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Full Length Research Paper

In vivo and *in vitro* protein profiling in *Acacia nilotica* (L.): A nitrogen fixing tree

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Present investigation was the first attempt which deals with the *in vivo* and *in vitro* comparative study of protein level in *Acacia nilotica* L., a nitrogen fixing tree. Protein was investigated in callus, seed, leaf and stem by means of SDS-PAGE. For obtaining the *in vitro* explant, the seeds were cultured on ½ MS medium under the *in vitro* condition. The cotyledonary nodal explants were taken from the *in vitro* seedlings and cultured in the MS medium supplemented in combination of 2, 4-D (0.4 mg/l) with BAP (0.25 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 seed following leaf and stem. In this study it was found that *A. nilotica* contained several protein bands of molecular weight 54.3, 44.1, 42.7, 40.1, 35.6, 31.2, 28.6, 24.7 and 19.5 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*

INTRODUCTION

Acacia nilotica L. is native to the drylands of tropic Africa. It is found in India, Australia and Africa. *Acacia nilotica* L. belongs to the family leguminoscae, commonly called as Babool or Kikar. Due to their ability of restore soil fertility, it is known as nitrogen fixing tree. Acacia species are a possible source of protein for human use (Bukhari, 2002). Acacias obtain their nitrogen from ground water rather than from atmosphere, but can produce more crude protein per ha than many grain crops (Prakash et al., 2001). At the molecular level, from the last century, many researches have been done on Acacia species. While, Protein and enzyme analysis of seeds in Acacia species (Ali, 1994). Seed protein analysis in Acacia species (Akkad, 2004), RAPD analysis in Acacia species (Rashmi et al., 2004), Genetic variability Analysis in *Acacia nilotica* L. (Ndir et al., 2008) and Extraction of DNA in *Acacia nilotica* L. (Sablok et al., 2009). However, no research has been done in Acacia species 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).

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MATERIALS AND METHODS

Establishment of aseptic seedlings

Mature seeds of *Acacia nilotica* L. were collected from Sirsi (Haatoj) District, Jaipur, Rajasthan. Prior to surface sterilization, seeds were treated in boiled water at 60°C for about 30 min and then soaked in distilled water for about 24 h. Then they were kept under running tap water for about 10 to 15 min followed by washing with 1% (v/v) Rankleen (Ranklem-India) for 2 min and rinsed with double distilled water for three times. Prior to inoculation, sterilized seeds were again sterilized with 0.1% (w/v) aqueous $HgCl_2$ for about 2 min followed by 2 to 3 rinsing with double distilled water in Laminar Air flow cabinet. These sterilized seeds were inoculated on half strength (Murashige and Skoog) MS salts medium in cultured bottles. After 7 to 10 days, seeds germinated and gave rise seedlings. These *in vitro* seedlings were used as source of explants.

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. *In vitro* cotyledonary node (1.0 cm) of 20 day old

Table 1. Protein profiling with intensities in *Acacia nilotica* L.

Marker molecular weight (KDa)	Leaf	Stem	Callus	Seed
61.5	-	-	+	+
60.9	+	-	++	++
58.1	-	+	+	+
43.9	-	-	+	+
42.3	+	+	++	+
40.7	+	-	+++	+++
35.6	+++	+++	+++	-
31.2	-	+	+	+
28.5	+++	+++	+++	+++
24.7	+	-	+	+
19.5	+	++	++	++

Strong intensity =+++, moderate intensity =++, weak intensity =+, Absent= -.

seedlings inoculated as explants for callus induction on MS medium supplemented with a series of 2, 4-D (0.4 mg/l) combination with BAP (0.2 mg/l).

The pH of medium was adjusted to 5.8 ± 0.2 before autoclaving at 1210 C for 15 min at 15 lb/in2. 20 ml of molten agar medium was poured into a culture bottle and plugged with nonabsorbent cotton. All cultures were incubated in 16 h /8 h photoperiod under light intensity of 50 µE/ m^2/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 and stem collected from disease free and healthy plant of *Acacia nilotica* L. The specimen was authenticated by the department of Botany, University of Rajasthan and the voucher specimen (Voucher No. RUBL 20432) was deposited for future reference in the Botany Department Herbarium. Leaves and stem were used as an *in vivo* sample for comparing protein content with green, friable calli from cotyledon 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 (SDS-PAGE) (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 SDS-PAGE, 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 seed (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 33 bands ranging from 14.4 to 66.2 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 33 protein bands, molecular weights 19.5, 28.5 and 42.3 KDa shown same protein banding pattern in callus , *in vivo* leaves, stem and seed 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 66.2 KDa. Seed only lacking at the molecular weight 35.6 KDa but leaves absent in 31.2, 58.1 and 61.1 KDa. Whereas, stem missing in the molecular weight of 24.7, 40.7, 43.9 and 60.9 KDa. From this we concluded that the highest numbers of protein bands were observed in callus followed by seed, leaves and stem. Regarding this experiment, no similar and contrary results were available in *Acacia nilotica* L. and Acacia species, 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. This paper presents and sheds light on the *Acacia nilotica* L. proteins which grow on the nile banks of Delta region and in arid regions. Therefore, very much attention should be given to arid species since they are under severing threats

