Theme:Post-Harvesting and Processing

Impact of invigouration treatments on viability of *Dendrocalamus hamiltonii seeds*

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Abstract

Bamboo flowers after a long interval and generally flowering is infrequent.. Moreover seeds are scarcely available and also they have a very short viability of 2-3 months. Generally they are stored under controlled condition of 4 degree Celsius in desiccators under anhydrous calcium chloride to maintain viability for longer period of time. However during storage, deterioration occurs. The aim of the study is to correlate membrane integrity deterioration with seed viability or physiology during storage upto 18 months in seeds of *Dendrocalamus hamiltonii* under both natural ageing i.e room temperature and controlled ageing i.e ageing in seed kept in desiccators at 4 degree celsius and to understand the effect of invigouration treatments on its viability. Invigouration treatments are beneficial treatments applied to the seeds that improves the germination or facilitates the delivery of seed and other material required at the time of sowing and storage. Various pre sowing i.e Infusion, Osmopriming and Fortification and pre storage treatments i.e Pelleting and Hardening were given to the seeds. Best presowing treatment was found to be with GA3 at 50 ppm that shows the germination percentage of 26% upto 18 months of ageing as compared to untreated seeds which loses complete viability at 12 months of ageing. While the best pre storage treatment was found to be 10% turmeric conc which shows 24.9% germination after 18 months of storage. Both pre storage and pre sowing treatments were statistically significant in improving the viability of seeds. Thus suggesting that invigouration treatments can improve the shelf life to a great extent.

Keywords: Invigouration, viability, hardening, pelleting, osmopriming, fortification, infusion.

Introduction

Bamboos are plants of global interest because of their distinctive life form, ecological importance and the range of uses and values they have for humans (Bystriakova *et al*., 2004). Atleast one third of human race uses bamboo in one way or the other. They are intermingled with the tradition and culture of rural tribal populations from times immemorial. As a renewable natural resource it plays a major role in the livelihood of rural people. The most peculiar feature of bamboo plant is its flowering which is a cyclic phenomenon and cycle varies between 5 to 120 years. This genetically controlled flowering is so profuse that a whole plant is transformed into a giant inflorescence. Since seeds serve as a best material for large scale plantation and germplasm conservation and they are viable for a very short duration of few months, considerable research has been conducted to understand the physical and chemical changes involved in loss of seed viability during storage. Due to all these factors, it is essential for bamboo researchers to study metabolism at various stages of storage, to increase their shelf life and devise methods to increase their viability Bamboos suffer from lack of post harvest treatment and technology for preservation and product development. Though the causes of deterioration of seed viability during storage has not been fully understood, scientists relate it to bioenergetics disturbance (Ching, 1982), damage to nucleic acids (Cheah and Osborne, 1978), loss of vitamins and hormones (Copeland, 2001, Bewley and Black,1982; Richa *et al.,*2000; 2006), and membrane deterioration. Biological membranes with a normal composition and

organisation regulate the transport of material into and out of the cell. They play a key role in maintaining seed viability and vigour. Seed viability was studied with TTC test and germination studies and their effects with ageing. The rate of leakage depends on the degree of cell membrane damage and repair in response to ageing(Simon,1978).Electrical conductivity measurements of seed leacheates are routinely used to determine seed vigour in a number of species (Pandey, 1992; Hampton and Tekrony, 1995). Leakage of sugars is considered as a less reliable index of membrane integrity than the leakage of electrolytes (Simon, 1974). Membrane damage that occurs during seed storage contributes to a loss of viability and vigour.. Seed deterioration is reported to accompany changes in enzyme activity during ageing (Aung and McDonald, 1995; Richa *et al*.,2006; Singh *et al*., 2010).Since high ß- amylase activity was present in seed stock of 99% germination while zero activity was detected in low viability, low vigour seed stock. Kobayashi *etal*.(1989) analyzed the endogenous level of gibberellins in shoots and ears of two dwarf rice (Oryze sativa) cultivars.

Seed invigouration is ascribed to beneficial treatments applied to the seeds prior to sowing, that improves germination or seedling growth or facilitates the delivery of seed and other materials required at the time of sowing. Various techniques are available, which enhance the vigor of seeds and these technologies are termed as seed invigoration/seed enhancement techniques (Moghadam and Mohammadi, 2013). Seed invigouration is an improvements in seed performance by post harvest treatment resulting in improved germinability , greater storability and better yield performance than the corresponding untreated lots (Ali *et al*., 1990). The treatments used to invigorate seeds include hydropriming; seed hardening; on-farm priming; osmopriming; osmohardenning;humidification; priming with plant growth regulators,polyamines, ascorbate, salicylicate, ethanol, osmolytes; coating technologies; and more recently pre-sowing dry heat treatments.Similar results on plant growth regulators and pre sowing treatments were done in our Lab in *D.strictus* (Richa *etal*. 2010; 2015).These treatments help in breaking dormancy and improving seedling density per unit area under optimal and adverse soil conditions. Induction and de novo synthesis of hydrolase such as amylases, lipases, proteases; and antioxidants (such as catalases, superoxide dismutase and peroxidase) are reported to be the basis of improved performance using these techniques. Seed priming can be performed by soaking simply in water, in a solution of salts, hormones, osmoprotectants, matric strain- producing materials, and other nonconventional means.

