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Micropropagation of camphor tree (*Cinnamomum camphora*)

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Abstract

Multiple shoots were induced from shoot tips and nodal segments of a 12-year-old tree of *Cinnamomum camphora* on Woody Plant Medium (WPM) supplemented with BA and kinetin. The nodal segments from the *in vitro* developed plantlets could be induced again to produce a large number of harvestable shoots. Harvested shoots were rooted *in vitro* in WPM supplemented with activated charcoal (AC) and IBA. The plantlets were transferred to thermocol cups after which they were replanted into polybags and then to field. These plants survived with over 90% success under field conditions and exhibited vigorous growth. This system could be utilized for large-scale multiplication of *C. camphora* by tissue culture.

Abbreviations: AC – activated charcoal; BA – 6-benzyl aminopurine; IBA – indole 3-butyric acid; KIN – Kinetin; MS – Murashige and Skoog's medium (1962); NAA – α-naphthalene acetic acid; WPM – Woody Plant Medium (McCown and Amos, 1979); Z – Zeatin

Introduction

Camphor tree, *Cinnamomum camphora* (L.) Nees and Eberm., a medium sized ever-green tree is native of China, Japan and Formosa and has been introduced and cultivated in many countries including India as an ornamental plant or as a source of camphor. It occurs sporadically in the wet evergreen forests of North-Eastern states of India. Camphor is formed in the oil cells distributed in all parts of the tree, though its concentration varies in different parts, being maximum in the roots. Leaves contain about 60–90% essential oil. This tree is the source of natural camphor used commonly in indigenous medicine and in a variety of sweetmeats. It is used as a plasticizer in the preparation of explosives and disinfectants. External application acts as a counter irritant in the treatment of muscular strains, rheumatic conditions, inflammations and is slightly antiseptic (CSIR, 1950; Bailey and Bailey, 1976; Grieve, 1979).

Camphor plants can be raised from seed, layers, branch cuttings and root suckers. Low seed set and seed germination hampers propagation by seeds. Micropropagation of camphor will be of immense importance as it allows obtaining a large number of plants from the very limited source available.

Preliminary work on the tissue culture of related species like *Cinnamomum verum* and *C. cassia* has been reported by Ravishankar Rai and Jagdish Chandra (1987) and Inomoto and Kitani (1989), respectively. Multiple shoot induction, *in vitro* rooting (Huang et al., 1998) and somatic embryogenesis has been reported (Jagdish Chandra and Umer Sherif, 1994, 1995) in *C. camphora*. This paper reports the protocol for micropropagation of *C. camphora*, which can be utilised for the large-scale multiplication of the species.

Materials and methods

Source of explant and surface sterilization

A 12-year-old mature tree growing in the experimen-

tal farm of Indian Institute of Spices Research; Calicut was the source of explant. Shoot tips and nodal segments were used as explant. The material was washed in running tap water to remove the dust particles. The explants were subjected to a stepwise disinfection process; treatment with fungicide (0.3% copper oxychloride) solution for 45 min, wiping with dilute wetting agent (Tween-20), treating with fungicide Savistin (0.3%) for 30 min, and washing several times in sterile double distilled water before taking them to the sterile airflow chamber. Surface sterilization was carried out by treating with 0.1% mercuric chloride solution for 4–8 min, depending on the maturity of the explant, and then washing with sterile water (4–5 times). Blot-drying the explants on sterilized blotting paper followed a final rinse with 70% alcohol for 1 min. Shoot tips and nodal segments of about 2 cm size were then inoculated into the medium. Contamination free cultures were transferred to fresh media in every 15 days initially and thereafter every 20 days.

Culture medium and conditions

MS basal medium (Murashige and Skoog, 1962) and Woody plant medium (WPM) (McCown and Amos, 1979) were used. For culture initiation MS basal medium, fortified with 2% (w/v) sucrose and supplemented with 2.32 μM kinetin was used. The responding cultures were used for the multiplication trials. For multiple shoot induction, WPM basal medium supplemented with 6-benzyl aminopurine (BA), kinetin and zeatin were used at different concentrations and combinations. Basal medium supplemented with indole 3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) was used for *in vitro* rooting. Activated charcoal (AC) also was incorporated in the medium for root induction. Medium was fortified with 2% (w/v) sucrose and gelled with 0.7% agar. The pH of the medium was adjusted to 5.8 and the media was sterilised by autoclaving at 1.5 lbs pressure and 121 °C for 20 min. The cultures were incubated at 25±2 °C under 14-h photoperiod and 30–35 μmol m^{-2} s^{-1} with Philips' cool white fluorescent lamps.

Observations were taken from ten replicates per treatment based on percentage of culture response, with regard to number of multiple shoots per culture, number of roots per shoot etc.

Hardening

Healthy rooted plantlets generated *in vitro* were transferred to thermocol cups containing sterile coirdust and covered with polybags and kept in incubation room for 40–60 days, for first stage of hardening. They were later transferred to polythene bags containing a mixture of soil, perlite and sand (1:1:3) and kept in nursery for second stage of hardening. After 6 months of growth in the nursery, the plants were transferred to the field.

