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EFFECT OF AGEING ON ENZYME ANALYSIS OF THREE SPECIES OF BOTH NATURALLY AGED AND CONTROLLED AGED BAMBOOSEEDS

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ABSTRACT

Bamboo seeds have a very low viability of short duration for 1-2 months. So bamboo seeds hold significant importance for northeastern Indians whose livelihood is completely dependent on Bamboos. In this study, Seeds were stored for period of 18 months in desiccators at 4°C in the presence of anhydrous Calcium chloride. while fresh seeds showed increase in the activity of α-amylase, β-amylase and catalase and their level decreases with ageing.Activity of α-amylase, β-amylase and catalase was assessed of all the three species of bamboos *Dendrocalamus strictus, Dendrocalamus hamiltonii and Bambusa bambos* till period of 18 months in both naturally and controlled aged seeds. Content of α-amylase increases in both naturally and controlled aged seeds with increase in time interval maximum content of α-amylase was found in *Dendrocalamus strictus* 93.24 in freshly aged seeds and β-amylase was maximum in 107.8 in *Dendrocalamus hamiltonii* and Catalase in 411.12 in *Bambusa bambos*. The study demonstrated the role of enzymes with ageing in both naturally ageing and controlled aged bamboo seeds and its correlation with seed deterioration and ageing.

KEYWORDS: Enzyme activity, α-amylase, β-amylase, Catalase

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INTRODUCTION

Bamboos are plants of global interest because of their distinctive life form, ecological importance and wide range of uses and values they have for humans.[1]Bamboos include some of the fastest growing plants in the world due to unique rhizome dependent system. Most bamboo species flower infrequently. Infact many only flowers as intervals as long as 60- 125 years. Flowering produces masses of seeds typically suspended from the end of branches. Seeds have a very low viability of few months so as to procure them and to study their enzymatic is tedious job. Seeds are stored in desiccator at 4° Celsius under calcium chloride. To study the enzyme effects under controlled conditions and compare them with naturally aged seeds. Decline in the activities of α amylases , β amylases , peroxidase and glutamate dehydrogenase with seed ageing of 6 months of bamboo seeds.[2] Seed deterioration in genebanks is normally predicted through assessments of seed viability, germination, vigour and integrity. There are many different methods available to assess seed ageing.[3] A germination test is the recommended method for testing seed deterioration in a genebank, because it is an accurate and reliable method. An accelerated ageing test and electrical conductivity test are commonly used to assess seed vigour and facilitate seed ageing research. Biochemical changes associated with seed ageing include impairment of protein synthesis, protein inactivation, changes in enzyme activities, protein hydrolysis and post-translational modifications.[4]So the study was conducted to analyse the effect of enzyme with ageing of seeds.

MATERIALS AND METHODS

The present study was conducted at Bamboo research lab, Panjab University, Chandigarh. Seeds were obtained from KFRI, Peechi . Seeds were authenticated in botany department , panjab university, Chandigarh.Identification was made based on the morphological observation using standard protocols and monographs. Seed Collection period: Sept-Oct,Seed Longevity: 1-2year, Seed Purity: 99%, Usual Germination: 60-90%, Characteristics: Evergreen, Ornamental, medicinal, Seed Counts per KG : 45000-50000 Prior to enzyme assaying the seeds were surface sterilized with 0.5% mercuric chloride ($HgCl_2$) for 2 min. and were washed thoroughly with running water followed by rinsing with distilled water. Each Seed species were placed equidistantly in pre- sterilized petriplated lined filter paper.The petriplates were placed in an incubator at 28°C+2. The enzyme extracts were made after 12 hours, 24 hours and 48 hours of germination. For this the seeds were deglumed, weighed and homogenized with 6 ml of buffer and a pinch of acid washed sand in cold pestle and mortar. The homogenate was centrifuged at 3000 rpm for 10 min. and the supernatant was retained and used for enzyme assaying. All the above mentioned steps were carried out at 1-4°C.