Material and Methods

Seeds of i.e. *Dendrocalamus hamiltonii,* were procured from KFRI, Peechi. The seeds of the species were stored in two lots: one set of seeds is subjected to natural ageing i.e stored at normal room temperature and and the other subjected to controlled ageing i.e stored at at 4°C (in a dessiccator over anhydrous Calcium chloride).

Various physiological and biochemical studies were carried out on these seeds at intervals of six months, for one and a half years to study the effects of ageing

1. 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.

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

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

3. TTC (2,3,5- triphenyltetrazolium chloride) Test (Viability Test)

(Steponuks and Lanphear, 1967)

4. Membrane Integrity (Duke and Kenyon, 1993)

In 10 ml of distilled water, add 20 seeds. After two hours, measure electrical conductivity. Boil it for 5 minutes and then cool it. Then measure the electrical conductivity.

6. Phospholipids estimation (Aemes,1966)

Phospholipid content were estimated by Aemes method

7. Electron microscopic studies

Seeds were crushed to form powder for TEM study,

i)Surface cleaning :Seeds were crushed to form powder and a suspension of lyophilized powdered seed was prepared in double distilled water

ii) Fixation and dehydration : Few drops of the suspension were deposited into a carbon-coated copper grid and immobilized for 1-2 min. After immoblization the excess solution was wicked off with filter paper and sodium phosphotungstate solution (0.2%, w/v) was added for negative staining. The grids were washed with double distilled water twice and dried after the few seconds.

8. α- amylase (Bernfeld, 1951)

α- amylase activity was calculated by determining the concentration of hydrolyzed starch substrate in a specific time by the method of Bernfeld (1951).

9. β- amylase (Shuster and Gifford,1962)

β amylase activity was calculated by determining the concentration of hydrolyzed starch substrate in a specific time by the method of Shuster and Gifford,(1962).

10. Endogenous level of Hormones

GA was determined by the method given by Chen Wen- shaw (1994) with a few modifications and Abscissic acid(ABA) and Auxins by (Nagar, 1996)

11. Seed Invigouration Treatments

i) Seed hardening (Rangaswamy *et al*., 1993)
ii)Seed pelleting (Sabir Ahmed , 1999)
iii)Seed infusion (Ells, 1963)
iv) Seed fortification(Ells, 1963)
v) Osmopriming (Ruan *et al*., 2002)

12. Statistical Analysis

Data were statistically analyzed using the software spss 14. Analysis of variance (ANOVA) was used to test the significance of variance sources, while LSD test (p= 0.05) was used to compare the differences among treatment means.

Results and Discussion

Over 18 months of ageing during storage period , the germinability declined from 81.2% in freshly harvested *Dendrocalamus hamiltonii* seeds to 20% after 6 months in naturally aged seed which became non viable after 12 months of natural ageing. However controlled stored seeds of*D. hamiltonii* remained viable even after 18 months of ageing with 15.3% of Germination percentage.Results of the TTC test indicated that viability was lower in naturally aged seeds than in controlled aged seeds, which is evident by G%.

TABLE 1:

Germination parameters in seeds of three species at different ageing intervals in both naturally aged and controlled aged seeds. (*DH: Dendrocalamus hamiltonii)*

Species		Freshly procured			6- month aged			12- monthaged			18 month aged		
		G%	V.I	E.I	G%	V.I	E.I	G%	V.I	E.I	G%	V.I	E. I
Naturally aged	DH	81.2	1236	3.5	20	114	1.4	0	0	0	0	0	0
Controlled aged	DH	81.2	1236	3.5	65.3	612	2.7	41	281.0	3.1	15.3	105.2	1.2

TTC (2,3,5,- Triphenyl tetrazolium chloride) activity (Viability test)

Species	Freshly procured	6 -monthaged		12- monthaged		18- monthaged	
		Naturally Aged	Controlled aged	Naturally aged	Controlled aged	Naturally aged	Controlled aged
DH	0.124	0.038	0.088	0.019	0.085	0.008	0.048

TABLE 2:

Membrane stability index (MSI) in seeds of three species at different ageing intervals							
Species	Freshly procured	6 -month aged		12 -month aged		18-month aged	
		Naturally aged	Controlled aged	Naturally aged	Controlled aged	Naturally aged	Controlled aged
DH	39.78	19.2	24.57	9.753	21.951	3.89	11.99

