I J R B A T, Issue (Special-17), June 2021: 394-401 e-ISSN 2347 – 517X
A Double-Blind Peer Reviewed & Refereed Journal Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© www.ijrbat.in

IN VITRO PROPAGATION USING NODAL EXPLANTS OF *CINNAMOMUM CAMPHORA*: AN IMPORTANT MEDICINAL TREE

Hemant Sharma
Dayanand PG College, Hisar (Haryana)-125001
E mail: hemantbotany@gmail.com

ABSTRACT:
Medicinal plants play an important role in human life to fight diseases since time immemorial. Over the past few years, the medicinal plants have received a wide acceptance due to the faith in herbal medicine in view of its lower side-effects as compared to allopathic medicine. *Cinnamomum camphora* (L.) Nees & Eberm is one of the important medicinal plants used in various systems of medicine. It is commonly known as Camphor tree or Kapur. It is an evergreen, aromatic, medium to large tree. Leaves are aromatic which give smell of camphor when crushed by hand. Camphor tree is used as a source of camphor and camphor oil. Both camphor and camphor oil have medicinal importance and commercial value. But conventional methods of propagation (by seeds, cuttings and layering) of camphor tree are very slow. In addition the long life cycle of this tree also hinders in conventional method of breeding. Therefore, to achieve their mass multiplication and propagation without any interruption, nodal explants of camphor tree were inoculated on MS and WPM supplemented with different concentrations (0.5, 1.0, and 2.0 mg/l) of BAP. Buds initiated after 10 days and 20 days of inoculation with 100% and 60% response on WPM and MS fortified with 1.0 mg/l BAP respectively. Shoots (2 cm height) rooted on half strength WPM fortified with 1.0 mg/l IBA. The rooted shoots were successfully transferred to field with 50% survival.

Abbreviations: **BAP:** 6-Benzyleaminopurine, **IBA:** Indole-3-butyric acid
WPM: Woody Plant Medium, **MS:** Murashige & Skoog's Medium

Key words: *In vitro*, *Cinnamomum camphora*, Camphor tree, WPM, Nodal explants.

INTRODUCTION

Medicinal plants play an important role in human life to fight diseases since time immemorial. The World Health Organization has estimated that up to 80% of people still rely on herbal remedies for their health care (1). All the major system of medicine, such as Allopathy, Homeopathy, Unani and Ayurvedic, use most of the drugs obtained from plants. Most of these medicines are actually the by-products of various processes of plants and each plant species produces its own characteristic chemicals. Over the past few years, the medicinal plants have received a wide acceptance due to the faith in herbal medicine in view of its lower side-effects as compared to allopathic medicine. With the passage of time, more and more plants with medicinal properties were brought to list, and at present more than 1500 species of plants are used in medicines. Among these, *Cinnamomum camphora* is one of the important plants used in various systems of medicine.

Cinnamomum camphora (L.) Nees & Eberm, popularly known as Camphor tree or Kapur belongs to family Lauraceae. It is an evergreen, aromatic, medium to large tree.

Leaves are aromatic which give smell of camphor when crushed by hand. Camphor tree is used as a source of camphor and camphor oil. Both camphor and camphor oil have medicinal importance and commercial value. Both have strong antiseptic, analgesic, antispasmodic, expectorant and stimulant properties (2). It is used in external applications as balms to relieve muscular strains, inflammations, arthritic and back pains. It is also used in treatment of cold sores and chill blains and used as a chest rub for bronchitis and other chest infections. Due to their commercial importance and extensive use in medicine there is a need to develop rapid and reliable methods of propagation of camphor tree. Because conventional methods of propagation (by seeds, cuttings and layering) of camphor tree are very slow. In addition the long life cycle of this tree also hinders in conventional method of breeding. Therefore, any alternative method has to be developed to achieve their mass multiplication and propagation without any interruption. And it will be possible by *in vitro* tissue culture technique.

MATERIALS & METHOD:

Explant:

Nodal segments were used as explants for the *in vitro* culture work.

