J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 95 (1 & 2) 2016 : 92-103

EFFECT OF PRESOWING TREATMENTS ON GERMINATION AND VIGOUR IN THE SEEDS OF *BAMBUSA BAMBOS*

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The pre-sowing invigouration treatments have been proven to be beneficial in improving vigour and viability of seeds. The present study was conducted to explore the role (if any) of invigouration treatments to improve the germination, seedling emergence, early seedling establishment and viability in bamboo seeds. The bamboo seeds were treated with various invigourating solutions (e.g. salicylic acid, ascorbic acid, KNO_3, GA_3, PEG etc), whereas untreated seeds were taken as control. The most effective concentrations in seed hardening treatments with different chemicals were found to be GA_3 (50ppm), resorcinol (10%), salicylic acid (10%), KH_2PO_4 (5%), $CaCl_2$ (5%), urea (10%) and ascorbic acid (10%). The most significant fortification treatment with different chemicals were found to be ascorbic acid (5%), GA_3 (50ppm) and KH_2PO_4 (5%). Whereas in case of osmopriming, the most significant treatment was found to be KCl (10%), KNO_3 (5%), $CaCl_2$ (10%) and PEG-6000 (5%) with which seeds show maximum vigour and viability of maximum even up to 24-months.

Key words : Fortification**,** germination, hardening, pre-sowing, priming, vigour.

Bamboos comprise the most diverse group of plants in the grass family. *Bamboos* are plants of global interest because of their unique life form, ecological significance and the extensive range of uses and values they have for humans (Bystriakova *et al.* 2004). As bamboos produce seeds after long time intervals, the knowledge on diverse aspects of seed propagation is very restricted. Bamboo seeds have very short viability of 1-3 months and are therefore useful as propagules for only a short period of time (Ravi kumar *et al.* 1998a, b; Rawat and Thapliyal 2003).

Seed deterioration is coupled with loss of membrane integrity, changes in enzymatic activities, decline in protein and nucleic acid synthesis, and lesions in DNA (McDonald 1999). These deteriorative changes have normally been related to activated oxygen species (AOS)-induced oxidative injury (Hendry 1993, Bernal-Lugo and Leopold 1998, McDonald 1999). Priming can turn round some of the ageing-induced deteriorative events, and thus improve seed performance (Taylor *et al.* 1998). So to conquer this problem, many seed invigouration treatments have been used to reduce the damage of ageing and improve their performance in many crops (Basra *et al.* 2003, Farooq *et al.* 2009).

Priming includes hydropriming, osmopriming, halopriming with or without the use of plant growth regulators (Wahid *et al.* 2008, Farooq *et al.* 2009). Priming is responsible to repair the age related cellular and subcellular damage of low vigour seeds that may accumulate during seed development (Bray 1995). Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA (Koehler *et al.* 1997). The intention of this study was to investigate the effects of ageing on the viability and vigour and to identify the suitable priming protocol for reversing the potency of low vigour bamboo seeds.

The purpose of these treatments is to curtail the time between planting and emergence (Farooq *et al.* 2006) and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment (Senaratna *et al.* 1988, Du and Young 2002, Shakirova *et al.* 2003). Such treatments coordinate emergence, which leads to uniform stand and improved yield (Harris *et al.* 2002). Improved seed performance has been achieved by incorporating plant growth regulators during pre-soaking, priming and other treatments in rice (Miyoshi and Sato 1997).

Received on February 24, 2016 www.indianbotsoc.org Accepted on April 01, 2016

Several workers (Pandey *et al.* 2000, Manjkhola *et al.* 2003, Ghimire *et al.* 2006) have reported use of plant growth regulators (PGRs) and other chemicals to stimulate and to coordinate seed germination. There are limited studies on bamboos' seed viability (Ravikumar *et al.* 1998, Richa *et al.* 2010).

