Research Article

Effect of Prolonged Storage on the Biochemical Constituents of Primary Metabolites in Three Species of Bamboo Seeds

Geetika Singh*, Richa, M.L Sharma
Department of Botany, Panjab University, Chandigarh, India.
***Corresponding author's E-mail:** geetika_phd@yahoo.com

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ABSTRACT

Bamboos popularly known as green gold is sufficiently cheap and plentiful to meet the vast needs of people. It plays major role in the livelihood of rural people. Seeds of bamboos are viable hardly for few months so understanding the biochemical metabolites and its effect with ageing was main aim of research work. The aim of the study was to estimate primary metabolites i.e. sugars, phenols and amino acids and antioxidant ascorbic acid in *Bambusa bambos, Dendrocalmus strictus and Dendrocalamus Hamiltonii* and its effect with ageing under prolonged storage conditions. The highest amount of sugars (7.99 mg/gmfwt), phenols (7.53 mg/gmfwt) and amino acids(7.56 mg/gmfwt) found in *Bambusa bambos* at 48 hours analysis in 18 months interval and ascorbic acid content maximum in fresh seeds of *Bambusa bambos* (3.67 mg/gmfwt) and its relationship of decrease in metabolites with the loss of viability with ageing after 18 months period.

Keywords: Primary metabolites, Sugar, Phenols, amino acids, Ascorbic acid.

INTRODUCTION

Bamboos belongs to the most diverse group pf plants in the grass family. 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]. There are more than 1500 different documented traditional uses of bamboo. In Asia, Bamboos are the essence of life for many communities and it is no exaggeration to speak of a Bamboo Civilization in the region. Bamboos can be propagated from rhizomes, culm or by multiplication of nursery raised seedlings. However seeds serve as best material for large scale plantations and germplasm conservation. But seeds have a very short viability of 1-2 months and therefore useful as propagules only for a short period of time. Seed quality is one of the factor for successful germination but its trait declines with ageing.

So the present study was aimed at determining the biochemical primary metabolites in the three species of bamboo seeds i.e. *Bambusa bambos, Dendrocalmus strictus and Dendrocalamus Hamiltonii* and their effect with ageing after 18 months period and its loss of viability during storage for such long period. Seed deterioration is reported to accompany changes in hydrolytic enzymes as well[2]. It was studied the physiological and biochemical changes in *Abelmoschus esculentes* seeds of different ages and concluded that germination decreases with seed ageing[3].

MATERIALS AND METHODS

For the quantitative estimation of primary metabolites, different protocols were used. 10 Seeds of each species i.e *Dendrocalamus strictus, Dendrocalamus hamiltonii and Bambusa bambos which* were stored in controlled conditions in desiccator at 4 degree Celsius were soaked and deglumed and weighed. Their homogenate was made in 10 ml of 80% ethanol using pestle and mortar with a pinch of acid washed sand. It was then centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant was used for the analysis of amino acids, sugars, total phenols and ascorbic acid.

For Estimation of Free Amino Acids[4]

Using glycine as standard. To 0.5 ml of homogenate added 5 ml of ninhydrin reagent and shaken properly. Then the mixture was boiled for 10 minutes in water and cooled thereafter absorbance was recorded at 570 nm on spectrophotometer.Glycine was taken as standard amino acid.

For Estimation of Total Sugars[5]

To 4 ml of chilled anthrone reagent 0.1 ml of ethanol extract was added. Tubes were shaken gently to mix the solution. these were covered with glass marbles and placed in water bath and cooled in ice water.

The absorbance of the blue green colored solution was taken at 625 nm in spectrophotometer against blank containing 80% ethanol extract.

For Estimation of Total Phenols[6]

To 1 gm of the seed material was extracted in 5ml of 0.3 N HCl in methanol. Centrifuge it, retain supernatant and repeat the extraction with residue.

Pooled the two supernatant and evaporate them. Dissolve the residue in 5 ml distilled water. 0.1 ml of aliquot in each case was taken to make final volume of 7 ml of distilled water.

Then 0.5 ml of Folin-Phenol reagent was added and solution shaken vigourously. After 3 min 1 ml of 35% sodium carbonate was added. The solution was shaken and allowed to stand for 1 hour. Absorbance was recorded at 630 nm.

