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COMPARATIVE STUDY ON DIFFERENTIAL PROTEIN EXPRESSION IN DIFFERENT PARTS OF CHICKPEA PLANTLET, DURING ITS GROWTH IN TISSUE-CULTURE

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ABSTRACT

Chickpea plant was selected to be grown through the tissue culture technique. The protein isolated from the different parts of the plant (shoot, root, stem, leaves) were analysed electrophoretically (Sodium Dodecyl Sulphate Poly acrylamide Gel Electrophoresis [SDS-PAGE]). The same species of the plant was grown outside the tissue culture cabinet and the latter was compared with that of grown inside. The present study was an effort to isolate proteins from root, shoot, stem and leaves and it was found that the protein content isolated from the leaves was found to be maximum rather than the other portion grown in tissue culture medium.

KEYWORDS: *Chick pea, Tissue Culture, SDS-PAGE*

INTRODUCTION

Chickpea (*Cicer arietinum*) is an ancient crop that belongs to the legume family. It is widely grown in Africa, the Middle East and India for centuries and is eaten as a dry pulse or green vegetable.[1]It is almost a small bush with diffused, spreading branches. Chickpea seeds germinate at an optimum temperature (28-33ºC) and moisture level in about 5-6 days. The process of germination begins with absorption of moisture and swelling of the seed.[2] It is a deep rooting crop with a strong ability to take up water from deep soil layers but requires stress during the latter part of life cycle to hasten maturity, reflecting its indeterminate growth habit.[3, 4]Literature survey had revealed that less work has been undertaken on this present subject. So efforts have been made to extract proteins from different portions and also compare its growth both in tissue culture medium and outside.

MATERIALS AND METHODS

Pre-measured seeds were taken and dipped into absolute ethyl alcohol for 10 second rinsed with purified water. The seeds were then exposed to 10% (w/v) aqueous calcium hypochlorite solution followed by treatment with sterile water. The sterilized seeds were incorporated aseptically into the tissue culture flask containing Murashige and Skoog media[5] in the laminar flow bench. These flasks were incubated in the plant tissue culture incubator maintaining temperature at 25^0 C and adjusting light periodically.

The growth rate was monitored every day in the defined condition and under normal condition. The parts of the chick pea plant like root, shoots, and leaves were collected and were kept in -20^0 C refrigerators at regular interval for 30 days.

Extraction of protein

Six mortar & pestles were precooled by placing them in -20°C extraction buffer and microfuse tubes were also precooled by placing them in ice box. Chick pea samples at different stages of growth were preserved in -20°C. The

samples were taken out one by one and crushed out in precooled mortar. Sufficient amount of buffer was added and homogenized (4°C, 5000 rpm, 20 min). The separated supernatant was taken and were marked S1 to S6.Tubes were immediately transferred to ice box. To these 50µl of protein extraction buffer was added and again centrifuged. Supernatant was again collected and transferred to fresh tubes, labelled P1 to P6.These tubes were kept in ice box.

S1: Root after 5 days

S2: Root after 15 days

S3: Shoot after 10 days

S4: Shoot after 25 days

S5: Leave after 15 days

S6: Leave after 30 days

Protein estimation by Biuret method[6]

This procedure was followed by taking Bovine Serum Albumin (BSA) (5mg/ ml) as standard protein and the absorbance was measured at 540nm. Extracted protein from chick pea plantlet at different stages of their development from different parts of the plant was expected to show differential expression of gene and hence protein which was detected by SDS-PAGE.

SDS-PAGE[7]

SDS-PAGE, a type of electrophoresis where proteins of different molecular weights present in a mixture(extracted protein) are separated in an electric field according to their molecular weight on net negative charge by migration towards the positive electrode through a PAG plate. The glass plates, spacer, comb were washed with ethanol and allowed to dry. Glass plates and spacer were assembled such that they become leak proof. The resolving gel (10%) was casted. In this gel the Tetra Ethyl Methylene Diamine(TEMED) and Ammonium per Sulphate(APS) were added just when it was ready to cast. The empty space between the glass plates above the resolving gel was filled with distilled water and allowed to stand in an upright position for about 25-30 min for polymerization. After the specified time gap the rest of the plate between the glass plates was filled with stacking gel (4%). The casting of stacking gel was done only when the resolving gel was polymerized. The comb was inserted in an upright position in the stacking gel and the glass plate was allowed to stand for about 1 hour. After the stacking gel was polymerized the comb was removed safely and the sample well formed in the stacking gel was with distilled water. The gel was ready now to load and run it protein. Samples were diluted to lowest protein concentration with extraction buffer & mixed well.160µl of diluted solution & 40µl of 5X loading dye (bromophenol blue) were mixed well.After mixing of samples, 15µl of samples were loaded on the SDS-PAGE & run for 1 hour.The gel was then stained with coomassie brilliant blue &destained by destaining solution to look for the bands.