Less expensive and easily practice-able invigouration methods like mid-storage hydration-dehydration treatments (Basu 1994) and short-term aerated hydration (Thornton and Powell 1992) to improve vigour, viability and field performance have been reported. The present study on bamboo seeds attempts to determine the most efficient pre-sowing treatments to gain information on the possible mode of action of such treatments in viability maintenance mechanism.

MATERIALS AND METHODS

Seeds of *Bambusa bambos* were procured from Kerala Forest Research Institute (KFRI), Peechi, Kerala, India. 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. These were later rinsed with distilled water 2-3 times. Ten randomly selected surface sterilized seeds were placed equidistantly in pre-sterilized petridishes (9.0 cm) lined with filter paper. The entire experiment was conducted in laboratory condition in seed germinator where temperature was maintained at $28^0C \pm 2^0C$. The seeds were observed daily, the number of seeds germinated and their respective root and shoot length were recorded for 14 days. Three replicates consisting of 10 seeds each per treatment were maintained. Germination test was carried out according to ISTA (1999) rules. The experiments were repeated after regular intervals of six months for one and a half years.

Germination percentage (G%) : Emergence of radical was considered as an indicator of germination. Number of seeds germinated was noted after every 24 hours for 14 days.

Vigor index (VI) (Abdul-Baki and Anderson, 1973) : Vigor index was calculated by the formula here under :

VI = G % x Root Length (cms)

A weighed quantity (200 g) of bamboo seeds were soaked in solution of GA_3 (10ppm, 20ppm and 50ppm), salicylic acid (2%, 5% and 10%), resorcinol (2%, 5% and 10%), KH_2PO_4 (2%, 5% and 10%), $CaCl_2$ (2%, 5% and 10%), urea (2%, 5% and 10%), ascorbic acid (2%, 5% and 10%), KCl (2%, 5% and 10%), KNO_3 (2%, 5% and 10%), and PEG-6000 (2%, 5% and 10%) for 24 hours used for invigouration treatments, while untreated seeds were taken as control. Then 10 best-looking seeds were selected randomly for germination experiments.

Various seed invigouration treatments :

a) Seed hardening (Rangaswamy *et al.* 1993) : Dry seed ---Soaking in water and/or dilute solution of GR and chemicals (10-20-50 ppm) for 18hours at 15-25^{0}C ---Shade drying (18hours) --- sun drying (14hours) to bring back to its original water content or weight --- Hardened seeds.

Chemicals for Hardening : GA_3, Resorcinol, Salicylic acid, KH_2PO_4, Water, Urea, and Ascorbic acid.

b) Seed fortification (Ells, 1963) : Seeds are soaked in solution of equal volume for duration of 6 hrs to raise the moisture content of the seed to 20-25% just enough for endogenous impregnation of the chemicals by exogenous application. Chemicals used for fortification : Ascorbic acid, GA_3, KH_2PO_4.

c) Osmopriming (Ruan *et al.* 2002) : *Osmolytes :* The salts used to prepare the osmotic solution are called osmolytes or osmoticants. The most common osmolytes are inorganic salts such as KNO_3 (Ells 1963) KCl (Cantiliffe 1981) and a chemically inert compound polyethylene glycol (PEG) 6000 (Ali *et al.* 1981). Use of PEG as a pre-sowing treatment has been much more successful in terms of germinability, uniformity of stand and subsequent seedling stand performance than the simple water soaking treatments. 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. Chemicals used for osmopriming (Pre-sowing) : $CaCl_2$, PEG-6000, KNO_3, KCl.

