Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
4(2):370-375
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

Effect of ageing on endogenous auxins, gibberellins and abscissic acid in three species of bamboo seeds in relation to loss of viability during storage

Geetika Singh[1]*, Richa[2] and M.L Sharma[3]

[1-3] *Department of botany, Panjab University, Chandigarh*

*Email: geetika_phd@yahoo.com

ABSTRACT

Bamboo seeds have a very low viability for short duration of 1-2 months. So the effect of growth regulator plays important role to understand its low viability. Three important growth regulators have been estimated from the fresh as well as aged seed samples stored for 18 months for both naturally and controlled aged seeds using HPLC technique. i.e auxins, gibberellins and abscissic acid. In this study, while fresh seeds showed decrease in the activity of ABA, which increases with ageing. While level of auxins and gibberellins showed decline in activity with ageing. Activity of auxins, gibberellins and abscissic acid was assessed of all the three species of bamboos *Dendrocalamus strictus, Dendrocalamus hamiltonii and Bambosa bambos* till period of 18 months. Both development and germination processes in seeds are regulated by hormonal interaction. Thus suggesting auxins, gibberellins and abscissic acid are critical in germination, promotory effect of IAA on germination.

KEYWORDS: Auxins, Abscissic acid, Germination, Gibberellin, Viability

INTRODUCTION

Bamboos are indeed one of nature's miracle and strength enables them to be put to diverse uses. Bamboos are extremely useful grasses which are in high demand throughout Asia. Bamboos can be propagated from rhizomes, cuttings or by multiplication of nursery raised seedling. However seeds serve as the best material for large scale plantations, germ plasm conservation and improvement of genotype. Seeds play vital role as they are source of food, fibre, spices, beverages, oils and drugs. Bamboo seeds have a very short viability of 1-2 months and therefore they are useful as propagates for only a short period of time. Plant hormones are the chemicals that affect flowering, ageing, root growth, promotion of stem elongation.

How to Site This Article:
Geetika Singh, Richa and M.L Sharma. (2016). Effect of ageing on endogenous auxins, gibberellins and abscissic acid in three species of bamboo seeds in relation to loss of viability during storage. *Biolife*, 4(2), pp 370-375. doi:10.17812/blj.2016.4225

Published online: 12 June, 2016

Even small quantities of these substances produce major growth changes. No study has been conducted on the endogenous hormones in bamboo seeds except the only work in our lab by Richa *et al.* (2006) who studied the endogenous levels of IAA, ABA in five bamboo species. The present study was aimed at understanding the amounts of endogenous level of hormones in seeds during ageing and its affect on the viability of seeds during storage in naturally ageing seeds and under controlled ageing seeds. Under controlled conditions seeds were kept at 4° C in desiccators under anhydrous calcium chloride.

MATERIALS AND METHODS

Endogenous levels of following plant growth hormones were estimated in seeds after regular intervals of six months. Prior to extraction seed were imbibed in distilled water for 12 hours.

Auxin (Nagar, 1996)

5 gm fresh weight of each species of seeds was separately homogenized in chilled 80% methanol three times. The homogenates were pooled and centrifuged at 10000 rpm at 5°C for 30 min.

The supernatants were concentrated in vacuum at 30°C and then applied to Polyvinyl-prryrolidone (pvp) columns. The columns (20 cm x1.5 cm, internal diameter (ID) were eluted with phosphate buffer (pH-8.0) and the resulting elutes were adjusted to pH 8.0 with 1N HCI and partitioned against diethyl ether (X3). The ether phases were evaporated in vacuum and taken up in methanol(HPLC grade) for the estimation of IAA.The partially purified methanolic extracts were filtered through 0.45 µm Millipore filters and injected into a 20 µl injector loop of a Lichrosorb RP 18 (10µM) column (250×4.6mm, ID) protected by a guard column. Elution was carried out by a gradient of 30-70 % methanol and finally with 100% gradient of methanol for 15 min. at a flow rate of 1ml/min. The column evaluated was passed through an ultraviolet (UV) detector at 254 nm, and IAA was measured by referring to an authentic standard of indole-3-acetic acid

