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EFFECT OF PRE-STORAGE AND PRE SOWING TREATMENTS ON PERFORMANCE OF AGEING *B. BAMBOS* SEEDS

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ABSTRACT
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. The aim of the study was to improve the performance of *Bambusa bambos* seeds which have a very short viability of 1-3 months. When seeds deteriorate, they lose vigour and become more sensitive to stresses upon germination. Eventually seeds lose the ability to germinate. With ageing seeds loses viability. Non availability of viable seed is a major drawback in enhancing productivity. It was found that bamboo with initial germination of 80.2% which reduces to zero after 12 months of ageing improve the germination upto 88% with the help of various treatments. 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 GA_3 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 effective in improving the viability of bamboo seeds. Thus suggesting that invigouration treatments can improve viability upto large extent.

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

INTRODUCTION

Bamboos are tall arborescent grasses belonging to family Poaceae, subfamily Bambusoideae and the 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.Infact, no other plant benefits us in so many ways as bamboos. They are distinguished from other member of the family by having woody culms, complex branching and generally robust rhizome system and infrequent flowering. There are 110 genera and 1500 species of bamboos distributed in tropical and temperate zone of different parts of the globe (Sharma, 1987). Tropical Asia can be referred to as the main centre of bamboo germplasm with as many as 45 genera and 750. Bamboo seeds, have very short viability of 1-3 months and are therefore useful as propagules for only a short period of time. The present study shall look into some aspects of this problem so that viability and related problems are understood. The present study is intended to study various physiological and biochemical factors (*i.e.* metabolites, enzymes, membrane integrity and levels of growth hormones) that lead to the loss of viability during storage. Loss in viability of seeds during storage is associated with loss in membrane permeability which results in leakage of cell contents. Invigouration treatments improve the viability upto 18 months.

MATERIAL AND METHODS

These invigouration treatments are physiological in nature. These treatments are classified as.

- Pre storage treatments for better storability
- Pre sowing treatments for improved field performance

There were two seed lots: one was treated with invigouration chemical and other untreated seed which served as control. Control seed lot was stored at normal temperature and humidity.

Pre-sowing treatments

Seed infusion (Ells, 1963)

It is a method of impregnation of seeds with bioactive chemical through organic solvents instead of water. This technique of infusion helps to avoid the damage caused to the seeds that suffer from soaking/seed coat injury. Simultaneously it also provides the protective, regulatory and selective functions of the chemical to improve the performance of seed with the help of organic solvents. Method: Desired solutes are dissolved in the organic solvent at desired concentration. Seeds are immersed in

solvent with desired soluted for 6 hrs. After the desired period, the solvent is evaporated by air or by vacuum desiccators for 30 minutes to hour or under ambient condition for 24 hour complete evaporation of solvent from the seed. The seeds are then ready for sowing. The depth and amount of chemical penetration into the seed vary with duration of treatment, amount of chemical reached the embryo increase with increase in time, concentration of soluted (higher the concentration more will be the penetration) and type of chemical compared to IAA, lesser quantity of GA_3 reaches the embryo lesser in a given time due to its higher molecular weight.

Chemicals for infusion: GA_3, IAA, IBA.
Organic solvent used for infusion: Acetone.

Seed fortification (Ells, 1963
It is an impregnation of required substance for invigourating the seed for improved production where the seeds are soaked either in water or dilute solutions of bioactive chemical such as micronutrients, growth regulators, vitamins and seed protectants.

Method
Seeds are soaked in solution of equal volume for duration of 6 hrs to raise the moisture content of the seed to 20-25%.

Chemicals used for fortification: Ascorbic acid, GA_3,KH_2PO_4.

Seed osmopriming (Ruan *et al*., 2002)
It is a pre-sowing treatment in which seeds are soaked in an osmotic that allows the seeds to imbibe water and go through the first stage of germination but does not permit radicle protrusion through through the seed coat. It is based on the principle of controlled imbibition of the seed to a level that permits pre- germinative metabolism to proceed, but prevents actual emergence of the radicle (Bradford, 1986).