Determination of α- amylase

α- amylase activity was calculated by determining the concentration of hydrolyzed starch substrate in a specific time.[5] To 1.0 ml of starch substrate added 0.5 ml of enzyme extract which forms the reaction mixture. At 0 time taken an aliquot of 0.2 ml of reaction mixture. Added 3 ml of KI solution and the absorbance of resulting blue colored solution at 620 nm. After 30 min. taken another aliquot of 0.2 ml of reaction mixture. Added 3.0 ml of KI solution and read blue- colour solution for absorbance at 620 nm.The enzyme activity was estimated by measuring the decrease in starch concentraration in the reaction mixture.

Determination of β- amylase

Reaction mixture containing 0.2 ml enzyme extract and 1 ml of freshly prepared starch solution was added and incubated the whole reaction mixture at 30°C for one hour.Reaction was terminated by adding 1 ml DNSA reagent. All test tubes were kept in boiling water bath for 10 min, cooled the tubes to room temperature and added 2 ml of distilled water to each tube and recorded the absorbance at 560 nm.[6]Control for every reaction mixture was read simultaneously.

Determination of Catalase

The reaction mixture (3 ml) containing 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2, 0.1 ml of enzyme extract. The reaction was stopped by adding 2 ml of titanium reagent. It was centrifuged at 10,000 rpm to 10 min.[7] The absorbance was read at 410 nm. The catalase activity was measured using the extinction coefficient 40 mM^{-1} cm^{-1} and expressed as μ Mol H_2O_2 reduced/sec/g FW.

Statistical analysis

The results of the data were statistically analysed and results were expressed in the form of standard error of all experiments by using graph pad software.

RESULTS

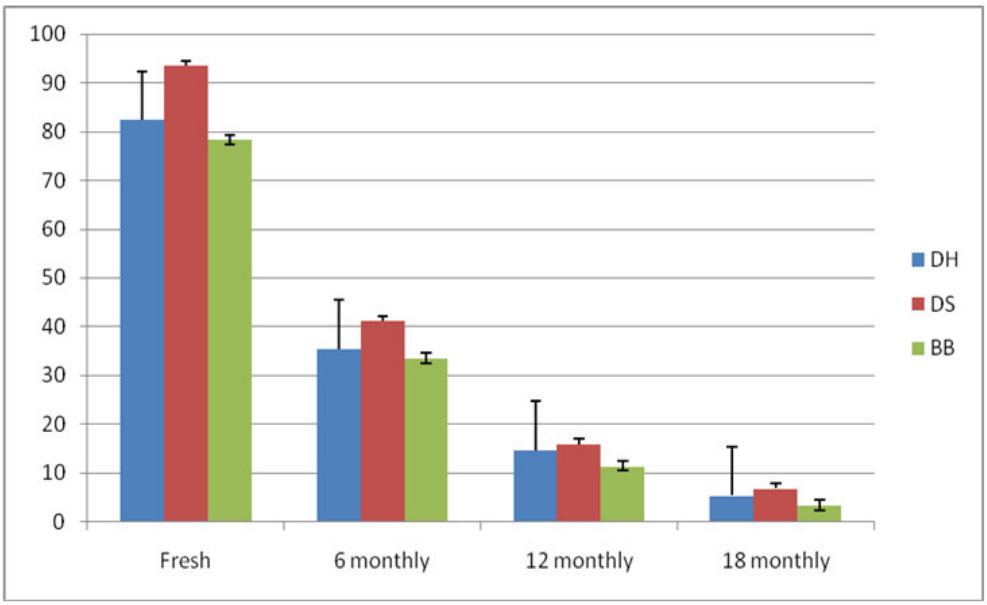
1.α-Amylase

α- amylase activity was estimated at three stages of germination i.e 12,24 and 48 hrs at 6-months of intervals till 18-months of natural and controlled stored ageing. In what follows, the result of changes in enzyme activity with ageing are presented.