Surface Sterilization of the Explants:

The nodal segments were washed in running tap water to remove all the dust particles. It was followed by washing with liquid detergent (Teepol) for 5 minutes and again, explants were washed several times with tap water to remove all the traces of detergent. Then explants were subjected to 0.2% streptomycin solution for 15-20 minutes before taking them to the sterile airflow chamber. In laminar air flow chamber surface sterilization was carried out by treating with 0.1% (w/v) mercuric chloride solution for 3-4 minutes and subsequently washed 3-4 times with sterile double distilled water to remove all the traces of mercuric chloride. Again explants were disinfected in a 70 % (v/v) ethyl alcohol for 1 min.

Culture media:

MS medium (9) with 3 % (w/v) sucrose and solidified with 0.8 % (w/v) agar-agar and Woody Plant Medium (7) with 2 % (w/v) sucrose and solidified with 0.6 % (w/v) agar-agar were used in the present investigation.

Inoculation of explants:

After surface sterilization, the further work was carried out under laminar air flow chamber. Before starting work, the floor of laminar air flow chamber was thoroughly wiped with rectified spirit and all the culture tubes, forceps and scalpels were placed in laminar air flow chamber. It was then sterilized by UV radiations for about 1hr. One segment of nodal explant was inoculated in each test tube having MS and WP basal medium as well as supplemented with different concentrations of growth regulator (BAP).

Culture Conditions:

The cultures were maintained at 25 ± 2°C under continuous illumination of 3500 lux of light from cool white fluorescent tubes.

Direct shoot regeneration:

Nodal explants having 2 cm in length were excised and cultured on MS or WP

medium supplemented with different concentrations (0.5, 1.0 and 2.0 mg/l) of BAP. Visual observations like percent response of explants, number of days required for shoot induction, number of shoots formed and shoot length were periodically recorded.

Rooting of *in vitro* shoots:

After *in vitro* regenerated shoots attained a height of 2 cm, they were excised and planted on half strength WP basal medium supplemented with auxins 1.0 mg/l IBA for rooting. WPM with double the usual conc. of sucrose was used for rooting. Visual observations like percent response of explants, number of days required for root induction, number of roots formed and root length were periodically recorded. When sufficient roots developed then the plantlets were taken out from the medium and transferred in the soil after hardening or acclimatization.

Hardening and acclimatization of plantlets in Soil:

The rooted plantlets were gently pulled out of the medium and washed in running tap water. Medium sticking to the root was carefully removed. The plantlets with well-developed roots were transferred to sterilized soil and sand mixture (1:1) in small plastic pots. The soil and sand mixture was sterilized in the autoclave at 121°C temperature at 15 psi for 20 minutes. To maintain high humidity around the plants, for initial 15 days covered them with transparent polythene bags and made small holes in them for air circulation. Plants were watered with ¼ WPM salt solution on alternate days. Then pots were transferred in Polyhouse.

RESULTS & DISCUSSION

Nodal explants cultured on MS & WP basal medium:

In MS basal medium, Nodal explants showed 40% response and buds were initiated after 28 days of inoculation. Node showed slightly enlargement of bud only. While in WP basal medium, Buds were initiated after 18 days of inoculation with 60% response in nodal explants. Single shoot appeared on explant (Figure 1 & 2).

The response of *Cinnamomum camphora* seems to be dependent on the explant source as well as on the media more precisely with respect to the type and balance of growth regulators (15). The results clearly suggest that WPM was more effective in giving better response in respect to time of bud initiation, shoot length, number of shoots and size of leaves and was suitable as the basal medium for *Cinnamomum camphora*. Similar observations have been reported in *Desmodium oojeinense* (6), *Cinnamomum camphora* (11), *Tinospora cordifolia* (13). The suitability of WPM over MS medium might be due to its low ionic strength which counteracts salt sensitivity of woody species (7). Further, MS basal medium (without growth regulator) was not much effective in inducing shoot buds. Similarly no shoot buds developed in *Crataeva nurvala* (16), *Cinnamomum camphora* (11) and *Tinospora cordifolia* (12) on MS basal medium.