RESULTS

The protein extracted from the plantlet was compared with that of BSA using the standard curve. The results are tabulated as below in Table [I]:

Table: 1

	Test Tube 1	**Test Tube 2**	**Test Tube 3**	**Test Tube 4**	**Test Tube5**
Standard protein solution	---------	0.2 ml	0.4 ml	0.6 ml	0.8 ml
Protein conc. (mg/ml)	0	1	2	3	4
Absorbance (at 540 nm)	0	0.06	0.11	0.158	0.198

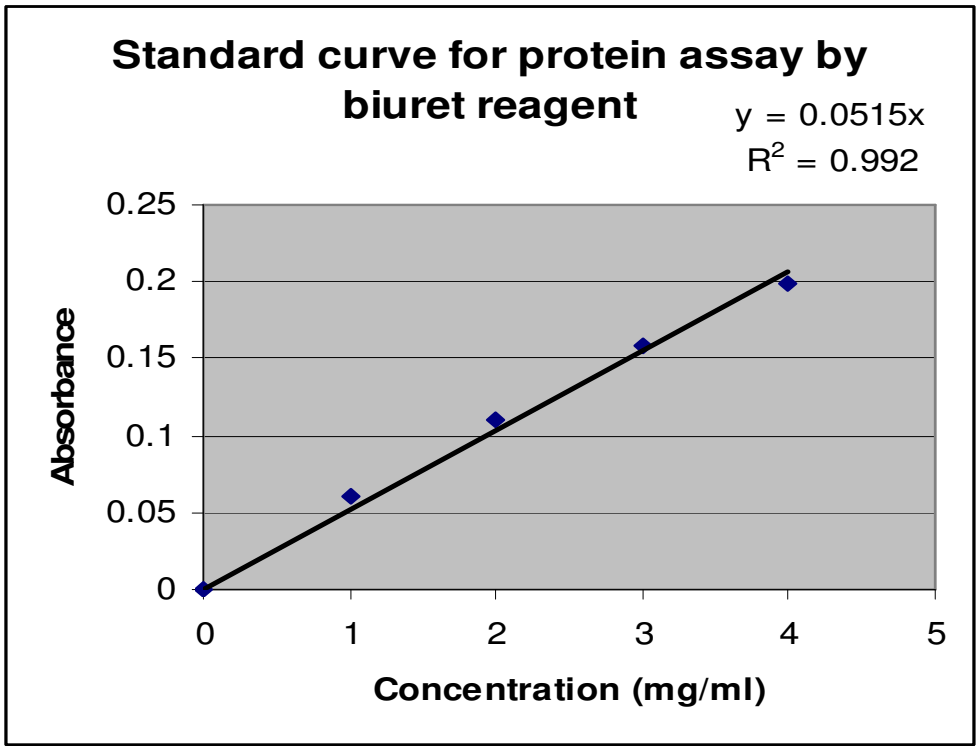

Fig. I: Standard curve for protein assay by biuret reagent

Determination of protein from the extraction of different parts

After extraction of protein from the different parts of the plant by protein extraction buffer, protein content was determined by biuret reagents. The resultant clear extract was taken 0.2 ml for protein activities.

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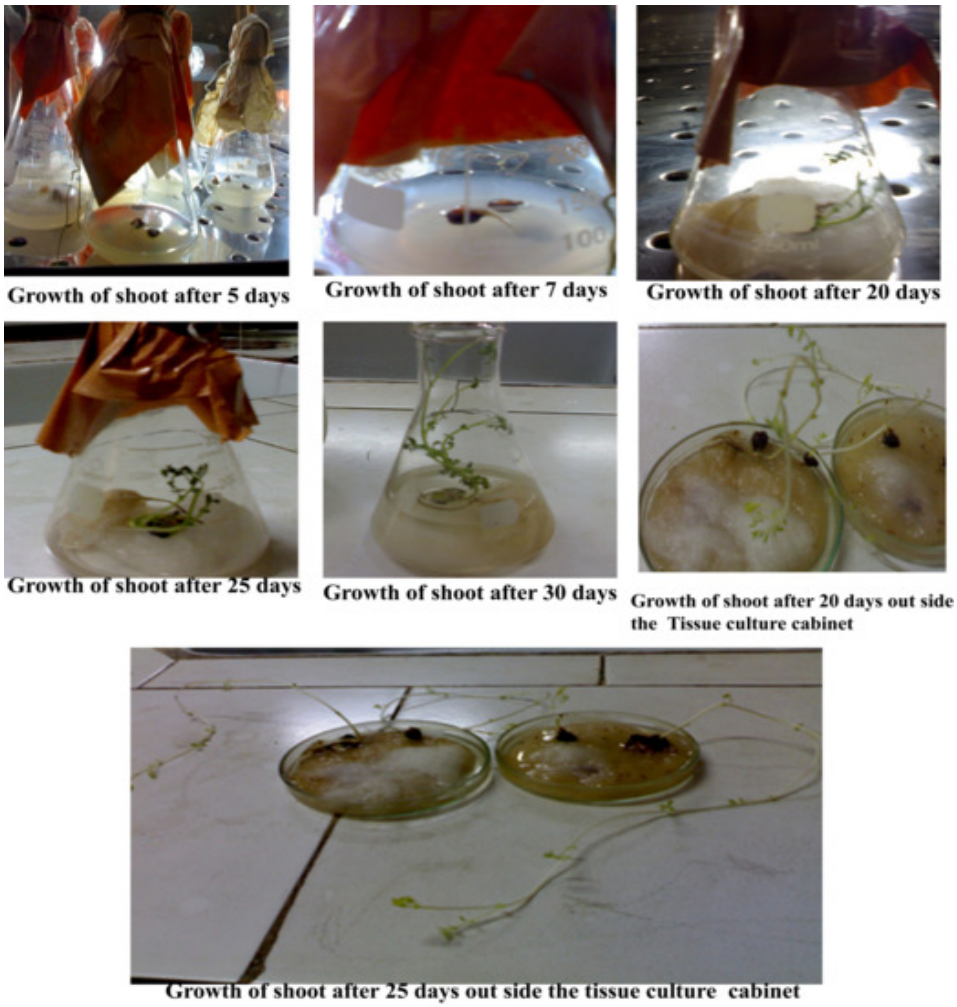

