Indian J. Plant Physiol., Vol. 11, No. 4, (N.S.) pp. 358-363 (Oct.-Dec., 2006)

ENDOGENOUS LEVELS OF PLANT GROWTH SUBSTANCES IN SEEDS OF FIVE BAMBOO SPECIES IN RELATION TO SEED VIABILITY

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Received on 17 July, 2006, Revised on 18 Sept., 2006

SUMMARY

Endogenous levels of auxins and ABA in seeds is found to be one of the major factors related to the loss in seed viability in stored bamboo seeds. This was examined during storage for one year, under controlled conditions, in 5 bamboo species by means of various viability tests. Endogenous levels of putative free indole acetic acid (IAA) and free and bound abscissic acid (ABA) were measured in freshly harvested seeds and in seeds stored for one year. In freshly harvested seeds, free IAA levels were higher in all species in comparison to one-year-old seeds. Seeds of *Thyrsostachys siamensis* showed highest viability (G% 76.6) and maximum content of IAA (2.90 µg g^{-1} fw) while the seeds of *Dendrocalamus strictus* showed lowest viability (G% 5.53) and lowest content of IAA (2.07 µg g^{-1} fw). Same pattern of IAA was observed in seeds stored for one year but the IAA levels were found to be reduced and so did the viability across all species. The free and bound ABA levels in freshly harvested seeds were maximum in *D. strictus*, with lowest viability. After one year, the amount of free ABA increased by about 58% while the bound ABA was found to be reduced by 78% in this species.

***Key words*: Abscissic acid, bamboo seeds, endogenous plant growth substances, indole acetic acid, seed viability.**

INTRODUCTION

Bamboo seeds are scarcely available due to infrequent flowering and possess short viability and literally no dormancy. Though bamboo seeds are best material for germplasm conservation and propagation, they are rendered useless in no time due to factors mentioned above. Seed storage may influence seed viability and reduce seed vigour the extent of change being dependent on internal metabolism and storage conditions (Dell Aguila 1987). Biochemical parameters are increasingly used as indicators of seed viability and vigour (Perl and Kretchmer 1988). However, different biochemical systems get affected by the wide range of deteriorative changes taking place in the seeds.

Endogenous plant growth substances (PGSs) play important role during seed development, reserve accumulation, storage, seed germination and subsequent growth. Karssen *et al.* (1989) emphasized the key role of endogenous gibberellins (GAs) in the control of seed germination. Study on the variation of PGS levels during seed germination in barley was carried out by Bewley and Black (1994). Lucke and Bessla (1997) analyzed the content of ABA in seeds of 40 species of orchids and reported that the higher ABA content adversely affected germination. However, IAA has promotory effect on germination because of its indirect effect through changes in membrane permeability, solublisation of carbohydrates and production of some precursors (such as GA) needed for germination (Ross *et al*. 1983).

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The present study was undertaken to find out whether seeds, subjected to storage for one year, show decrease during storage in the levels of free auxins or increase in ABA levels as compared to freshly harvested seeds. For this study, seeds of five bamboo species of varying viability were studied with an aim to understand the factors leading to deterioration of seeds.

MATERIALS AND METHODS

The freshly harvested seeds of *Bambusa bambos*, *Dendrocalamus membranaceus*, *Gigantochloa albociliata*, and *Thyrsostachys siamensis* were procured from Royal Forest Department, Bangkok, Thailand while those of *Dendrocalamus strictus* were procured from FRI, Dehradun. The seeds were stored for one year at 4ºC in a desiccator containing $CaCl_2$.

The seeds of all species were divided into two lots, *i.e.* freshly harvested (FH) seeds and stored (S) seeds and were independently subjected to various investigations.