TABLE 3:

Electrical conductivity (m.mhos/cm) at 48 hrs of imbibitions in seeds at different stages of ageing							
Species	Freshly procured	6 -month aged		12 -month aged		18-month aged	
		Naturally aged	Controlled aged	Naturally aged	Controlled aged	Naturally aged	Controlled aged
DH	20.17	33.15	19.98	41.18	29.103	53.67	36.12

TABLE 4:

Electrolyte leakage (m/ mhos) in seeds at different naturally ageing intervals					
Species		Freshly procured	6 -month aged	12-month aged	18-month aged
Naturally ageing	DH	65.9	81.6	81.0	88.5
Controlled ageing	DH	65.9	75.4	77.9	80.6

Phospholipid content (mg/g(dm)) activity in seeds at different ageing intervals

Species	Freshly procured	6 -monthaged		12 -monthaged		18- monthaged	
		Naturally aged	Controlled aged	Naturally aged	Controlled aged	Naturally aged	Controlled aged
DH	4.15	3.11	4.03	2.16	3.29	1.70	2.80

Statistically, significant difference in MembraneStabilityIndex was observed with decrease in Germination percentage in both natural and controlled aged seeds.After 18 months of controlled storage, seeds of *D. hamiltonii* showed value of MSI i.e 11.99. Measure of electrical conductivity is a good indicator of viability of seeds. In the present study , a gradual increase in the electrical conductivity was observed with the increasing hours of imbibitions of seeds as well as with seed ageing. Statistically , significant increase in electrical conductivity was observed with decrease in G% in seeds. After 18 months of controlled storage, seeds of *Dendrocalamus hamiltonii* has minimum value of electrical conductivity i.e 36.129 (at 48 hours). A gradual increase in electrolyte leakage was observed with increase in time interval of ageing and decrease in G%.Membrane damage has been considered as a common early event in seed deterioration. Damage to the cellular membranes during ageing results in the inability of membranes to reorganize before the loss of metabolites. Deterioration was found to be maximum in the seeds of *D. hamiltonii(* stored for 18 months under controlled conditions and G%= 15.3). In the same way, controlled ageing caused a slow progressive decrease in total phospholipid (PL)contents whereas heavy losses were observed in the naturally ageing seeds which results in the impairment of germination capacity (to zero viability after natural ageing of one year). TEM observations of seed structure were focused on the arrangement and characterization of epidermal cells, their periclinal and anticlinal walls as well as the characters of the interspace.

TEM observations of seed coat

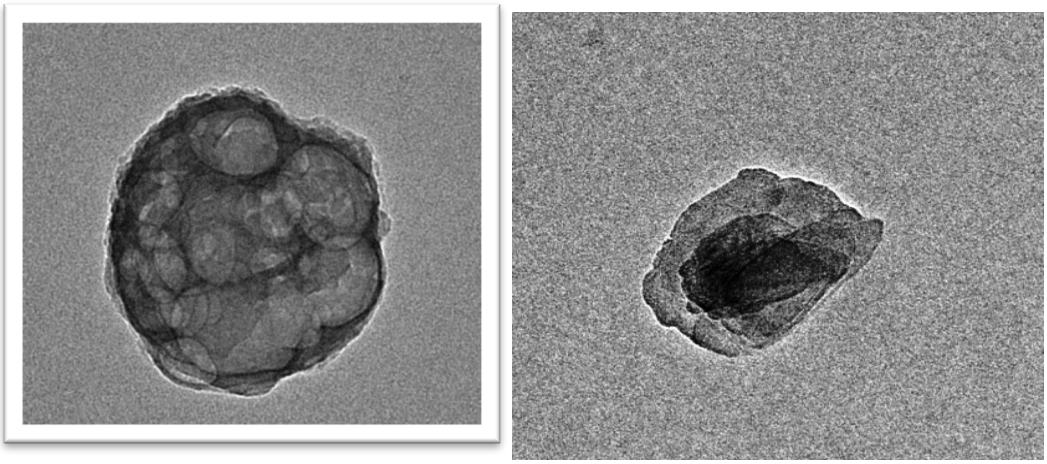

Fig 1: Transmission Electron microscopic photographs showing fresh (one month) (Left) and non viable (Right) seed membrane taken after one year of *Dendrocalamus hamiltonii* (C).(Scale bar= 100nm).

TEM observations of seed coat were focused on the arrangement and characterization of cells and characters of interspace. The freshly procured seeds showed compact structure as compared to non viable seeds which showed broken structure. Microscopic level of study has been found to be of great help in determining the relationship of ageing and membrane degradation.

