International Journal of Education and Science Research Review
IJESRR
E-ISSN 2348-6457
Volume-5, Issue-4 July 2018
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

IN VIVO AND IN VITRO ESTIMATION OF PROTEIN CONTENT

Lalita Yadav

Associate Professor, Botany

Plant Tissue Culture and Biotechnology Lab, Govt. Dungar College,

Bikaner (Rajasthan) INDIA

ABSTRACT

Callus cultures of medicinally important plants ***Cocculus pendulus*** and ***Tinospora cordifolia*** were established on MS medium supplemented with suitable combination and concentration of growth regulators***. T.cordifolia*** showed its growth through inflorescence explant in case of ***C.pendulus*** growth represented by nodal segment(explant). Plant parts according to seasons (summer and winter) as well as calli of both the plant species were analyzed for protein content.Unorganized tissues established and multiplied were harvested at their maximum growth indices i.e. eight weeks and analyzed for protein content by **Lowry's method** (1951). The study of their many roles and varying activities is called 'proteomics'.

KEYWORDS: Callus, Medicinally Important Plants, Explant, Proteomics, Growth Indices.

INTRODUCTION

Plants are one of the important sources of medicines. The medicinal properties of plants associated with their chemical constituents. Proteins or polypeptides are organic compounds derived from primary metabolic pathway act as a structural component and biocatalyst which yield energy when required. Protein called antibodies help to protect the body against infections behave as an enzymes ensure that food is being properly digested.Many proteins are enzymes that catalyze biochemical reactions and generally responsible for regulating the cellular machinery and determined the phenotype of an organism. Protein accomplish their task in the body by three dimensional tertiary and quaternary interaction between various substrates. The high quality of proteins reported in many families like Chenopodiaceae, Polygonaceae and Amaranthaceae and from many food legumes.

The experimental plants ***Cocculus pendulus*** and ***Tinospora cordifolia*** are versatile medicinal plants having a great medicinal value in Ayurvedic and Unani system of medicine belonging to family **'Menispermaceae'**. ***C. pendulus*** commonly known as '**Jaljamini**' is a straggling scandent shrub, thermophilous or desert plant. C. pendulus is a rich source of chemical constituents some of them possess biological properties and highly effective against tuberculosis and leprosy. It is also useful in other human diseases viz. Gout, eczema, cough, opthalamia, general debility etc. Plant is traditionally used in India to treat fever or malarial fever.

T. cordifolia is a herbaceous vine, considered as detoxifying herb or rejuvenative herb known as '**Amrita'** habitat ranging from climbing shrub to large perennial.The adaptogenic constituents are found in all plant parts used to making different types of drugs and contain several primary and secondary metabolites. There are evidence hints that ***T.cordifolia*** may have anticancer, antidiabetic, cholesterol lowering and liver protective action and considered to be a unique herb build up the body own defense mechanism**.** ***T.cordifolia*** also used in veterinary folk medicine (**Ethnoveterinary** uses).

Cocculus pendulus and ***Tinospora cordifolia*** have been selected for *in vivo* and *in vitro* study of protein. The main objectives of this studies is to encourage attempts to commercially produce plant products from tissue culture. Bryne and Koch (1962),Coelho (1994), Staba(1963), Martin(1980).

MATERIAL AND METHOD

Fresh plant parts (stem, leaves, fresh pods and dried seeds) of selected plant species were collected from different areas of **Bikaner** in month of **January** and **June**. Parts were separated,dried, powdered and used for estimation of protein content. Five replicates were taken for each plant part and mean value was calculated.

Unorganized cultures with profuse callusing were established using nodal segment in ***C. pendulus*** and floral buds in ***T. cordifolia*** on **Murashige and Skoogs** medium supplemented with 2mg/L BAP + 1mg/L 2,4-D in ***C.pendulus***, 2mg/L BAP + 1.5mg/L IAA in ***T. cordifolia***. These cultures were maintained for a periods of six months by frequent subculturing at interval of 6 to 8 weeks at 26 ± 1ºC, 55% relative humidity and diffused light conditions (3000 lux). The growth indices were calculated at different intervals of 2,4,6, 8 and 10 weeks.

Unorganized tissues were harvested at their maximum growth indices i.e. **eight weeks**. Five such replicates were analyzed for calculating mean value. Plant parts as well as tissue samples harvested at their maximum **GI** were analyzed separately for their Protein content by **Lowry' s metods (1951).**

REAGENTS

A. 2% Sodium Carbonate (Na_2CO_3) in 0.1N Sodium Hydroxide (NaOH)
B. 0.3% Copper Sulphate ($CuSO_4.5H_20$) in 1% Potassium Sodium tartarate
C. Alkaline Copper solution (mix 50ml of A and 1 ml of B shortly before assay)
D. Phenol Reagent (Folin- Ciocalteau Reagent) 1.0 normal
E. Standard Protein solution: Bovine serum albumin (BSA) was mixed at a rat of 1mg/ml in 0.1 N NaOH
F. Working standard: Dilute 10 ml of the standard protein solution to 50 ml with distilled water in a standard flask, 1 ml of this solution contains 200 mg proteins

PROCEDURE

Each of the plant parts and tissue samples were homogenized separately in 10% cold TtriChloro Acetic acid TCA (10 mg:5ml) and centrifuge at 5000 rpm for 10 minutes. Supernatant discarded and pellets were saved. Pellets were again suspended in 5ml of 10% cold TCA and recentrifuged for 10 minutes. Supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1 N NaOH. 0.1ml of this solution was used for protein estimation.