Germination Studies : Ten randomly selected seeds were surface sterilized with 0.5% mercuric chloride ($HgCl_2$) for two minutes followed by thorough washing in running water. These were later rinsed with distilled water. The seeds were placed equidistantly in presterilized Petri-dishes (09.0 cm) lined with filter paper. The filter paper was moistened and the petridishes were maintained at 28ºC ± 2ºC in dark in B.O.D. incubator. The experiment was run in triplicate. Emergence of radicle was considered as an indicator of germination. Number of seeds germinated were noted daily. Number of seeds germinating each day were recorded up to a period of 13 days. The emergence index, speed of germination and the coefficient of velocity of germination were calculated using the following formulae:

Emergence index (E_1) was calculated by the following formula given by Baskin (1969).

E = n1/dnl + n2/dn2 + n3/dn3 …… nx/dnx

Where n = number of seeds emerged on the day (1^{st}), dn = number of days from the day of sowing, dnx = number of days to the final count

Speed of germination was calculated by the formula, S.G. = n/t given by Maguire (1962); where n = number of seeds emerged on the day, t = time or days from sowing

Coefficient of velocity of germination (CVG): CVG was calculated by the formula given by Kotowski (1926).

CVG = Sum of n/Sum of (nt) x 100

Where n = number of seeds emerged on the day, t = time or days from sowing

Viability test : The sterilized seeds were imbibed for 6 h and the embryos were gently taken out and immersed in 0.1% Triphenyl-tetrazolium chloride solution for 23 hrs. in the dark. The embryos from the seeds of both lots developed red or purple colour, the intensity depending on the state of the seeds. For quantitative estimation of the response, the reduction product formed as TTC-formazon was extracted with dimethyl formamide. The extinction was read at 485nm using the procedure given by Malik and Singh (1980).

Extraction for indole acetic acid (IAA): Endogenous levels of IAA were determined by the method of Nagar (1995). Freshly harvested seeds of all five bamboo species were used for the estimation of endogenous IAA levels. Each sample of 5 g fresh wt. was separately homogenized in chilled 80% methanol three times. The homogenates were centrifuged at 10,000 g at 5ºC for 30 min. and pH of the extract was maintained at 6.5 by checking it on a pH meter.

PVP column chromatography: The supernatants were concentrated in vacuo at 30ºC and then applied to polyvinylpyrrolidone (PVP) columns. The columns (20cm x 1.5cm, internal diameter) were eluted with phosphate buffer (pH 8.0) and the resulting eluates were again adjusted to pH 8.0 with IN HCl and partitioned against peroxide free-diethyl ether (x3). The ether phases were discharged. The remaining aqueous fractions were adjusted to pH 3.0 and partitioned against diethyl ether (x3). The ether phases were evaporated in vacuo and taken up in methanol (HPLC grade) for the estimation of IAA.

Estimation of IAA by HPLC : The partially purified methanolic extracts were filtered through 0.45µm Millipore filters and injected into a 20µl injector loop fitted over a Lichrosorb RP18 (10µm) column (250 x 4.6 mm, ID) protected by a guard column. Elution was carried out by a gradient of 30-70% methanol (5min) followed by 70-100% methanol (5 min) and finally with pure methanol for 15 min at a flow rate of 1 ml/min. The column eluates were passed through an ultraviolet (UV) detector set at 254 nm, and the IAA was estimated by measuring the peak area and comparing it with the standard curve of indole-3-acetic acid (Sigma Chemical Co., St Louis, USA).