Effect of BAP on nodal segments cultured on MS Medium supplemented with BAP:

In MS medium, both at 0.5 and 1.0 mg/l BAP response of nodal explants was 80%. But bud initiation occurred after 22 and 20 days of

inoculation on 0.5 and 1 mg/l BAP, respectively. However, nodal explants on 2 mg/l BAP gave 60% response after 24 days of inoculation (Figure 3 & 4). Only single shoot appeared at each node at all the concentrations (Plate 2). Among these, cultures raised on 1 mg/l BAP gave best response in terms of time taken for bud initiation.

Effect of BAP on nodal segments cultured on WPM supplemented with BAP:

Nodal explants gave 100% response on BAP supplemented WPM. At all the concentrations of BAP single shoot appeared from each explant. However, the shoot length was maximum at 1 mg/l BAP. At this concentration, bud was initiated after 10 days of inoculation. At higher level bud initiation was delayed and bud appeared after 13 days. Further, the shoot length decreased at higher level i.e. 2.0 mg/l (Figure 3 & 4). The shoots formed on BAP supplemented medium were green, healthy, having dark green shiny leaves.

The growth regulators applied externally during *in vitro* studies might disturb the internal polarity and change the genetically programmed physiology of explants resulting in organogenesis from the explant. Therefore, in *Cinnamomum camphora,* BAP was reported to promote early bud initiation and was effective as bud inducer. Similar observations have been made in *Cinnamomum camphora* (11) and *Tinospora cordifolia* (13). Khan et al. (5) and Balaraju et al., (3) showed that BAP was an efficient growth regulator for shoot multiplication in *Datura metel* and *Pterocarpus santalinus*, respectively.

Formation of shoot buds on BAP containing cultures is also influenced by the concentration of BAP in the medium. BAP at 1mg/l was optimum for shoot bud formation in the present study and higher concentration (2mg/l) was inhibitory. Similar observations have been made in *Cinnamomum camphora* (11).

Effect of IBA on rooting of *in vitro* developed shoots:

In vitro developed shoots on different media were excised when attained a height of 2 cm and inoculated on WPM without and with 1.0 mg/l IBA for rooting. In the present investigation, rooting of shoots occurred when medium was supplemented with IBA. The result is summarized in Table 1. The effect of IBA on rooting was also observed in *Bacopa monnieri* (8) and *Chlorophytum borivilianum* (14). No rooting was observed on half-strength or full-strength WP basal medium having 4% (double the normal concentration) of sucrose. However, healthy and elongated roots were produced at 1 mg/l IBA in 80% of shoots within 30-35 days (Plate 3A). The promotive effect of IBA has also been reported for *in vitro* rooting in many other investigations (11, 12 and 13). Further, the low salt medium (half strength) was effective in root formation in present investigation as well as in *Withania somnifera* (4), *Maerua oblongifolia* (10), *Cinnamomum camphora* (11) and *Tinospora cordifolia* (12). However, Kumari & Shivanna (6) reported root initiation in *Desmodium oojeinense* in full strength medium.

Hardening and transfer of plantlets to the field: In the present investigation on

Cinnamomum camphora, for successful acclimatization to natural conditions and normal growth a careful and gradual transfer of *in vitro* regenerated plantlets were necessary. Therefore, shoots with well-developed roots were gently pulled out of the medium and transferred them to plastic cups having sterile soil and sand mixture (1:1) and irrigated with ¼ strength WP salt solution. High humidity was maintained for initial 15 days with the help of polythene bags and thereafter, these pots were exposed to natural conditions for 3-4 hrs daily (Plate 3B). After about a month the plants were shifted to pots in Polyhouse where they grew normally with 50% survival rate. After two months these plants were transferred to the field from Polyhouse. The survived plants grew normally. Successful acclimatization and field transfer of *in vitro* regenerated plantlets have also been reported in many other studies (11, 12 and 13).