Table 1
Levels of α- amylase (Expressed as ∆ O.D. per gram F.Wt. per unit time) at different stages of ageing

			Fresh			
Species	12Hrs		24 Hrs		48 Hrs	
DH	71.23		74.45		82.34	
DS	76.67		83.23		93.24	
BB	69.87		74.35		78.23	
			6-Monthly			
Seeds Ageing under controlled conditions				Seeds Ageing under Natural conditions		
DH	49.15	56.72	59.34	34.07	35.16	35.46
DS	57.23	59.43	59.78	39.57	39.98	41.21
BB	48.7	49.12	50.12	31.87	33.42	33.56
			12-Monthly			
DH	27.76	30.13	30.67	14.54	14.56	14.65
DS	29.87	30.15	30.45	15.67	15.89	15.91
BB	25.45	26.7	27.67	11.23	11.34	11.40
			18-Monthly			
DH	17.6	18.67	18.76	5.23	5.26	5.42
DS	15.56	15.78	15.89	6.17	6.45	6.79
BB	11.32	11.45	11.56	3.28	3.32	3.37
			LSD at 5%			
Species				0.121		
Storage time				0.161		
Species x Storage time				0.321		
Storage time x Anaysis Hrs				0.278		

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



Levels of α-amylase in seeds of three species of bamboos at different stages of ageing

D. hamiltoni

In freshly harvested seeds, after germination of 12hrs with 81.2% germination, the activity of α- amylase was found to be 71.23 and it increases to 74.45 and 82.34 after 24 and 48 hrs respectively. Activity of a- amylase decreased to 34.07, 35.16 and 35.46 after 12hrs, 24hrs and 48 hrs of imbibitions respectively in naturally aged seeds, activity of a-amylase was 49.15, 56.72 and 59.34 after 12, 24, and 48 hrs of germination respectively in controlled aged seeds which is more in naturally aged seeds. After 18-months of ageing, activity of a-amylase was 5.23, 5.26, and 5.42 in naturally aged seeds (when germination was zero) whereas it was 17.6, 18.67 and 18.76 in controlled ageing seedswhich were still viable.

D. strictus

In freshly harvested seeds after imbibitions for 12 hrs with 70% germination, the activity of a-amylase was found to be 76.67 and it increase to 83.23 and 93.24 after 24 and 48 hrs respectively. .Activity of α- amylase decreases to 39.57 after 12 hrs imbibitions 39.98 and 41.21 after 24 hrs 48 hrs of imbibitions respectively in seeds under natural ageing of 6- months (G%=13.3). After 18- months of ageing , activity of α- amylase was 6.17, 6.45 and 6.79 in naturally aged seeds whereas it was 15.56, 15.78 and 15.89 in controlled ageing seeds.

B.bambos

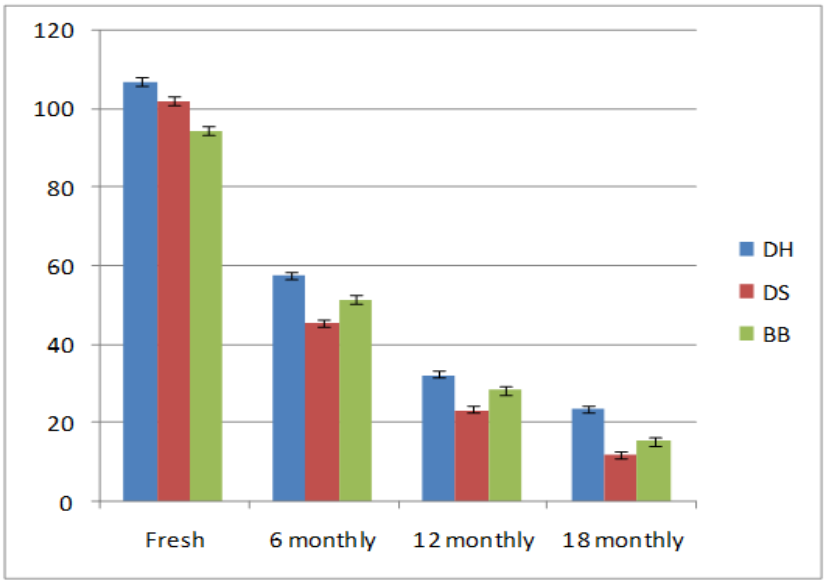
In freshly harvested seeds, after mbibitions for 12hrs with 80.2 % germination , the activity of α- amylase was found to be 69.87 and increased to 74.35 and 78.23 after 24 and 48 hrs respectively. After 18- months of ageing, activity of α- amylase was 3.28, 3.32 and 3.37 in naturally aged seeds whereas it was 11.32, 11.45 and 11.56 in controlled ageing seeds Activity of α- amylase decreased to 31.87, 33.42 and 33.56 after 12 hrs, 24 hrs and 48 hrs of imbibitions respectively in seeds under natural ageing of 6-months (G%=20) Thus showing the content increases with ageing as its less in 6 months and with increase in time period level of enzyme increases of *α-amylase.* Content was more in naturally ageing than controlled ageing.

