleic acids metabolism

Table 5 TTC activity in seeds of three species of bamboos

Species	Fresh
Dendrocalamus hamiltonii	0.124
Dendrocalamus strictus	0.116
Bambusa bambos	0.189

Maximum TTC activity was in *B. bambos* and Minimum in *D. strictus* which can be correlated with Germination percentage (Table 5)

Table 6 Levels of α-amylase (expressed asΔ O.D. per gram F.Wt. per unit time) in seeds of three species of bamboos

	Fresh		
Species	**12Hrs**	**24 Hrs**	**48 Hrs**
Dendrocalamus hamiltonii	71.23	74.45	82.34
Dendrocalamus strictus	76.67	83.23	93.24
Bambusa bambos	69.87	74.35	78.23

Table 7 Levels of β-Amylase expressed asΔ O.D. per gram F.Wt. per unit time) in seeds of three species of bamboos

	Fresh		
Species	**12Hrs**	**24 Hrs**	**48 Hrs**
Dendrocalamus hamiltonii	102.34	105.6	107.8
Dendrocalamus strictus	99.34	99.89	102.3
Bambusa bambos	89.23	93.42	94.53

Table 8 Levels of catalase (CAT) (µmol H_2O_2 reduced/gfwt) in seeds of three species of bamboos

	Fresh		
Species	**12Hrs**	**24hrs**	**48hrs**
Dendrocalamus hamiltonii	284.14	290.15	325.5
Dendrocalamus strictus	299.12	300.23	311.26
Bambusa bambos	398.23	401.23	411.12

Studies on the effect of ageing on enzyme activity in bamboo seeds are limited. Ravikumar *et al*. (1998) studied the various changes associated with ageing in seeds of the thorny bamboos *Bambusa bambus*. They reported that enzymes acid phosphatase, alkaline phosphatase and peroxidase showed decline in their activity after ageing. *Dendrocalamus strictus* seeds when kept at 42±1ºC and 100% RH for 1 to 8 days showed a loss in viability of seed, reduction in sugar, starch, proteins and lipids, decrease in the activity of peroxidase and alkaline phosphatase and an increase in total free amino acids and the activity of amylase, confirming degradation of stored reserves (Richa *et al*., 2006, 2010). Decline in the activities of α and βamylases, peroxidase and glutamate dehydrogenase with seed ageing of 6 month of bamboo seeds of *Dendrocalamus membranes* and *Cephalostachyum pergracile* was reported by Richa *et al*. (2010). Thus the study suggested level of all the three enzymes in all the three species of bamboos seen in Table 6,7 and 8.

CONCLUSION

The present study is intended to understand the physiology of fresh bamboo seeds of three species so that it can be used to improve the germination percentage by subjecting seeds to different physiochemical environments i.e invigouration treatments to improve viability of seeds and to determine various physiological factors (i.e Metabolites, enzymes, membrane integrity) that lead to the loss of viability during storage. It is good alternative for propagation which is desirable due to its high demand and depletion in natural forests due to unscientific large scale extraction by the rural population for meeting their increased requirements.

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