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Comparative Studies of Protein Patterns in Five Mulberries (*Morus Alba* L.) Cultivars Through SDS-PAGE

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ABSTRACT

The present study is aimed to analyze the protein patterns in five mulberry varieties viz., V_1, $S_{13,}$ $S_{36,}$ M_5 and ATP. The patterns of protein bands observed in 3nd, 5th ,7th and 9th leaves of the said varieties from the apex. Different stages of leaf protein bands using 12% of Sodium Dodecyl Sulphate poly Acryl amide gel electrophoresis (SDS-PAGE) indicated a distinct pattern of seven protein bands and some additional bands with poor resolution observed in different leaf growth stages. The study demonstrated the variability in leaf protein content. It is high significant in 3rd leaves and less significant in7th and 9th leaves.. Gradually the number of protein bands decreased from 3rd leaves to 9th leaves in five different varieties. Protein band patterns of 45kDa, 86 kDa were common in all stages of mulberry varieties.

Key words: Mulberry leaf protein profiles, SDS-PAGE,

Introduction

Mulberry has immense economic importance in silk industry due to its foliage, which constitutes the chief food for the silkworm, *Bombyx mori* L, improves food value. Mulberry leaves are very rich in protein (15-35%), minerals (2.42-4.71% Ca, 0.23-0.97% P), metabolisable energy (1130-2240 Kcal/Kg), and absence of anti-nutritional factors (Omar *et al.,* 1999; sanchez, 2002, 2000; saddul *et al.,* 2003; sarita *et al.,* 2006). Mulberry 3 rd leaves containing highest amino acids and high moisture content. As young worms require leaves of more water content for easy ingestion, thus they should be fed with young leaves. While late age silkworms fed with mature leaves as they have strength to cut and ingest the hard leaves. Mature leaves do not have sufficient protein and water.

Nearly 70 percent of the silk produced by silk by silk worm is directly derived from proteins of mulberry leaves. The cocoon shell weight relative to the total amount of mulberry leaves consumed by the silk worm (Machii & Katagiri 1991). It is therefore possible that an increase in the protein level of mulberry leaves may lead to improvement in cocoon productivity.

Proteins are the building blocks of an organism. Protein quantification studies by SDS-PAGE reveal the molecular weight of the protein in the leaves. Protein bands appear and disappear on different stages of mulberry leaves. Here we compare the variation of four stages of the mulberry leaves protein molecular weight bands. In the upper (35 -68 kDa) and low molecular weight zones (below the 29 kDa) compare to the leaf stages of bands.

Materials and Methods

Mulberry varieties of V_1, S_{13}, S_{36}, M_5, and ATP were obtained from the R S R S (Regional Sericulture Research Station), Ananthapuramu, India. Mulberry leaves from one year old plants were used to test from the top of the plant i.e., from 3rd, 5th, 7th, and 9th leaf collected the different mulberry varieties. Method of Lowry *et al.,* (1951) was adopted for the quantitative estimation of protein and was expressed in mg/g.

Preparation leaf extract

500mg of leaf sample from the genotypes from different varieties. Sample extracted from the apex of 3rd, 5th, 7th, and 9th leaf. They were washed well to clean surface dust. The plant samples powdered by using liquid nitrogen and extracted in 50ml. Tris buffer grind with all stages of leaves and the homogenate was centrifuged at 5000 g for 10 minutes, the supernant was taken out and used as leaf extract, the concentration of leaf extract is 1mg/ml.

Estimation of total leaf protein by Lowry's method

The content of proteins was estimated according to Lowry *et al.,*(1951).The protein sample solution was taken in a test tube. To this sample 5ml of alkaline solution was added mixed thoroughly and allowed to stand at room temperature

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for 10' then 0.5ml of diluted Folin's reagent 1:1 was added rapidly mixed and allowed for incubation for 30'. The colors developed was read at 750 nm against reagent was calculated from a standard curve prepared from bovine serum albumin. A standard graph was plotted b taking concentration of protein in the X-axis and absorbance in the Y-axis. From the graph, the amount of protein present in the test tubes was calculated. Blank was prepared by using distilled water.

