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Analysis of Protein from Pea (*Pisum sativum*) and Gram (*Cicer arietinum*) by Electrophoresis and Paper Chromatography

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Abstract: Protein is not only essential compound of animal but it is also important constitute of plants. Plants are good source of proteins. These proteins are mostly analyzed and identified from the fruits, seeds or any other parts of plants which are mostly used in diet but there are many other types of protein which are synthesized by other parts also, if one can know about synthesis of those proteins at different growth periods of different parts of plant then one may get interesting results. In this study stem of two plants were used for the analysis of protein i.e Pea (*Pisum sativum*) and Gram (*Cicer arietinum*). Molecular weight of protein was analyzed by SDS/Polyacrylamide Gel Electrophoresis and protein was stained with Lawsone dye by paper chromatography. Lawsone dye is protein dye which is mostly used for the staining of hair, skin and nails protein but in this research same dye was used for the staining of plant protein through paper chromatography.

Key words: Plant protein, lawsone dye, molecular weight of protein

INTRODUCTION

Protein is not only functional or structural molecule of animals and plants (Stayanarayana and Chakrapati, 2007; Jain *et al*., 2006; Vasudevan and SreeKumari, 2005) but it can also be used in the diagnosis and treatment. Plants are good source of proteins. These proteins are mostly analyzed and identified from the fruits, seeds or any other parts of plants which are mostly used in diet but there are many other types of protein which are synthesized by other parts also, if one can know about synthesis of those proteins at different growth periods then one may get interesting results (Schiltz *et al*., 2005).

In this research work two plants i.e Pea (*Pisum sativum*) and Gram (*Cicer arietinum*) were grown and protein was analyzed by SDS/Polyacrylamide Electrophoresis Gel and Paper Chromatography. In Paper Chromatography Lawsone dye was used which is obtained from Henna (*Lawsonia inermis*). It is a shrub or small tree. Henna leaves contain glucoside colouring matter Hennotannic acid which are generally known as Lawsone (Wallis, 2005; Willaim, 2002; Dhumal *et al*., 2005; Osman and Van Noort, 2003; Kang and Lee, 2006). Dried leaves of Henna are used in cosmetic and medicine such as Cardio inhibitor, hypertensive, intestinal anti spasmodic and uterine sedative effect etc. Lawsone is also used for the treatment of allergic reaction (Rund *et al*., 2007; Nikkel *et al*., 2001; Hazra, 2002). It is grown in Pakistan, India, North Africa, China, Egypt, Florida, Sudan, Iran, Malagasy and Australia (Syed, 1989). Henna leaves extraction is used in Pakistan and India for the adornment of hands and feet of Bride and ladies on special occasion such as engagement, wedding ceremony and eid festivals etc (Petkewich, 2006; Brancaccio *et al*., 2002; Ali and Sayeed, 1988). This means that the Henna leaves contain dye which stain the protein of hair, nails, hands and feet (Syed, 1989; Petkewich, 2006). Pea (*Pisum sativum*) and Gram (*Cicer arietinum*) belong to Fabaceae family (Ali, 1977). In this study protein was analyzed from three different growth stages of stem of Pea and Gram.

MATERIALS AND METHODS

Collection of pea and gram samples: Gram and Peas were sown in first week of December 2008 by drilling method on the experimental field of Institute of Plant Sciences, University of Sindh, Jamshoro. Plot size for each crop was 10 x 10 meters and normal agronomic practices were performed from sowing till harvest. Stem samples were collected at three different periods, that is, after 2 months (pre-flowering Stage), after 3 months (flowering Stage) and after 4 months (fruiting Stage) denoted P-1, P-2 and P-3 for Peas and G-1, G-2 and G-3 for Gram. At each growth stage stem samples were collected from lower, middle and upper part of the same plant and were replicated four times.

Preparation of solution:

- **Stacking gel buffer:** Tris (6.0 g) dissolve in 48 ml of 1 M HCl and adjust pH-6.8 and make final volume to 100 ml with water.

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Resolving gel buffer stock (Tris HCl, pH-8.8): Take 36.3g Tris and add 48 ml of 1 M HCl and adjust pH-8.8 and make final volume 100 ml with distilled H_2O.

Stacking gel buffer stock (Tris-HCl, pH-6.8): Take 6.0 g Tris, 48.0 ml 1 M HCl and make final volume 100 ml with distilled H_2O.

Staining solution: Take 1.25 g of Coomassie Brilliant Blue (G-250) and add 200 ml methanol and 35 ml Glacial acetic acid make final volume to 500 ml with distle water. Filter the solution for removing of undissolved material and store at room temperature.

Destaining solution: Take 75 ml of Glacial acetic acid and 50 ml of Methanol, mix it and make the final volume to 1L.

Sucrose (40%, w/v): Dissolved 40 g of sucrose in 100 ml of distilled water.

0.01% Bromophenol blue solution: 10 mg of Bromophenol blue dissolved in 100 ml distilled water.