Extraction of abscisic acid (ABA): The endogenous level of ABA was determined by the method described by Nagar (1996). Each seed sample of 5 g fresh wt. was separately homogenized in chilled 80% methanol (20 ml/g) containing butylated hydroxytoluene (BHT) at 100 mg/l. The homogenates were stored for 24h in the dark at 4ºC and then vacuum filteration was done. The residues were re-extracted five times with chilled methanol, filtered and centrifuged at 10,000 g at 5ºC. The supernatants were dried in vacuo, dissolved in 2 ml of 0.1M potassium phosphate buffer (pH 8.8) and then applied to insoluble polyvinyl-pyrrolidone (PVP) columns. The columns (20 cm x 1.5 cm ID) were eluted with phosphate buffer (pH 8.0) and the resulting eluates were adjusted to pH 2.5 with IN HCl and partitioned against peroxide-free diethyl ether (x5) containing BHT (100 mg/I). The combined organic phases were evaporated to dryness in vacuum, taken up in methanol (HPLC grade) and used for free ABA estimation. The remaining aqueous fractions were hydrolyzed at pH 11.0 for 1h at 60ºC, cooled, adjusted to pH 2.5 and partitioned against diethyl ether containing BHT (x5). The ether phases were evaporated in vacuo and taken up in methanol (HPLC grade) for the estimation of bound ABA (Harris and Dugger 1986).

Estimation of ABA by HPLC: For HPLC, the partially purified methanolic extracts were filtered through 0.45µm Millipore filters and injected into a 20µl injector loop fitted over a Lichrosorb RP18 (10µm) column (250 x 4.6 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 1 ml/min. The solvents were filtered through 0.45µm whatman membrane and degassed under vacuo prior to use. The column eluates were passed through an UV detector at 2.54 nm and the ABA was measured by comparing with standard curve of ABA (Sigma Chemical Col., St. Louis, USA).

RESULTS

Per cent germination values of freshly harvested seeds to all five species of bamboo were significantly higher as compared to seeds stored for one year. Emergence index (EI), speed of germination (SG) and coefficient of velocity of germination (CVC) were all higher in freshly harvested seeds than stored seeds. All the five samples of seeds had varying viability (Table 1), with *Thyrsostachys siamensis* having the highest germination percentage (76.6%) and *D. strictus* with the lowest (53.3%). Vigour test was performed for all the five species to see the embryo response in terms of formazon formation. The highest vigour at the time of harvest was observed in *T. siamensis* and lowest in *D. strictus* (Table 2). Vigour test showed considerable decline in vigour during storage. After one year, deterioration in stored seeds was observed in all the viability parameters including G%. Maximum loss of viability was found in *D. strictus* (88.6%) while minimum values were observed in *Bambusa bambos* (71.6%) and *T. siamensis* (71.5%). Other parameters of seed vigour such as E, SG, CVG and vigour test showed similar trend across species (Table 1).

Corresponding levels of the PGSs, IAA and ABA, in freshly harvested and stored stage were estimated. Endogenous content of free IAA was measured in both freshly harvested and stored seeds. The amount of IAA in freshly harvested seeds of all the five species was much higher than in seeds stored for one year. Amongst the freshly harvested seeds of various species, the IAA levels were observed to be maximum in *T. siamensis* (2.90 µg/g fw) followed by *B. bambos* (2.58 µg/g fw), *G. albociliata* (2.49 µg/g fw) and *D. membranaceus* (2.12 µg/g fw) and least in *D. strictus* (2.07 µg/g fw) (Table 2). In stored seeds again, the maximum level of IAA was in *T. siamensis* and the minimum in *D. strictus* seeds, which correspond to their viability levels.

Table 1. Germination parameters in both freshly harvested and stored (12 months bamboo seeds)

Species	Mean G%		Emergence index (EI)		Speed of germination (SG)		Coefficient of velocity of germination (CVG)	
	FH	S	FH	S	FH	S	FH	S
Bambusa bambos	70±0.1	18.5±0.1	0.58	0.21	3.46	1.08	33	20
Dendrocalamus membranaceus	63.3±0.07	15±0.2	0.43	0.13	2.68	0.68	29	19
Gigantochloa albociliata	70±0.1	20±0.0	0.45	0.21	2.53	0.95	25	16
Thyrsostachys siamensis	76.6±0.12	20. ±0.2	0.45	0.25	2.84	1.28	27	20
Dendrocalamus strictus	53.3±0.08	6.7±0.7	0.28	0.11	1.81	0.45	24	23

