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In vivo and *in vitro* protein profiling in *Tinospora cordifolia*- An anti-diabetic plant

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Abstract-

Present investigation was the first attempt which deals with the *in vivo* and *in vitro* comparative study of protein level in *Tinospora cordifolia* (Willd.)., a nitrogen fixing tree. Protein was investigated in callus, root, leaf and stem by means of SDS-PAGE.. The nodal explants were taken from the *in vitro* seedlings and cultured in the MS medium supplemented on 2.5 mg/l of BAP and callus were initiated on 2,4-D (0.5 mg/l) . Though some differences were observed in the protein contents of *in vivo* and *in vitro* samples, the data proved that protein content in callus was higher than the stem following leaf and root. In this study it was found that *Tinospora cordifolia* (Willd.)., contained several protein bands of molecular weight 116, 110, 100, 68, 65, 63,61, 60, 45, 43, 36, 33, 32, 30, 29, 27, 23, 18.5, 14 kDa. These results indicate that the intensity of protein bands was high in *in vitro* sample compared to *in vivo* samples.

Key words: SDS-PAGE, *in vitro*, *in vivo*, callus, BAP, 2,4-D.

INTRODUCTION

Plant is a large, glabrous, deciduous, climbing shrub. The stem structure is fibrous and the transverse section exhibits a yellowish wood with radially arranged wedge shaped wood bundles, containing large vessels, separated by narrow medullary rays. The bark is creamy white to grey, deeply left spirally and stem contains rosette like lenticels. The leaves are membranous and cordate in shape. Flowers are in axillary position, 2-9 cm long raceme on leaflet branches, unisexual, small and yellow in color. Male flowers are clustered and female are usually solitary. The seeds are curved. Fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter [4]. While, Protein and enzyme analysis of stem in *Tinospora cordifolia*. (Aranha et al., 2012). RAPD analysis in *Tinospora cordifolia* (Gyana et al., 2006),. However, no research has been done in Tinospora for comparion of protein profile *in vitro* and *in vivo*. Although, other medicinal plant species were examined for their protein *in vivo* and *in vitro viz*. *Bacopa monnieri* (Mohapatra and Rath, 2005); *Boerhaavia diffusa* (Sharma, 2006).

MATERIALS AND METHODS

Establishment of aseptic seedlings

Nodal explant of *Tinospora cordifolia* were collected from nursery of University of Rajasthan , Jaipur. Then they were kept under running tap water for about 10 to 15 min followed by washing with 1% (v/v) Teepole (Ranklem-India) for 2 min and rinsed with double distilled water for three times. Prior to inoculation, sterilized nodal were again sterilized with 0.1% (w/v) aqueous HgCl2 for about 2 min followed by 2 to 3 rinsing with double distilled water in

Laminar Air flow cabinet. These sterilized nodal explant were inoculated on WPM medium in cultured bottles. After 18 to 20 days, bud initiation started and after it multiplication occures.

Callus induction and maintenance

The Murashige and Skoog (MS) medium was prepared by adding 3% sucrose as a carbon source and 0.8% (w/v) agar as a solidifying agent. Leaf as explants for callus induction on MS medium fortified with a series of 2, 4-D (0.6 mg/l). The pH of medium was adjusted to 5.8 ± 0.2 before autoclaving at 121° C for 15 min at 15 lb/in2. 20 ml of molten agar medium was poured into a culture bottle and plugged with non-absorbent cotton. All cultures were incubated in 16 h /8 h photoperiod under light intensity of 50 μE/ m2/s provided by cool, white and fluorescent light at 25 ± 2°C with 55% relative humidity. Each treatment performed using eight replicates and the experiment was repeated at least thrice.

Protein content determination

Leaves, stem, root collected from disease free and healthy plant of *Tinospora cordifolia*. The specimen was authenticated by the department of Botany, University of Rajasthan. Leaves, stem and roots were used as an *in vivo* sample for comparing protein content with green, friable calli from leaves obtained after 28 days. For protein estimation these samples were lyophilized, macerated in 80% ethanol and elucidated by the method of Lowry et al., (1951). Protein in the unknown sample was estimated at 660 nm using bovine serum albumin as standard and expressed per gm fresh weight basis.

Analysis of protein profile by SDS-PAGE

Electrophoresis has become a useful tool for the characterization of plant proteins. Protein profiles were studied by sodiumdodecylsulphate polyacrylamide gel electrophoresis (SDSPAGE) (Laemmli, 1970). A vertical slab gel apparatus as described by Studier (1973), Desatronic 3000/200 power supply and Frigostat, West Germany, were used during the electrophoretic work. In SDSPAGE, proteins are treated with sodium dodecyl sulfate (SDS) before electrophoresis so that the charge density of all proteins is made roughly equal. When these samples are electrophoresed, proteins are separated according to mass. The protein bands were visualized by transilluminator and photographs were taken for comparison of results.

