Indian J. Applied & Pure Bio. Vol. 35(2), 133-138 (2020). **ISSN: 0970-2091**

In vitro indirect somatic embryogenesis in *Naravelia zeylanica* (L.) DC.

Praveen Dhar T.

Department of Botany,
St. Stephen's College, Pathanapuram, Kollam-689695 (India)
Email:dharpraveent@gmail.com

Abstract

Naravelia zeylanica is a medicinal plant belonging to the family Ranunculaceae. The plant has diverse medicinal values and commonly used to cure rheumatism. In the present investigation in vitro regeneration potentialities were tested using inter nodal explants. Callus derived from the inter nodal explants showed somatic embryogenesis in different hormonal concentrations. MS medium supplemented with 2,4-D, BAP, Kinetin, IAA and NAA alone and in combination showed callusing potential and somatic embryogenesis.

Naravelia zeylanica (L.) DC., belongs to Ranunculaceae consist of about fifty genera and about 2000 species[3]. The plant is useful on vitiated conditions of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers. The roots and stems have a strong penetrating smell[18]. In Kerala *Naravelia zeylanica* is used for regulating intestinal worms, skin related diseases, leprosy, toothache and headache[16]. In India, over 7,500 species of plants are estimated to be consumed by 4,635 ethnic communities for health care needs. Over 1,700 species of plants are fully documented in terms of their biological properties and over 10,000 herbal drug formulations are recommended for a range of health conditions[14]. When compared to traditional agricultural growth techniques, plant tissue culture production of medicinal plants offers a number of unique advantages like possibility of year round continuous production of plant medicinal compounds under highly controlled conditions[10].

In vitro propagation techniques offer an option for the study and conservation of rare, threatened and endangered medicinal plants[7]. In vitro culture helps in clonal propagation and conservation of germplasm. Micro propagation is the process of vegetative growth and multiplication from plant tissues or seeds inside artificial conditions. Tissue culture methods ensure a good and regular supply of medicinal plants, using minimum space and time[12]. Most of the regenerated plants produced in the tissue culture by the way of somatic embryogenesis, mainly differ from the parent plant according to one or several parameters, due to which they are selectable[2]. The era of somatic embryogenesis began after 60 years of theory of totipotency by Haberlandt, is now one of

the progressive areas of research in plant science and biotechnology. Various authors have described protocols for propagation of *Tylophora indica* (Asclepiadaceae) by using different methods such as somatic embryogenesis through leaf explants[5,8] axillary bud multiplication through nodal segment (organogenesis)[4].

The inter nodal explants were isolated from the plant kept in the green house. The explants were washed thoroughly under running tap water for 30 minutes. It was followed by washing in 10% labolene for 15 minutes and then in running tap water for 30 minutes. Further sterilization was done within the laminar air flow chamber using 0.1% Mercuric chloride ($HgCl_2$) (w/v). The sterilized explants were washed thrice, for 5 minutes in each wash using autoclaved double distilled water. After proper sterilization, the inter nodal explants were inoculated in Murashige and Skoog medium containing different hormonal concentrations.

The cultures were maintained at a temperature of 25 ± 1°C with a relative humidity of 50-70 % and incubated at 16 hours photoperiod at a light intensity of 3000 lux from cool fluorescent tubes. Callus obtained from inter nodal explants were sub cultured on MS medium supplemented with 2,4-D, BAP, Kinetin, IAA and NAA alone and in combination with to assess the regeneration potential of callus and somatic embryogenesis. Cultures were maintained by sub-culturing at a regular period of 25 days.