Results and discussion

Culture initiation

Establishment of contamination free shoots and nodal explants and the culture response was dependant on the selection of suitable explants. Partially mature shoots with a light green colour were suitable for the induction of best results. Contamination free shoots were transferred to fresh media in every 15 days initially and thereafter every 20 days. It is very important that subculture interval should not exceed more than 20 days at the initial stages specifically, since it adversely affected further response of the tissue. Bud break was observed within 20–30 days. The responding cultures were transferred to multiplication medium with a different combination of growth regulators. The requirement of cytokinin for the initiation of primary cultures was reported earlier (Huang et al., 1998).

Multiplication of shoots

Woody Plant Medium was more effective in giving suitable responses and hence used in the multiplication trials. Response of explants to multiple shoot induction medium was vigorous. The lateral shoots started growing within 30 days (Figure 1.1) and they produced multiple shoots from every node. Proliferation and production of large number of shoots from the shoot tips and nodal explants were observed within 40–60 days. The shoot tip with axillary buds appeared like a clump and a number of buds of size ranging from 0.1 to 1 cm could be carefully separated from the cluster and this could be used for the induction of further multiplication.

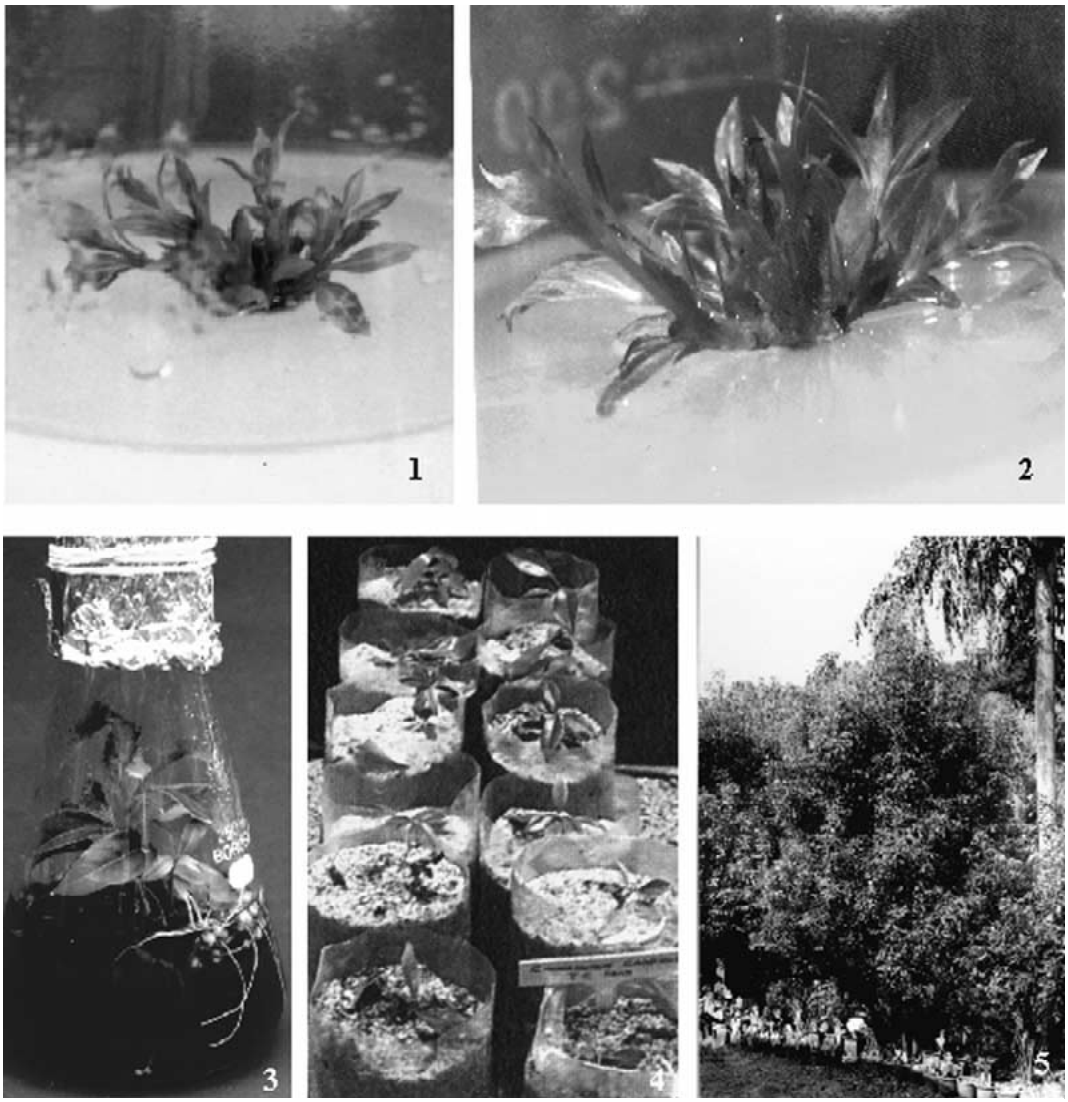

Figure 1. In vitro responses in camphor. 1. Induction of axillary bud growth *in vitro*; 2. Multiple shoots; 3. Root induction from *in vitro* derived shoots; 4. Hardening of plantlets in soil; 5. A view of field growing tissue cultured camphor trees.

