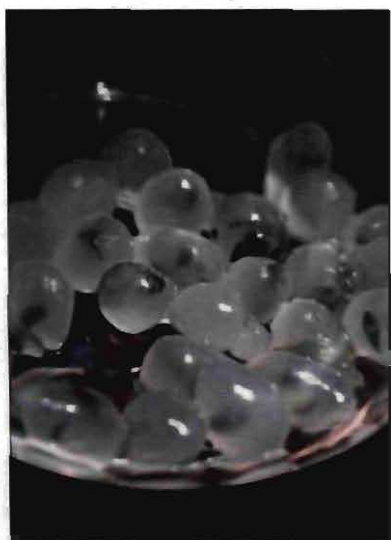
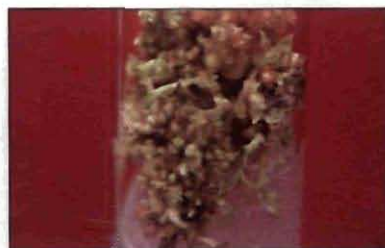




Protocols for Micropropagation of Spices and Aromatic Crops

Edited by

Nirmal Babu K
Ravindran P N
Peter K V



IISR PUB-31



Indian Institute of Spices Research
Calicut - 673012, Kerala, India

Front Cover

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1. Conversion of root meristem into shoot in vanilla
2. Plant regeneration in ginger
3. Somatic embryogenesis in cinnamon
4. Synthetic seeds in camphor

2

3

4

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Indian Institute of Spices Research
(Indian Council of Agricultural Research)
Calicut - 673012, Kerala, India

*“To those in the distant past
who made India the Land of Spices”*

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FOREWORD

India is the legendary land of spices, and spices have influenced the course of history and human civilisation. In the western world, once, black pepper was ranked on par with gold in value and it was the search for the source of 'black gold' that led to many ocean expeditions which culminated in the discovery of sea routes, continents and islands. It was the same lure that led Vasco-da-Gama to discover the sea route to India and his landing on the Malabar coast in 1498, opening a new chapter in the history of our nation.

*India holds a prominent position in the World Trade of spices contributing to 12.92% of the global trade in value and 34.22% in volume. Currently India is producing 20-23 lakh tonnes of spices worth Rs. 5,200 crores. However, the low productivity of various spices in India is because of various constraints. The more serious among them are the foot rot disease of black pepper caused by *Phytophthora capsici*, the 'Katte' disease of cardamom, the rhizome rot and bacterial wilt diseases of ginger etc. Non availability of disease free planting material is a major bottleneck in increasing the production and productivity of spices. It is in this context that the frontline research work of the Indian Institute of Spices Research assumes great significance. The IISR has developed tissue culture protocols for a large number of spices including black pepper, cardamom, ginger, turmeric, vanilla, large cardamom and many important herbal and seed spices. These protocols are now available for scaling up and commercialisation. Developing synseed technology for conservation and exchange of germplasm in the vegetatively propagated spices is another major achievement of IISR. The present compilation gives protocols for the micropropagation of 31 spices. This is the first time that such a publication on protocols of tissue culture for spices and aromatic plants has been brought out, based on the work of a single institution. I take this opportunity, to congratulate the Indian Institute of Spices Research, Calicut and the scientists concerned for this significant contribution.*

I wish them all success.