Statistical Analysis : Data was statistically analyzed using the software GENSTAT Discovery edition. 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

Hardening (Table 1) : Germination percentage (G%) of freshly procured seeds of *Bambusa bambos* was 83.3% which decreased to 20% after 6-months and became zero or completely non-viable after 12-months interval. But with application of hardening treatments results indicated that with ageing G% did not decrease drastically like naturally ageing seeds. In hardening, GA_3 was applied in three different concentrations i.e. 10, 20 and 50ppm. G% observed was more than control with the application of GA_3 at 20 and 50 ppm (90% in both conc.) in fresh seed lot whereas after 6-monthly controlled storage G% became 60, 60 and 63.3 at 10, 20 and 50ppm concentration respectively and all the results were statistically significant at 5% level. After 12-months of ageing, when seeds became non-viable, the hardened seeds showed G% of 36.7% with 50ppm concentration whereas after 18-monthly interval it maximum G% was also in case of 10ppm and 50ppm i.e. 20%. Statistically, the most effective concentration of GA_3 for maintaining the germination percentage up to 18-months was found to be 50ppm. Resorcinol was applied in three different concentrations i.e. 2%, 5% and 10%. In fresh seed lot, the most effective treatment concentration was found to be 5% and 10% where G% was 83.3% in both cases same as that of control while after 6-monthly interval same concentrations was observed effective i.e. maximum G% was observed at these concentrations i.e. 53.3 at both concentrations which was almost 2.5 times more than control. After 12-monthly interval, same concentrations of resorcinol were effective with G% of 29.6 and same concentrations were again effective after 18-months of controlled ageing with G% of 15.6 in both the cases, where seeds became non-viable even after 12-months of ageing. Results were statistically significant in both these concentrations in all the three 6-monthly trials. With treatment of salicylic acid as hardening material to fresh seed lot of *Bambusa bambos*, maximum of 90 germination percentage was observed in case of 10% concentration. At 6-months of ageing, G% was found to be 46.7%, 53.3% and 53.3% at 2%, 5% and 10% concentrations respectively which is more than double as compared to G% of control. After 12-months of ageing, the untreated seeds had zero G% while in treated seeds G% was 25.9, 29.6 and 29.6 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 13.6, 15.6 and 15.6 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of salicylic acid for maintaining the germination percentage up to 18-months was found to be 10%. Hardening with KH_2PO_4 resulted in maximum of 83.3, 90 and 83.3 germination percentage at 2%, 5% and 10% concentrations respectively, which became 46.7%, 60% and 53.3% at same concentrations respectively after 6-months of controlled ageing which was significantly higher than control. After 12-months of ageing, G% reduced to 25.9, 33.3 and 29.6 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 13.6, 17.5 and 15.6 at 2%, 5% and 10% concentrations respectively whereas in case of untreated seeds (control) G% was zero even after 12-months of ageing. Statistically, the most effective concentration of KH_2PO_4 for maintaining the germination percentage up to 18-months was found to be 5%. Hardening with $CaCl_2$ resulted in same G% as that of control in case of fresh seeds lot at 2%, 5%, and 10% concentrations respectively which was maximum i.e. 83%. At 6-months of ageing, it was 40%, 46.7% and 40% which is more than double than control at same