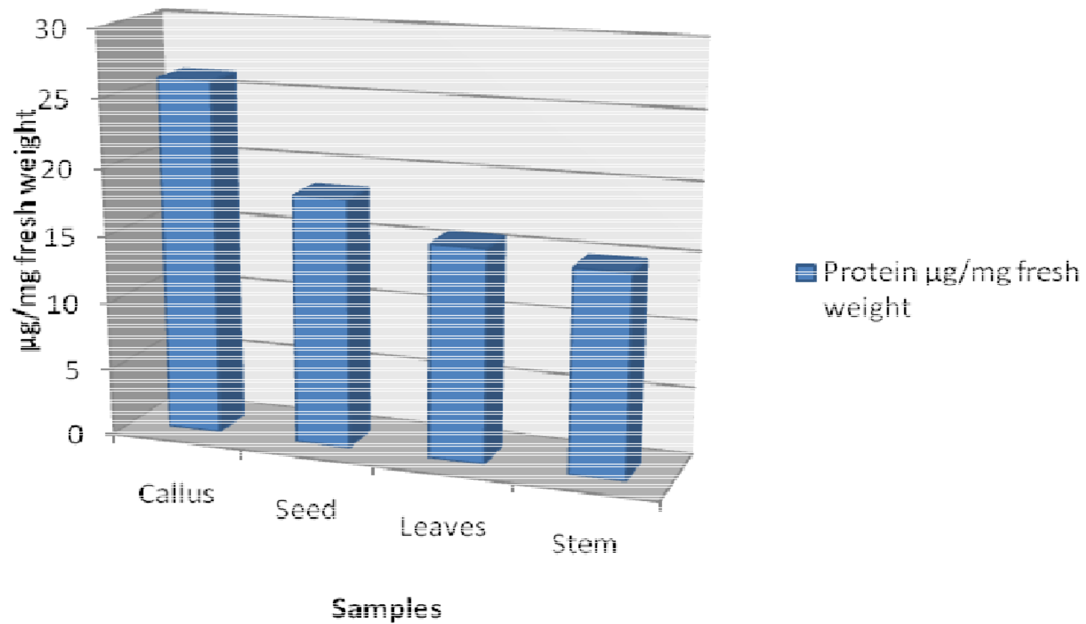

Figure 1. Protein estimation in *Acacia nilotica* L.

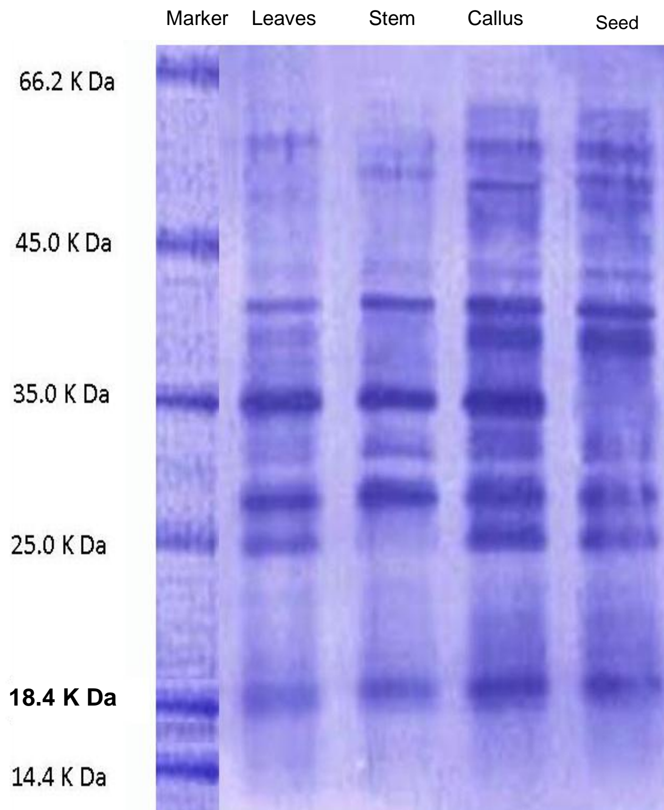

Figure 2. Protein analysis via. SDS-PAGE in *Acacia nilotica* L.

these valuable plants which showed some signs as an important resource for desert ecosystem and arid environment.

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