(Dr. R.S. Paroda)

October 1997



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INTRODUCTION

Spices are part of human history and civilisation. In medieval West, India was always associated with the land of maharajas, diamonds, ivory and spices. India still maintains its pre-eminent position in the global trade of spices. Currently India produces 23 lakh tonnes of spices valued Rs. 5200 crores, and we ourselves consume over 90% of the production. We receive Rs. 700 crores by way of export of spices. The latest list of ISO recognises 109 spices, but only a few are important commercially such as black pepper, cardamom, ginger, turmeric, large cardamom, cumin, coriander, fennel, fenugreek, garlic, saffron and celery. In spite of the rich diversity and importance, the productivity of spices is low in India. We are envisaging a production of 29.35 lakh tonnes during 1996-97 and 37.4 lakh tonnes by 2000 AD; and the projected export by 2001 AD is two lakh tonnes. To achieve even these moderate targets, considerable research support is required. The research and development agencies must work jointly on war footing towards a Targetted Constraint Alleviation Programme. These TCAPs are to remove the major constraints facing spices production in the country such as diseases (foot rot and slow decline of pepper, katte virus of cardamom, rhizome rot and bacterial wilt of ginger etc.), insect pests, drought, and malnutrition. One major constraint limiting spices production in the country, is the insufficiency in disease free quality planting material. Use of high quality and disease free planting materials is the first major requirement for the enhancement of productivity of spices.

It is in this background that the present publication from IISR assumes significance. Tissue culture is a practical solution to produce pest and disease free planting material on a large scale and the technology is being made use of in many horticultural and ornamental plants. The Indian Institute of Spices Research during the past one decade of existence (earlier as NRCS) developed tissue culture protocols for about 31 spices and these technologies are now available for scaling up and commercial production. This publication embodies these protocols in an abridged form for the use of industry and scientists. Developing successful tissue culture protocols for a crop is a time consuming process and developing such protocols for 31 spices by a single group is really, a great feat. These protocols are also useful in crop improvement, conservation of genetic resources and exchange of germplasm. I am sure, this publication will have considerable appeal to the tissue culture scientists in general and in particular spice workers, traders and manufacturers.

K.L. Chadha

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October 1997



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PREFACE

Indian spices flavour foods in over 130 countries in the world. India is also the largest producer, exporter and consumer of spices. With emphasis on natural colour and fragrance, spices are receiving global attention in fast food industry.

Incidence of Phytophthora foot rot and stunted disease in black pepper, 'katte' viral disease in cardamom and rhizome rot in ginger and turmeric are still major diseases affecting production and productivity. Indian spices industry is, however facing certain constraints and problems. Non-availability of quality disease / pest free planting materials is one of the major production constraints. The Indian Institute of Spices Research, Calicut, has been working on development of protocols for micropropagation of spices since 1985. These protocol are useful for rapid multiplication of elite planting materials and also to genenerate variability through callus culture for screening against biotic and abiotic stresses. The production of quality planting materials of important spice crops. The present bulletin carries information on protocols for tissue culture of thirty one spices.

I am sure that the publication will be of great use to biotechnologists and to those involved in R&D activities in spice crops

October 1997

S. P. Ghosh
S.P. GHOSH

Abbreviations

MS	-	Murashige and Skoog medium (Murashige & Skoog, 1962)
WPM	-	Woody Plant Medium (McCown & Amos, 1978)
BA	-	N ⁶ -benzyladenine
NAA	-	α - Naphthalene acetic acid
Kin	-	Kinetin
2,4-D	-	2, 4-dichlorophenoxy acetic acid
IBA	-	Indole 3-butyric acid
HgCl ₂	-	Mercuric chloride



Defined in the Oxford English Dictionary as “one or other of various strongly flavoured or aromatic substances of vegetable origin, obtained from tropical plants, commonly used as condiments,” spices are aromatic, dried roots, bark, buds, seeds, berries and other fruits. The word ‘spice’ derives from the Latin word *species*, meaning specific kind and later, goods or merchandise.