Osmolytes
The salt used to prepare the osmotic solution are called osmolytes or osmoticants. The most common osmolytes are inorganic salt such as KNO_3 (Ells1963) KCI and a chemically insert compound polyethylene glycol (PEG) 6000. Use of PEG as a pre-sowing treatment has been much more successful in terms of germinability, uniformity of stand and subsequent crop performance than the simple water soaking treatment. In cotton, seed priming with KCl enhanced the speed of germination and activities of antioxidant enzymes (Afghani *etal*., 2012). PEG regulates entry of water into the seed, without carrying injury and advances germination to more or less fixed level determined by the water potential. Seed priming is a technique widely used to overcome the germination related problems in different crops (Dahamarudin and Arvin, 2013).

Chemicals used for osmopriming (Pre-sowing) PEG-6000, KNO_3, KCI.

Pre-storage Treatments
There were two seed lots of *Bambusa bambos*: one treated with invigouration chemicals and other included untreated seeds stored at desiccators at 4° C. The experiment was conducted after intervals of 6 months upto 18 months.

Seed hardening (Rangaswamy *et al*., 1993)
Pre- sowing hardening of seed is one of the best method that result in modifying the physiological and biochemical nature of seed so as to get the hardening is the result of extensive physiological reorganization induced by dehydration process. It can only be done with water and dilute solutions of growth regulating compounds and chemical to induce early germination, better root and seedling growth and also to enhance the yield potential of crop varieties.

Principle
During hardening process, a number of physiochemical changes occur and modifies the protoplasmic characters and increasing the physiological activity of the embryo and associated structures. Eventually due to more absorption of water due to increase in the elasticity of cell and development of a stronger and efficient root system.

Method
Dry seed were soaked in water and/or dilute solution of growth regulators and chemical (10-20-50) for 1-2 days at 15-25°C. Then followed by shade drying (1-2 days) and sun drying (1-2 days) to bring back to its original water content or weight forming hardened seeds.

Chemicals for Hardening
GA_3, IAA, Salicylic acid, Albizzia leaf powder, Clay, Calcium oxychloride.

Seed pelleting (Sabir Ahmed, 1999)
It is the process of enclosing the seed inside a small quantity of inert material just large enough to produce a globular unit of standard size.

Method
Before imposing seed treatments, the seeds were manually cleaned and subjected for processing. The three basic steps involved in seed pelleting are stated as stamping, coating and rolling. The materials needed for pelleting are seed, adhesive and filler materials. The seed are uniformly coated with adhesive and chemicals mentioned in correct quantity initially. Then the filler materials(Ash) are sprinked on the coated seeds and are rolled on the filler material for effective and uniform coating. Seeds are introduced into a pan with an amalgam of pelleting materials (Calcium chloride, Calcium carbonate, turmeric powder, cementing adhesives and gum arabic) are used to form the pellet.

As we shake the seeds while spraying with water followed by the addition of the pelleting materials with binder (Diammonium phosphate and ZnSO4@ 10g and 10mg respectively) for 100g of seeds after drying to the original moisture content). The wet seed attract and become coated with the dry pelleting material and the pellet gradually increase in size with each turn of coating while shaking. Longer rotation times with greater amounts of pelleting materials lead to greater pellet size and roundness. At the end of the pelleting process, a binder is added to harden the outer layer of the pellet.

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 difference among treatment means.

RESULTS AND DISCUSSION

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. 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. 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 invigouration 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 invigouration techniques have been found to be very promising for seeds of many species including bamboos. However, little information of invigouration 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 invigouration methods like mid-storage hydration and dehydration treatments (Basu,1976) and short-term aerated hydration (Thornton and Powell, 1992) to improve vigour, viability and field performance have been reported.