Table 2

Levels of β- amylase (Expressed as ∆ O.D. per gram F.Wt. per unit time) at different stages of ageing

Fresh						
Species			12Hrs	24 Hrs		48 Hrs
DH			102.34	105.6		107.8
DS			99.34	99.89		102.3
BB			89.23	93.42		94.53
6-Monthly						
Seeds Ageing under controlled conditions				Seeds Ageing under Natural conditions		
DH	86.12	87.23	87.9	57.23	57.37	57.61
DS	72.34	73.4	74.32	42.34	43.2	45.6
BB	87.67	88.98	90.12	51.23	51.34	51.56
12-Monthly						
DH	65.34	65.39	65.49	32.31	32.38	32.45
DS	46.12	46.23	46.52	23.12	23.41	23.49
BB	59.13	59.28	59.34	27.34	28.38	28.44
18-Monthly						
DH	47.56	47.67	47.84	23.4	23.244	23.64
DS	21.34	21.48	21.51	11.67	11.87	11.99
BB	39.23	39.25	39.56	15.12	15.23	15.45
LSD at 5%						
Species				0.0819		
Storage time				0.1084		
Species x Storage time				0.2167		
Storage time x Anaysis Hrs				0.1877		

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



Levels of β amylase in seeds of three species of bamboos at different stages of ageing

2. β- amylase

Level of β amylase were estimated after 12, 24 and 48 hrs of germination at 6 months till interval of 18 months of naturally and controlled aged seeds. Increase of enzyme level with ageing was found . increase was more prominent in naturally ageing as compared to controlled aged seeds.

D. hamiltonii

In freshly harvested seeds, imbibitions for 12hrs, activity of β-Amylase was 102.34 at 81.2% germination and

10.65 and 105.6 and 107.8 after 24 and 48 hrs of imbibitions respectively . Activity of β- amylase decrease to 57.23, 57.37 and 57.61 after 12hrs, 24 hrs and 48hrs of imbibitions respectively in seeds aged for 6 months (G%=16.7) under natural ageing conditions.

D. strictus

In freshly harvested seeds, imbibitions for 12hrs, activity of β- amylase was 99.34, 99.89 and 102.3 after 24 and 48hrs of imbibitions respectively. Activity of β- amylase decreased to 57.23 after 12hrs imbibitions, whereas 57.37 and 57.61 after 24hrs and 48 hrs of imbibitions respectively. In seeds aged for 6-months under controlled conditions.

B. bambos

In freshly harvested seeds, imbibitions for 12hrs, the activity of β- amylase was 89.23 at 83.3% germination and 93.42 and 94.53 after 24 and 48 hrs respectively. Activity of β- amylase decreased to 51.23,51.34 and 51.56 after 12hrs,24hrs and 48hrs of imbibitions respectively in seeds aged for 6 months (G%=18) under natural ageing conditions. After 18-months of ageing , activity of β- amylase was 23.4 , 23.24 and 23.64 in naturally ageing seeds and 47.56, 47.67 and 47.84 in controlled ageing seeds. Thus showing the content increases with ageing as its less in 6 months and with increase in time period level of enzyme increases of *β-amylase.*