India was once the legendary land of spices. It was the lure of Indian spices that attracted the ancient Egyptians, Romans, Arabs and the rest of the world to India. It was in search of spices, Vasco da Gama landed in the Malabar coast of India which changed the course of Indian history. Spices have played a pivotal and colourful role in the history of mankind. As one looks back across the 5000 years of recorded history, one realises that the Western world knew nothing of spices, tea or coffee. India supplied them with flavour and piquancy to their food and drink; aroma and fragrance to mask the unpleasant aroma of their food and body. The spices were so precious and indispensable that 'Kings sent expeditions in search of them; merchants risked life and fortune to trade in them, wars were fought over them, whole populations were enslaved; the globe was explored and much far reaching changes were brought about by the restless and ruthless competition'

Some of the important spices and aromatic plants are pepper, cardamom, ginger, turmeric, vanilla, cinnamon, cassia, camphor, mint, celery, fennel, marjoram, thyme, oregano, ocimum, parsley etc. They acquire their characteristic odour from volatile constituents in the plant material. Spices and aromatic plants are extensively used either directly or after extraction of volatile oils as condiments and flavouring materials. They are extensively used in flavouring food and confectionery, perfumery and soaps and also as colouring materials. Many of these groups of crops have medicinal properties and are used in many Ayurvedic and Unani medicines.

Spices are of considerable importance for India and are a major source of foreign exchange among agricultural commodities. India has been traditionally known as the land of spices and has a pre-eminent position in the production and export of these crops India accounts for about 47% of the global trade. India is a rich repository of spices and over 100 species of spices are grown in about 2 million hectares in the country with an annual production of 2.02 million tones fetching Rs. 7,000 crores of revenue and Rs. 1184 crores worth of foreign exchange. Spices play a crucial role in the economy of the states in which they are grown.

Spices and Aromatic crops being crops based on quality of the produce, it is essential to identify genotypes with high quality attributes and use the same genotypes for commercial planting. In this context, micropropagation protocols come in handy in multiplying the elite genotypes and production of disease free planting material. These techniques could also be used for multiplication of biomass and *in vitro* conservation of genetic resources of these crops. The plant regeneration protocols developed can be used for induction and exploitation of somaclonal variation for crop improvement especially in spices with limited variability in germplasm.

Protocols for micropropagation of some of the major spices and aromatic crops were standardised at the Indian Institute of Spices Research, Calicut. A condensed version of these protocols are presented in this book.

1. **Plant species:** BLACK PEPPER (*Piper nigrum* L.)
2. **Family:** Piperaceae
3. **Origin and distribution:** Black pepper is native to the tropical evergreen forests of SouthWest India. It is cultivated commercially in India, Indonesia, Malaysia, Brazil, Sri Lanka and Vietnam^{1,21}.
4. **Accessions in the germplasm:** 3000
5. **Uses:** Black pepper and white pepper are used mainly in flavouring of processed food, perfumery and in seasoning meat products. In medicine, it is used as an aromatic stimulant in cholera, as an antipyretic in malarial fever and arthritic diseases^{1&3}. The active principle in pepper is piperine, responsible for the biting taste.
6. **Explants for micropropagation:** Shoot tip and nodal segments for clonal propagation stem and leaf tissues for plant regeneration and shoot tips for *in vitro* conservation.
7. **Culture conditions and media :** Surface sterilisation with 0.1% $HgCl_2$ for 5-10 min. Incubation at $22\pm 2^\circ C$ with 14 h photoperiod of 3000 lux.
 - a) $\frac{1}{2}$ WPM + 3 $mg\ l^{-1}$ BA + 1 $mg\ l^{-1}$ Kin for multiple shoots and callus regeneration.
 - b) $\frac{1}{2}$ WPM without growth regulators for *in vitro* rooting
 - c) $\frac{1}{2}$ WPM + 15 $g\ l^{-1}$ sucrose + 15 $g\ l^{-1}$ mannitol for *in vitro* conservation by slow growth
8. ***In vitro* responses:** Multiple shoots could be induced from shoot tips and nodal explants in 60 days and callus regeneration from leaf, shoot tip and stem explants was induced when cultured on $\frac{1}{2}$ WPM + 3 $mg\ l^{-1}$ BA and 1 $mg\ l^{-1}$ Kin within 90 days. Shoot tip-cultures could be maintained up to 1 year without subculture in $\frac{1}{2}$ WPM + 15 $g\ l^{-1}$ sucrose + 15 $g\ l^{-1}$ mannitol^{2, 13, 14, 17, 22,23}.

