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SDS PAGE Analysis of Bamboo Seed Proteins and Its Comparative Evaluation with Ageing in Different Species.

Geetika Singh*.

Assistant Professor, M.C.M DAV College, Sector 36, Chandigarh, India.

ABSTRACT

SDS PAGE has been proved as one of the most important parameters for studying total protein as well as storage proteins in plants. However the consistency and accuracy of the protein based studies depend completely on the protein isolation.. Three different species of were compared for their protein analysis from the seeds of three different species of bamboo plant (*Dendrocalamus strictus, Bambusa bambos, Dendrocalamus hamiltonii)* under both natural and controlled conditions after six months of ageing upto 18 months period. Controlled condtions seeds were kept at 4 degree celsius in desiccator. The analysis was determined by the yield of protein as well as the clarity in the resolution of protein bands separated on Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). In the study, it was found that content of proteins decreases with ageing in all the three species. Using the technique of SDS-PAGE there werediscovered a lot of protein fractions. The profile of these fractions varies from the wide distribution along the entire molecular masses spectrum at the beginning of the germination to a poorly represented profile of protein fractions

Keywords: Bamboos, SDS PAGE, Protein isolation, Seed protein, Isolation method

Corresponding author

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INTRODUCTION

Bamboos are indeed one of nature's miracles and their strength and structure enables them to be put to diverse uses. Bamboo can be extremely important in providing vegetative cover to deforested areas. It produces leafy mulch to the soil surface, its foliage provides shade and protection against rains, and its habit of producing new culms from rhizomes enable the culms to be procured without disturbing the soil. Perhaps the most peculiar feature of this plant is its flowering which is cyclic phenomenon and depending on its species, cycle varies between 5 to 120 years. Vegetative propagation in bamboos is practised through offsets but these rhizomes and propagules are cumbersome. Micropropagation is an expensive technology and therefore seed serve as the best source of propagation on large scale. Seeds serve as the best material for large – scale plantation, germplasm conservation and improvement of genotype. When seeds deteriorate, they lose vigour and become more sensitive to stresses upon germination. Eventually seeds lose the ability to germinate. bamboo seeds, have very short viability of 1-3 months and are therefore useful as propagules for only a short period of time. The present study shall look into some aspects of this problem so that protein content of seeds decrease with ageing and how content is more under controlled conditions compared to natural conditions.

MATERIALS AND METHODS

The protein content in the seeds were estimated by using the method given by Bradford assay (Bradford, 1976), using bovine serum albumin as standard.1g of seeds were crushed in 3 ml of the homogenization buffer and centrifuged at 5000 r.p.m. for 30 minutes. The supernatant was collected and used for quantitative and qualitative analysis of proteins.

Procedure for quantitative estimation

Reagents

The kits for Bradford assay *i.e.* Bio- Rad protein assay bufferor dye reagent was used. The dye was diluted with distilled water in the ration of 1:4 and then filtered through Whatman #1 filter paper. It could be stored up to 2 weeks at 4 degree Celsius. Standard Bovine Serum Albumin solution (10mg/100 ml distilled water) was prepared

Procedure

0.1 ml of the protein extract was taken in a test tube and 5 ml of Bio- Rad protein reagent prepared was added. This was allowed to vortex and stand for 10 minutes.The blank was prepared containing 0.1 ml of the homogenization buffer.The absorbance was read at 595 nm within 1 hour. A reference curve was prepared by using 0.1, 0.2, 0.4, 0.6, 0.8,1 concentration of BSA and reading the absorbance at the same wavelength (595nm) as in above.The amount of proteins in the different samples was calculated by using above reference curve and the readings were expressed in g/100g fresh weight.

Procedure for qualitative estimation

For qualitative studies of proteins in the bamboo shoots SDS-PAGE (Sodium dodecyl sulphate – Polyacrylamide Gel Electrophoresis) was used.