Enzymatic Analysis

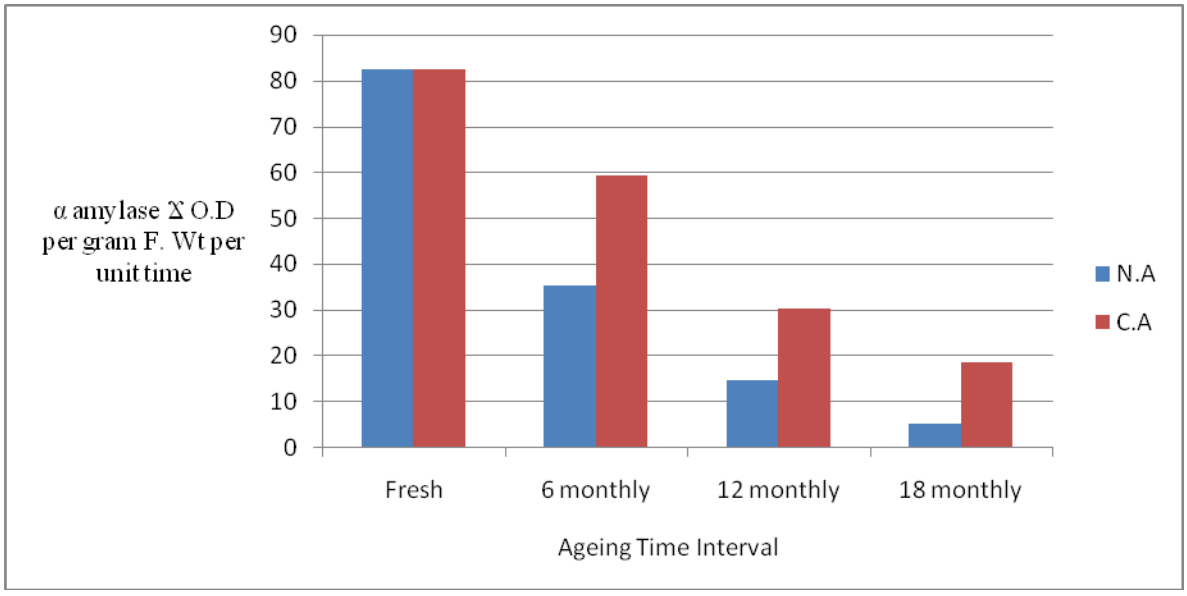

Fig 2 :Changes in α-Amylase (Δ O.D. per gram FWt per unit time) in seeds (at 48 hrs analysis) at different time intervals in naturally aged and controlled aged seedsdi

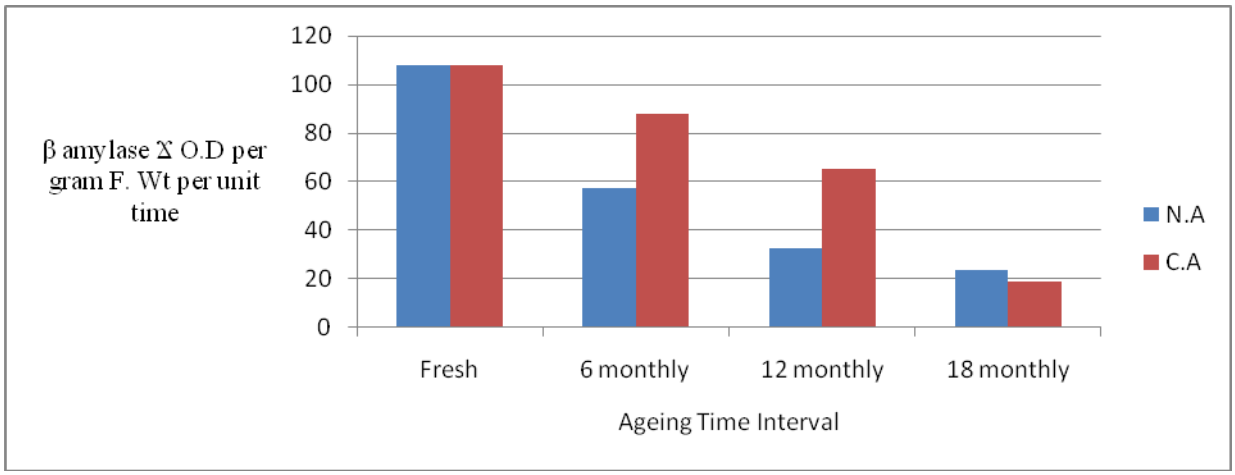

Fig 3 :Changes in β-Amylase (Δ O.D. per gram FWt per unit time) in seeds (at 48 hrs analysis) at different time intervals in naturally aged and controlled aged seedsdi

TABLE 5:

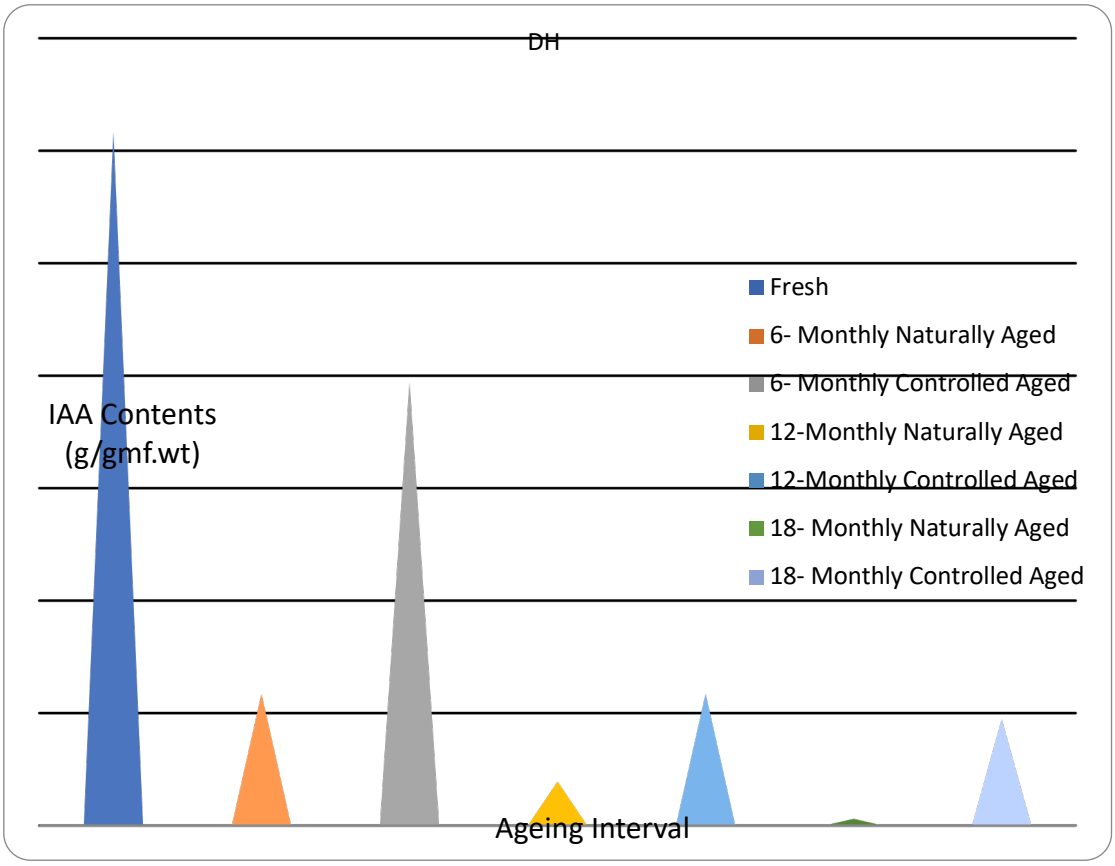

Fig 5: Changes in IAA contents(µg/gm f.wt) in seeds at different stages of ageing