Abscissic acid (ABA) (Nagar, 1996)

Each seed species sample of 5 gm freshly homogenized in chilled 80% methanol (20 ml/g) containing butylated hydroxytoluene (BHT) at 100 mg/l. The homogenates were stored for 24 hours in the dark at 4°C and then filtered in vacuum. The residues were re-extracted five times with chilled methanol, filtered and centrifuged at 10,000 rpm at 5° C. The supernatants were dried in vacuum, dissolved in 2 ml of 0.1 M potassium phosphate buffer (pH-8.8) and then applied to polyvinyl- pyrrolidone (PVP) columns. The columns (20cmX1.5 cm, internal diameter (ID) were eluted with phosphate buffer (pH-8.0) and the resulting evaluates were adjusted to pH 2.5 with IN HCI and partitioned against peroxide –free diethyl ether (X5) containing BHT (100 mg/l).The combined organic phases were evaporated to dryness in vacuum, taken up in methanol (HPLC grade) and used for free ABA estimation. For HPLC, the partially purified methanolic extracts were filtered through 0.45 µl injector loop Lichrosorb RP 18 (10um) column (250X4.6 mm, ID) protected by a guard column. Elution was carried out with methanol (HPLC grade) in 30 mm acetic acid (HPLC grade) at a flow rate of ml/min. The solvents were filtered through 0.45µm pore size whatman membranes and degassed under vacuum prior to use. The column elutes were passed through an UV detector at 254 nm and ABA was measured by referring to an authentic standard of ABA.

Gibberellins (Chen Wen-shaw, 1994)

The equivalent of 3 gm dry weight of each bamboo seeds was homogenized and extracted four times with 80% (V/V) methanol (500 ml). The methanol extract was concentrated in vacuum and the residue was diluted with H_2O to 2 ml mixed 0.5 gm Celite, dried in a gentle air stream, and loaded onto a SiO_2 partition column (prepared from 5 gm of deactivated woelm SiO_2 slurried in 95/5 V/V ethyl acetate n-hexane). This was first eluted with 80 ml ethyl acetate /hexane (95/5V/V) to remove free Gas and then with 100% methanol (150 ml) to remove highly water soluble GAs and GA conjugates. The Ethyl acetate/hexane elute was neutralized with 0.1 N NH_4OH and then dried in vacuum. Both were adjusted to pH-8 with 0.1N NaOH and then each was extracted three times: the fraction containing free Gas with hexane, and the fraction containing GA glucosyl conjugates with ethyl acetate. The aqueous phase was diluted with H_2O to 100 ml adjusted to pH 3.0 with 3N HCI, and extracted three times with ethyl acetate. The ethyl acetate was evaporated in vacuum to give an acidic ethyl acetate fraction. The acidic ethyl acetate was then chromatographed on Lichrosorb RP 18 (10 µm) HPLC column (250X44.6, ID) eluting with a linear gradient of methanol (30%100%) in 1% aqueous acetic acid, run in 25 min. flow rate 2.0 ml/min.

RESULTS AND DISCUSSION

In the present study, the endogenous level of IAA and *B. bambos* (12.47ug/gm.f.wt) and so was the G% in these species in both fresh seeds and those ageing under controlled & natural ageing conditions at all the ageing intervals. Depletion of IAA content with ageing may be due to degradation of IAA (Alba *et al.*, 1998) or its inactive conjugate formation (Bartel *et al.,* 2001); Taiz and Zeiger, 2002). Auxins are different from other plant hormones and signalling agents except cytokinins in one respect i.e., they are required for seed viability. So far, no mutants lacking either auxins and cytokinins have been found suggesting that mutations that eliminate them are lethal (Taiz and Zeiger, 2002).Attenuation of growth substances like auxins and gibberellins appear to be responsible for the loss of viability of bamboo seeds also (Richa and Sharma 1994; Warrier *et al*., 2004).A marked decreased (about 95.8%) was seen in endogenous levels of GA in naturally ageing seeds while in controlled stored seeds decrease was nearly 90.9% after 18-months of ageing. Gibberellins play an important role in seed germination as they act upon aleurone cells to release α-amylase (Filner and Varner, 1967; Taiz and Zeiger, 2002) along with a number of other hydrolases .GA acts as a precursor for the breakdown of de novo synthesis of proteinases (Jacobson and Varner, 1967) thus facilitating the release of amino acids for de novo enzyme synthesis. Briggs (1973) reported that GA_1, and GA_3 were the major gibberellins released during seed germination, while GA_4 and GA_7 were also detected in considerable amounts. Thus decline in the GA levels during ageing could be an important reason for decrease in various germination parameters, as the reserve mobilization of seeds is affected, which in turn affects the seedling growth. Role of gibberellins in seed germination is well documented.