For Estimation of Ascorbic Acid[7]

Plant tissue is homogenized in 6 % Trichloroacetic acid and the homogenate was centrifuged at 8000 rpm.

The supernatant was used as extract for estimation. To 4 ml of extract 2 ml of 2% Dinitro-phenlyhydrazine was added followed by 1 drop of 10% Thiourea Mixture was boiled for 15 min in water bath and cooled at room temperature. 5 ml of chilled Sulphuric acid was added at zero degree Celsius. The absorbance was recorded at 530 nm.

TTC test was conducted[8], Seeds were taken in 3 replicates with 2 seeds. All the replicates were then placed in small glass tubes containing 3 ml of 0.1 M phosphate buffer with 0.5 % TTC. Then they are incubated for 20 hours at 28 degree Celsius in darkness. The TTC solution was drained. Seeds were washed twice and 2 ml of ethanol was added. Tubes were kept in water bath at 95 degree Celsius until complete evaporation of the ethanol. 4 ml of ethanol was added again and tubes were shaken vigourously and absorbance due to formazon was recorded at 520 nm against ethanol.

Data was statistically analysed and experiment was conducted in completely randomized block design mean of three treatments with standard error was applied.

RESULTS AND DISCUSSION

Total sugars were found to be maximum in *Bambusa bambos* after 18 months interval of all the three species of bamboos. These results were analysed after 48 hours of soaking the seeds. Total Sugar content was found to be less in fresh seeds. It increased with ageing of seeds. It was 2.44, 2.78 and 4.33 mg/gfwt in *Dendrocalmus hamiltonii, Dendrocalmaus strictus and Bambusa bambos* in fresh seeds and 7.56, 7.60, 8.89 mg/gfwt after 18 months of ageing respectively as shown in Figure 1.

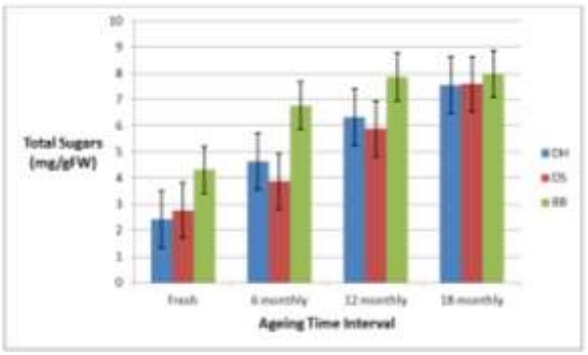

Figure1: Changes in Total sugars (mg/gFW) in seeds of three species of bamboos at different ageing intervals

In Freshly harvested seeds soaked for 48 hours, the amount of total phenols. It was 0.58, 0.27 and 0.26 mg/gfwt in *Dendrocalmus hamiltonii, Dendrocalmaus strictus and Bambusa bambos* and 2.45, 2.48, 1.99 mg/gfwt after 18 months of ageing respectively as shown in Figure 2.

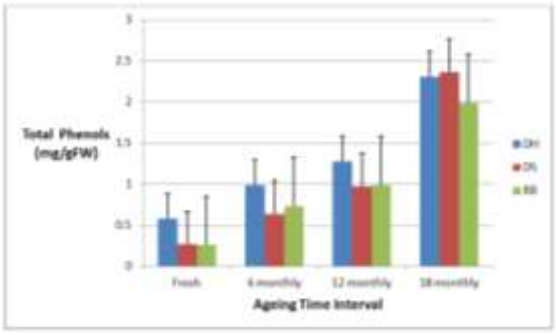

Figure 2: Changes in total phenols (mg/gFW) in seeds of three species of bamboos at different ageing intervals

Total amino acids was also found to be more in *Bambusa bambos*. It was 2.01, 1.35 and 4.33 mg/gfwt in *Dendrocalmus hamiltonii, Dendrocalmaus strictus and Bambusa bambos* in fresh seeds and 6.99, 7.11, 7.53 mg/gfwt after 18 months of ageing respectively as shown in Figure 3.

The amount of Ascorbic acid was more in freshly harvested seeds as compared to 18 months old seeds. So content of ascorbic acid decreases with ageing.