Seed invigouration studies were presently done only on seeds of *Bambusa bamboos* as the seeds of other species were not available in sufficient quantity. In this study, freshly procured seeds of *Bambusa bambos* with germination percentage of 80.2% were used. Germination percentage of these seeds declined progressively to 20% after 6-months and became zero after 12-months in non-treated, naturally aged seeds. The results of seed vigour treatments are given in Table 1) to v) and briefly discussed below.

1) Infusion

Table 1: Effect of seed infusion (pre-sowing) treatments on germination percentage and vigour index of *Bambusa bambos* 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	10 ppm	82.2*	1274	56.4*	854.1	34.7	609	23.3	341
	20 ppm	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
LSD#		Treat 5.12	Treat x 9.12	Treat 3.12	Treat x 6.32	Treat 2.1	Treat x 3.14	Treat 1.12	Treat x 1.9

 # Least significant differences of means (5%level)

*** Significant figures**

2) Fortification

Table 2: Effect of seed fortification (pre-sowing) treatments on germination percentage and vigour index of *Bambusa bambos* 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
LSD#		Treat 2.5	Treatx 4.3	Treat 1.8	Treat x 3.12	Treat 1.6	Treat x 2.5	Treat 1.2	Treat x 2.3

3) Osmopriming

Table 3: Effect of seed osmopriming (pre-sowing) treatments on germination percentage and vigour index of *Bambusa bambos* 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
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
LSD #		Treat 4.5	Treat x 8.3	Treat 3.2	Treat x 5.4	Treat 1.5	Treat x 3.12	Treat 0.98	Treat x 1.2

Pre- sowing treatments

In pre-sowing treatments, Infusion with GA_3, IAA and IBA, Fortification was done with Ascorbic acid, GA_3 and Dipotassium phosphate (KH_2PO_4) at various concentration. Osmopriming was done with potassium chloride (KCl), potassium nitrate and polyethylene glycol -6000(PEG-6000). In control seeds, germination percentage of freshly procured seeds was 80.2 which decreased to 20% after 6 months and become zero after 12 months. Fortification of fresh seeds with ascorbic acid (10% conc.) showed germination percentage of 86.4 (control 80.2%). Of all the treatments, best results were obtained by fortification with GA_3 at 50 ppm concentration after 18 months of storage (G%=23.4) when the seeds in control lost viability after 6 months. Improved seed performance has been achieved by incorporating plant growth regulators during pre-soaking, priming and other treatments in many crops (Jeong *et al*., 1994). Bhatt *et al.* (2000) found significant reduction of MGT and substantial improvement in germination when 100 ppm GA_3 was used as pre-treatment in *Myrica esculenta*. In *Bambusa bambos*, Krishnaveni *et al*. (2010) found that fortification with 1% KNO_3 increased seed germination by 29%, than control. In case of pre-sowing treatments, various treatments at various concentrations were presently given in Osmopriming Fortification and Infusion. During osmopriming maximum germination percentage (86.2) was seen with treatment of PEG-6000 at 5% concentration (control of 80.2%). After 18 months, best results were seen with Potassium chloride at 10% concentration with germination percentage of 18 (control zero%). Infusion with GA_3 showed best results at 50 ppm concentration with germination percentage of 86 over control of 80.2% and vigour index of 1447 and even after 18 months, treated seeds still showed a viability of 26% as compared to untreated seeds whose viability reduces to zero.

According to Fredj *et al*. (2013), the best germination percentage of coriander (*Coriandrum sativum*) was obtained by soaking seeds in NaCl at 4 g/L for 12 hrs. Movaghatian and Khorsandi *et al*.(2014) suggested that seed priming by salicylic acid (0.00001 mM) improved all germination characteristics (percentage of germination, radicle and plumule lengths and seed vigour) in ajwain (*Trachyspermum ammi)* under salt stress. The positive effect of priming on seed germination in many species is attributed to the induction of biochemical mechanisms of cell repair (Di Girolamo and Barbanti 2012). In the present study out of all the pre sowing treatments, Infusion showed the best result after 18 months with GA_3 at 50 ppm concentration as compared to any other treatment. Fariman *et al*., (2011) observed that seed priming enhances percentage and rate of germination in *Echinacea purpurea*.