GA is known to stimulate the production of numerous hydrolyses, (notably α-amylase) by the aleuronic layer of germination cereal grains. In the present study, endogenous levels of GA were seen to decrease with increase in seed ageing suggesting

thereby depletion of endogenous hormones along with other derivative changes, as factors for loss viability

Table-1. Endogenous IAA levels (expressed as µg/gm.f.wt) in seeds of three species of bamboos at different stages of ageing.

Species	FRESH SEEDS	6-Monthly		12-Monthly		18-Monthly	
		Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds
DH	12.34	2.34	7.89	0.78	2.34	0.12	1.89
DS	11.89	2.03	7.03	0.66	3.12	0.29	2.13
BB	12.56	2.12	7.99	0.61	3.82	0.22	1.19

DH: Dendrocalamus hamiltonii; DS: Dendrocalamus strictus; BB:Bambosa bambos

Table-2. Endogenous GA_3 levels (expressed as µg/gm.f.wt) in seeds of three species of Bamboos at different stages of ageing.

Species	FRESH SEEDS	6-Monthly		12-Monthly		18-Monthly	
		Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds
DH	67.82	6.78	35.67	0.99	21.34	0.30	4.89
DS	51.34	4.91	31.67	0.74	15.12	0.29	2.13
BB	89.56	11.12	45.99	1.61	25.82	0.45	11.19

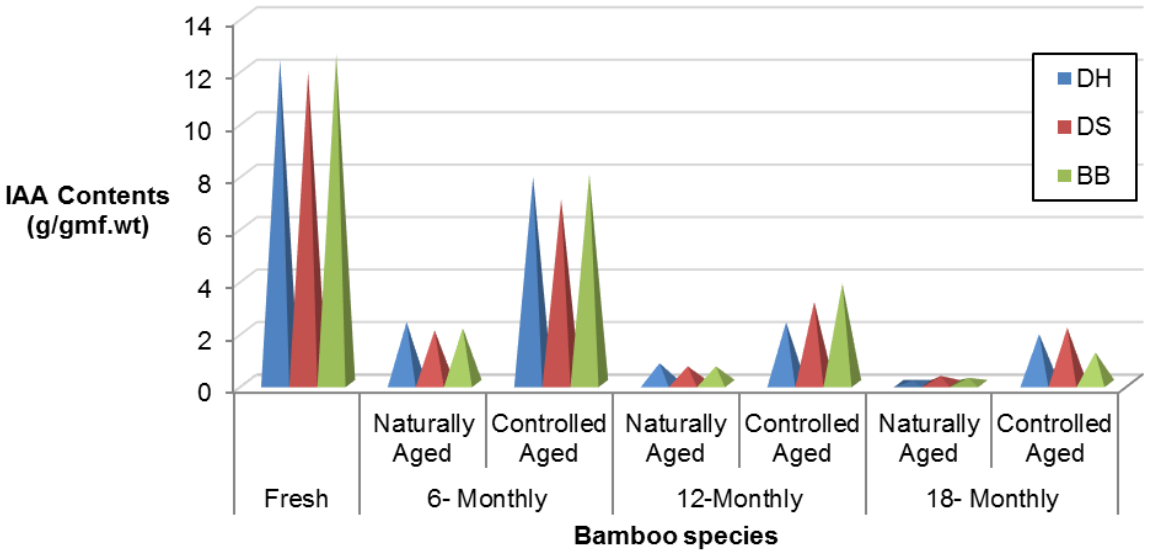
DH: Dendrocalamus hamiltonii; DS: Dendrocalamus strictus; BB:Bambosa bambos

Table-3. Endogenous ABA levels (expressed as µg/gm.f.wt) in seeds of three species of Bamboos at different stages of ageing.