CONCLUSION:

Cinnamomum camphora or Camphor tree is a source of camphor and camphor oil and widely used as a medicinal plant in several formulations. But, the conventional methods of propagation (by seeds, cuttings and layering) of camphor tree are very slow. In addition the long life cycle of this tree also hinders in conventional method of breeding. Therefore, any alternative method has to be developed to achieve their mass multiplication and propagation without any interruption. And it will be possible by *in vitro* tissue culture technique. Therefore, in this research paper, the effort is done to achieve the mass multiplication of this important medicinal tree via *in vitro* propagation of nodal explants.

REFERENCES:

Afolayan, A.J. and Adebola, P.O. (2004) *In vitro* propagation: A biotechnological tool capable of solving the problem of medicinal plants decimation in South Africa. African J. Biotech., 3(12):683-687.

Anonymus (1986) Useful plants of India. CSIR (Publication and Information Div.), New Delhi.

Balaraju, K., Agastian, P., Ignacimuthu, S. and Park, K. (2011) A rapid *in vitro* propagation of red sanders (*Pterocarpus santalinus* L.) using shoot tip explants. Acta Physiol. Plant., 33:2501-2510.

Kannan, P.; Ebenezer, G.; Dayanandan, P.; Abraham, G.C. and Ignacimuthu, S. (2005) Large scale production of *Withania somnifera* (L.) Dunal using *in vitro* techniques. Phytomorphology, 55(3, 4):259-266.

Khan, S., Tyagi, P., Kachhwaha, S. and Kothari, S.L. (2010) High frequency plant regeneration and detection of genetic variation among micropropagated plants of *Datura metel* L. Phytomorphology, 60:1-8.

Kumari, M.M.V. and Shivanna, M.B. (2005) Callus mediated regeneration of *Desmodium oojeinense* Roxb. Phytomorphology, 55(3, 4):171-177.

Lloyd, G.B. and McCown, B.H. (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot-tip culture. Proc. Int. Plant Prop. Soc., 30:421-427.

Mohaptra, H.P. and Rath, S.P. (2005) *In vitro* studies of *Bacopa monnieri* - an imp. medicinal plant with reference to its biochemical variation. Indian J. Expt. Biol., 42(4):373-376.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-497.

Rathore, J.S.; Rathore, M.S. and Shekhawat, N.S. (2005) Micropropagation of *Maerua oblongifolia* - a liana of arid areas. Phytomorphology, 55(3,4):241-247.

Sharma, H. and Vashistha, B.D. (2010) *In vitro* propagation of *Cinnamomum camphora* (L.) Nees & Eberm using shoot tip explants. Ann. Biol., 26:109-114.

Sharma, H. and Vashistha, B.D. (2015). *In vitro* plant regeneration through callus in Giloy (*Tinospora cordifolia*) (Willd.) Miers ex Hook. f & Thoms.). Indian J. Science, 12(34):59-68.

Sharma, H.; Vashistha B.D.; Singh, N. and Kumar, R. (2015) *Tinospora cordifolia* (willd.) miers ex hook. f & Thoms. (menispermaceae): rapid *in vitro* propagation through shoot tip explants. International Journal of Recent Scientific Research, 6(2):2714-2718.

Sharma, U. and Mohan, J.S.S. (2006) *In vitro* clonal propagation of *Chlorophytum borivilianum* Sant. et Fernand., a rare medicinal herb from mature floral buds along with inflorescence axis. Indian J. Exp. Biol., 44(1):77-82.

Soulange, J.G.; Ranghoo-Sanmukhiya, V.M. and Seeburrum, S.D. (2007) Tissue Culture and RAPD analysis of *Cinnamomum camphora* and *Cinnamomum verum*. Biotechnology, 6(2):1-6.

Walia, N.; Sinha, S. and Babbar, S.B. (2003) Micropropagation of *Crataeva nurvala*. Biol. Plant., 46(2):181-185.