Internodes inoculated on MS medium supplemented alone with 2,4-D and BAP produced light brown, friable and sticky callus after 25 days. Inter nodal explants on 1mg/l NAA produced pale green callus and in combination of IAA and Kinetin showed poor callus proliferation. In IAA hormone alone, maximum callus proliferation was obtained in 0.5 mg/l and the callus was brownish, friable. MS medium containing 2, 4-D in combination with NAA produced callus yellowish friable callus. Lower and higher concentration of 2, 4-D along with different concentrations of NAA produced more callusing. The callus morphology varied from pale yellow friable to brownish yellow friable. BAP in increased combination with NAA showed green to brown semi friable callus proliferation. Lower to higher concentration Kinetin in combination with BAP showed callus proliferation. Inter nodal explants inoculated on MS medium augmented with BAP and IAA in combination also greenish yellow semi friable and compact callus proliferation. In this combination callus morphology varied from yellowish green compact to green compact. Various forms of somatic embryos such as globular, heart shaped, torpedo, and early dicot embryo were observed directly from the inoculated explants indirectly via callusing of explants. The frequency of differentiation of these structures varied with the stage of the zygotic embryos and the culture medium. With BAP along with NAA, the globular, heart, torpedo shaped and early dicotyledonous embryoids quantity was noticed as higher. The quantity of somatic embryo regeneration was almost equal in 2,4-D:NAA, BAP:NAA,KIN:BAP and BAP: IAA hormonal concentrations (Table: 1 & Plate: 1).

