Indian J.L.Sci.2(1) : 17-19, 2012

EVALUATION OF FEW BIOCHEMICAL PARAMETERS OF THE IN VITRO GROWN CALLUS TISSUE OF *Aquilaria agallocha* ROXB

ABHIJIT TALUKDAR[1]
Department of Herbal Technology, Rajiv Gandhi Govt. Polytechnic, Itanagar, Ar.P, India
E-mail : abhijittalukdar@yahoo.co.uk

ABSTRACT

Biochemical parameters in respect to total phenol, total sugar, reducing sugar nitrogen and crude protein content were studied in the in vitro grown callus tissue and the plant material of *Aquilaria agallocha*. The percentage content of all the biochemical components except total phenol content were found to be more in the in vitro grown callus tissue than that present in the plant tissues used as explants. Total phenol was recorded to be 2.10 % in the plant material where as in the callus it was 0.03 % only. Total sugar and reducing sugar in the callus tissue were found to be 2.64% and 0.89% respectively where as in the plant tissue it was 1.32 % and 0.47% respectively. Similarly higher nitrogen and protein content were reported in the callus compared to that of plant material.

KEYWORDS : *Aquilaria agallocha*, biochemical parameters, callus

Aquilaria agallocha Roxb, called as Agar tree or Sanchi tree and generally known in commerce, as Eagle wood tree is a valuable and wonderful tree associated with historical and cultural heritage of Assam. It is an evergreen tree of medium to large size, belonging to the family Thymeliaceae. The plant is distributed in the Southern and South- east Asian nations comprising of India, Bangladesh, Bhutan, Burma, Indonesia, Thailand and Cambodia. In India it is mainly confined to the North-eastern region and distributed in the hills of almost all the states of the region.

The plant is of great commercial and economical value due to an aromatic oil that accumulates in its infected wood and obtained by hydro-distillation. Agar oil is an excellent perfume retainer and highly prized by European perfumers and the Middle-East for mixing with their best grade of scents. The oil also has several medicinal values. It has been used as a stimulant cardial tonic, carminatives, antiasthmatic, aphrodisiac and astringent. It also acts as a preventive against microbes, fleas and lice. The wood is used for making fumigators, pastilles and agarbatties. Biochemical components of the leaf and the bark consist of alkaloid, polyphenol, saponin, flavonoid, glycoside, amino acids etc. (Dash et al., 2008).

Although natural propagation could be carried out by seeds very easily but in vitro propagation is quite difficult, because of the exudation of phenols by the excised edge of the explants into the culture medium. This results in browning of the medium, leading to cell necrosis due to phenol toxicity.

The present study was undertaken to make a quantitative estimation of the different biochemical constituents like total phenol, total sugar, reducing sugar, nitrogen content and crude protein content, synthesized in the in vitro grown callus tissue and to make a comparative analysis with that of the in vivo grown plant tissue which was used as explants to raise the callus cells. Since callus cells are grown under controlled physical and chemical environment, variations are likely to be observed in the content of their biochemical parameters.

MATERIALS AND METHODS

Matured callus tissue and young leaf tissue were taken as materials for the biochemical study. Comparative and quantitative evaluation between the in vitro grown callus tissue and in vivo grown plant tissue, used as explants were studied on the following parameters- total phenol, total sugar, reducing sugar, nitrogen content and crude protein content. 1 gram of the fresh material was used for extraction against all studies.

Callus was induced from leaf as well as shoot tip explants in MS medium supplemented with 2,4-D (6 mg/l) +Kn (2 mg/l), and maintained through regular subculture after every 6 weeks (Talukdar and Ahmed, 2001). Fresh and matured white callus was used to carry out the biochemical studies.

Total phenol estimation of the callus tissue and the plant material was carried out by Folin-Ciolalteau Reagent method. Identification of the phenol present in the callus

[1]**Corresponding author**

and the plant material of A. agallocha were done following the methods given by Sadasivam and Manikam, (1992) .

Estimation of total sugar was done by Phenol-sulphuric acid method described by Mahadevan and Sridhar, (1986) using the Reagents- Phenol (5%) and Sulphuric acid (96%).Reducing sugar estimation of the plant material and callus tissues was done by Dinitrosalycilic acid method using the reagents -DNS reagent and 40 % Roschelle Salt.

Nitrogen content of the callus and the plant material was studied with standard method using Microkzeldahl. Crude protein content was estimated by microkzeldahl method for nitrogen estimation. Multiplying the total nitrogen value with 6.25 gives the crude protein content, including the non-protein nitrogen.

RESULTS AND DISCUSSION

Morphological variations have been observed in cultured callus after different period of growth, which is supposed to indicate differences in biochemical and other characteristics. Some of the biochemical parameters viz. total phenol, total and reducing sugar, nitrogen content, and crude protein were estimated for the plant tissue and the fully grown callus tissue to make a comparative study between them.

The high and the complex polyphenol content, makes the plant difficult to establish in a culture medium. However phenol exudation can be checked and 80 - 90 % survivality of the cultures could be achieved by using antioxidants like citric acid, ascorbic acid and activated charcoal. They can be used with the culture medium as an ingredient or as a pre-treatment solution of the explants. Callus was successfully raised in the MS medium with combined supplementation of 2,4-D and kinetin in various concentrations. Highest callus growth was observed in the treatment 2,4-D (6 mg/l) and kinetin (2 mg/l). It recorded 10.49 grams of fresh weight of callus biomass after 105 days of inoculation from leaf explants (Talukdar and Ahmed, 2001). In vitro studies of *A. agallocha* was also reported by Qi and Sethi, (1989) and He et al., (2005) where they have successfully established cell suspension culture and induced shoots in MS medium supplemented with BAP (1.3

Table1: Biochemical constituents of the in vitro grown callus tissue and the plant material of *A. agallocha*

S. No.	Biochemical constituents	*In vitro* grown Callus (content in % age)	Plant material (content in % age)
1	Total phenol	2.10	0.03
2	Total Sugar	2 64	1 32
3	Reducing Sugar	0.89	0.47
4	Nitrogen	1.04	0.36
5	Crude Protein	6.5	2.27

micromol/L) respectively.

