Embryonic Callus Induction of *Lobelia nicotianifolia* Roth: A Medicinal Wild Tobacco.

Naikawadi Vijay B[1], and Naikawadi Vikas B[*2]

[1*]DEPARTMENT OF BOTANY, POST GRADUATE RESEARCH CENTER
PROF. R.M COLLEGE AKURDI PUNE: 411 0044. MS INDIA
[2*]DEPARTMENT OF BOTANY, POST GRADUATE RESEARCH CENTER
CHANDMAL TARACHAND BORA COLLEGE, SHIRUR (GHODNADI),
SHIRUR – 412 210, DIST. PUNE.MS INDIA

Abstract: *Lobelia nicotianifolia* (Campanulaceae ; Rann tambaku) is a rare high altitude wild tobacco species. This species is generally found in monsoon season. Ethno medicinally and local tribal people use this plant for treatment of cold and respiratory problem. This plant contains active metabolites lobeline, which comes under piperidine alkaloids. Natural population hamper due to low seed viability as well as germination rate. Biotechnological intervention through tissue culture play important role for conservation and rare endanger medicinal plant. MS media supplemented permutation and combination of cytokinin and auxins with 30 % sucrose plus application of cytokinin and auxin for induction of embryonic calli through leaves explant. This protocol would be beneficial for improvement of clonal propagation for sustainable source of lobeline.

Keywords: Alkaloid, Ayurvedic system of medicines. Mass propagation, conservation, medicinal plant.

I. Introduction

Western Ghats is the unique source of rich and varied store house of endemic elite medicinal plant species. *Lobelia nicotianifolia* is found in high altitude of Western Ghats (Bapat et al., 2008; Tamboli et al.2012). In traditional systems of medicines, various plant extracts by the local people have been used in treating brain related (Austin 2008). *Lobelia nicotinaefolia* is a source of piperidine alkaloid of lobeline group (Szőke 1994; Felpin and Lebreton 2004; Pullaiah; 2006). Ancient literature and preclinical research has justified its importance, aerial plant parts are antispasmodic and used on asthma, bronchitis as well as on fever, sciatica, back pain speedy healing of wounds (Jegan et al 2008). Roots paste are mainly useful on treatment of eye diseases (Udayan et al 2008; Wabale et al 2011).

Lobelia species have regained popularity and the supply of plant parts for from the natural sites, it is resulting in a dwindling natural resource base (Tamboli et al. 2011). Therefore, there is need to conserve and propagate this shrub on large scale (Tamboli et al 2012; Kaur et al. 2020). Micro propagation is a worldwide proven method for mass scale clonal production of various medicinal plants (Nikule et al., 2020). Very meager information is available on *in vitro* clonal propagation of *L. nicotianifolia.* The objective of this study was establishment embryonic callus from leaf explant.

II. Material and Methods

2.1 Preparation of Plant material and culture conditions

The juvenile leaves were used as explant (Savitribai Phule Pune University 18.5530° N, 73.8265° E). Sterilization and preparation of explants procedure was followed by Naikawadi et al., 2016; Nikam et al., 2013; Nitnavare et al., 2011 and Nikule et al., 2020 with some slight modification. Sterilized explants were grown on MS (Murashige and Skoog 1962) full strength with combination of cytokinin and auxins concentrations (0.5 to 2.0 mg/l Benzyladenine: BA) (0.5mg/l 2,4 D, IAA, NAA and IBA). Embryogenic callus were transferred onto fresh parental medium supplemented with full strength MS+2.0 mg/l BAP + MS+0.5 mg/l 2,4 D. Nature in terms of embryo, colour, texture and morphology of callus culture was recorded after 4th week of incubation.

III. DATA ANALYSIS

All experiments were set up a completely randomized design (CRD) was used in all experiments with 15 replicates and repeated at least three times. Data were subjected ANOVA followed by Duncan's multiple range test (DMRT) at the 5% probability level.