Black pepper cultures are plagued by endogenous bacteria contaminating cultures. Use of antibiotics in the culture media help in reducing bacterial contamination.

9. **Hardening and field performance:** *In vitro* rooted plantlets were transferred to a mixture of garden soil, sand and vermiculite



Production of multiple shoots



Micropropagated plants in pots

(1:1:1) with 80% success when kept in humid chamber for 20 days for hardening. Preliminary evaluation of micropropagated plants, based on vegetative characters after 2 years in the field, indicated that micropropagated plants are uniform and are similar to their parental stocks.

10. **Applications:** The protocols could be used for clonal multiplication of elite genotypes, creating somaclonal variation for crop improvement and for *in vitro* conservation and exchange of germplasm.

1. **Plant species:** INDIAN LONG PEPPER (*Piper longum* L.).
2. **Family:** Piperaceae.
3. **Origin and distribution:** Occurring in hotter parts of India, from central Himalayas to Assam, Khasi and Mikir hills and ever-green forests of the Western Ghats from Konkan to Travancore¹.
4. **Accessions in the germplasm:** 20
5. **Uses:** Fruits are used as spice and also in pickles and preserves. Alkaloids like piperine, pipartine are present in the fruit. The roots and thicker parts of the stem are dried and used as an important drug (Pipalmul) in the Ayurvedic and Unani systems of medicine¹.
6. **Explants for micropropagation:** Shoot tips and nodal segments for clonal propagation; leaf, stem and roots for plant regeneration and shoot tips for *in vitro* conservation
7. **Culture conditions and media :** Surface sterilisation with 0.1% HgCl₂ for 5-10 minutes. Incubation at 22±2° C with 14 h photoperiod of 3000 lux.
 - a) WPM + 3 mg l⁻¹ BA + 1 mg l⁻¹ Kin for multiple shoots and callus regeneration
 - b) 1/2 WPM without growth regulators for rooting.
 - c) WPM + 20 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for slow growth.
8. **In vitro responses:** WPM with 3 mg l⁻¹ BA and 1 mg l⁻¹ Kin induced shoot regeneration from leaf, stem and root explants directly as well as from callus. *In vitro* rooting was induced on growth regulator free medium. Slow growth could be induced in WPM + 20 g l⁻¹ sucrose + 10 g l⁻¹ mannitol without growth regulator in sealed culture tubes^{2,13,14,17,22,23}.
9. **Hardening and field performance:** Over 95% of the plantlets could be established in a mixture of garden soil, sand and vermiculite (1:1:1) by keeping them in humid chamber for 20 days. Preliminary observations on two year old plantlets in the field indicated that



Organogenesis from shoot explants



Tissue cultured plants in field

tissue cultured plants flowered early and have more number of axillary shoots initially.

10. **Applications:** The protocols could be used for clonal multiplication of elite genotypes, creating somaclonal variation for crop improvement and for *in vitro* conservation and exchange of germplasm

1. **Plant species:** JAVA LONG PEPPER (*Piper chaba* Hunt.).

2. **Family:** Piperaceae.

3. **Origin and distribution:** Native of South East Asia, *P. chaba* (Java long pepper) is cultivated in Malaysia, Indonesia and Singapore¹.

4. **Accessions in the germplasm:** 2

5. **Uses:** The fruits are used as spice and also in pickles, preserves and in indigenous medicine. The product of this spice is known as long pepper. Fruits have stimulant and carminative properties, stem has properties similar to *Pipli* from *P. longum*. It contains alkaloids piperine and pipartine^{1,17}.