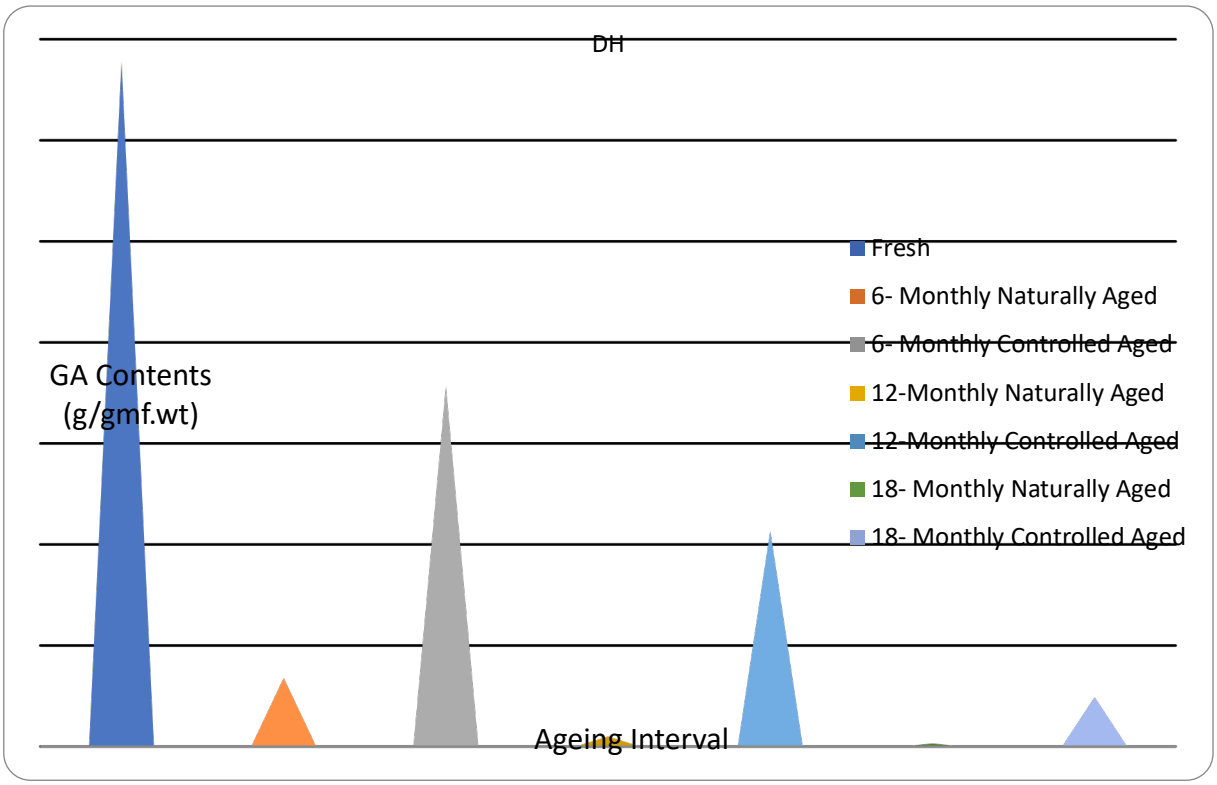

Fig 6:Changes in GA contents(µg/gm f.wt) in seeds at different stages of ageing

Abscissic Acid (ABA)

:Endogenous ABA levels (expressed as µg/gmfwt) in seeds at different stages of ageing