Acrylamide-Bisacrylamide stock solution: Take 30.0 g of Acrylamide and 0.8 g of bisacrylamide dissolved in 100 ml distilled water. Filter the solution through filter paper.

1.5% (w/v) Amonium Persulphate (APS): Dissolved 0.15 g of APS in 10 ml of distilled water.

N, N, N', N'- Tetramethyl Ethylene Diamine (TEMED): Supplied by manufacturer.

Staching gel (2.5%): Take 2.5 ml of acrylamide-bisacrylamide stock solution, 5 ml of stacking gel buffer stock solution, 1.5 ml of 1.5% APS, 0.015 ml TEMED and 11 ml of distilled water, make final volume 20 ml.

Resolving gel: Take 7.5 ml of acrylamide-bisacrylamide stock solution, 3.75 ml of resolving gel buffer stock solution, 1.5 ml of 1.5% APS, 0.15 ml of TEMED, 17.25 ml of distilled water and make final volume 30 ml.

Sample preparation: Take 0.2 ml of each sample, add 0.1 ml of sucrose (40%, w/v) solution, add one drop of glycerol (Sawhney and Randhir, 2006).

Procedure for electrophoresis: Load 0.1 ml of each sample and marker on the top of glass tubes, only one sample should be loaded onto each of the tube. Supply 3 mA/tube current, tracking dye migrate to about 1 cm from the bottom end of the tube, this will take 1-2 h. Turn off the power supply, remove the gel from the tube. Place the gel in staining solution for 3-4 h and then destained in destaining solution until background of the gel is clear. Calculate the molecular weight of Protein of samples against the molecular weight of marker (Sawhney and Randhir, 2006; Schiltz *et al*., 2005; Zor and Selinger, 1996; De St Groth *et al*., 1963).

Extraction of protein: 1 g of each plant portion was grinded in pestle mortar with 1.0 ml of 0.1 M Phosphate buffer pH 7.5. These samples were kept overnight for complete extraction of protein. These were centrifuged at 16.163 g for 20 min and repeat it for 20 min more. The supernatant was used for protein analysis and pellet was discarded (Sawhney and Randhir, 2006).

Preparation of spray solution: 2 g of Lawsone dye in 100 ml of alcohol and 7% acetic acid (1:1) was prepared (Ali and Sayeed, 1988).

Selection of suitable pH for staining through paper chromatography: Protein extractions of all above three plants and Bovine Serum Albumin were placed on Chromatographic paper (Whatman-1) at point 1-2 and 1-4 respectively. Lawsone dye 2% (alcoholic) solution of pH-2, 3, 4, 5, 6 and 8 was sprayed on spots. The maximum darkness of the spots was selected for the staining. Place also solution of glucose, Galactose, Maltose, Starch, Rapeseed oil and Olive oil on chromatogram and sprayed with 2% Lawsone dye solution (Sawhney and Randhir, 2006; Ali and Sayeed, 1988).

RESULTS

Molecular weight of protein by SDS/PAGE method: Plants (Pea and Gram) were used for the determination of molecular weight of protein by SDS/PAGE. Electrophoresis is an Electochromatographic technique which is utilized for the separation of proteins, in which charged particle move in electric field (Sawhney and Randhir, 2006; Osset *et al*., 1989). In SDS-PAGE Coomassie dye was used for the staining of protein bands on the electrophoresis gel (Atta *et al*., 2004). Protein concentration in different bands in the gel was determined from three growth stages of stem of Pea i.e P-1, P-2 and P-3 and stem of Gram i.e G-1, G-2 and G-3 by using total protein marker (standard) for the calculation of molecular weight. Protein assigned all expected compartment fractions of the samples (Fig. 1, 2 and Table 1, 2).

Table 1: Electrophoresis determination of molecular weight of protein by SDS/PAGE from three stages of stem of Pea

Bands	P-1 Molecular weight	P-2 Molecular weight	P-3 Molecular weight
1	58.05 KDa	58.05 KDa	48.96 KDa
2	50.00 KDa	48.96 KDa	43.91 KDa
3	42.28 KDa	33.36 KDa	35.00 KDa
4	24.60 KDa	24.60 KDa	23.83 KDa
5	20.13 KDa	19.71 KDa	19.13 KDa
6	15.00 KDa	15.00 KDa	14.58 KDa

Table 2: Electrophoretic determination of molecular weight of protein by SDS/PAGE from three growth stages of stem of Gram

Bands	G-1 Molecular weight	G-2 Molecular weight	G-3 Molecular weight
1	48.96 KDa	48.96 KDa	39.46 KDa
2	41.86 KDa	41.50 KDa	28.52 KDa
3	35.89 KDa	35.98 KDa	18.42 KDa
4	24.80 KDa	25.36 KDa	15.60 KDa
5	.....	14.28 KDa	.....