4) Pelleting

Table 4: Effect of seed pelleting (pre- storage treatments on germination percentage and vigour index of *Bambusa bambos* 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 Oxychloride	2%	82.1*	1254	56.7	839	33.1	483	16.7	231
	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
LSD#		Treat 5.1	Treat x 9.12	Treat 3.4	Treatx 6.12	Treat 2.12	Treat x 3.12	Treat 1.1	Treat x 1.01

5) Hardening

Table 5: Effect of seed hardening (pre- storage) treatments on germination percentage and vigour index of bamboo seeds of *Bambusa bambos* 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
LSD #		Treat 4.12	Treat × 8.1	Treat 2.78	Treat × 5.43	Treat 2.13	Treat × 3.67	Treat 1.14	Treat × 1.8

Pre- storage treatments

In pre-storage treatments, Pelleting with calcium oxychloride, calcium carbonate and turmeric powder and Hardening with GA_3, IAA, Albizzia leaf powder, clay and calcium oxychloride was tried at various concentrations

In hardening treatments, significant increase was observed in germination rate, root length and shoot length in treated seeds over the untreated seeds even after 18 months. The maximum germination percentage (88%) and V.I (1448) was recorded in seeds treated with GA_3at 50ppm concentration while the control seeds showed only 80.2 germination. Hardening treatments after 18 months of storage, resulted in maximum germination percentage of 23% and V.I 443 with GA treatment at 50 ppm concentration. Thus various hardening treatments were effective in increasing the G% and V.I. Pelleting, treatment with 10% turmeric powder showed 24.9% and V.I = 360.8 after 18 months of storage when the viability of untreated seeds reduced to zero.

Thus all the pre-storage treatments were found to be effective in increasing the viability. Statistically, the most effective concentration of GA_3 for maintaining best germination and VI up to 18-months was found to be 50ppm and turmeric powder at 10% concentration. Analysis of variance (ANOVA) was applied to the germination parameters (G%) to find out which treatment and concentration was statistically significant in enhancing various germination parameters while LSD test (0.05) was used to compare the differences among treatment means. Seeds stored under controlled conditions showed initial germination of 80.2% which

reduced to 20% after 6 months and reduced to 0 after 12 months. Fariman (2012) observed that in brinjal, bavistin (1%) recorded significantly high germination percentage (85.70%) and seedling vigour index (887) than the other treatments ($ZnSO_4$, $MnSO_4$, DAP and control) followed by Arappu leaf powder (250g/kg) at the end of 12months of storage period. All these examples suggest that exogenous application of growth regulators increases the viability of seeds.

The present study has shown that both pre-storage and pre-sowing treatments are effective in increasing the viability and germination percentage of bamboo seeds for longer duration of storage. Pelleting with turmeric powder at 50 ppm concentration has been found to be the best pre-storage treatment while Infusion with GA_3 at 50 ppm is the best pre- sowing treatment. Thus treatment with GA_3 at 50 ppm conc. is most effective in maintaining viability of seeds for longer periods perhaps because this growth regulator makes up for the deficiency of endogenous growth hormones in the seed ageing.

CONCLUSION

Statistically, the most significant (at 5% level) treatment concentration in fortification was shown by GA_3 at 50 ppm , in Osmopriming with KCl at 10% conc. and Infusion again by GA_3 at 50 ppm. It can be concluded that bamboo seeds undergo age induced physiological changes and germination percentage decreases with ageing, age deterioration brings about membrane damage Invigouration treatments are commercially important to increase the seed longevity and make the scarcely available bamboo seeds useful for longer period of time.

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