6. **Explants for micropropagation:** Shoot tips and nodal segments for micropropagation, and *in vitro* conservation. Leaf and stem explants for plant regeneration.

7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl_2 incubation at $22 \pm 2^\circ \text{C}$ with 14 h photoperiod of 3000 lux.

a) WPM + 3 mg l^{-1} BA + 1 mg l^{-1} Kin for multiple shoots and callus regeneration

b) WPM without growth regulators for *in vitro* rooting.

c) WPM + 20 g l^{-1} sucrose + 10 g l^{-1} mannitol for slow growth.

8. ***In vitro* responses:** WPM with 3 mg l^{-1} BA + 1 mg l^{-1} Kin induced shoot regeneration from leaf and stem explants, directly as well as from callus. *In vitro* rooting could be induced on growth regulator free medium. Slow growth could be induced in WPM + 20 g l^{-1} sucrose + 10 g l^{-1} mannitol without growth regulator in sealed culture tubes^{2,14,22,23}.

9. **Hardening and field performance:** Over 95% of the plantlets could be established in a mixture of garden soil, sand and vermiculite (1:1:1) by keeping them in humid chamber for



Piper chaba



Plantlets from callus

20 days. Preliminary observations on two year old plantlets in the field indicated that tissue cultured plants flowered early and have more number of axillary shoots initially.

10. **Applications:** Protocols can be used for clonal multiplication of elite genotypes. Regenerants from callus can be utilised for crop improvement by exploiting somaclonal variation. Safe exchange of germplasm can be done using *in vitro* culture.

1. **Plant species:** BETELVINE (*Piper betle* L.)
2. **Family:** Piperaceae.
3. **Origin and distribution:** Betel vine is native to Malaysia and is cultivated in India, Philippines, Indonesia. It thrives well under tropical forest conditions with an annual rainfall of 225-475 cm upto an altitude of 900m¹.
4. **Accessions in the germplasm:** 15
5. **Uses:** Betel leaves are used as a masticatory and for chewing. It is digestive, stimulant and carminative. Medicinally it is useful in catarrhal and pulmonary affections. Active principle in oil of betel is chavibetol (isomer of eugenol). Leaves contain large amount of vitamin B and essential amino acids¹⁹.
6. **Explants for micropropagation:** Shoot and nodal segments for clonal propagation and *in vitro* conservation. Leaf and shoot tissue for callus regeneration.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂ incubation at 22±22°C with 14 h photoperiod of 3000 lux.
 - a) WPM + 3 mg l⁻¹ BA + 1 mg l⁻¹ Kin for multiple shoot production and regeneration.
 - b) Growth regulator free WPM for rooting.
 - c) 1/2 WPM + 20 gl⁻¹ sucrose + 10 gl⁻¹ mannitol for slow growth
8. **In vitro responses:** Explants from shoot, leaf and root tissues developed multiple shoots and regenerated into plantlets either directly or through intervening callus phase on WPM supplemented with 3 mg l⁻¹ BA and 1 mg l⁻¹ Kin. The excised shoots developed good root system on growth regulator free medium of the same composition. Slow growth could be included in 1/2 WPM+20gl⁻¹ sucrose+10 gl⁻¹ mannitol in sealed culture tubes^{2,9,13,14,22,23}.
9. **Hardening and field performance:** The plantlets were planted out in a mixture of garden soil, sand and vermiculite (1:1:1) in humid chamber for 15-20 days and transferred to soil with 80% success.
10. **Applications:** Clonal propagation of elite



Induction of multiple shoots



Micropropagated plant in field

genotypes for production of disease-free planting material, exploitation of somaclonal variation for crop improvement and *in vitro* conservation of germplasm.