Total phenol content of the plant material was found to be 2.10 %, whereas the amount of phenol present in the mature callus tissue was 0.03 %, (Table,1) which was quite negligible. However the amount of phenol released into the culture medium was 0. 84 %. Phloroglucinol was identified to be the major phenol present in the leaf of *A. Agallocha.*. This was identified through TLC with standard compound as control and further confirmed by running the sample in a UV-Vis. Spectrophotometer which showed an absorbance maximum at 271 nm.

Polyphenol study was necessary because it was found to be an interfering substance in the successful establishment of callus culture. About 40 % of the total phenol content in the plant material was found to be released by the explants into the medium. Study on polyphenol content in *A. Agallocha* was also reported by Dash et al., (2008). They have reported presence of tannins as the major phenol in the methanolic extract of leaf and bark of the plant. Phenols in other plants like *Cynodon dectylon* was reported by Sachdeva and Bhatia ,(1979) and Citrus (Singh, 2000).

Total sugar and reducing sugar contents were found to be higher in callus then that in the plant tissue. Total sugar in the callus was 2.64 %, but half of this was recorded in the plant tissue which was 1.32 %. Reducing sugar in callus tissue was 0.89 % and in plant material it was however 0.47 % (Table,1). Total and reducing sugar in the in vitro grown callus tissue was found to be comparatively more than that in the plant material used as explants. Studies on total sugar and reducing sugar were reported in the tissue

cultures of Banana (Madamba et al., 1977), Sugar beet (De Greef and Jacab, 1979). In sugar beet tissue culture De Greef has reported higher percentage of total and reducing sugar in callus culture in comparison to the plant material, which is in accordance to our finding. Similarly, Roy ,(2000) also reported higher content of total and reducing sugar in the callus of several tomato variants.

Higher nitrogen and protein content was also noticed in the callus tissue. Nitrogen content in the callus was found to be 1.04 % and that in plant tissue it was 0.36 %. Crude protein content was 6.5 % and 2.27 % in callus tissue and plant material respectively (Table,1). The high amount of these biochemical components in the callus tissue is believed to be more because they are grown in a medium enriched with the exogenous salts, sugars, nitrogen source and vitamins.

Nitrogen and protein content in the plant material was studied by different workers in woody species. Studies on nitrogen content by microkjeldahl method and subsequent conversion of nitrogen to protein was reported in 90 different plants including *Ficus religiosa* by Yeoh and Wee, (1994). In *Ficus religioisa* the nitrogen and protein content was reported to be 0.90 % and 4.49 % respectively.

REFERENCES

Dash M., Patra J. K. and Panda P.P., 2008. Phytochemical and antimicro-bial screening of extracts of *Aquilaria agallocha* Roxb. African J. of Biotech., **7 (20)**: 3531-3534.

De Greff, and Jacob M., 1979. In vitro culture of the sugarbeet, description of cell line With high regeneration capacity. Plant Sci. Lett. ,**17**: 55-61.

He M.L., Qi S.Y. and Hu L.J., 2005. Rapid in vitro propagation of medicinally important *Aquilaria agallocha.* J. of Zhejiang University Science, 6B **(8)**: 849-852.

Madamba L.S.P., Baes A.U. and Mendoza J.R., 1977. Effect of maturity on some bioche-mical changes during ripening of banana (*Musa sapientum*). Fd. Chem.,**2**:177-183.

Mahadevan A. and Sridhar R.,1986. Estimation of total soluble sugar- Methods in physi-ological plant pathology: 147-148.

Qi S. and Sethi K.L. 1989. Accumulation of secondary metabolite in cell suspension culture of *Aquilaria sinensis* (Lour.) Gilg. Thymeliaceae. In: Bhattacharyya S.C. & Sen N.(Ed.) Proceedings of the 11th Int. Cong. Of Essential oil Fragrance and Flavours. New Delhi India.

Roy A. K., 2000. Genetics of certain characters in Tomato. Ph. D Thesis. Gauhati University, Guwahati.

Sachdeva S.K and Bhatia M. S., 1979. Chemotaxonomic studies in *Cynodon dectylon* (L)Press. Complex 1-data on free amino acids, soluble sugar, acid invertase activity and total proteins. Proc Indian Acad. Sci., (Part 11) **3**: 189-193.

Sadasivam S. and Manikam A.,1992. In: Biochemical methods for agricultural sciences:187.

Singh I. P., 2000. Comparative Evaluation of Tissue Culture Regenerated Plantlets and Seed lings of Citrus Species. Ph. D. Thesis, Gauhati university, Guwahati.

Talukdar Abhijit and Ahmed G.U., 2001. In vitro induction and growth characteristics of callus of *Aquilaria agallocha* Roxb. from North-eastern region of India. J. of Microbiol. Biotech. & Env. Sci., **3** (1-2): 53-57.

Yeoh H.H. and Wee Y.C., 1994. Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species. Food Chem., **49**: 245-250.