concentrations respectively. After 12-months of ageing when control seeds became non-viable, treated seeds recorded as 22.2, 25.9, and 22.2 of G% at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 11.7, 13.6 and 11.7 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of $CaCl_2$ for maintaining the germination percentage for 18-months was found to be 5%. Urea as hardening material resulted in increasing G% to 90 at 10% concentration which became 46.7%, 53.3% and 53.3% at 2%, 5% and 10% concentrations respectively after 6-months of controlled ageing where increase in G% was more than double from control. After 12-months of ageing, G% reduced to 25.9, 29.6 and 29.6 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 13.6, 15.6 and 15.6 at 2%, 5% and 10% concentrations respectively as compared to zero in control. Statistically, the most effective concentration of urea for maintaining the germination percentage upto 18-months was found to be 10%. Hardening with ascorbic acid resulted in maximum of 83.3, 83.3 and 90 germination percentage at 2%, 5%, and 10% concentrations respectively, which maintained to 46.7%, 53.3% and 53.3% at same concentrations respectively after 6-months of controlled ageing approximately double than untreated seeds. After 12-months of ageing, G% was found to be 25.9, 29.6, and 29.6 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 13.6, 15.6 and 15.6 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of ascorbic acid for maintaining the germination percentage up to 18-months was found to be 10%. The performance of hardened seeds revealed that the increase in G% was considerably higher in hardened seeds as compared to control (untreated seeds). Similar results were reported by Zheng *et al.* 2002, Basra *et al.* 2004, Farooq *et al.* 2006 and Vigneshwari *et al.* 2006. Seed hardening technique has been used to reduce the emergence time, to get synchronized emergence, to improve emergence rate and to have better seedling stand in many horticultural (Rudrapal and Nakamura 1998, Bradford *et al.* 1990, Khan 1992) and field crops such as wheat, maize (Dell' Aquilla and Tritto 1990, Chowdhary and Baset 1994, Basra *et al.* 2002) and more recently rice (Lee and Kim 1999, 2000; Basra *et al.* 2003, 2004, 2005; Farooq *et al.* 2006). Our results of hardening with $CaCl_2$ solution was the most efficient for maintaining the viability of bamboo seeds (Farooq *et al.* 2006). The beneficial effect of seed hardening is primarily due to pre-enlargement of the embryo (Bradford *et al.* 1990), biochemical changes like enzyme activation (Lee and Kim 2000; Basra *et al.* 2005), faster production of emergence metabolites (Lee and Kim 2000 and Basra *et al.* 2005), and genetic and structural repair (Dell'Aquilla and Tritto 1990). From the present investigations, it may be concluded that employing seed hardening treatments in bamboo improved the germination significantly and maintenance of viability for longer period (more than 18-months), which resulted in improved growth and yield.

Fortification (Table 2) : Seed fortification is a seed enrichment technique by which the seeds are invigourated. In the present study with seed fortification of bamboo seeds, were enriched with various nutrient solutions at different concentration (Table-2). The results revealed that G% were higher when fortified seeds were used as compared to untreated seeds. Seeds fortified with ascorbic acid resulted in maximum of 83.3, 90 and 83.3 germination percentage at 2%, 5% and 10% concentrations respectively whereas after 6-month G% was 50%, 56.7% and 53.3% at same concentrations respectively which was more than double from untreated seeds. After 12-months of ageing, G% maintained to 26.7, 30, and 26.7 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 16.7, 20 and 16.7 at 2%, 5% and 10% concentrations respectively. While untreated seeds completely lost their viability after 12-months of ageing. Statistically, the most effective concentration

of ascorbic acid for maintaining the germination percentage up to 18-months was found to be 5%. Fortification with GA_3 resulted in increasing G% of fresh seeds even to 90% at all the concentrations, which maintained to 60%, 63.3% and 63.3% at 10, 20, 50ppm concentrations respectively after 6-months of controlled ageing which was almost three times higher than untreated seeds (control). After 12-months of ageing, G% recorded was 30, 36.7 and 36.7 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% was 20, 23.3, and 20 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of GA_3 for maintaining the germination percentage up to 18-months was found to be 50ppm. KH_2PO_4 fortified seeds resulted in maximum of 83.3, 90 and 90 germination percentage at 2%, 5% and 10% concentrations respectively. After 6-months of ageing, G% was 53.3%, 56.7% and 53.3% at same concentrations respectively almost more than double from control. After 12-months of ageing, G% became 26.7, 30, and 26.7 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 16.7, 20 and 20 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of KH_2PO_4 for maintaining the germination percentage up to 18-months was found to be 5%.

Our results are corresponding with the findings of Rengel and Graham (1995), Yilmaz *et al.* (1997) and Jerlin *et al.* (2005) who also observed differences between fortified and normal seeds' germination percentage. It can be concluded that fortified seeds would improve the grain yield in poor soils by producing stronger seedlings than those produced by normal seeds. The advantageous effects of enhanced germination and vigour of the seedling due to fortification of the seeds with nutrient solutions like Ascorbic acid and KH_2PO_4 have been demonstrated by several workers (Vanangamudi and Kulandaivelu 1989 and Kajamaideen *et al.* 1990). Similar results due to KH_2PO_4 treatment were also reported in pine (Biswas *et al.* 1972), *Peltophorum ferugeneum* (Mukhopadhyay *et al.* 1990), *Casuarina equsetifolia* (Kajamaideen et al. 1990), *Albizia lebeck* (Roy 1992) and *Acacia nilotica* (Palani *et al.* 1995). Such an increase in germination and vigour was observed in the present study with the seeds fortified with KH_2PO_4 (5%), ascorbic acid (5%) and GA_3 (50ppm) also corroborated by Vanagamudi and Karivaratharaju (1986), Ponnusamy and Vijaya (1997) and Jerlin *et al.* (2005). Our results of fortification of seeds with GA_3 and KH_2PO_4 were even more encouraging than seed hardening.

