Research in Plant Biology, 3(5): 37-40, 2013 ISSN : 2231-5101
www.resplantbiol.com

Short Communication

An efficient method for callus induction of an important medicinal plant (*Sarcostemma brevistigma*) from stem segments

Palanivel, S[1], Chanthuru, A[2]*, Yogananth, N[2], Bhakyaraj, R[3] and Parvathi, S[1]

[1]Department of Botany, Government Arts College, Karur
[2]Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119
[3]St. Joseph College of Agricultural Sciences and Technology, Songea, Tanzania, East Africa
*Corresponding author: researchchand@gmail.com

The present study was undertaken to evaluate the most suitable concentration of growth regulators i.e., IAA, NAA, 2,4-D with BAP and Kin for callus induction. Stems were proved to be the best explant for culture, which were grown on MS basal medium with different concentration of various growth regulators. The standard plant tissue culture protocol for callus culture was adopted. The highest efficiency of callus formation was observed in the medium containing different concentration of 2, 4-D and BAP. *In vitro* generated callus can be used as a source for the isolation of secondary metabolites from *Sarcostemma brevistigma*.

Key words: *Sarcostemma brevistigma*, Asclepiadaceae, callus induction, stem explants

Sarcostemma brevistigma Wight & Arn (Asclepiadaceae) grows throughout India and other tropical regions of the world. According to folklore, this plant extract is used for the treatment of asthma. A fraction of this plant extract has been reported to have anti-allergic and anti-inflammatory activities (Saraf and Patwardhan, 1988). It is found to be active as anti rheumatic, anti-allergy, antiemetic and bronchodilator (Kirtikar and Basu, 1993). Dried stem is an emetic employed in leprosy patients. Roots have been used in snake bite and rabies (Jain *et al*., 2009). Due to its medicinal importance, a number of biologically active compounds have been isolated from this plant. The plant contains malic acid, succinic acid, reducing sugar, sucrose, traces of tannin and alkaloid a phyto sterol, α and β- amyrine, lupeol and lupeol acetate and β-sistosterol.

Due to overexploitation and misuse of medicinal plants, we are faced with the problem of losing our precious plant resource in the future. Vegetative propagation is also limited by the lower number of individuals that can be obtained simultaneously from a single plant (Sakaguchi and Kan, 1982). With the above mentioned difficulties, callus culture has been an alternative and efficient source for the production of secondary metabolites. The objectives of this study was to investigate the influence of plant growth

Received: 19.7.2013; Revised: 05.10.2013; Accepted: 08.10.2013

regulators on induction and growth of *S. brevistigma* callus culture as a starting point to produce bioactive compounds in plant cell culture.

Materials and Methods

Young stem were collected from mature field grown healthy plant of *Sarcostemma brevistigma* maintained in green house at J J College of Arts and Science, Pudukkottai, Tamil Nadu, India and washed thoroughly under running tap water and then treated with a few drops of Tween-80 and 1% Savlon for 10 minutes with constant shaking. This followed by successive three washing with distilled water to make the material free from savlon. Again the explants were washed with 70% ethyl alcohol for few seconds and washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and disinfected with 0.1% $HgCl_2$ for 2 minutes and washed with sterile distilled water for 5-7 times. Then, the explants were placed in sterile Petri plates before inoculation. The sterilized explants were injured all over the surface and used for callus induction.

The excised explants were cultured on MS (Murashige and Skoog, 1962) medium augmented with different concentrations of auxins like IAA, NAA and 2, 4-D (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) along with cytokinins like KIN or BAP (0.5 mg/l) , 3% sucrose and 0.8 to 1% agar with pH adjusted to 5.8 before the addition of agar. Culture tubes containing medium were autoclaved at 121°C for 15 lbs/inch2 for 15 min. All the inoculated cultures were incubated in growth room in controlled conditions at a temperature of 25 ± 2°C, 16 h light/8 h dark photoperiod and continuous illumination was provided by cool white fluorescent tubes at 2000 lux. Each experiment was repeated thrice. Analysis of variance was carried out and the differences between the treatments were determined by DMRT at 5% level of significance using SPSS (SPSS ver. 16.0).

Results and Discussion

In general, media containing high auxin and low cytokinin concentrations promote cell proliferation resulting in callus formation (Slater *et al*., 2003). In this present study, callus cultures were established from young stem explants of *Sarcostemma brevistigma* cultured on MS medium + B5 - vitamins with varying concentrations of auxins like IAA, NAA and 2, 4-D along with cytokinins like KIN or BAP (0.5 mg/l). It is in agreement with the findings of Narayanaswamy, 1977 who reported that the composition of nutrient medium is an important factor for successful initiation of callusing from different explants.

Maximum amount of callusing (85.23%) was observed on the medium supplemented with combination of 2,4-D and BAP (2.5 mg/l 2, 4-D, and 0.5 mg/l BAP) after 3 weeks of culture initiation (Fig 1 and Plate 1). Similar observations were reported by Chintalwar *et al.,* (2003) in *Tinospora cordifolia* and Sathyanarayana *et al*., (2008) in *Leptadenia reticulate.* Minimum response (22.45%) was obtained in IAA 1.0 mg/l and BAP 0.50 mg/l combination. All the calli derived from stem explants were pale green and friable in nature.