Species	FRESH SEEDS	6-Monthly		12-Monthly		18-Monthly	
		Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds	Naturally aged seeds	Controlled aged seeds
DH	59.82	169.8	76.67	218.99	99.34	265.30	128.89
DS	67.34	203.54	89.67	287.8	134.12	299.29	135.13
BB	39.56	109.12	53.99	139.61	87.82	165.45	101.19

DH: Dendrocalamus hamiltonii; DS: Dendrocalamus strictus; BB:Bambosa bambos

Figure-1. Changes in IAA contents (µg/gm f.wt) in seeds of three species of bamboos at different stages of ageing in seeds of three species of bamboos



DH: Dendrocalamus hamiltonii; DS: Dendrocalamus strictus; BB:Bambosa bambos

Figure-2. Changes in GA contents (µg/gm f.wt) in seeds of three species of bamboos at different stages of ageing in seeds of three species of bamboos

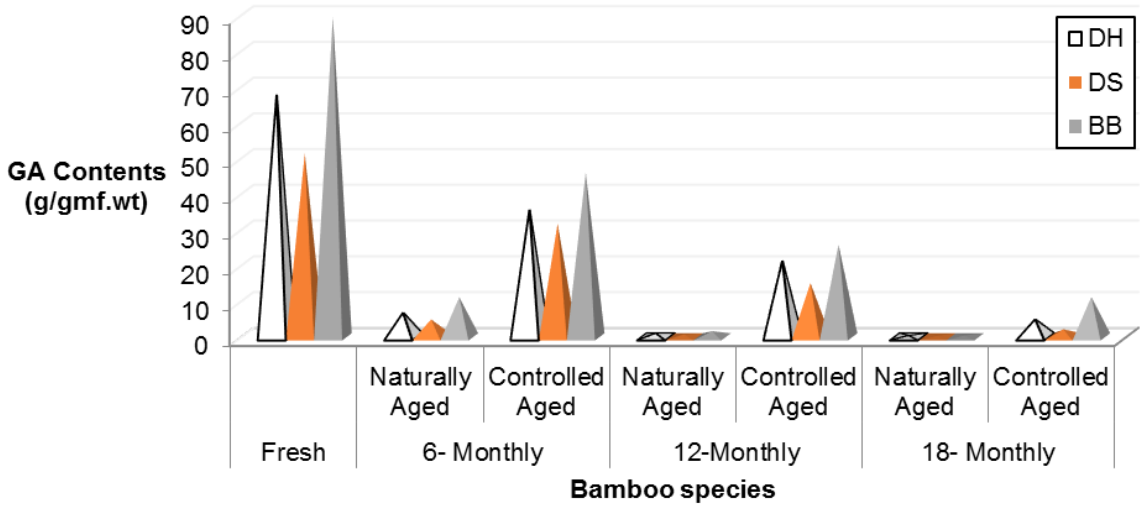
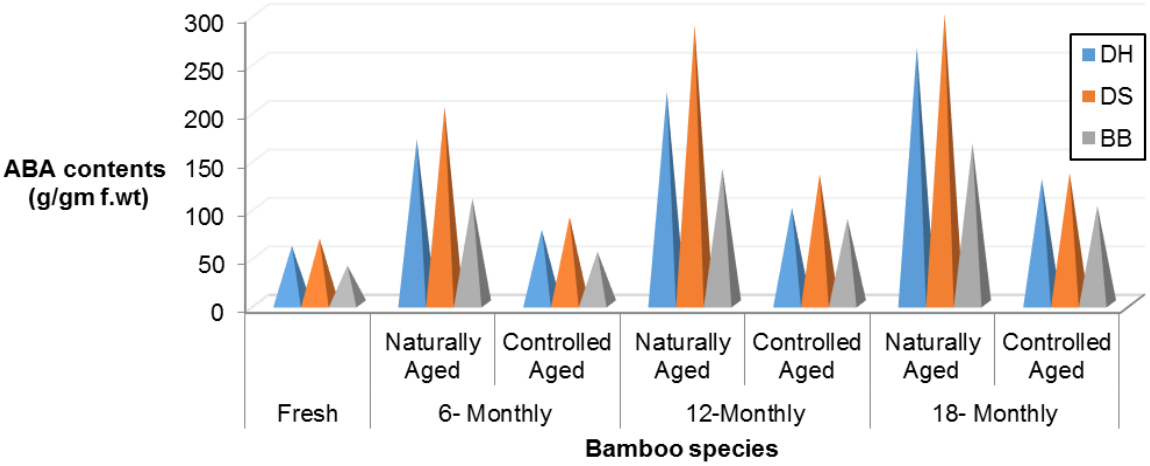
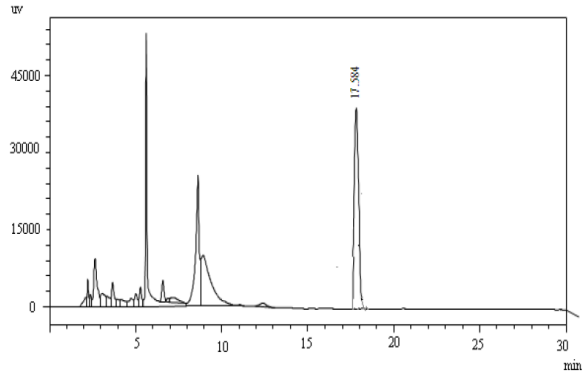