SDS –PAGE according to Laemmli Procedure

A gel size of 12 cmx15.5cm×0.5 mm was made for which 19cmx 17 cm glass plates were used.The glass plates were cleaned, dried and stored in dust free boxes. Three spacers of 0.5 mm thick were smeared with wax (Metroark 211 compound) on both surfaces and placed on the boundary of the three sides of the glass plate, leaving the upper side opened. Another cleaned plate having a notch of 2 cm deep and 14 cm wide was placed with its notched side facing towards the open side above the earlier plate. The assembled plates were held with fold-back clamps. It is made sure that the vertical spacer are in contact with the horizontal one at the bottom. A small line was marked at 4 cm below the top of the notched plate. That was for the indication of the level of separating gel when it was poured.

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The mixture was immediately poured into the assembled plate by using a pipette up to the mark and overlaid with 2 ml of 50 % 2- propanol. The gel was left to polymerize for 30 minutes.When the gel has polymerized the liquid from the upper part of the gel was removed with a strip of Whatman 3 mm paper.

The mixture was poured above the separating gel and a polystyrene comb was inserted. The edges were overlaid with 50 % 2-propanol and the gel as left to polymerize for about 1 hour. When the gel has polymerized, the comb was removed and top of the gel cleaned with a strip of whatmann 3 mm paper. The clamps and the horizontal spacer at the bottom were removed with the aid of a spatula. The space between the plates at the bottom was cleaned by using a thin spatula covered with tissue paper. The glass set having the gel was clamped to the electrophoresis tank, the lower chamber of which has been filled with electrode buffer. 20 µl of each sample was loaded into the bottom of each well by suing a micropipette fitted with a long narrow tip. The well on the left corner was loaded with the standard protein marker. A medium ranged molecular weight protein marker (Bangalore Genie) was used in the present study. The electrodes were connected to the power supply and the gel was run at 150 V at room temperature until the tracking dye crossed the stacking gel. The voltage was then increase to 200V. The current was stopped when the tracking dye was left for about 1 cm to reach the bottom. The assembled plates were removed from the electrophoresis chamber. The upper plate was separated with the aid of a spatula by pressing from one corner. This was followed by removal of the stacking gel by using a scalpel. The gel was then preceded for silver staining.

Staining procedure

The gel was soaked in 50% methanol for 60 minutes.The gel was then ringed in 200 ml of distilled water twice for 10 minutes each.0.8 g of silver nitrate was dissolved in 4 ml of distilled water. To make the staining solution, the above solution was added to a mixture of 21 ml 0.36% NaOH and 1.4 ml of 14.8 M Ammonium hydroxide . The volume was then raised to 100 ml.The gel was soaked in the staining solution for 15 minutes in the dark.The staining solution was discarded and the gel ringed twice in 200 ml distilled water for 5 minutes each.The developing solution was prepared by adding 1 ml of 1% citric acid and 100 µl of 38% formaldehyde in 200 ml of distilled water.The gel was then soaked in the developing solution until the bands become visible. The bands can usually be seen in 2-15 minutes. After bands of desired intensity were observed, the gel was rinsed in 3 changes of about 200 ml of distilled water.

RESULTS AND DISCUSSION

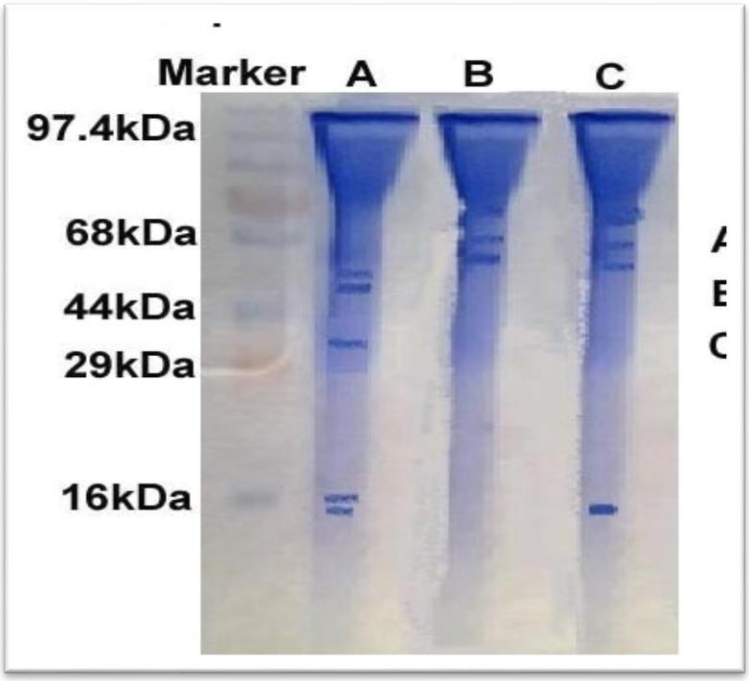

Fig 1: Fresh seeds SDS PAGE of fresh seed proteins of A)*B. bambos* B)*D. strictus* and *C) D. hamiltonii*