Osmopriming (Table 3) : Seed priming is a technique by which seeds are partially hydrated to a point where germination processes begin but radicle emergence does not occur (Bradford 1986). Typical responses to priming are faster to germination and emergence over all environments and wider temperature range, leading to better crop stands and hence improved yield and harvest quality, especially under sub-optimal and stress condition growing conditions in the field (Halmer 2004).

In osmopriming, KCl was applied in three different concentrations i.e. 2%, 5% and 10%. G% was same in all three concentrations by application of KCl (83.3%) in fresh seed lot whereas after 6-monthly controlled storage G% recorded as 46.7, 46.7 and 53.3 at 2%, 5% and 10% concentration respectively which was more than double as compared to control. After 12-months of ageing, maximum G% was observed in case of 5% and 10% concentration i.e. 36.7 whereas after 18-monthly interval, maximum G% was at concentration of 10% i.e. 20%. Statistically, the most effective concentration of GA_3 for maintaining the germination percentage for 18-months was found to be 10%. With application of KNO_3 as osmolyte, results were very encouraging like that of KCl. KNO_3 was applied in three different concentrations i.e. 2%, 5% and 10%. In fresh seed lot, the most effective treatment concentration was found to 5% where G% was 90%, which was more than control, while after 6-monthly interval same

Table 1 : Effect of hardening treatments on germination percentage and Vigour index of bamboo seeds

S.No.			Fresh		6-monthly		12_Months		18_MONTHS	
	Treatment	Conc.	G %	Vigour Index	G %	Vigour Index	G %	Vigour Index	G %	Vigour Index
1	GA_3	10ppm	83.3	1316.14	60*	924	36.7*	554.17	20*	288
		20ppm	90	1449	60*	942	33.3	526.14	17.5	257.25
		50ppm	90*	1611	63.3*	1107.75	36.7*	631.24	20*	312
2	Resorcinol	2%	83.3*	1291.15	46.7*	709.84	25.9	388.5	13.6	193.12
		5%	83.3	1274.49	53.3*	799.5	29.6	438.08	15.6	218.4
		10%	83.3*	1291.15	53.3*	810.16	29.6*	444	15.6*	221.52
3	Salicylic acid	2%	83.3	1257.83	46.7*	691.16	25.9*	378.14	13.6	187.68
		5%	83.3	1282.82	53.3	804.83	29.6*	441.04	15.6	219.96
		10%	90*	1431	53.3*	831.48	29.6*	455.84	15.6*	227.76
4	KH_2PO_4	2%	83.3	1241.17	46.7*	681.82	25.9*	372.96	13.6	184.96
		5%	90*	1359	60*	888	33.3*	486.18	17.5*	241.5
		10%	83.3	1274.49	53.3*	799.5	29.6*	438.08	15.6	218.4
5	$CaCl_2$	2%	83.3*	1291.15	40*	608	22.2*	333	11.7	166.14
		5%	83.3	1257.83	46.7*	691.16	25.9*	378.14	13.6*	187.68
		10%	83.3	1241.17	40*	584	22.2*	319.68	11.7	159.12
6	Urea	2%	83.3	1257.83	46.7*	691.16	25.9*	378.14	13.6	187.68
		5%	83.3	1282.82	53.3*	804.83	29.6*	441.04	15.6*	219.96
		10%	90*	1395	53.3*	788.84	29.6*	432.16	15.6	215.28
7	Ascorbic acid	2%	83.3	1257.83	46.7*	691.16	25.9*	378.14	13.6	187.68
		5%	83.3	1282.82	53.3*	804.83	29.6*	441.04	15.6	219.96
		10%	90*	1413	53.3*	820.82	29.6*	449.92	15.6*	224.64
8	Control (Untreated Seeds)		83.3	1367.9	20	114	0	0	0	0
	LSD#		Treat.	Treat. X Conc.	Treat.	Treat. X Conc.	Treat.	Treat X Conc.	Treat.	Treat. X Conc.
			5.17	8.954	3.25	5.629	3.25	5.629	1.806	3.127