Figure-3. Changes in ABA contents (µg/gm f.wt) in seeds of three species of bamboos at different stages of ageing in seeds of three species of bamboos

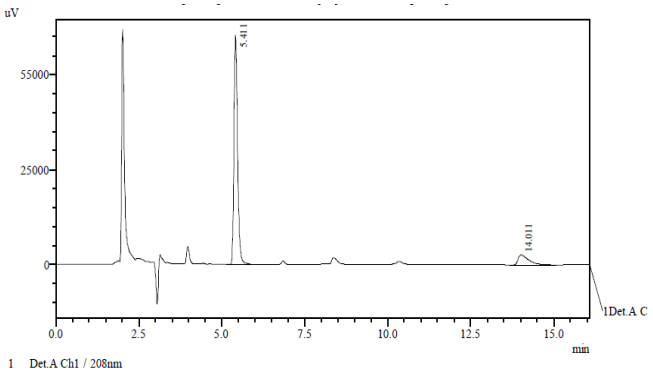


DH: Dendrocalamus hamiltonii; DS: Dendrocalamus strictus; BB:Bambosa bambos

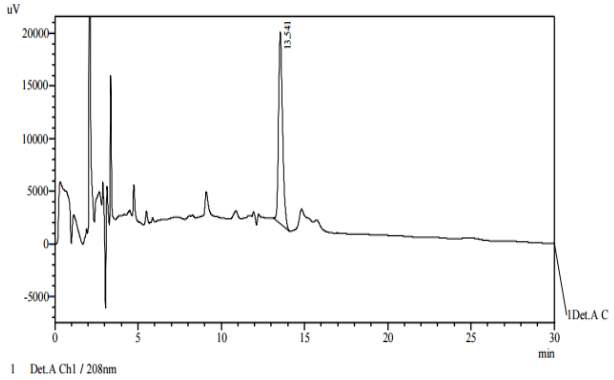
Figure-4. HPLC chromatogram showing standards of IAA, ABA and GA



Standard of IAA



Standard of GA



Standard of ABA

Figure-5. HPLC chromatogram showing the Presence of IAA, GA and ABA contents (µg/gm f.wt) respectively in seeds of bamboos