RESULTS AND DISCUSSION

During the present set of experiment, the total protein estimated in callus, *in vivo* leaves, stem and roots (Figure 1) after that to find out the molecular weight of the total protein these were subjected to SDS-PAGE analysis. The proteins were found to be composed of a total of 62 bands ranging from 14.4 to 116.0 KDa were recognized (Figure 2). Protein profiles further showed variability on the basis of presence or absence and intensities of protein bands with banding pattern (Table 1). SDS-PAGE is considered as a reliable method of genetic characterization because electrophoretic patterns of the protein fractions are directly related to the genetic background of the proteins and can be used to certify the genetic make-up (Rehana et al., 2004). In order to estimate the variability at genetic level, SDS-PAGE banding pattern of the gel using total protein was investigated. Overall out of 62 protein bands, molecular weights 14, 18.5, 23, 27, 29, 30, 36, 60, 61, 63, 65, 68 KDa shown same protein banding pattern in callus , *in vivo* leaves, stem and root samples but with variation in

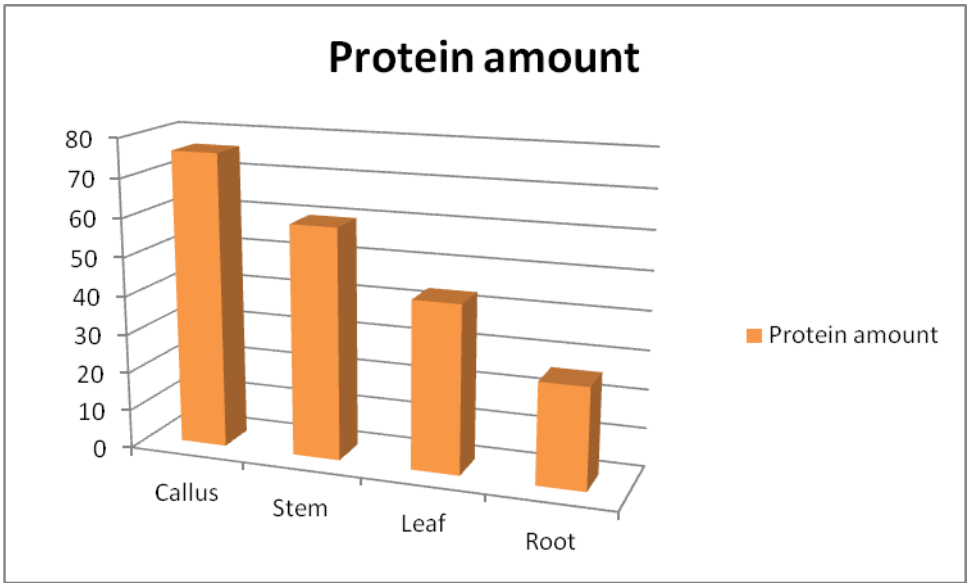
intensities. Left behind protein banding pattern exhibited a considerable range of variability with regard to their mobilities and intensities. Callus exposed at protein molecular weight ranging from 14.4 to 116.0 KDa and only lacking at 32 KDa. Root lacking at the molecular weight 30, 32, 43, 45, 100, 110 and 116 KDa but leaves absent in 32, 33, 110 and 116. Whereas, stem missing in the molecular weight of 33 and 110 KDa. From this we concluded that the highest numbers of protein bands were observed in callus followed by stem, leaf and root. Regarding this experiment, no similar and contrary results were available in *Tinospora cordifolia* , but in other plants, analogous results were reported in *Artemisia vulgaris* (Kumar and Ranjitha, 2009), *Glycine max* (L.) Merr (Radhakrishnan and Ranjitha, 2009), and *Plumbago zeylanica* L. (Rout et al., 2010). No research has been done contrary to these results.

Table 1- Protein profiling with intensities in *Tinospora cordifolia.*

Protein Bands	Callus	Stem	Leaf	Root
14	+++	+++	+++	+++
18.5	+	+	+	+
23	++	++	++	++
27	+++	+++	+++	+++
29	+	+	+	-
30	+	+	+	
32	-	+	-	-
33	+	-	-	+
36	+++	+++	+++	+++
43	++	++	++	-
45	+++	+++	+++	-
60	+++	+++	+++	+++
61	+++	+++	+++	+++
63	+++	+++	+++	++
65	++	++	++	+
68	++	++	++	+
100	+	+	+	-
110	++	-	-	-
116	+++	+++	-	-

+++ - Strong intensity, ++ - Moderate intensity, + - Weak intensity, - - Absent.

Figure 1- Protein amount in different plant parts



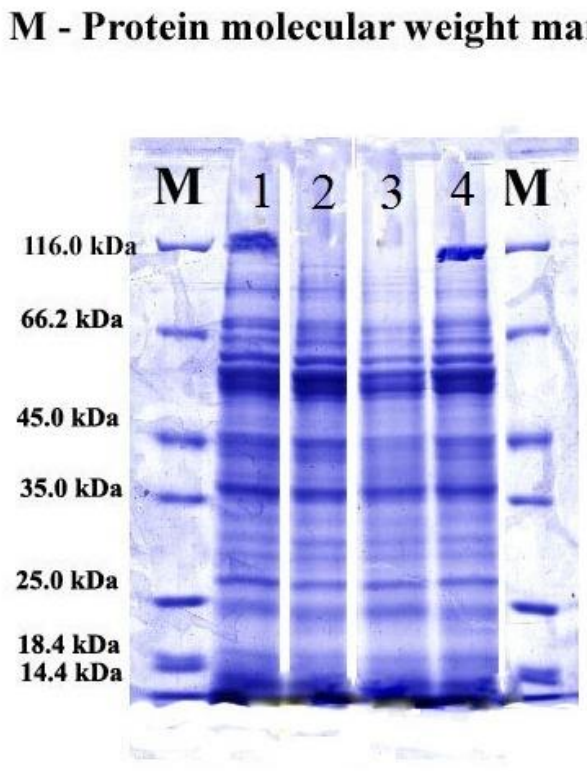


Figure 2- Protein analysis via. SDS-PAGE in *Tinospora cordifolia*

(Well 1- stem, Well 2- Leaf, Well 3- Root, Well 4- Callus)

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