Quest Journals
Journal of Research in Environmental and Earth Sciences
Volume 8 ~ Issue 6 (2022) pp: 29-31
ISSN(Online) :2348-2532
www.questjournals.org

Research Paper

Regeneration of Nicotiana plumbaginifolia Viv. through in vitro stem culture

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Abstract
Ornamental plants with colorful, showy and fragrant flowers significantly play an important role in landscape of a garden, park, or other such amenity area. Nicotiana plumbaginifolia Viv.is cultivated for ornamental purposes. Plant tissue culture technique is helpful in clonal propagation of plants and understanding morphogenetic studies. Indirect organogenesis of N. plumbaginifolia has been observed in vitro through seedling stem explants on MS medium supplemented with Kn. Kn, a potent cytokinin, played an important role in shoot regeneration. The regenerated shoots are tried to induce roots on different auxins however shoots successfully rooted on MS medium with NAA. 72 % of rooted shoots were hardened using cocopeat and soil mixture 1:1.

Key words*: Nicotiana plumbaginifolia, Indirect organogenesis, Kn, NAA, Hardening*

Received** 26 May, 2022; **Revised** 05 June, 2022; **Accepted** 08 June, 2022* *© The author(s) 2022.* ***Published** with open access at **www.questjournals.org

I. Introduction

N. plumbaginifolia Viv. (Solanaceae) is a native of Mexico and West Indies. It is an erect sticky-glanded herbaceous plant with simple alternate leaves on upper and whorled rosette leaves with sheathing leaf base on the lower side of the stem. The plant is cultivated for ornamental purposes[1]. Experimental studies have been done in various fields Viz. cytogenetics, physiology and tissue culture using different parts of the plant material. Adventitious shoot buds by using leaf explants have been achieved through direct and indirect organogenesis by Poornima and Shivamurthy[2]. Point mutations distributed in plasma membrane regions of the *N. plumbaginifolia* has been studied by Morsomme *et al.* [3]. Barfield *et al.* [4] have used the plant for stable haploid cell line production and also for cryopreservation studies. Molecular and pathological studies have been carried like influence of genotype and environment on *de novo* differentiation of flowers[5] and pleiotropic drug resistance- type ATP- binding cassette transporter and role in plant pathogen defense[6] respectively. Clonal propagation using seedling stem explants have been successfully carried out through indirect organogenesis.

II. Material and Methods

Seeds after dehiscence from *N. plumbaginifolia* were collected from a healthy garden growing plant in Mysore, Karnataka. Seeds were wetted using tween-80 for 8 min followed by surface sterilization using 1.5% w/v bavistin for 3 min and subsequently with freshly prepared 0.2% w/v mercuric chloride for 3 min. The sterilants were removed by repeated washings with sterile tap water. 2-3 rinses with sterile distilled water was carried under aseptic condition. The seeds were inoculated aseptically on MS medium supplemented with growth regulator Kn (kinetin), 0.5 mg/l. Seeds germinated after ten days, the seedling stems were excised after twenty five days and used as explants for inducing organogenesis. The seedling stems (1.0 – 1.5 cm) were excised and inoculated on MS medium with 0.2 mg/l NAA and Kn (1.0 mg/l). The medium contained 3.0 % w/v sucrose as carbohydrate source and 0.8 w/v agar as gelling agent, pH of the medium was adjusted to 5.8 before autoclaving. The cultures were incubated at 20$\pm$2°C under cool day light fluorescent tubes (50 M $m^{-2}s^{-1}$) for 14 hr photo period.

III. Results

Smooth light brown nodular callus initiated after four days of explant inoculation. The 13 days older callus was sub cultured on the fresh MS medium supplemented with Kn (1.5 mg/l). Adventitious shoot buds regenerated on the callus after 18 days. Shoots of 1.5-2.0 cm length with 3-4 pairs of leaves were obtained after 45 days.

The shoots obtained were separated from the clumps and were inoculated on MS basal medium for three days and subsequently transferred to MS medium with NAA (1.5 mg/l). Roots were initiated from the shoots after ten days of culture. Roots reached a length of 2.5 cm after twenty days. The rooted shoots after a month were transferred to plastic cups containing sterile coco peat and soil mixture. The miniature plants were kept covered with holed polythene cover under laboratory condition. The plants were regularly supplied with quarter strength MS basal medium and were incubated for fifteen days. The plant lets were then transferred to plastic pots containing coco peat and soil mixture 1:1 under green house condition.

IV. Discussion

Medium with cytokinins are effective in adventitious shoot formation from seeds[7]. Ohyma and Oka[8] found that mulberry seeds when germinated on MS medium containing cytokinin (2Cl-4PU), could stimulate shoot formation strongly. In the present study Kn proved to be most effective cytokinin in influencing seed germination as well as indirect shoot induction on callus. In *Convolvulus arvensis*[9] the stem cultures were more dependent on 2,4-D to form proliferative callus and Kn for shoot induction. However in *Phyllanthus urinaria*[10] NAA has been successfully used for callus induction and Kn for shoot regeneration. Similarly in *N. plumbaginifolia* vigorous callus production was possible by the use of NAA and more number of adventitious buds could be induced using Kn. Instead of Kn, BAP could also induce shoot buds but it takes longer period for morphogenesis and also number of shoots formed are lesser. The rooting of shoots on MS medium supplemented with different auxins like NAA, IAA and IBA were tried, however IAA and IBA were less potent in inducing roots like in case of *Quisqualis indica* [11] . Even though NAA could induce roots, shoots after intial growth on basal MS medium and NAA supplementation could induce more roots. Dhar and Upreti [12] have successfully used two step rooting procedure in *in vitro* raised shoots of *Banhinia vahilli*. IBA and NAA have been used successfully for root induction. Shoots on MS medium with Kn and adenine sulphate were initially cultured on MS medium with Kn and NAA, then were transferred to the medium without growth regulators. Hardening of plants were carriedout using cocopeat and soil mixture in 1:1 ratio, 70±2 plants survived. Similar studies have been carried out in *Aloe vera* [13].

V. Conclusion

Ornamentals are important components and popular subjects in bio-aesthetic planning. Micropropagation provides a useful method for the mass propagation of many plants including ornamentals. Kinetin, a cytokinin successfully induced germination of seeds and also indirect organogenensis forming numerous shoot buds, NAA, an auxin induced roots successfully. The micropropagated plants could be hardened using coco peat and soil mixture.

Acknowledgement

The authors thank the Principal, Botany Department, Sree Siddaganga College of Arts, Science and Commerce, Tumakuru for providing research facilities.

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