QUANTITATIVE ESTIMATION

Five concentrations (0.2,0.4,0.6,0.8 and 1ml) from the working standard solution (F) were taken in series of test tubes. In another set of test tubes 0.1ml and 0.2ml of the sample extracts were taken and the volume was raised

up to 1 ml in all the test tubes. A test tube with 1ml of water served as the blank. 5ml of the reagent C was added to each test tube including the blank and allowed to stand for 10 minutes. Then 0.5 ml of reagent D was mixed and incubated at room temperature (about 25°C) for 30 minutes until the blue colour developed.

The spectronic colorimeter (**Bausch and Lomb**) was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively. A regression curve was worked out of various concentrations of the standard solutions their respective absorbance, which followed the **Beer's law**.

RESULT AND DISCUSSION

Among all the plant of ***C. pendulus and T. cordifolia***, analyzed for protein content, seeds showed minimum amount in both plant species (0.27 mg/g.d.w. in ***C.pendulus***, 0.39 mg/g.d.w. in ***T. cordifolia***). Maximum amount of protein was estimated in stem of ***C.pendulus*** (0.48mg/g.d.w.) and leaves of ***T.cordifolia*** (0.72mg/g.d.w.). In both the plant species protein content of fruits (0.30mg/g.d.w. in ***C.pendulus*** and 0.42 mg/g.d.w.in ***T.cordifolia***) was little higher than seeds but less than leaves and stem. Callus showed comparatively less amount of protein (0.46mg/g.d.w. in ***C. pendulus*** and 0.50mg/g.d.w. in ***T.cordifolia***) Fruiting takes place in winters only in both plant species hence amount could not be compared with fruits and seeds of summer but stem and leaves collected in summer and winter, showed that amount in summer is more by 0.01 to 0.03 digits than that of winter.(Shown in Table 1.1)

Present study shows that amount of protein content in ***C. pendulus*** and ***T. cordifolia*** is not so high that these can be considered as good source of protein for living beings. As unorganized tissues Contains less amount of protein than vivo parts it can not be considered as positive response at the cost of callus production.

TABLE NO. 1.1

PROTEIN CONTENT (mg/100g.d.w.) IN *C. PENDULUS* AND *T. CORDIFOLIA IN VIVO* AND *IN VITRO*

PLANT NAME	SEASON	PLANT PARTS				
		Stem	Leaves	Seeds	Fruits	Callus (8 weeks)
C.pendulus	winter	0.48±0.02	0.35±0.02	0.27±0.04	0.30±0.03	

	Summer	0.48±0.04	0.36±0.04	-	-	0.46±0.04
T.cordifolia	Winter	0.63±0.03	0.72±0.03	0.39±0.02	0.42±0.04	0.50±0.02
	Summer	0.63±0.04	0.74±0.03	-	-	

Values are mean of five replicates ±SD

REFERENCES

Agarwal, M. (2007). Effect of seasonal variation on phytochemical products of ***Maytenus*** spp. – medicinal plants of arid zone. Ph.D. Thesis,M. D. S. Univ., Ajmer, Rajasthan.

Bansal, H. C. (1974). Induced variability for protein quantity and quality in**barley**. Ind. J. Genet. Plant Bread. 34 A:657-661.

Chand, S. and Arora, S. K. (1968). An estimation of protein, ascorbic and mineral matter content in some indigenous exotic varieties of gram (***Cicer arietinum).***Curr. Sci. 37: 237-238.

Grover, S. and Nag. T. N. (1984). Nutritive value of dried zygophyllaceous plants growing in Rajasthan. Chemical Composition, Cop. Physiol. Ecol. 9 (4) 1250-252.

Key, D. E. (1979). Crop and food digest NO. -3- Food legume, Tropical Products Institute, London. Pp. 266.

Kaur, A. (1997). Influence of plant growth regulators on the production of primary and secondary products in Cotton varieties growing *in vitro*.Ph.D. Thesis M. D. S. Univ. Ajmer, Rajasthan.

Nag et al. (1979). Phytochemical studies of ***Tribulus alatus, T. terrestris*** and ***Agave wightii*** contents of primary and secondary products. Comp. Physiol. Ecol. 4: 157-160.

Nickell, L. G. (1982). Submerged growth of plant cell. In: Advances in Applied Microbiology, Umbreit, W. W. ,(Ed.). Academic Press , New York. 4: 213-236.

Noaman, M. M. and Taylor, G. A. (1988) . Vegetative protein in relation to grain protein in high and low grain protein winter wheats. Euphytica 48 (1) 1-8.

Ottman, M. J.; Doerge, T. A. and Martin, E. C. (1996). Late season water and nitrogen effect on Durum quality. Forage and Grain pp.95-108.

Rao, H. R. (2007). Phytochemical studies of medicinally important desert trees *in vivo* and *in vitro*. Ph.D. Thesis M. G. S. Univ. Bikaner, Rajasthan.

Reddy, A. (2005). Biochemical investigation of economically important plant of arid zone of Rajasthan-Cassia angustifolia- in vivo and in vitro. Ph.D. Thesis M. G. S. Univ. Bikaner, Rajasthan.

Singh, V.; Mathur, K.; Sethia, M. and Nag, T. N. (1987). Nutritional aspect of dried terrestrial plants growing in arid zone of Rajasthan. Chemical Composition, Geobios 14 : 274-276.

Tulecke, W. (1965). Growth of tissue in higher plants in nutritional experiment in Air Force Rep. AMRLTER 65 101 Boyce Thompson, Institute for Plant Science, Yonkers 37.