In order to select the most suitable medium for multiplication, different combinations were tried and the results are given in Table 1. WPM was suitable as the basal medium for favourable response. BA alone and in combination with kinetin proved superior to kinetin and zeatin for multiple shoot production. WPM with 13.32 μM BA and 4.65 μM kinetin gave best results (Figure 1.2) and was used for further multiplication. Medium supplemented with zeatin either alone or in combination with kinetin was superior in terms of shoot elongation and leaf size. Though this has an added advantage of getting sufficiently elongated shoots for rooting, due to high cost of zeatin, it cannot be recommended for large scale multiplication as it increases the cost of tissue cultured plants. Hyperhydricity of culture in the medium

Table 1. Effect of cytokinins on multiple shoot induction

WPM+growth regulators (μM)	Response (%)	No. of multiple shoots Mean±S.D.*
Kin (2.32)	83	–
Kin (4.65)	75	–
Kin (9.29)	68	–
BA (2.22)	60	7.1±2.5
BA (4.44)	80	12.1±3.6
BA (8.87)	80	12.6±3.2
BA (13.3)	70	9.5±2.3
BA (4.44)+Kin (4.65)	87	12.8±3.4
BA (4.44)+Kin (9.29)	83	12.2±3.5
BA (8.87)+Kin (4.65)	70	11.2±1.1
BA (13.3)+Kin (9.29)	85	13.9±2.8
BA (13.3)+Kin (4.65)	85	20.0±4.8
Z (2.28)	83	–
Z (4.56)	89	–
Z (9.12)	62	–
Z (4.56)+Kin (4.65)	85	3.2±1.6
Z (9.12)+Kin (9.29)	70	7.1±2.2
Z (9.12)+Kin (4.65)	30	3.5±1.2
Z (4.56)+Kin (9.29)	40	6.5±1.4

*Mean of 10 replications; '–' single shoot growth.

containing higher levels of BA was reported by Huang et al. (1998) but in the present study it was negligible.

In vitro *rooting*

Different media combinations were used for *in vitro* rooting and the results are given in Table 2. Rooting of *in vitro* generated shoots (1–1.5 cm) could be achieved in 20–30 days by the incorporation of activated AC (2.0 g l^{-1}) to the medium. In all the media combinations with AC thick, healthy, elongated roots were developed (Figure 1.3). WPM alone and in combination with AC is used as control and prominent root system was produced within 90 days. Though root system is prominent in all combinations with AC, WPM with 2.46 μM IBA gave 1–4 roots/shoot within 20 days of culture. Medium without AC

Table 2. Effect of auxins and charcoal on rooting

WPM+additives (μM)	Response (%)	Av. Root length* (cm)	Av. no. of root/shoot*
WPM	100	5.0±1.6	1.2±0.9
AC (2)	100	11.0±2.1	1.7±0.8
IBA (4.9)	40	5.6±1.5	1.7±0.2
IBA (4.9)+AC (2)	100	8.2±2.1	1.5±0.2
IBA (2.46)	100	13.0±2.6	2.6±0.7
IBA (9.8)	60	1.7±1.2	1.3±0.1
IBA (9.8)+AC (2)	82	3.1±1.1	1.7±0.2
NAA (5.37)	–	–	–
NAA (5.37)+AC (2)	83	2.9±1.1	1.1±0.1
NAA (10.74)	–	–	–
NAA (10.74)+AC (2)	75	3.9±1.2	1.0±0.1
IBA (2.46)+NAA (2.69)	–	–	–
IBA (2.46)+NAA (2.69)+AC (2.0)	70	5.1±0.6	1.02±0.7
IBA (4.9)+NAA (5.37)	20	2.4±0.3	1.1±0.2
IBA (4.9)+NAA (5.37)+AC (2)	80	5.8±1.2	1.1±0.7

*Mean of 10 replications taken after 90 days.

responded differently. NAA alone and in combination with IBA induced callusing in 85% of the cultures. Rooting of multiplied shoots in this species was achieved in a medium with 0.5 μM NAA (Huang et al., 1998).

Hardening and field planting

In vitro generated plantlets could be established with 70% success on initial hardening in the incubation room. These plantlets could be established with over 90% success under the nursery conditions (Figure 1.4). Hardened plants have been planted in the field, established with 100% success. The micropropagated plants exhibited vigorous growth under field conditions (Figure 1.5).

Conclusion

This protocol, with a high rate of multiplication, can be successfully utilized for micropropagation of camphor, a medicinally important tree, on a large scale, more so, as it allows obtaining a large number of plants from the very limited source available.

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