Least significant differences of means (5% level). *Significant figures.

concentration was observed effective i.e. maximum G% was observed at same concentration i.e. 53.3 which was almost three times than control. After 12-monthly interval, concentrations of 5% and 10% of KNO_3 were effective with G% of 30% whereas in control G% was zero and same concentrations were again effective after 18-months of controlled ageing with G% of 16.7 in both the cases. It was observed that osmopriming with $CaCl_2$ resulted in maximum of 83.3% germination percentage at all three concentrations i.e. 2%, 5%, and 10% which maintained to 46.7%, 46.7% and 53.3% at same concentrations respectively after 6-months of controlled ageing which is more than double from control. After 12-months of ageing, G% was 26.7, 30, and 30 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% recorded was 13.3, 13.3 and 16.7 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of $CaCl_2$ for maintaining the germination percentage was 2% and 10% concentration was effective up to 18-months. Priming with PEG-6000 resulted in maximum of 83.3, 90 and 83.3 germination percentage at 2%, 5%, and 10% concentrations respectively which maintained to 46.7%, 53.3% and 46.7% at same concentrations respectively after 6-

Table 2 : Effect of fortification treatments on germination percentage and Vigour index of bamboo seeds

S. No.			Fresh		6_Monthly		12_monthly		18_monthly	
	Treatment	Conc.	G%	Vigour Index	G%	Vigour Index	G%	Vigour Index	G%	Vigour Index
1	Ascorbic acid	2%	83.3	1291.15	50	760	26.7	392.49	16.7	232.13
		5%	90*	1377	56.7*	850.5	30*	444	20*	280
		10%	83.3	1282.82	53.3	804.83	26.7	397.83	16.7	235.47
2	GA_3	10ppm	90	1377	60	900	30	444	20	280
		20ppm	90*	1386	63.3*	955.83	36.7	557.84	23.3	328.53
		50ppm	90*	1386	63.3*	955.83	36.7*	572.52	23.3*	337.85
3	KH_2PO_4	2%	83.3	1266.16	53.3	794.17	26.7	392.49	16.7	232.13
		5%	90*	1386	56.7*	856.17	30*	447	20*	282
		10%	90	1377	53.3	799.5	26.7	395.16	20	280
4	Control (Untreated Seeds)		83.3	1367.9	20	114	0	0	0	0
	LSD#		Treat.	Treat. X Conc.	Treat.	Treat. X Conc.	Treat.	Treat X Conc.	Treat.	Treat. X Conc.
			5.97	10.33	3.51	6.079	1.95	3.377	0.985	1.706

Least significant differences of means (5% level). *Significant figures.

Table 3 : Effect of osmopriming treatments on germination percentage and Vigour index of bamboo seeds