The fresh weight and dry weight of callus of the *S. brevistigma* is as shown in Figs 2, 3 and 4. The maximum growth rate in terms of fresh weight (FW) (3.52 ± 0.07g) and dry weight (DW) (0.83 ± 0.01g) was observed in the combination of 2, 4-D 2.5 mg/l and KIN 0.5 mg/l. Minimum growth rate 1.05 ± 0.09 g fresh weight and 0.18 ± 0.00 g dry weight was obtained in 3.0 mg/l IAA and 0.5 mg/l BAP combination. The results obtained were agreement with the previous report by Yogananth *et al*., (2012) in *Dregea volubilis*.

Among the various concentrations of auxins tested, 2, 4-D with BAP was more effective for callus induction than NAA (Fig 3) and IAA (Fig 4) as a source of auxin in stem explants tested. Hooker and Nabors (1977) recorded an interaction between the

type and concentrations of plant growth regulators, and explant source, a combination of 2,4-D and BAP resulting in a higher callus production than the combinations of IAA or NAA with KIN.

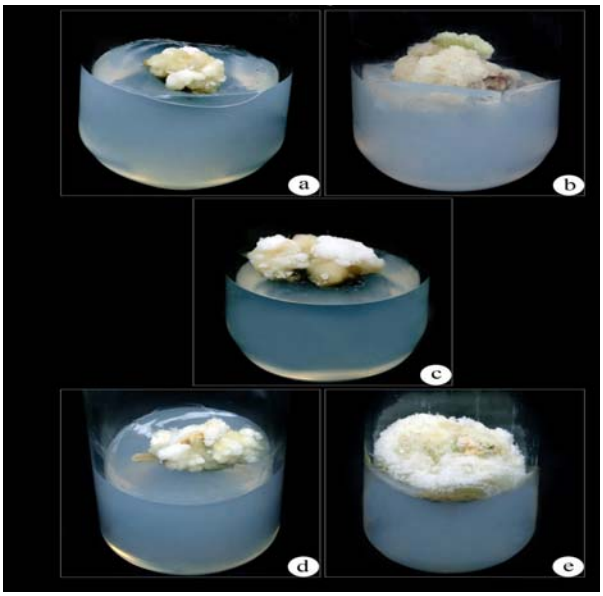

Plate 1: Effect of various concentrations of 2,4-D with Kin on Callus induction from stem explants. a) Callus derived from 2, 4, D 1.0 mg/l and BAP 0.5 mg/l; b) Callus derived from 2, 4, D 1.5 mg/l and BAP 0.5 mg/l; c) Callus derived from 2, 4, D 2.0 mg/l and BAP 0.5 mg/l; d) Callus derived from 2, 4, D 2.5 mg/l and BAP 0.5 mg/l; e) Callus derived from 2, 4, D 3.0 mg/l and BAP 0.5 mg/l