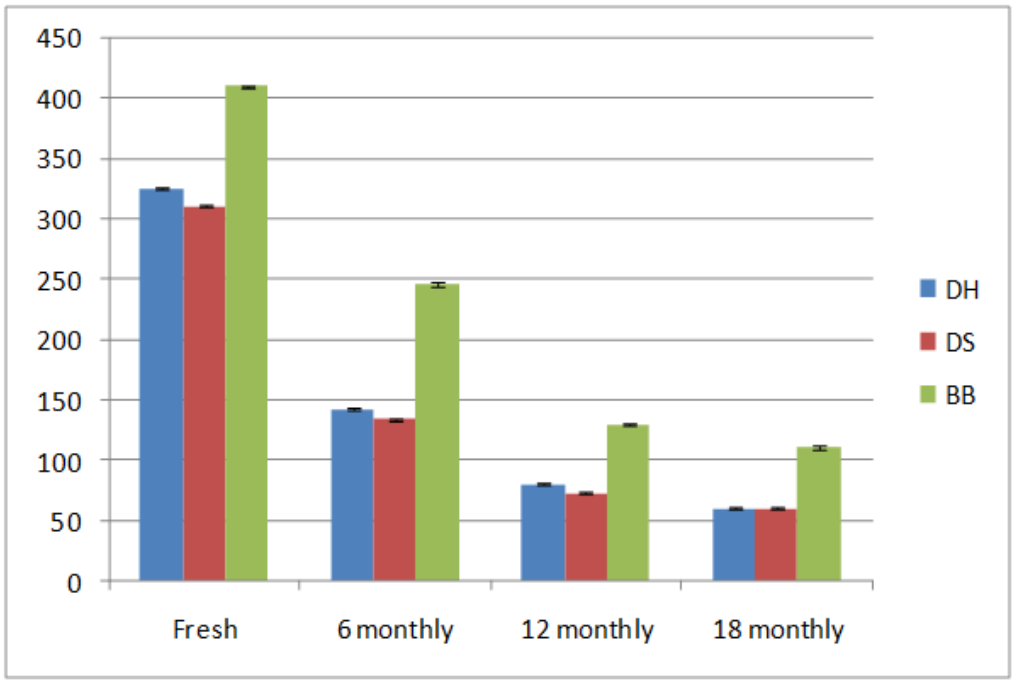
3. Catalase

Catalase activity was estimated at three stages of germination i.e. 12, 24 and 48 hrs at 6-monthly intervals of ageing up to 18-months of natural and controlled ageing in all the species. The results are presented below. Ageing seeds after 12, 24 and 48 hrs of imbibitions respectively.

Table 3
Levels of catalase (Expressed as ∆ O.D. per gram F.Wt. per unit time) at different stages of ageing

Fresh						
Species			12Hrs		24hrs	48hrs
DH			284.14		290.15	325.5
DS			299.12		300.23	311.26
BB			398.23		401.23	411.12
6-Monthly						
Seeds Ageing under controlled conditions				Seeds Ageing under Natural conditions		
DH	256.13	267.12	289.12	137.80	139.78	142.3
DS	213.45	231.23	243.34	114.5	128.9	134.5
BB	303.4	312.4	324.5	245.6	237.8	246.7
12-Monthly						
DH	189.3	191.3	192.3	78.9	79.3	80.2
DS	234.5	236.7	238.4	69.8	72.1	73.2
BB	256.4	278.3	290.3	123.4	125.6	130.2
18-Monthly						
DH	131.29	139.0	145.32	56.7	57.6	60.2
DS	114.5	118.6	126.7	53.45	59.7	61.23
BB	208.7	211.5	220.8	102.3	107.6	111.2
LSD at 5%						
Species				0.1317		
Storage time				0.1743		
Species x Storage time				0.3485		
Storage time x Anaysis Hrs						

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



Levels of catalase in seeds of three species of bamboos at different stages of ageing

D. Hamiltonii

In freshly harvested seeds, after germination for 12hrs, activity of catalase was 284.14 (at 81.2 % germination) and 290.15 and 325.5 after 24 and 48 hrs of imbibitions respectively. In seeds aged for 6-months of natural ageing, activity of catalase decreased to 137.80 after 12hrs germination and was 139.78 and 142.3 after 24hrs and 48hrs of imbibitions respectively.