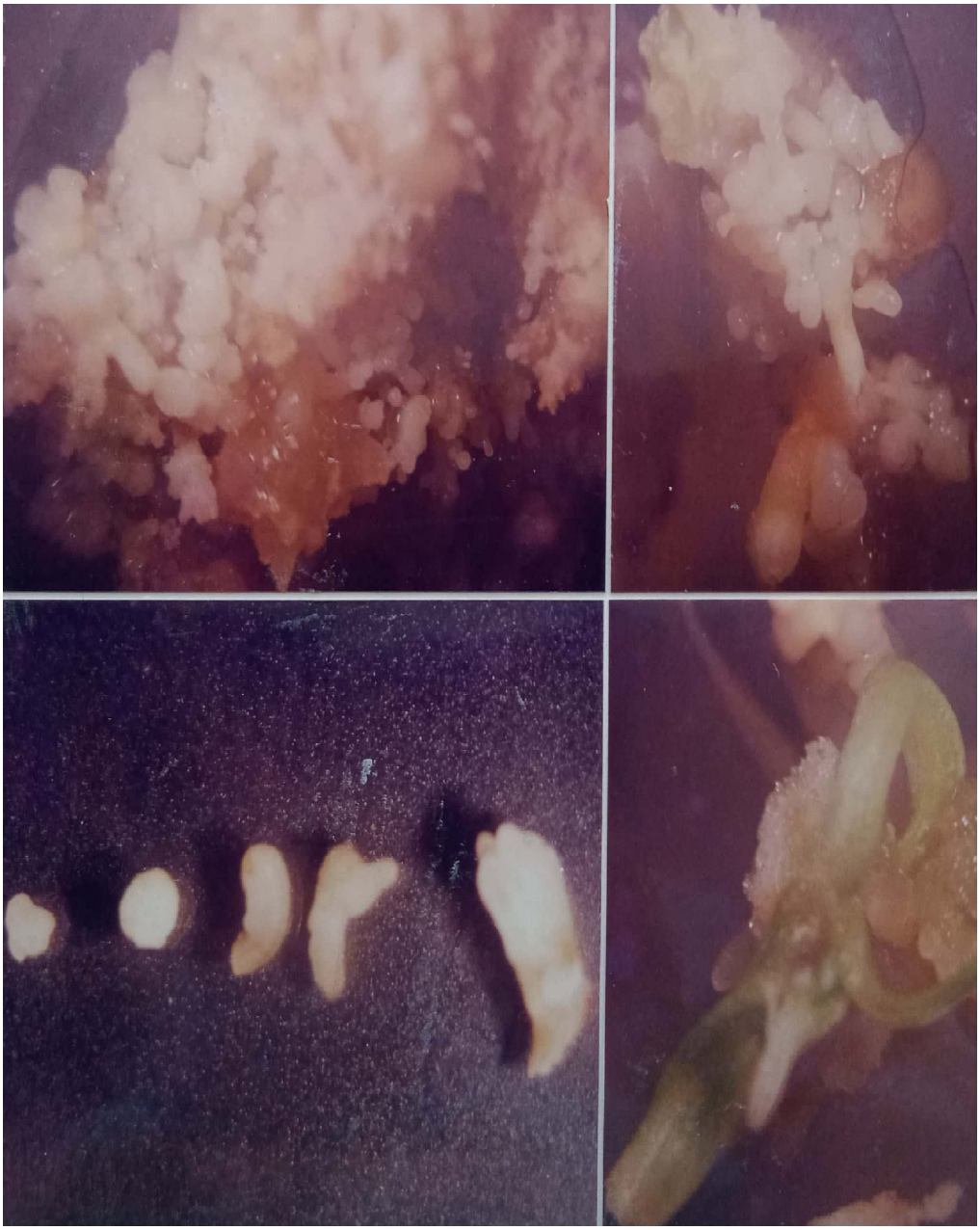

Plate 1 : Showing the stages of Somatic enbryogenesis in *Naravelia zeylanica*

Table-1 Response of inter nodal explants on callusing and somatic embryogenesis (III subculture) in *Naravelia zeylanica*

Hormone Concentrations (mg 1^{-1})	Morphology of Callus	Quantity of somatic embryoids	Quantity embryo regeneration
2,4- D : NAA			
1:5	Pale yellow, friable	64 ± 4	12± 0.77
1:2	Yellow, semi friable	56 ± 4	10± 0.34
2:1	Brownish yellow, semi friable	66 ± 3	12± 0.69
5:1	Pale yellow, semi friable	70 ± 4	09± 0.78
10:1	Pale yellow, semi friable	72 ± 4	11± 0.88
BAP: N A A			
5:05	Green, semi friable	73 ± 2	14± 0.78
5:10	Green, semi friable	72 ± 3	11± 0.77
5:15	Green, semi friable	78 ± 2	12± 0.59
5:20	Brown, semi friable	79 ± 2	11± 0.89
5:25	Green, semi friable	81 ± 3	15± 0.67
5:30	Green, semi friable	81 ± 3	14± 0.90
KIN: BAP			
1:5	Brown, friable	79 ± 2	11± 0.78
1:2	Greenish brown, semi friable	76 ± 4	10± 0.78
2:1	Brown, semi friable	78 ± 3	11± 0.89
5:1	Greenish brown, semi friable	79 ± 2	09± 0.79
10:1	Greenish brown, semi friable	80 ± 3	11± 0.80
BAP: IAA			
5:05	Greenish yellow, semi friable	81 ± 4	11± 0.83
5:10	Greenish yellow, semi friable	76 ± 3	11± 0.84
5:15	Greenish yellow, semi friable	56 ± 3	12± 0.89
5:20	Greenish yellow, compact	78 ± 3	12± 0.79
5:25	Green, compact- semi friable	78 ± 4	10± 0.79
5:30	Green, compact- semi friable	79 ± 3	11± 0.86

Tissue culture methods have been successfully employed for large scale multiplication of a number of woody plants[19]. Callus induction was found to be best in MS media solidified with 10 g/l agar and supplemented with 1-5 mg/l NAA. Further, addition of kinetin (1-5 mg/l) has resulted in more active callus formation. The colour of

calluses ranged from brown to green, greenish yellow and yellow[11]. It may be explained that the specific growth hormones at appropriate concentrations can play major role to induce callus besides the other factors[1]. Induction of callus in *Adhatoda vasica* and *Ageratum conyzoides* were carried out using different explants viz. leaves, nodal and inter nodal stem segments. These explants were tried with various concentration and combinations of phytohormones. In *A. vasica*, stock callus developed from nodal segment on MS-medium supplemented with BAP (0.5 mg/l) and NAA (2.5 mg/l) which was used for further experimentation[13]. The highest frequency of callus induction is achieved on MS medium containing $CaSiO_3$. However, plant or root regeneration potential of rice calluses is cultivar depended. Similarly, effects of Si on plant or root development depend on reed (*Phragmites australis*) genotype used for callus induction[10].