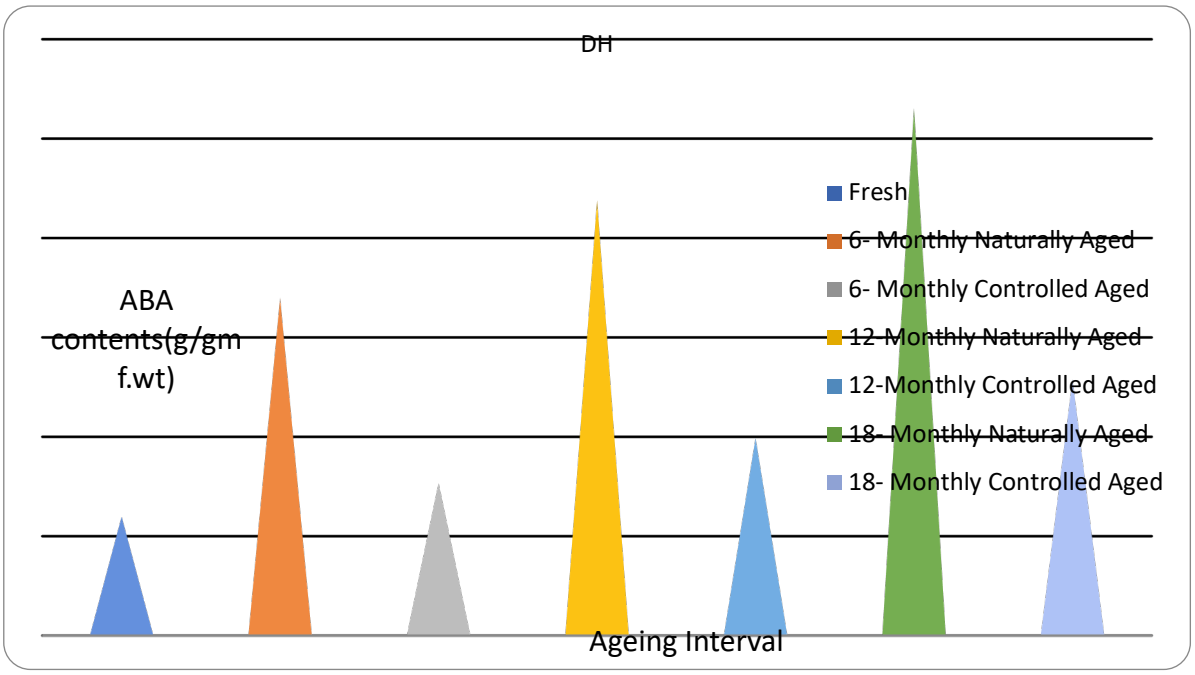

Fig 7:Changes in ABA contents(µg/gm f.wt) in seeds at different stages of ageing

Decrease of α amylase activity (86%) was observed in seeds of *Dendrocalamus hamiltonii* at 48 hours of analysis after 18 months. In case of controlled stored ageing seeds, decrease of enzyme activity (84%) was observed in seeds of *Dendrocalamus hamiltonii* at 48 hours of analysis after 18 months. Decline (85.3%) was observed in the seeds of *D. hamiltonii* 48 hours of analysis after 18 months of natural ageing. In case of controlled stored ageing seeds decrease of β enzyme activity (84%) was observed in seeds of *Dendrocalamus hamiltonii* at 48 hours of analysis after 18 months. After 18 months , decrease in enzyme activity of naturally aged seed lot was seen in seeds of *Dendrocalamus hamiltonii*.It was observed that there is direct correlation between viability and growth regulators. There is decrease in the concentration of growth promoters(IAA and GA) and increase in growth inhibitor (ABA). It was observed that controlled ageing seeds shown minimum crucial concentration of growth promoters(IAA and GA) was observed which made them viable for longer period of ageing than naturally ageing seeds where growth promoters concentration decreased drastically just after 6 months of ageing . And the concentration of growth inhibitor (ABA) was increased exponentially in naturally aged seeds than the controlled ageing seeds. in naturally ageing seeds of, *D. hamiltonii* , the value of concentration of IAA(0.12), GA (0.30) which was quite lower than controlled ageing seeds and in ABA(265.30) which has very high concentration than the controlled ageing seeds as shown in Fig 5, 6 and 7.

TABLE 5:

Seed Vigour Treatments

i) Infusion

: Effect of seed infusion (pre-sowing) treatments on germination percentage and vigour index of seeds.

Treatment	Conc.	Freshly procured		6 -month aged		12-monthaged		18 -month aged	
		G%	V.I	G%	V.I	G%	V.I	G%	V.I
GA_3	10ppm	82.2*	1274	56.4*	854.1	34.7	609	23.3	341

	20ppm	84.3*	1309	56.3*	868.2	34.8	594	23.3	360
	50ppm	86*	1447	60	984	40*	704	26*	443
IAA	10ppm	83.1	1291.15	54.3*	798	25.4	390.2	16.4*	234.5
	20ppm	83.1	1264	53.1	790	25.2	395	20*	282
	50ppm	85.2*	1312	54.0*	795.2	27.4*	413	16.4	244.4
IBA	10ppm	88.2*	1480	53*	826.15	25.4*	405	15.4	215
	20 ppm	84.2	1338	48	780	23.2	349	16*	237.38
	50 ppm	84.2*	1339.2	48*	785	23.1	352	15.2*	220.1
Control		80.2	1014	20	108	0	0	0	0

TABLE 6:

ii) Fortification

: Effect of seed fortification (pre-sowing) treatments on germination percentage and vigour index of seeds.

Treatment	Conc.	Freshly procured		6- month aged		12-monthaged		18- month aged	
		G%	V.I	G%	V.I	G%	V.I	G%	V.I
Ascorbic acid	2%	82.1*	1281	50.1	755	25.4	390	16.7	232.13

	5%	86.4	1318.2	56.8*	848	30*	441	20*	281
	10%	82.4	1284.2	53.3	818.5	26.1	397.2	20.1	234.2
GA_3	10ppm	82.4	1280	56.4	845.2	30	444	20.1	280
	20ppm	83.2*	1264	60*	901	36.7*	556.2	23.3*	328.5
	50ppm	82.8	1285	60.2*	910.2	36.2	572.4	23.4*	337.4
KH_2PO_4	2%	82.4*	1265	52.4	781.2	25.4	390.2	16.7	231.4
	5%	88.1*	1348	56.7	856	26.7	392.4	20*	282
	10%	86.2	1332	52.4	784.2	30	447	20*	280.2
Control		80.2	1014	20	108	0	0	0	0

TABLE 7:

iii) Osmopriming

:Effect of seed osmopriming (pre-sowing) treatments on germination percentage and vigour index of seeds

Treatment	Conc.	Freshlyprocured		6-month aged		12-month aged		18-month aged	
		G%	V.I	G%	V.I	G%	V.I	G%	V.I
KCl	2%	82.3*	1272	48.2	720	28.2	420.1	16.2	230
	5%	82.3	1282	48.0	724	34.5*	528	16.2*	231
	10%	83.1*	1290	52.1*	820	34.2	525	18*	260

KNO_3	2%	83.2*	1295	46.2	709	26.2	397	14.5	201
	5%	83.1	1291	52.1	824.5	30*	448	14.5	211
	10%	85.2*	1340	52.4*	830.1	30*	442	15.6*	230
PEG-6000	2%	83.2*	1257	45.7	702	25.4	390.1	12.2	150
	5%	86.2*	1382	53.1*	845	28.5*	415	14.2*	209
	10%	82.1	1280	44.2*	690	28.4	412	13.8*	188.2
Control		80.2	1014	20	108	0	0	0	0