Results and Discussion

The quantitative estimation of proteins bands separated the 3rd, 5th,7th and 8th leaf was carried out, also the qualitative analysis was done by SDS-PAGE . Electrophoresis analysis separated 10-12 bands in different leaf stages of five mulberry varieties. About 12 clearly detectable mulberry protein bands over a wide range of molecular weight 18.4 kDa to 97.4 kDa were recognized (Figure 1to 4).The number of protein bands gradually decreased from 3rd leaf to 9th leaf. Especially, in the bands migrating in the upper (35 -68 kDa) and low molecular weight zones (below 29 kDa) figure 1to 4.

It is observed that, 15 kDa, 18kDa, 45kDa, 68kDa were found common through figure1 of the five mulberry varieties. The 15 kDa small subunit is common for all of the five mulberry varieties.

15kDa, 25kDa, 45kDa, 67kDa, 84kDa, 86kDa were common in all five varieties in figure 2. 15kDa, 21kDa, 32kDa, 45kDa,84 kDa, 86 kDa were common in all five varieties in figure3 leaf from top. Protein band 68kDa was absent in figure 4 leaf in s13 variety being present in remaining four varieties.86kDa,45kDa bands are common in all stage of five mulberry varieties. Leaf protein profile of variety s_{13} revealed five major proteins (poly peptides). While 64, 55 and 44kDa proteins were present with quantitative variations. In s_{13} 44kDa protein was the major component of the leaf protein (Muniswamy *et al.,*). Two mulberry varieties *Morus alba* var. kokuso-27 and *Morus indica* var. kanva-2 were compared with *Morus alba* var. Balady in their effects on the protein banding patterns of 5th -instars larvae of *Bombyx mori.* Kanva-2 produced bands at 251,74 and 8kDa; and Balady was characterized by bands at 38 and 11 kDa(Somia S E1-Akkad *et al.,*) In mulberry variety S_{34} significant decrease in the quantity of 55kDa protein was observed in Gamma-rays dosage from 7kr to 10kr.(Reddy Muniswamy *et al.,*)

Rubisco (Ribulose-1, 5-biphosphate carboxylase/ oxygenase) is a major soluble chloroplast protein, which can account for up to 50% of total protein in mulberry leaf. The large sub unit of the native enzymes has a molecular weight of 55 kDa. Rubisco is the most abundant protein on the earth, which captures CO_2 in the first reaction of photosynthesis. It is a large molecule having a molecule mass of about 560 kDa, consisting of eight copies of two types of sub units within this holoenzyme.

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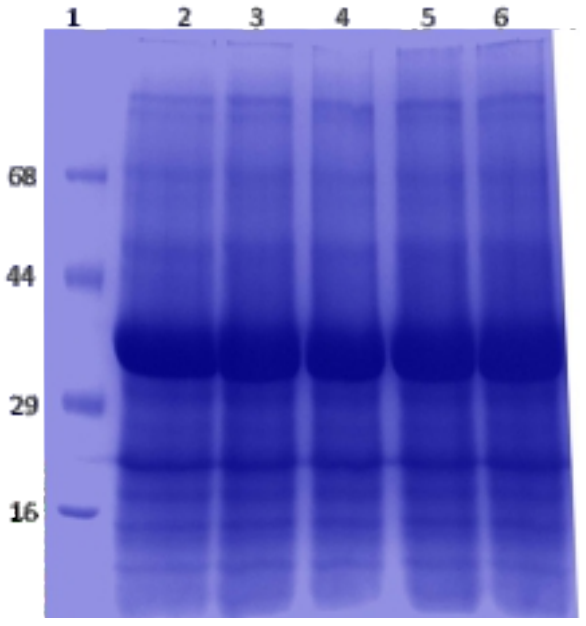