IV. RESULTS AND DISCUSSION

Total twelve kinds of permutation and combinations of phyto-hormones one cytokinins and four auxins were used. Well sterilized and disinfected leaf lamina was gave well survival response under control conditions. The explant grown on without growth regulators and growth medium does not give any sign of differentiation. Low concentration of cytokinins with combination of auxins show poor callus induction and results were obtained after 20 to 21 days incubation. The juvenile leaf explant showed notable response for callus induction compare to the medium grown leaves Table.1 and Fig. 1. The outside of leaf lamina showed well embryonic structure development. Nature of callus show significant differences in terms of snowy, whitish, green, globular, hard, compact and regenerative on various types of growth regulators. Culture condition and incubation period also affect the callus growth. After 2 weeks of incubation, cut end of leaf lamina surface showed the some snowy callus cells. After 3rd week of incubation, well organized globular, heart shaped and torpedo structure were observed. As data depicted in table.1, % of explants responding for embryonic callus, no. of embryo/explant, no. of days for embryonic callus induction. Significant finding were noted on MS + 2 BA + 0.5 2,4-D, 87.6±1.1% of explants responding for embryonic callus, 14.111±0.2 no. of embryo/explant, 12.1±0.02 no. of days for embryonic callus induction. The morphological features of well grown 28th days callus culture show Light green callus with globular embryonic structure Fig. 1. The calli was grown on MS medium with growth hormones more than 28 days, calli become dark and necrotic in nature and responded for further growth and development. The callus was showed low moisture percentage and compact with well differentiation of embryonic structure. Maintained callus show constant regenerative capacity under control condition without any sign of abnormalities.

Callus culture had been regularly sub cultured more than 2 years on parental medium with same culture conditions. Similar findings were noted in other medicinal and ornamental plant species Cho et al., 2003, *Ostericum koreanum,* Becerra et al., 2004, *Passiflora edulis,* Kim et al., 2005 *Pinellia tripartite*, Zhu et al., (2018) peony, Duan et al., (2019) *Isodon amethystoides*. Mass production of embryo in callus would be beneficial for further plantlets development under control conditions.

Table: 1 Effect of cytokinins and auxins with MS medium on leaf explant of *Lobelia nicotianifolia* for embryonic callus induction.

Concentration of Growth regulators	% of explant responding for embryonic callus	No. embryo/ explant	No. of days for embryonic callus induction	Nature of Callus or embryo
0.5 BA + 0.5 2,4-D	29.33±0.68 ^{f}	4.026±0.09 ^{ef} *	18.044±0.16^{cd}	Profuse, snowy, white to pale yellow callus with some embryonic structure
0.5 BA + 0.5 IAA	22.67±1.13 ^{fg}	3.067±0.33^{f}*	21.756±0.21 ^{b}	Slight green callus with some globular structure
0.5 BA + 0.5 NAA	17.67±0.83^{h}	2.133±0.26^{fg}**	20.778±0.24^{bc}	Dark green, globular callus with some globular structure
0.5 BA + 0.5 IBA	24.67±1.40 ^{fg}	3.067±0.33^{f}**	24.311±0.14^{a}	Profuse callus with some heart shaped structure
1 BA + 0.5 2,4-D	48.67±0.83^{de}	6.286±0.15 ^{cd} **	14.111±0.22^{f}	Light green to dark green callus
1 BA + 0.5 IAA	31.00±0.82^{ef}	4.830±0.18^{ef}	12.111±0.16^{fg}	Yellowish to faint green embryonic calli.
1 BA+ 0.5 NAA	27.33±1.29^{f}	3.482±0.11^{f}*	16.089±0.22^{de}	Dark green callus with without any differentiation.
1 BA + 0.5 IBA	22.67±1.55 ^{fg}	6.048±0.09^{cd}***	18.156±0.11^{cd}	Dark green callus with some globular structure
2 BA + 0.5 2,4-D	87.67±1.10^{a}	14.111±0.22^{a}***	12.111±0.02^{fg}	Light green callus with globular embryonic structure
2 BA + 0.5 IAA	67.33±1.55 ^{B}	9.024±0.12^{b}*	14.022±0.17^{f}	Pale yellow, slight green callus with some globular structure
2 BA+ 0.5 NAA	55.67±1.55^{c}	8.095±0.05^{bc}*	16.000±0.16^{de}	Light green callus with some heart embryonic structure
2 BA + 0.5 IBA	52.00±0.98 ^{cd}	8.048±0.11 ^{bc}**	14.489±0.16^{f}	Compact Dark green callus with torpedo embryonic structure