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TABLE 8:

iv)Pelleting

: Effect of seed pelleting (pre- storage treatments on germination percentage and vigour index of seeds

Treatment	Conc.	Freshly procured		6 -month aged		12 -month aged		18-monthaged	
		G%	V.I	G%	V.I	G%	V.I	G%	V.I
Calcium Oxychlorid	2%	82.1*	1254	56.7	839	33.1	483	16.7	231

e	5%	83.3	1256	56.7	839	36.7*	530	16.5	242
	10%	83.4*	1304	60*	914	33.3	512	20*	280
Calcium Carbonate	2%	83.3*	1274	54*	900	36.7	530	15.5	212
	5%	82	1286	56	856	38.3*	604	15.6	230
	10%	83*	1307	60*	924	34.1	512	18*	241
Turmeric powder	2%	83.3	1274	55.7	856.17	31.1	490	23.1*	330.8
	5%	84.3*	1320	60*	888	33.1	514	20	292
	10%	86.7*	1369.7	61.7*	930	36.4*	560	24.9*	360.8
Control		80.2	1014	20	108	0	0	0	0

TABLE 9:

v) Hardening

: Effect of seed hardening (pre- storage) treatments on germination percentage and vigour index of seeds.

Treatment	Conc.	Freshly procured		6 -month aged		12-month aged		18-month aged	
		G%	V.I	G%	V.I	G%	V.I	G%	V.I
GA_3	10ppm	84.4	1378	63.1	1000	33.1*	521.6	16.1	240
	20ppm	86.7*	1362	63.0	1042	33.8	540.9	15.1	312

	50ppm	88*	1448	66.1*	1152	38.0*	708	23*	443
IAA	10ppm	83.3	1306	46.7	804	30.1	448	16.1	238
	20ppm	85.7	1308	53.3*	803	32.8	483	13.3	186
	50ppm	86.0*	1335	53.1*	840	33.3*	490	16.7	235
Albizzia leaf powder	2%	83.1*	1280	49.1	755	33.3	496	20	282
	5%	83.3	1285	50.2	756	40	584	20.1	333
	10%	86.4*	1308	53.2*	760	40.1*	621	20.3	342.1
Clay	2%	80.1	1288	53.2	814	34.5	445	16.1	238
	5%	80.1*	1286	56.7*	830	35.1*	448	13.4	233
	10%	83.1*	1311	56.8*	880	36*	590	20*	280
Calcium oxychloride	2%	83.1	1281	53	804	33.3	540	13.1	180
	5%	82.4	1241	50	740	36.1	543	13.2	183
	10%	86.1*	1308	56*	867	40*	596	16*	212
Control		80.2	1014	20	108	0	0	0	0

Seed quality is one of the key factors affecting the successful germination, but this seed trait inevitably declines during prolonged storage. Poor seed quality generally shows decline in its ability to germinate and emerge into seedling, leading to problems in successful establishment of plantation. Assessing the quality of long-term stored seeds is usually costly and time consuming. Seed producers and buyers need rapid tests that can provide information on the physiological quality of commercially produced seeds. Ageing studies enable us to understand the possible causes of seed deterioration at fast rate, and thus help in formulating counteractive seed treatment and appropriate storage practices.Seed invigoration techniques are the most important developments to help rapid, uniform germination, emergence of seeds and to increase seed tolerance to adverse environmental conditions to maintain viabililty for longer periods (Harris *et al*., 2001). Seed invigoration techniques have been found to be very promising for seeds of many species including bamboos. However, little information of invigoration studies on bamboo seeds is available, and more information is required before its use as a routine practice in seed technology. Less expensive and easily practicable invigoration methods like mid-storage hydration and dehydration treatments (Basu,1976) and short-term aerated hydration (Thorton and Powell, 1992) to improve vigour, viability and field performance have been reported. Germination percentage decline progressively to 20% after 6-months and became zero after 12-months in non-treated naturally aged seeds.

Among all the treatments GA3 (50ppm) is the most effective statistically in maintaining best germination and VI over control at 5% level of significance in both INFUSION , FORTIFICATION

AND HARDENING ,While KCl (10% conc) is the statistically most effective in case of OSMOPRIMING and TURMERIC POWDER (10 % conc) in case of PELLETING. Seed viability could be retained for a greater period by storing the seeds under controlled conditions. The decline in vigour and viability of seeds is recoverable to some extent by the appropriate application of various seed invigouration treatments.

From the results of present study, it can be concluded that bamboo seeds undergo age induced biochemical and physiological changes, similar to that of cereals. Age induced deterioration brings about membrane damage, causes leakage of reserve food material and enzyme degradation. Change in the optimum levels of plant hormones was also observed .

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