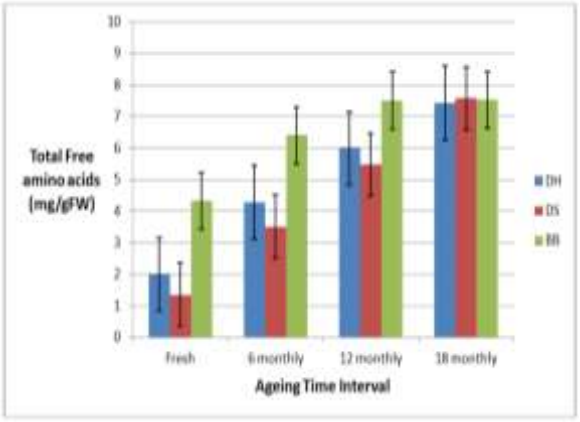

Figure 3: Changes in total amino acids (mg/gFW) in seeds of three species of bamboos at different ageing intervals

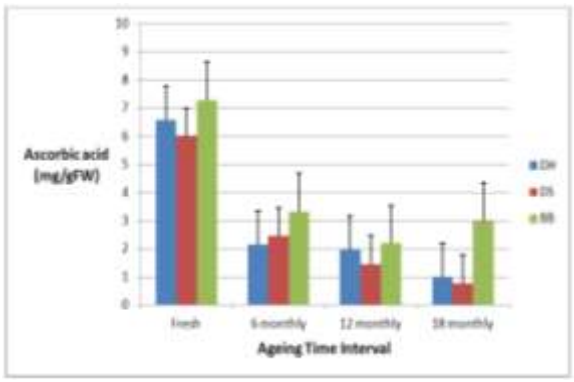

Figure 4: Changes in Ascorbic acid (mg/gFW) in seeds of three species of bamboos at different ageing intervals

So with ageing the content of total sugars, total phenols and total amino acids increases with ageing and ascorbic acid which is an antioxidant decreases with ageing as can be seen through TTC test which suggests the viability decreases with increase in metabolites as shown in Figure 5.

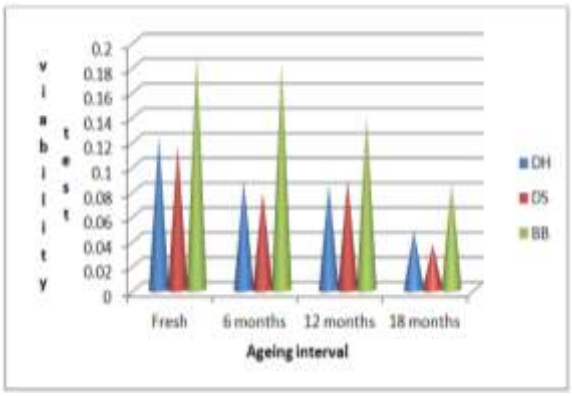

Figure 5: TTC activity in seeds of three species of bamboo at different ageing intervals

It was reported a decline in the amount of non reducing sugars in aged seeds[9]. There are reports that during ageing of seeds there is a decrease in the amounts of several constituents like proteins, nucleic acids, fats[10]. A decrease in the maount of total soluble sugars in aged wheat seeds[11], while decrease in reducing sugars with storage in Glycine max seeds was observed[12].

It was reported a decline in metabolites like carbohydrates, proteins and reducing sugars and increase in amino acids in ageing *Zea mays* seeds[13].

Decrease in starch was observed in pigeon pea seeds during ageing[14]. Many suggests that total carbohydrates contents increased with the ageing due to activity of hydrolytic enzymes and decrease in membrane integrity.

CONCLUSION

It can be concluded that all the three species of bamboos contain total sugars, phenols, amino acids and Ascorbic acid. Out of all the three *Bambusa bambos* has the maximum metabolites after prolonged storage of 18 months.

These results are suggestive of primary metabolites of commercial importance and result in great interest in plant pharmaceuticals and its decrease with ageing after 18 months interval and effect of biochemical factors in loss of viability can be seen through TTC test. Maximum content of primary metabolites and Viability was also maximum in the same species. This correlates the relationship between viability of seeds with presence of primary metabolites.

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