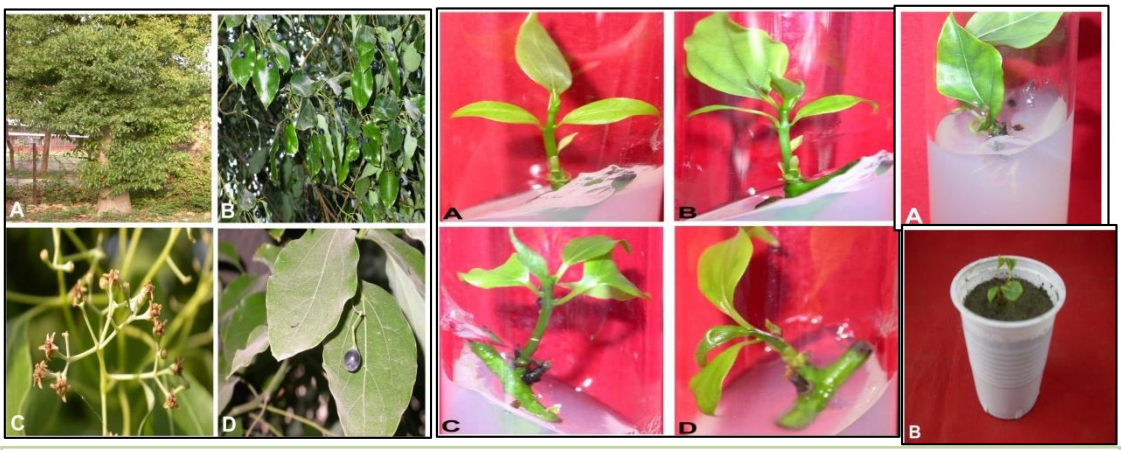

Plate1. (A-D) Morphology of *Cinnamomum camphora:* showing a tree and branch with flowering & fruiting.
Plate2. (A-D) Nodal segment on WPM supplemented with 0.5, 1.0 & 2.0 mg/l BAP showing shoot induction.
Plate3. (A) Rooting of *in vitro* shoot (B) Hardening of plantlet in pot.

Table. 1: Effect of IBA on rooting of *in vitro* shoots.

Treatment	Days required for root initiation	% root induction	Number of roots per shoot	Av. Root length (cm)
WPM	–	–	–	–
WPM + 1.0 IBA	32	80	2.1 ± 0.8	1.9 ± 0.4