S. No.			Fresh		6_monthly		12_monthly		18_monthly	
	Treatment	Conc.	G%	Vigour Index	G%	Vigour Index	G%	Vigour Index	G%	Vigour Index
1	KCl	2%	83.3	1299.48	46.7	705.17	26.7	397.83	16.7	235.47
		5%	83.3	1282.82	46.7	705.17	36.7*	535.82	16.7	230.46
		10%	83.3*	1324.47	53.3*	804.83	36.7*	535.82	20*	282
2	KNO3	2%	83.3	1291.15	46.7	709.84	26.7	405.84	13.3	191.52
		5%	90*	1395	53.3*	836.81	30*	450	16.7*	237.14
		10%	83.3	1307.81	53.3	804.83	30	444	16.7	233.8
3	CaCl2	2%	83.3	1282.82	46.7	705.17	26.7	397.83	13.3	187.53
		5%	83.3	1257.83	46.7	691.16	30	447	13.3	183.54
		10%	83.3*	1299.48	53.3	820.82*	30	456*	16.7*	240.48
4	PEG-6000	2%	83.3	1257.83	46.7	691.16	26.7	395.16	13.3	183.54
		5%	90*	1386	53.3*	804.83	30*	447	16.7*	235.47
		10%	83.3	1324.47	46.7	728.52	30	438	13.3	194.18
5	Control (Untreated Seeds)		83.3	1367.9	20	114	0	0	0	0
	LSD#		Treat.	Treat. X Conc.	Treat.	Treat. X Conc.	Treat.	Treat X Conc.	Treat.	Treat. X Conc.
			4.957	8.586	3.305	5.724	1.836	3.18	0.966	1.674

Least significant differences of means (5% level). *Significant figures.

months of controlled ageing which was more than double from control. After 12-months of ageing, G% recorded as 26.7, 30 and 30 at 2%, 5% and 10% concentrations respectively whereas after 18-months G% was 13.3, 16.7 and 13.3 at 2%, 5% and 10% concentrations respectively. Statistically, the most effective concentration of PEG-6000 for maintaining the germination percentage up to 18-months was found to be 5%.

It is seen that with these invigouration treatments we have been able to maintain vigour and viability of seeds of bamboos up to even more than 18-months whereas these seeds have very short viability of 1-3 months and -7 months under controlled conditions (Ravikumar *et al.* 1998a, b; Rawat and Thapliyal 2003). So, these invigouration treatments are very effective tools for maintaining the viability of seeds like bamboos whose have very short viability.

These findings support the earlier work on improved germination rate and percentage by hormonal treatment in wheat by Al-Hakimi and Hamada (2001) and by Richa and Sharma (1994) in bamboo seeds. The earlier and synchronized germination might be attributed to increased metabolic activities in the primed seeds (Afzal *et al.* 2002, Shakirova *et al.* 2003, Basra *et al.* 2003, 2004, 2005, 2006; Farooq *et al.* 2005, 2006). Vigour enhancement by the application of priming solution might be due to increased cell division within the apical meristem of seedling root, which caused an increase in plant growth (Sakhabutdinova *et al.* 2003).

In practice, invigouration is followed by dehydration of seeds to their initial moisture content while retaining the benefit of the treatment. Germination of invigourated seeds is less sensitive to temperature and oxygen deprivation than that of untreated seeds (Corbineau *et al.* 1994, Ozbingol *et al.* 1999). Metabolic events, such as protein, RNA and DNA syntheses (Bray 1995), DNA replication (De Castro *et al.* 2000) or tubulin accumulation (De Castro *et al.* 1995), are associated with invigouration. Our results are further confirmed that invigouration treatments can improve the germination of aged seeds and their storability (Fujikura and Karssen 1995, Bailly *et al.* 2000).

CONCLUSION

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. 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. Statistically, the most effective concentrations in seed hardening treatments with different chemicals were found to be GA_3 (50ppm), resorcinol (10%), salicylic acid (10%), KH_2PO_4 (5%), $CaCl_2$ (5%), urea (10%), and ascorbic acid (10%). Statistically, most significant (at 5% level) treatment concentrations in fortification with different chemicals were: ascorbic acid (5%), GA_3 (50ppm), and KH_2PO_4 (5%). While in case of osmopriming, statistically, the most significant (at 5% level) treatment was found to be KCl (10%), KNO_3 (5%), $CaCl_2$ (10%) and PEG-6000 (5%).

Authors are thankful to University Grants Commission (UGC), New Delhi, India for providing financial help for the research work.

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