This synergestic effect of NAA and BA combinations was also observed in European linden and in *Echinacea purpurea*[7]. Inter nodal explants was found to be most suitable for the initiation of callus. Addition of Si as sodium silicate (Na_2SiO_3) to the modified MS medium promotes the growth of callus obtained from stem nodal and root explants of *P. australis* while its effect on somatic embryogenesis is explants dependent: it stimulates embryogenesis of root calluses, but it does not influence this process in stem nodal callus. Soares *et al.*,[17] evaluated the effect of Si source on shoot multiplication of *Cattleya loddigesii*. The highest number of shoots is observed on the modified Knudson C medium containing 5.0 mg L^{-1} K_2SiO_3. In *Ajuga multiflora*, addition of Si to MS medium containing 2iP and IAA enhanced adventitious shoot regeneration by increasing the activity of antioxidant enzymes such as SOD, POD, APX, and CAT[15].

References :

1. Ananthi, P., Ranjitha Kumari, B.D. and Ramachandran, A. (2011) *International Journal for Biotechnology and Molecular Biology Research, 2* (3): 51-55.
2. Corredoira, E., Cano, V. and I. Barany (2017) *Journal of Plant Physiology, 213:* 42-54.
3. Cronquist, A. (1981) An integrated system of classification of flowering plants, New York, Columbia University Press.
4. Faisal, M., N. Ahmad and M. Anis (2007) *Plant Biotechnology Report, 1:* 155–161.
5. Jayanthi, M. and P.K. Mandal (2001) *In Vitro Cellular and Developmental Biology- Plant, 37:* 576-580.
6. Koroch, K., H.R. Juliani, J. Kapteyn and J. E. Simon (2002) *Plant Cell Tissue and Organ culture, 69:* 79-83.
7. Mallon, R., R.J. Oubina and M.L. Gonzalez (2010) *Plant Cell, Tissue and Organ Culture, 101:* 31-39.
8. Manjula, S. A. Job and G.M. Nair (2000) *Indian Journal of Experimental Biology, 38*(10): 1069-1072.
9. Mathe, C., A. Mosolygo, G. Suranyi, A. Beke, Z. Demeter and V. R. Toth (2012) *Aquatic Botany. 97:* 57–63.
10. Matthys, K., Julsing, J.Q. Wim and K. Oliver. (2007) The Engineering of medicinal plants: Prospects and limitations of medicinal plant biotechnology. In: Oliver Kayser and Wim J. Quax. (eds) Medicinal

Plant Biotechnology from basic Research to Industrial Applications. WILEY-VCH Vergal GmbH & Co. KGaA, Weinheim,. 1-8.

11. Oselebe, O. and E.E. Ene-Obong (2007) *Tropicultura. 25* (1): 37-43.
12. Prakash, S. and J. Van Staden (2007) *South African, Journal of Botany, 73:* 60-63.
13. Renu, S. and B. Nidhi (2011) *International Journal of Research in Plant Science, 1*(1): 1-8.
14. Shanker, D., K. Ved and P. Singh (1997) Conserving a natural resource need for a national programme on medicinal Plant Conservation Biodiversity and tropical forest The Kerala scenario Pushpangathan P & Nair K S S (eds) STEC Kerala.
15. Sivanesan, I., and B. R. Jeong (2014) *Science World Journal. 10 :* 23-29.
16. Sivarajan, V. V. and I. Balachadran (1958) Ayurvedic drugs and their plant sources Oxford and IBH publishing Co Pvt Ltd 128-129.
17. Soares, J. D. R., M. Pasqual, A. G. de Araujo, E. M. De Castro, F. J. Pereira and F. T. Braga (2012) *Acta Science Agron. 34:* 413–421.
18. Warrier, P. K., V. P. K. Nambiar and C. Ramankutty (1995) Indian medicinal plants, a compendium of 500 species. *4 :* 100.
19. Yogesh, T. J., A. Ramakanthan, C. H. Pandya, J. Subramani and D.P. Batt (1999) *Plant Biochemistry. and Biotechnology 8:* 103-109.