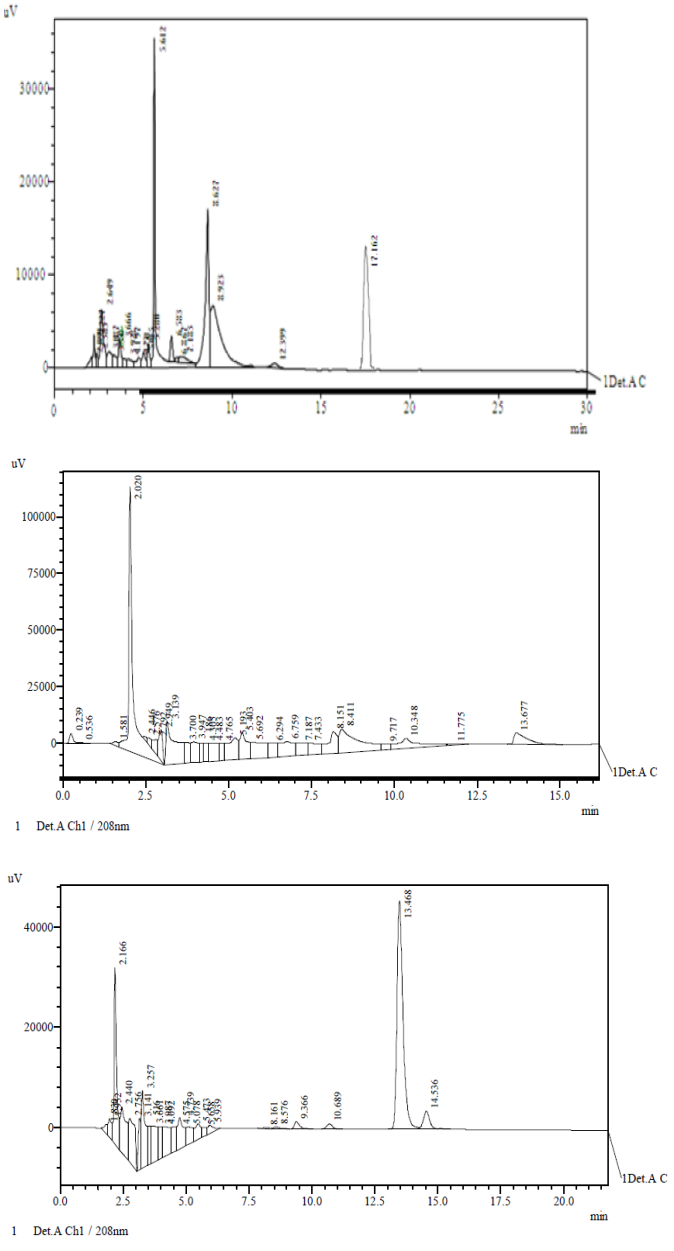
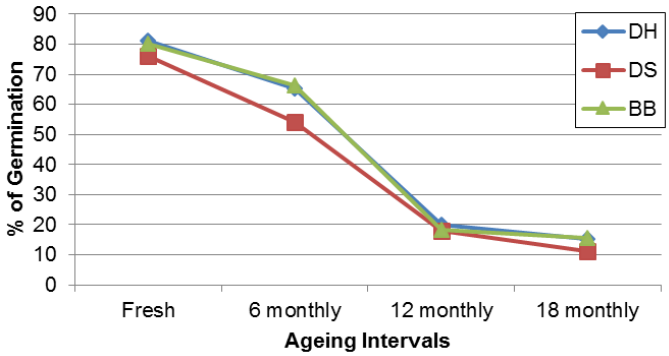


Figure-6. Decrease in Germination percentage with ageing in all the three species of bamboo seeds.