*callus mediated shoot formation, **callus formation, *** extensive callus formation. Values represent mean ± SE calculated on three independent experiments, each based on minimum 15 replicates. Means followed by same letters within columns are not significantly different at 5% level (DMRT).

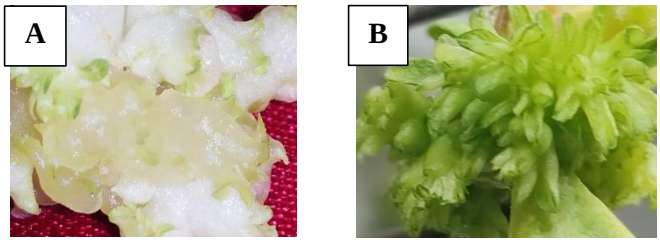

Fig.1 A. one week old embryonic callus developed on MS + 1.0 mg/l BA+ 0.5 mg/l 2, 4 D., **B.** 28th days old embryonic callus MS + 2.0 mg/l BA+ 0.5 mg/l 2,4 D.

V. CONCLUSION

An efficient embryonic callus was obtained from leaf explant with moderate concentration and combination of cytokinins with auxins on full strength MS medium. This protocol does not depend on natural seedlings resource and would be beneficial for mass clonal production in short period of time.

VI. ACKNOWLEDGMENT

Authors are grateful to Board of College and University Development (BCUD), University of Pune, for their financial support and to the authorities of Chandmal Tarachand Bora College, Shirur, Pune, for all infrastructures facility to conduct this present study.

REFERENCES

[1] Austin DF (2008) *Evolvulus alsinoides* (Convolvulaceae). An American herb in the old world. J Ethnopharmacol 117:185–198

[2] Bapat VA, Yadav SR, Dixit GB. (2008) Rescue of endangered plants through biotechnological applications. Natl. Acad. Sci. Lett.; 31:201–210.

[3] Becerra, D., Forero, A. & Góngora, G. (2004) Age and Physiological Condition of Donor Plants Affect *in vitro* Morphogenesis in Leaf Explants of *Passiflora edulis* f. flavicarpa .Plant Cell, Tissue and Organ Culture 79, 87–90. https://doi.org/10.1023/B:TICU.0000049440.10767.29

[4] Cho, DY., Lee, EK., Lee, S. et al (2003). Enhanced somatic embryogenesis and plant regeneration in leaf explant cultures of *Ostericum koreanum* on medium of varying pH. Plant Cell, Tissue and Organ Culture 75, 215–222. https://doi.org/10.1023/A:1025824904491.

[5] Duan, Y., Su, Y., Chao, E. et al (2019). Callus-mediated plant regeneration in *Isodon amethystoides* using young seedling leaves as starting materials. Plant Cell Tiss Organ Cult 136, 247–253. https://doi.org/10.1007/s11240-018-1510-x.

[6] Felpin FX, Lebreton J (2004) History, chemistry and biology of alkaloids from *Lobelia inflata*. Tetrahedron 60:10127–10153.

[7] Jegan G, Kamalraj P, Muthuchelian K. Medicinal plants in tropical evergreen forest of Pachakumachi Hill, Cumbum Valley, Western Ghats, India. Ethnobot Leafl 2008; 12: 254-260.

[8] Kaur, H., Lekhak, M. M., Chahal, S., Goutam, U., Jha, P., Naidoo, D.,Kumar, V.,2020. *Nardostachys jatamansi* (D. Don) DC: An invaluable and constantly dwindling resource of the Himalayas. South African Journal of Botany, 135, 252-267.Pullaiah T. Encyclopedia of world medicinal plants. Delhi, India: Regency Publications; 2006, p. 1264-1265.