1. **Plant species :** *Piper colubrinum* Link.
2. **Family:** Piperaceae
3. **Origin and distribution:** It is a South American species distantly related to *Piper nigrum* L and is the only species reported to be totally immune to *Phytophthora* foot rot of black pepper¹³.
4. **Accessions in the germplasm:** 1
5. **Uses:** *Piper colubrinum* is resistant to major diseases and pests of black pepper viz. foot rot caused by *Phytophthora*, slow decline caused by *Radopholus similis* and *Meloidogyne incognita*, hollow berry of black pepper caused by pollu beetle¹³.
6. **Explants for micropropagation:** Shoot tips and nodal segments for micropropagation and *in vitro* conservation. Leaf and stem tissues for callus regeneration.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂. Incubation at 22±2°C with a 14 h photoperiod of 3000 lux.
 - a) WPM + 3 mg l⁻¹ BA + 1 mg l⁻¹ Kin for multiple shoot induction and regeneration from callus
 - b) WPM without growth regulators for *in vitro* rooting.
 - c) WPM + 20 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for slow growth.
8. **In vitro responses:** This species could be successfully micropropagated from leaf, stem and root tissues. They developed multiple shoots on WPM supplemented with cytokinins. Direct organogenesis as well as through callus phase was obtained on WPM + 3 mg l⁻¹ BA and 1 mg l⁻¹ Kin. Shoots developed excellent root system on hormone free medium. Slow growth could be included in WPM + 20 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in sealed culture tubes^{2,14,17,19,22}.
9. **Hardening and field performance:** *In vitro* rooted plantlets were kept in humid chamber for 15-20 days in a mixture of garden soil, sand and vermiculite (1:1:1) and transferred to soil with 85% success. Tissue cultured plants showed vigorous growth with



Plant regeneration from leaf tissues



Micropropagated plants in field

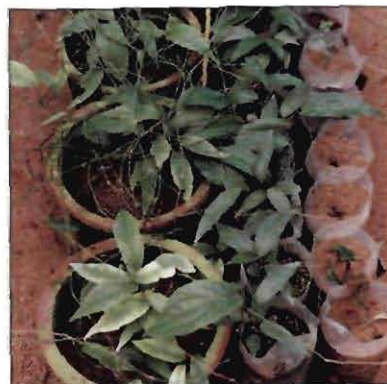
production of more axillary branches and early flowering after two years of field evaluation.

10. **Applications:** Grafting of black pepper on *P. colubrinum* root stocks is an important aspect of *Phytophthora* foot rot disease management. These protocols will help in large scale production of *P. colubrinum* root stocks and in attaining the ultimate objective of transfer of resistance from *P. colubrinum* to cultivated black pepper through genetic engineering.

1. **Plant species:** *Piper barberi* Gamble.
2. **Family:** Piperaceae.
3. **Origin & distribution:** *P. barberi* is a rare and almost extinct species of *Piper*¹⁶.
4. **Accessions in the germplasm:** 7
5. **Uses:** A rare and endangered species related to black pepper assumed to have medicinal properties.
6. **Explants for micropropagation:** Leaf tissue, shoot tips and nodal segments were used for multiplication and shoot tips for *in vitro* conservation
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14 h photoperiod at 3000 lux.
 - a) WPM + 3 mg l⁻¹ BA + 1 mg l⁻¹ Kin for multiple shoots and plant regeneration.
 - b) WPM without growth regulator for *in vitro* rooting.
 - c) WPM + 25 g l⁻¹ sucrose + 5 g l⁻¹ mannitol for conservation by slow growth.
8. ***In vitro* responses:** Multiple shoots (10-20) could be induced in 60 days of culture in WPM supplemented with 3 mg l⁻¹ BA and 1 mg l⁻¹ Kin. Regeneration of plantlets from the cut end of the petiole in 75% of the cultures, was also obtained. The number of plants regenerated ranged from 5-15 in 60 days of culture. Growth regulator free medium was better suited for rooting of excised shoots. *P. barberi* cultures could be maintained *in vitro* under slow growth in ½ WPM + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol upto 1 year^{2,13,14,17,18,22}.
9. **Hardening and field performance:** The *in vitro* rooted plantlets could be transferred to soil with 80% success, after hardening in a humid chamber for 15-20 days in a mixture of garden soil, sand and vermiculite in equal proportions
10. **Applications:** The protocols can be used for micropropagation, rapid multiplication and conservation of this endangered species.