D. strictus

In freshly harvested seeds, after germination for 12hrs, activity of Catalase was 299.12 (at 76%) germination and 300.23 and 311.26 after 24 and 48 hrs of imbibitions respectively. In seeds aged for 6-months (G%) of natural ageing, activity of catalase decreased to 114.5 after 12hrs imbibitions and was 128.9 and 134.5 after 24 hrs and 117.93 after 24 and 48 hrs of imbibitions respectively After 18-months of ageing, activity of catalase was 53.45, 59.7 and 61.23 in naturally aged seeds and 114.5, 118.6 and 126.7 in controlled ageing seeds after 12, 24 and 48 hrs of imbibitions respectively.

B. bambos

In freshly harvested seeds, after germination for 12hrs, activity of catalase was 398.23 (at 80.2% germination) and 401.23 and 411.12 after 24 and 48 hrs of imbibitions respectively. After 6-months (G%) of ageing, controlled aged seeds showed decrease in catalase activity to 303.4, 312.4 and 324.5 after 12, 24 and 48 hrs of germination respectively. After 18-months of ageing activity of Catalase was 102.3, 107.6 and 111.2 in naturally aged seeds and 208.7, 211.5 and 220.8 in controlled ageing seeds after 12, 24 and 48 hrs of imbibitions respectively.Thus showing the content increases with ageing as its less in 6 months and with increase in time period level of enzyme increases of *catalase* and increase is more in naturally aged seeds than controlled aged.This increase may be attributed to increase in deterioration of seed with ageing and increase in permeability with ageing and loss of integrity.

DISCUSSION

In the present study we found that after 12 and 18 months of ageing, activity of α amylase was prominenty reduced in bambos *bambos* in naturally aged seeds whereas it was in minor change in controlled aged seeds. After 48 hours of imbibitions while the activity of catalase in case of 18 months aged seeds where germination is reduced to zero it was more prominent in *Dendrocalamus hamiltonii* naturally ageing while in controlled ageing *Dendrocalamus strictus* showed major effects . Level of β amylase was reduced in *Dendrocalamus strictus* prominently in both naturally ageing and controlled ageing. Level of enzyme activity was more in naturally ageing as compared to controlled ageing. Our results were in conformation with the work done earlier. Correlations was found between loss of viability and decline in enzyme activity of aged wheat seeds.[8] Decreased α -amylase activity in aged wheat seeds occurred as this enzyme was synthesized at a reduced rate by the aleurone. In corn seeds, loss of seed viability was accompanied by decrease in the activity of enzymes like oxidases (catalase, peroxidise and phenolase), hydrolase (amylase), cytochrome oxidase , glutamic acid decarboxylase.[9] Thus there is a decrease in the activity of several hydrolytic enzymes with ageing.[10] Decrease in ageing can be very vital factor which lead to loss of germination and loss of viability. Seed deterioration is directly related to enzyme activity. It showed that decrease in enzyme activity can be vital factor for loss of germination.The activity of amylases is one of the important factors related to seed germination since they act upon starch to release utilizable form of substrate thus facilitating germination.

CONCLUSION

Seed aging is a complex biological trait and difficult to monitor. It summarizes the recent development of tools for assessing seed aging and reveals seed deterioration with aging. These research efforts will provide useful methods to supplement traditional germination tests, enhancing the monitoring of seed deterioration for long-term conservation of *ex situ* seed germplasm and how enzyme levels decreases with ageing. The role of enzymes α –amylase, β amylase and catalase and its activity reduces with ageing in all the three species of bamboo seeds. So their role in physiology of seeds can be ascertained.). Present investigation revealed that activities of α –amylase, β amylase and catalase (CAT) were significantly decreased with the increased in the duration of storage leading to deterioration of seed viability. Age associated decline in enzyme activity and seed deterioration are closely related.

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CONFLICT OF INTEREST

The Authors declares no conflict of interest.

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