Fig. II: Growth of shoot of the chickpea plantlet inside and outside the tissue culture cabinet

Table [II]: Determination of protein from the extraction of different parts

	Sample S1	Sample S2	Sample S3	Sample S4	Sample S5	Sample S6
Absorbance (at 540 nm)	0.148	0.316	0.262	0.462	0.528	0.648
Conc. (mg/ml)	2.87	6.14	5.09	8.97	10.25	12.58

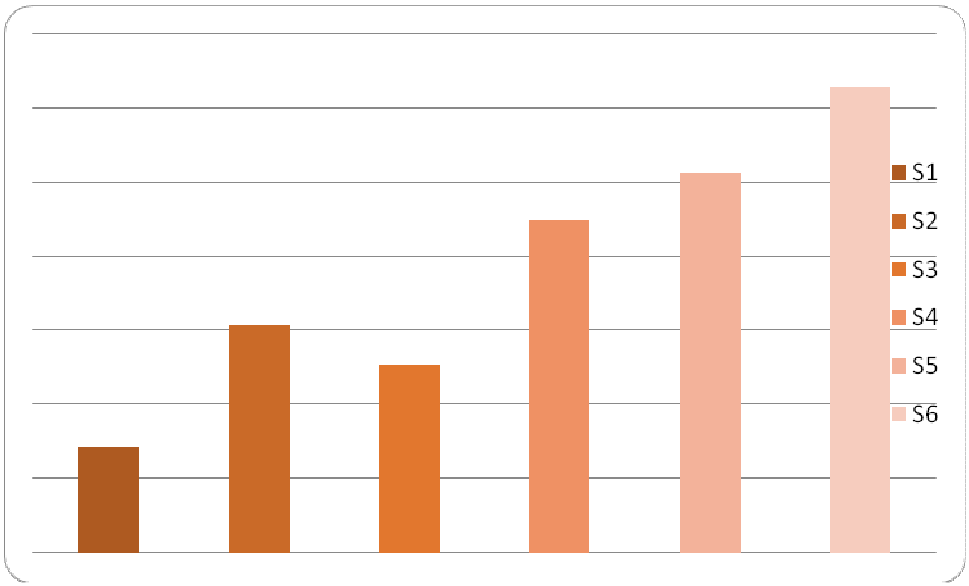

Fig. III: Comparative study of the differences of protein from the extraction of different parts

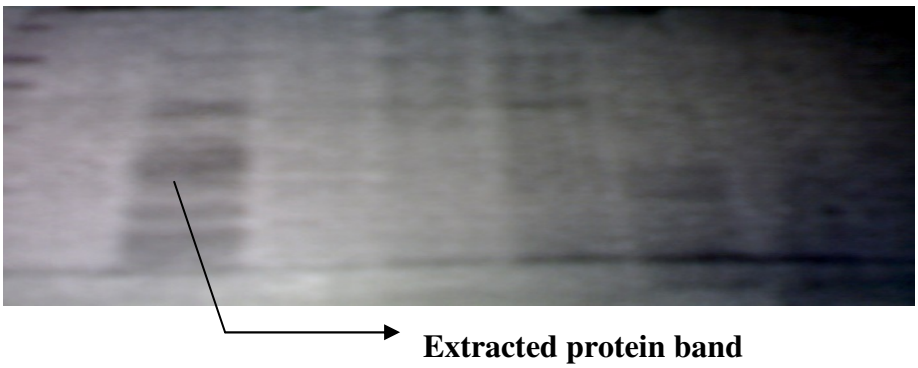

Fig. IV: SDS-PAGE of extracted protein

CONCLUSION

From the above study a conclusion can be drawn that protein isolated from the leaves of the chickpea plantlet is maximum which was clear from the bands that was electrophoretically obtained through SDS-PAGE. As protein itself is neutral in nature so for its separation through electrophoresis it has to be a charged particle which can be made through SDS-PAGE covering a layer on the protein molecule.

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