[9] Kim, S.W., In, D.S., Tae, K.H. et al (2005). Somatic embryogenesis and plant regeneration in leaf and petiole explant cultures and cell suspension cultures of *Pinellia tripartita*. Plant Cell Tiss Organ Cult 80, 267–270. https://doi.org/10.1007/s11240-004-1018-4.

[10] Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–505

[11] Naikawadi, V. B., Ahire, M. L., Lahiri, A., Nikam, T. D. (2016). In vitro propagation and cell cultures of memory tonic herb *Evolvulus alsinoides*: a best source for elicited production of scopoletin. Applied microbiology and biotechnology, 100, 3463-3476.

[12] Nikam, T. D., Ghorpade, R. P., Nitnaware, K. M., Ahire, M. L., Lokhande, V. H., Chopra, A., (2013). Micropropagation and non-steroidal anti-inflammatory and anti-arthritic agent boswellic acid production in callus cultures of *Boswellia serrata* Roxb. Physiology and Molecular Biology of Plants, 19, 105-116.

[13] Nikule HA, Nitnaware KM, Chambhare MR, Kadam NS, Borde MY, Nikam TD (2020). In-vitro propagation, callus culture and bioactive lignan production in *Phyllanthus tenellus* Roxb: a new source of phyllanthin, hypophyllanthin and phyltetralin. Sci Rep. Jun 30;10(1):10668. doi: 10.1038/s41598-020-67637-8. PMID: 32606305; PMCID: PMC7327055.

[14] Nitnaware KM, Naik DG, Nikam TD (2011). Thidiazuron-induced shoot organogenesis and production of hepatoprotective lignan phyllanthin and hypophyllanthin in *Phyllanthus amarus*. Plant Cell Tissue Organ Cult.;104:101–110.

[15] Patil, J., Ahire, M., Nitnavare, K., Panda, S., Bhatt, V., Kavi, P., Nikam, T. (2013) *In vitro* propagation and production of cardiotonic glucosides in shoot cultures of *Digitalis purpurea* L. by elicitation and precursor feeding. Appl Micobiol Biotechnol; 97: 2379-2393.

[16] Ramawat, K., Merrilion, J (1999). Biotechnology-secondary metabolites. Mohan Primlani for Oxford and IBH Delhi. 243–247.

[17] Szőke É (1994) In vitro culture and the production of lobeline and other related secondary metabolites. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 28, Medicinal and aromatic plants VII. Springer, Berlin, Heidelberg, pp 289–327.

[18] Tamboli A, Rub R, Ghosh P, Bodhankar S (2012). Antiepileptic activity of lobeline isolated from the leaf of *Lobelia nicotianaefolia* and its effect on brain GABA level in mice. Asian Pac J Trop Biomed. Jul;2 (7):537-42. doi: 10.1016/S2221-1691(12)60092-6. PMID: 23569966; PMCID: PMC3609340.

[19] Tamboli A, Khan I, Bhutkar K, Rub R (2011) Systematic Review On Phytochemical And Pharmacological Profile Of *Lobelia nicotianaefolia* Roth E & S. Pharmacologyonline 3: 7-22.

[20] Udayan,P.S., Harinarayanan, M.K., Tushar, K.V., Balachandran,I., (2008). Some common plants used by Kurichiar tribes of Tirunelli forest, Wayanad district, Kerala in medicine and other traditional uses. Indian J Tradit Knowl; 7(2): 250-255.

[21] Wabale, A.S., Kharde, M.N., Salunke, K.J., (2011) Costus speciosus Lobelia nicotianaefolia *Urginea indica* (Koeing.) J. E. Sm., Roth. And Kunth- the important ethnomedicinal plants from the Western Ghats. Asian J Exp Biol Sci; 169-170.

[22] Zhu, X., Li, X., Ding, W. et al (2018). Callus induction and plant regeneration from leaves of Peony. Hortic. Environ. Biotechnol. 59, 575–582. https://doi.org/10.1007/s13580-018-0065-4.