Figure 1: 3rd leaf

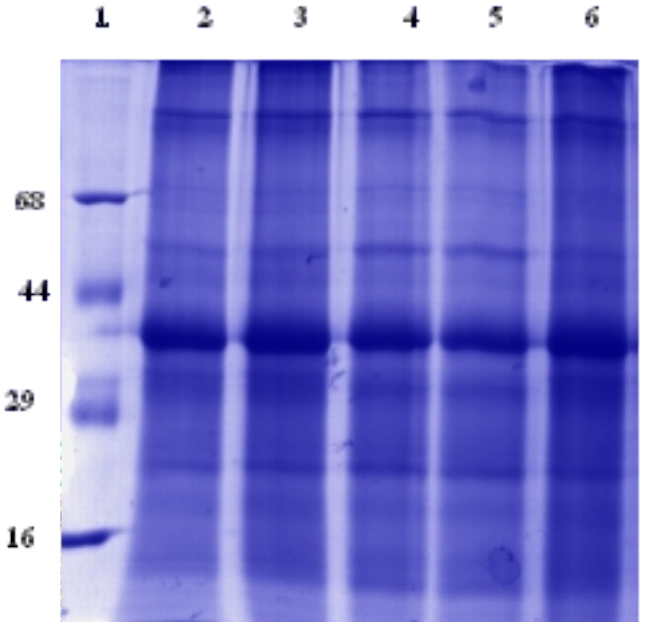
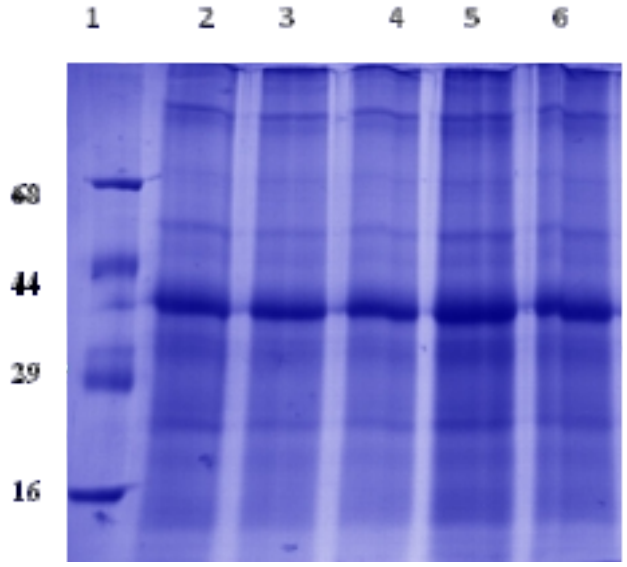


Figure 2: 5th leaf

SDS-PAGE was carried out on 12% poly Acrylamide gel and stained with Coomassie brilliant blue. Lane-1 Standard molecular weight protein marker. Lane2 V_1, Lane 3S_{13}, Lane 4S_{36}, Lane 5 M_5, Lane 6 Anantha.



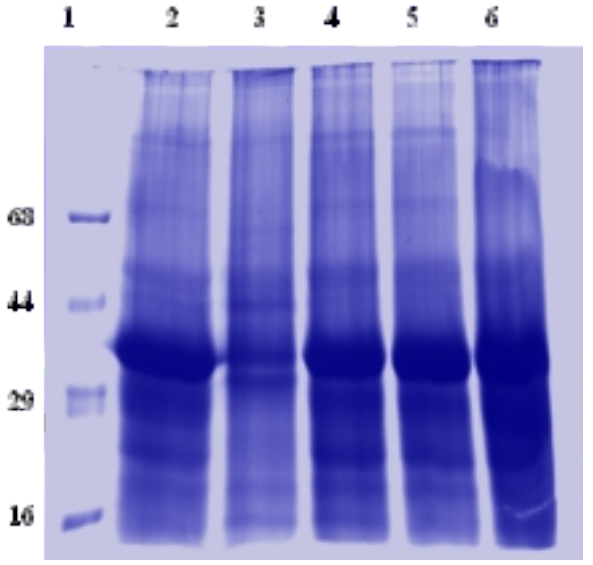


Figure 3: 7th leaf ***Figure 4: 9 th leaf***

SDS-PAGE was carried out on 12% poly Acrylamide gel and stained with Coomassie brilliant blue. Lane-1 Standard molecular weight protein marker. Lane2 V 1, Lane 3S13, Lane 4 S36, Lane 5 M5, Lane 6 Anantha

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