(-) No Response

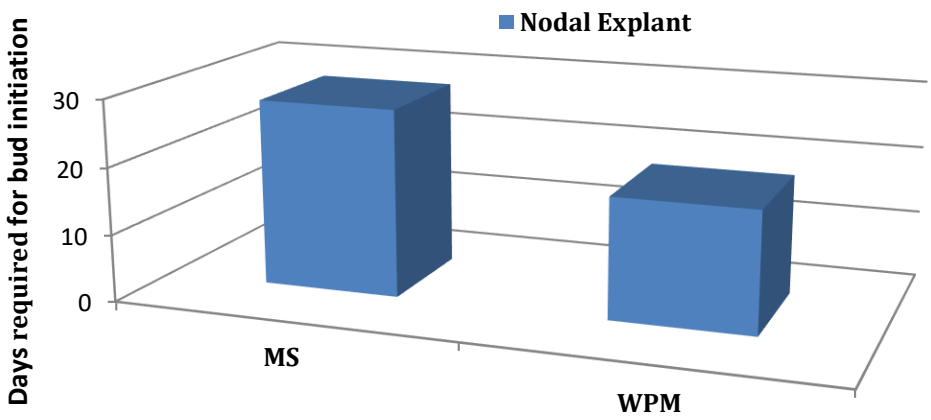

Figure 1. Response of nodalexplant on MS & WP basal medium

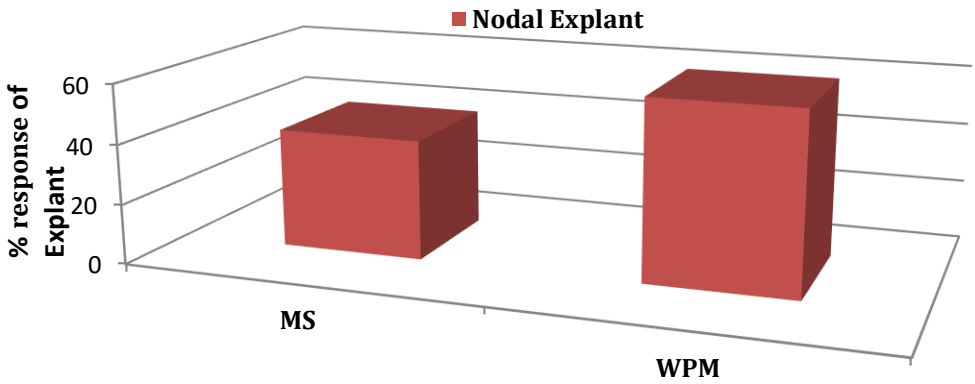

Figure 2. Response of nodalexplant on MS & WP basal medium

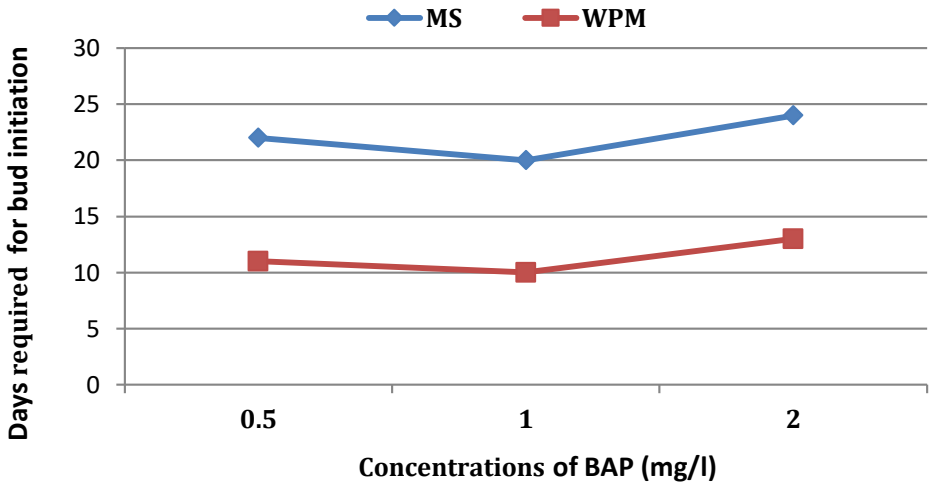

Figure 3. Effect of different concentrations of BAP on nodal explant

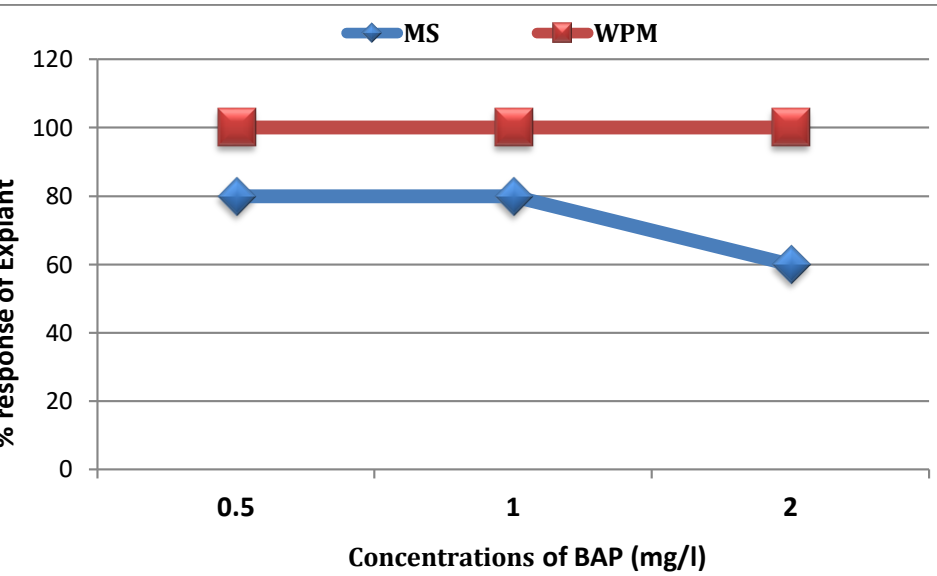

Figure 4. Effect of different concentrations of BAP on nodal explants