DH: Dendrocalamus hamiltonii
DS: Dendrocalamus strictus
BB: Bambosa bambos

(Taiz and Zeiger, 2002). ABA influences many other aspects of plant development by interacting usually as an antagonist with GA and other hormones (Taiz and Zieger, 2002). A marked increase of about 400% was seen in endogenous level of ABA in naturally ageing seeds while in controlled stored seeds increase was nearly 280% after 18-months of ageing .Abscissic acid can suppress GA mediated genes which encode hydrolytic enzymes in germinating seeds and in the seedling growth (Hilhorst, 1995). This ABA hormone, which is, involved in short term effects i.e; stomatal closure and long term responses i.e seed maturation, dormancy etc. Consequently, a high endogenous tolerance by modulation of genes expression and storage proteins synthesis, allowing maturation of gene expression and storage proteins synthesis, allowing maturation to proceed, while inhibiting precocious germination (Prewein *et al*. 2006). ABA has an essential role in the physiological processes that affect seed survival and reproduction.

CONCLUSION

It was found that the level of auxins and gibberellins decreases with ageing in both naturally and controlled aged seeds while content of ABA decrease with ageing. The decrease was more prominent in naturally aged than in controlled aged seeds. Minimum amount of IAA, GA and ABA required to maintain viability of bamboo seeds is 2.34, 6.78 and 169.8 µg/gm f.wt in *Dendrocalamus hamiltonii*.,2.03, 4.91 and 203.54 µg/gm. f.wt in *Dendrocalamus strictus* and 2.12, 11.12 and 109.12 in *Bambosa bambos* respectively. Below these amount viability reduces to zero after 6 months under naturally ageing seeds. It can also be concluded that under controlled conditions level of hormones reduces slowly as compared to naturally ageing and therefore viability could be enhanced for longer duration under controlled conditions.

Acknowledgement:

The senior author is thankful to ICMR, Govt. of India for providing the necessary grant for assistance.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1]. Alba C. M., Forchette., S.M ., Quesada., M.A., Valpuesta, V. and H.A. Tiger. 1998. Localization and general properties of developing peach seed coat and endosperm peroxidase isoenzyme *J. Plant Growth Regul.* 17(1): 7-11.

[2]. Bartel, B., S. LeClere, M. Mabidin and B.K Zolman. 2001. Inputs to the active indole-3-acetic acid pool: de novo synthesis, conjugate hydrolysis and indole-3-butryric acid B-oxidation. J. Plant Growth Regul. 20:198-216

[3]. Briggs, D.E. (1973). Hormones and carbohydrates metabolism in germinating cereal grains. In: *Biosynthesis and its control in Plants*. Milborrow, B.V.(Ed.) London. Academic Press, pp. 219-277.

[4]. Chen, Wen Shaw (1994). Gibberellins in Buds of *Euphoria longana* Lam. During different stages of growth. *Bot. Bull. Sin.* **35:**39-43.

[5]. Hilhorst, H.W.M. (1995). A critical update on seed dormancy. In. Primary dormancy. *Seed Science Research*. 5:61-73.

[6]. Nagar, P.K. (1996). Changes in endogenous abscisic acid and phenols during winter dormancy in tea (*Camilla sinensis*. (L.) O. Kuntze) Acte Physiological Plantarum, **18:**33-38.

[7]. Prewein, C., Endemann, M., Reinohl, V., Salaj., J. Sunderlikova V. and Wihelm, E. (2006). Physiological and morphological characteristics during development of pedunculate oak (*Quercus robur* L.) zygotic embryos. *Trees*20:53-60.

[8]. Richa and M.L. Sharma. (1994). Enhancing the germination of stored bamboo seeds using plant growth regulators. *Seed Sci. and Technol.,* **22(2):**313-317.

[9]. Richa, M.L. Sharma, and Neeru Bala. (2006). Studies on endogenous levels of plant growth hormones in relation to seed viability in some bamboo seeds. *Indian J. Pl. Physiol*., **11(4):**358-363.

[10]. Taiz, L. And E. Zeiger (2002). Plant Physiology III Panima Publishing Corporation, New Delhi/ Banglore.

[11]. Warrier, R.R., Sivakumar, V., Anandlakshmi, R., Vijayachandran, S.N., Mahadevan, N.P. and Singh, B.G. (2004). Improving storability of *Bambusa arundinacea* (Retz.) Wild seeds. *Journal of Bamboo and Rattan.***3:**375-382.

****