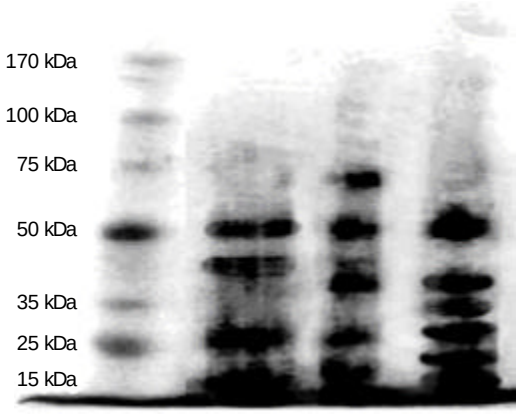

Fig. 1: Lane 1: Total protein; Lane 2: Protein from P-1; Lane 3: Protein from P-2; Lane 4: Protein from stem of P-3

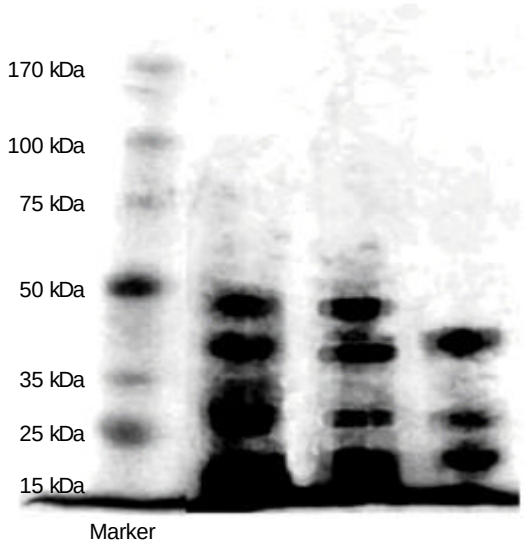

Fig. 2: Lane 1: Total protein; Lane 2: Protein of G-1; Lane 3: Protein from G-2; Lane 4: Protein from G-3

DISCUSSION

Protein from Three Stem Growth Stages of Pea (P-1, P-2 and P-3): Determination of molecular weight of protein in three stages of growth of stems of Pea (P-1, P-2 and P-3), (Table 1 and Fig. 1): In P-1 and P-2 only two types of bands (58.05 KDa and 24.60 KDa) are identical but all others are different but in P-1 and P-3, there was not any same protein band which means that protein of P-2 have similarity but protein content in P-3 was totally different from P-1 and same observations were seen by Schiltz *et al.* (2005) that as plant grew, it synthesized different types of protein at all stages and at the time of seed filling types of protein become changed. So there is great need of the research for protein synthesis that occurs during the growth of plant (Pandey *et al.*, 2006).

Protein from three stem growth stages of gram (G-1, G-2 and G-3): In case of Gram, four bands appeared at G-1 (Fig. 2, Table 2) and at G-2 only three bands appeared and one band is same as in G-1 but two bands have been changed in G-2 sample i.e 25.36 KDa and 4.28 KDa but in case of G-1 and G-3 there was totally different protein bands appeared and same observation was seen in G-2 and G-3. Result of Spectrophotometric methods and Electrophoresis shows that from first stage to the third stage of growth of plants, not only concentration of protein changed but types of protein was also changed (Pandey *et al.*, 2006; Muilerman *et al.*, 1982; Righetti and Drysdale, 1974).

Paper chromatography for protein: Paper Chromatography is a useful technique in which cellulose paper (Whatman-1) is used (Sawhney and Randhir, 2006). In this research work paper chromatography was used for the assessment of staining of Proteins, Carbohydrates and Lipids with Lawsone dye at the different pH i.e 2, 3, 4, 5, 6 and 8 (Ali and Sayeed, 1988) and then same dye was used for the staining of anatomical sections of plants. Extraction of three plants rachis of Siris, stem of Pea (P-1, P-2 and P-3) and stem of Gram (G-1, G-2 and G-3) were used. Chromatogram shows that protein stained with Lawsone dye at all pH but spot of protein were darker at acidic pH which contrasts with the study of Ali and Sayeed (1988) who reported that darker spots appeared at alkaline pH while the results of Catherine (2005) agrees with the present study while when samples of Carbohydrates (Glucose, Galactose, Maltose and Starch) and Lipids (Rapseed oil and Olive oil) were placed on Chromatogram and same Lawsone dye solution (of all pH) were sprayed, but spots did not appear in both cases i.e Carbohydrates and Lipids, so it is proved that Lawsone dye only stains protein molecules and it will not stain any other molecule and gave good results at acidic pH. In this research work pH-4 was used for staining of protein through anatomical sections of all above plants.

Conclusion: Thsi study was conducted to analyze the protein from plants through growth stages, as Pea and

Gram grow, the protein concentration increases and also types of protein changes at all stages. Paper Chromatography shows the results that Lawsone stain protein at acidic pH, that is why pH-4 is suitable for the staining of protein. In this study new and low cost Lawsone dye was introduced, in future this can also be used in Electrophoretic methods for the staining of protein.

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