Table 2. Change in mean G%, (µg/g fw), endogenous ABA (µg/g fresh wt.) and endogenous IAA ((µg/g fw) in both freshly harvested and stored bamboo seeds

Species	Freshly harvested seeds					Seeds stored for 12 months				
	Mean G%	Formazon	Endog. ABA		Endog. IAA	Mean	Formazon	Endog. ABA		Endog. IAA
			Free	Bound				Free	Bound	
Bambusa bambos	70±0.1	1.286	0.214	0.035	2.58	18.5±0.1	0.365	0.361	0.018	0.66
Dendrocalamus membranaceus	63.3±0.07	1.176	0.178	0.031	2.12	15±0.2	0.298	0.391	0.011	0.25
Gigantochloa albociliata	70±0.1	1.292	0.177	0.031	2.49	20±0.0	0.427	0.353	0.017	0.39
Thyrsostachys siamensis	76.6±0.12	1.33	0.202	0.029	2.90	20. ±0.2	0.378	0.322	0.015	1.01
Dendrocalamus strictus	53.3±0.08	0.988	0.302	0.041	2.07	6.7±0.7	0.112	0.476	0.009	0.21

The content of free ABA was found to be more than bound ABA in seeds of all the five species in both freshly harvested and stored seeds (Table 2). The level of free ABA was more in stored seeds of all species than in the freshly harvested seeds. Level of free ABA was highest in both the freshly harvested and stored seeds. Level of free ABA was highest in both the freshly harvested and stored seeds of *Dendrocalamus strictus* with lowest germination %. Level of free ABA after storage of one year became lowest in *Thyrsostachys siamensis* with highest G%. The bound ABA levels also decreased after storage. It was minimum in *D. strictus* and maximum in *B. bambos* in both freshly harvested and stored seeds.

DISCUSSION

Seed vigour is the index of all the parameters of germination, while germination percentage (G%) is the most widely used indicator of viability. In the present study, using these parameters, seeds of *Thyrsostachys siamensis* may be considered with maximum vigour while

those of *D. strictus* with least vigour, both at the time of procurement and after storage for one year under controlled conditions. After one year of ageing, seeds of all the five species underwent deterioration but were still viable. Renewal of viability of such partially viable seeds is now possible with the application of PGSs (Saxena and Pakerriah 1985, Saxena and Maheshwari 1986, Richa and Sharma 1994, Richa *et al*. 2000).

Germination, a highly orchestrated process in seeds, is regulated by the interaction of PGSs especially GA, IAA and ABA (Khan 1982, Karssen 1995, Taiz and Zeiger 2002). Plant growth substances interact directly or at the molecular level after acting upon response system and cause biochemical or physiological responses (Karssen 1995). Auxins are critical in germination because of their effect on cell elongation, thus contributing towards radicle emergency. The promotory effect of IAA in germination may be attributed to its indirect effect through changes in membrane permeability, solubilisation of carbohydrates and production of some precursors (such as GA) needed for germination (Ross *et al.* 1983). The level of free ABA (inhibitor in the seeds) was maximum in stored seeds as compared to the fresh ones. Likewise, the level of free ABA was highest in *D. strictus* with lowest viability. The promoter, IAA was maximum in the freshly harvested seeds which decreased after storage. IAA, which is responsible for viability was found to be maximum in *T. siamensis* and minimum in *D. strictus*, in both freshly harvested and stored seeds.

The investigation on the levels of endogenous IAA and ABA, both in freshly harvested and stored seeds in all five species, give indication of the role of these plant growth substances in processes related with germination and further growth. The deterioration in seeds during storage may be attributed to changes in the level of promoters and inhibitors (Joshi *et al.* 1980, Singh and Nayyar 2000), thereby affecting the enzyme activity, membrane integrity and changes in the levels of macromolecules. It may, however, be noted that IAA and ABA identification is only tentative as these are based only on HPLC retention times.

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