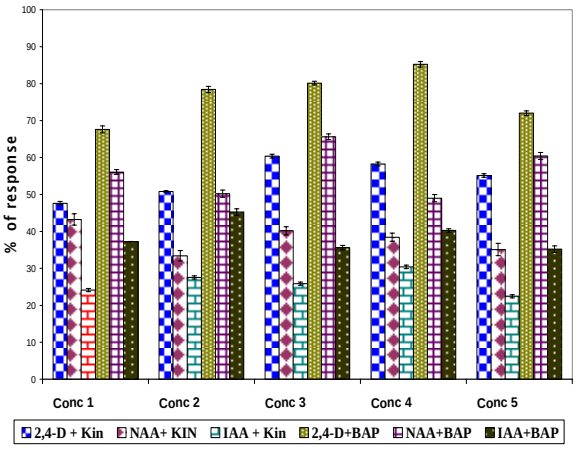

Fig 1: Percentage of callus induction

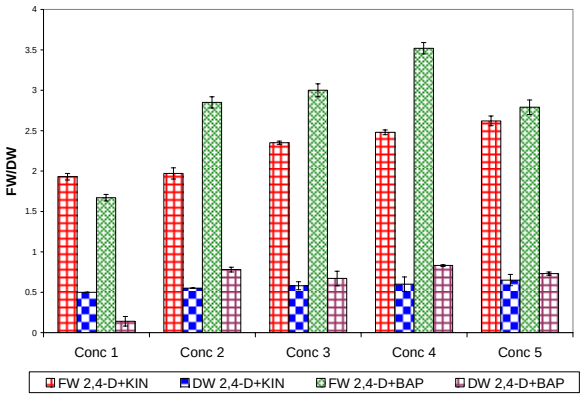

Fig 2: Effect of 2,4-D on callus induction

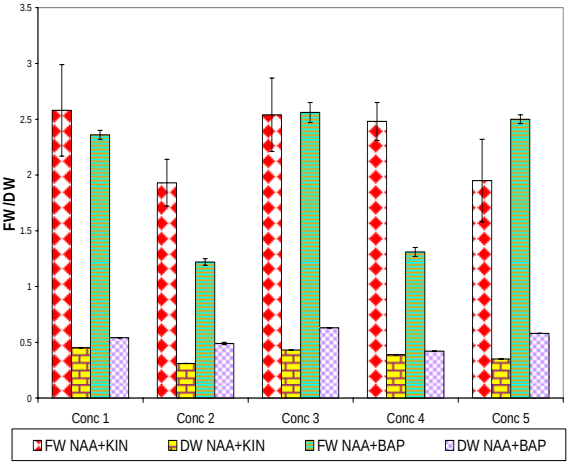

Fig 3: Effect of NAA on Callus induction

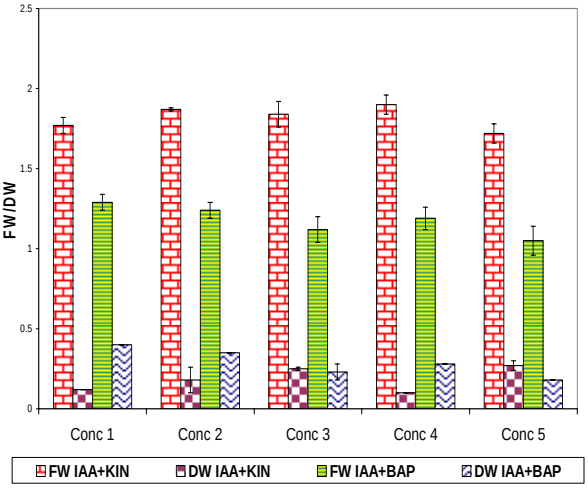

Fig 4: Effect of IAA on Callus induction

Krens and Jamar (1989) reported that auxins were more effective when combined with low BAP levels (0.05 mg/l). George, (1996) reported that the 2,4-D shows effect on the RNA metabolism by inducing the transcription of messenger RNA capable of coding proteins required for the growth and hence, promoting a chaotic cell proliferation, i.e., callus formation. *In vitro* generation of callus can encourage *in vitro* mass production of bioactive compounds of health benefits from *Sarcostemma brevistigma* plant.

References

Chintalwar, S.V.A., Bapat, G., Gupta, J. and Roja, G. 2003. Protoberberine alkaloids from callus and cell suspension cultures of *Tinospora cordifolia*. *Pharmaceutical Biology*. 41(2): 81-86.

George, E.F. 1996. Plant propagation by tissue culture: part 1: the technology. Edington: Exegetics, 574 p.

Hooker,, M.P. and Nabors, M.W. 1977. Callus initiation, growth, and organogenesis in sugarbeet (*Beta vulgaris* L.*). Z. Pflanzenphysiol*. 84: 237-246.

Jain, G.C., Hemant Pareek, B.S., Khajja, Kusum Jain, S., Jhalani, S., Agarwal, D. and Sameer Sharma, 2009. Modulation of di- (2- ethylhexyl) phthalate induced hepatic toxicity by *Apium graveolens* L. seeds extract in ratsl. *African Journal of Biochemistry Research*. 3(5): 222-225.

Kirtikar, K.R. and Basu, B.D. 1993. Indian Medicinal Plants. Bishen Singh Mahendra pal Singh, Dehradun, 3(2):1683-1684.

Krens, F.A. and Jamar, D. 1989. The role of explant source and culture conditions on callus induction and shoot regeneration in sugar beet (*Beta vulgaris* L.). *J Plant Physiol*. 13(6): 651-655.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-97.

Narayanaswamy, S. 1977. Regeneration of Plants from Tissue Cultures. In: J. Reinert and Y.P.S. Bajaj (Eds.) *Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture*. Springer-Verlag, Berlin Heidelberg New York, 179-248.

Sakaguchi, M. and Kan, T. 1982. Japanese researches on *Stevia rebaudiana* (Bert.) Bertoni and stevioside. *Ci Cult*. 34: 235–248.

Saraf, M.N. and Patwardhan, B.K. 1988. Pharmacological studies on. *Sarcostemma brevistigma* W. *Indian Drugs*. 26: 49-. 53.

Sathyanarayana, N., Rajesha, R., Vikas, P.B. and Bharath Kumar, T.N. 2008. Somatic embryogenesis and plant regeneration from stem explants of *Leptadenia reticulate* (Rez) Wight and Arn. *Indian journal of Biotechnology*. 7: 250-254.

Slater, A., Scotee, N. and Wand-Fowler, M.R. 2003. Plant Biotechnology (the genetic Manipulation of Plants). Oxford University Press.

Yogananth, N., Palanivel, S., Parvathy, S., Chanthuru, A. and Bhakyaraj, R. 2012. Effect of different plant hormones on callus induction in Leaf explant of *Dregea volubilis* Benth. (Asclepiadaceae). *Journal bioscience Research*. 3(3):198-202.