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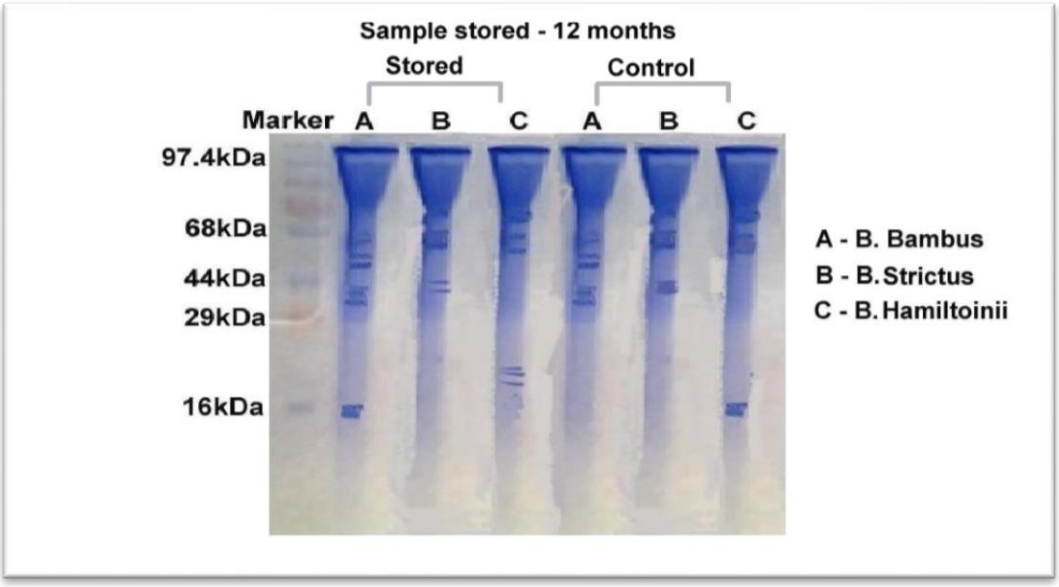

Fig 1a: SDS PAGE of one year old stored(dry freezer) and naturally aged seed proteins of A) *B. bambos* B) *D. strictus* and C)*D. hamiltonii*

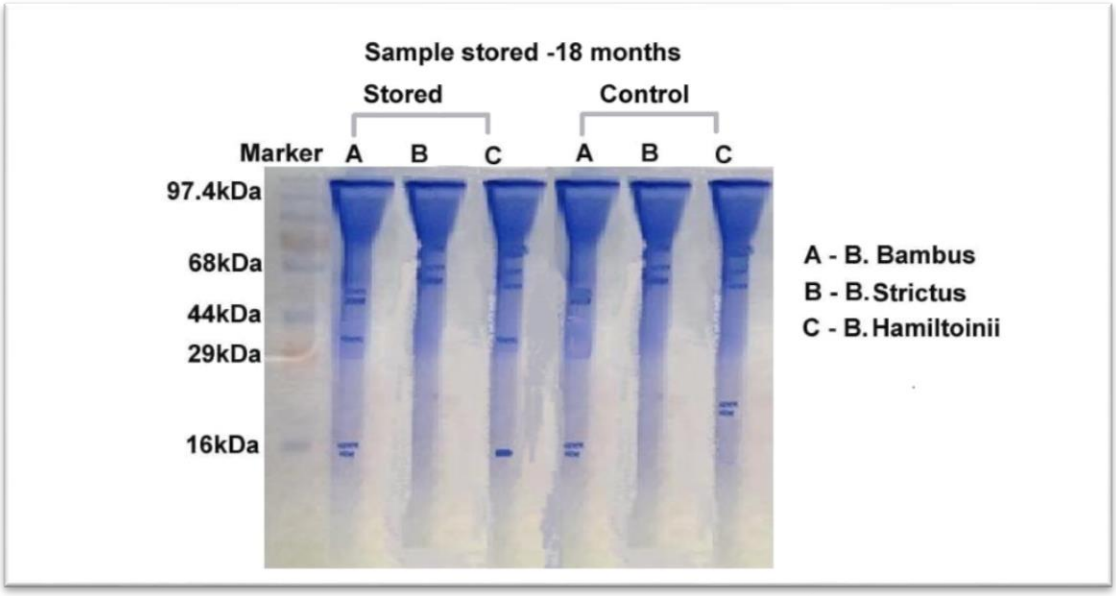

Fig 1b: SDS PAGE of 18 Months old stored(dry freezer) and naturally aged seed proteins of A) *B. bambos,* B) *D. strictus* and C) *D. hamiltonii*

In electrophoregram of fresh seeds, all the major proteins of 97.4,44,29 and 16kda expressed in high intensity.(Fig 1) In one year aged seeds, electrophoregram profile of the proteins in the seeds of all the presently studied bamboo species showed a wide renge of proteins with variable molecular weight ranging from 16-97kda .Molecular weight of 97.4 KDa polypeptide was present in low intensity in seeds of *D.hamiltonii, D. strictus* whereas these were not unable to expresss in seeds of *B. bambos* (Fig 1a).In naturally aged seeds of *D. strictus* it expressed in medium intensity.Band of 29kda protein expressed in high intensity in all the controlled ageing seeds while in naturally aged seeds it expressed in low intensity only in *D. strictus* whereas it was not expressed in *D. hamiltonii and B. bambos*. It also expressed in medium intensity controlled ageing seeds of all the species except in seeds of *D. hamiltonii* where it expressed in low intensity. Polypeptide of 68kda expressed in medium intensity controlled ageing seeds of *D. hamiltonii D.strictus* seeds, this polypeptide expressed in low intensity. In case of natural ageing seeds of *D. hamiltonii, D. strictus*. In case of polypetide of molecular weight 44kda, it expressed in high intensity in *D. hamiltonii,* in medium intensity in *B.*

bambos. Polypeptide of molecular weight neither in controlled ageing seeds nor in naturally aged seeds.It was observed that polypeptides of different molecular wiight expressed in varying internsities which elucidated the behaviour of different proteins with decrease in vigour and viability with ageing. After one year analysis polypeptide of 97.4kda expressed in low intensity in controlled stored seeds whereas unable to expressed in high intensity all the three species of controlled stored seeds whereas in naturally aged seeds it expressed in low intensity only in *D.strictus* whereas it was not expressed in *D. hamiltonii and B.bambos.* After 18-months of ageing, polypeptide of molecular weight 29kda and 16kda were not expressed in any of molecular weight 29kda and 16kda were not expressed in any of the three species neither in controlled ageing seeds nor in naturally aged seeds It has been observed that protein oxidation can cause modification of amino acid side chains, backbone fragmentation, protein dimerization or aggregation, and the unfolding or altered conformation of proteins(Hawkins and Davies, 2001). These structural changes alter the functional activity of the modified proteins such as their ability to modulate gene expression, cell signalling, apoptosis and necrosis. Reactive intermediates from protein peroxides can induce chain reaction that cause damage to other intracellular targets such as DNA lipids and other proteins(McDonald, 1999). Protein modification are often associated with ageing and diseases(Stadtman, 1992). However, protein oxidation may provide a mean by which reactive oxygenspecies following imbibition of mature dry seeds (Job *et al.,* 2005).While some seed lots retain their full germination capacity during prolonged storage, other deteriorate very rapidly, often with a characteristic abrupt drop in the germination capacity (Kaewnare *et al.,*2008). A major cause of deterioration in these seeds is probably due to the oxidation of intracellular macromolecules (lipids and proteins). However, it is very difficult to prove causes and effects of the mechanistic features underlying seed deterioration.

CONCLUSION

There are a lot of well expressed polypeptide fractions in the seeds of bamboos. During germination the variety of the proteins tend to decrease, more evidently in the phosphate buffer extracts, which is normal because of the transformations suffered by the proteins. The number and quantity of proteins with high molecular mass decreases during ageing.

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