Production of multiple shoots



Tissue cultured plants in pots

1. **Plant species:** **CARDAMOM** (*Elettaria cardamomum* Maton.).
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** Cardamom grows wild in the rain forests of southern India and Sri Lanka at altitudes between 750-1500 m. It is now cultivated widely in India, Sri Lanka, Guatemala, Tanzania and Vietnam²¹.
4. **Accessions in the germplasm:** 265
5. **Uses:** Cardamom spice obtained by drying the fully developed fruit capsules is used on a world wide basis for domestic culinary purposes. The active principles in cardamom are α - terpenyl acetate and 1, 8 cineole. Tincture of cardamom is used in medicine for flatulence and stomach disorder. It is used as mouth refreshner and digestive aid^{1,3,21}.
6. **Explants for micropropagation:** Rhizome bits with vegetative buds for micropropagation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% $HgCl_2$, incubation at $22 \pm 2^\circ C$ with 14 h photoperiod of 3000 lux.
 - a) MS + 1 $mg\ l^{-1}$ NAA for rooting.
 - b) MS + 1 $mg\ l^{-1}$ BA + 0.5 $mg\ l^{-1}$ NAA for multiplication and rooting.
 - c) MS without growth regulators for rooting
 - d) $\frac{1}{2}$ MS + 15 $g\ l^{-1}$ sucrose + 15 $g\ l^{-1}$ mannitol for slow growth.
8. **In vitro responses:** Multiple shoots upto 8 could be induced on MS with NAA within 90 days of culture and subsequently in every 70 days. Plants could be regenerated on MS + BA and NAA. Rooting was better in growth regulator free medium especially in liquid medium. Cardamom cultures could be maintained upto 1 year on $\frac{1}{2}$ MS + 15 $g\ l^{-1}$ sucrose + 15 $g\ l^{-1}$ mannitol in sealed culture tubes. *In vitro* raised plantlets were established in soil with 80% success after hardening in a humid chamber for 30 days, in a mixture of garden soil, sand and vermiculite in equal proportions^{12,13,15,17,22}.
9. **Hardening and field performance:** After 5 years of field evaluation, tissue cultured plantlets performed similar to that of the control.



Induction of multiple shoots



Tissue cultured plants in field

10. **Applications:** The protocols can be used to multiply elite genotypes, producing disease free planting materials and production of somaclones to exploit somaclonal variation for crop improvement. The slow growth method is ideal for *in vitro* conservation of germplasm.

1. **Plant species:** LARGE CARDAMOM (*Amomum subulatum* Roxb.).
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** Large cardamom is native of Eastern Himalayas and is cultivated in India, Nepal, China and South East Asia^{1, 21}.
4. **Accessions in the germplasm:** 15
5. **Uses:** The seeds are used in preparation of sweet meats. In medicine, they are fragrant adjuvants to other stimulants, bitter and purgative. The oil extracted from them is applied to eyelids to reduce inflammation. Cineole contributes to its pungency while terpenyl acetate towards the pleasant aroma^{1, 19}.
6. **Explants for micropropagation:** Vegetative buds and rhizome bits with buds for micropropagation and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14 h photoperiod of 3000 lux.
 - a) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA⁻¹ for micropropagation.
 - b) 1/2 M S + 10 gl⁻¹ sucrose + 10 gl⁻¹ mannitol for *in vitro* conservation by slow growth.
8. **In vitro responses:** Multiple shoots (5-10) could be induced from vegetative buds explants in MS with BA (1mg l⁻¹) and IBA (0.5 mg l⁻¹) within 60 days of culture. Plantlets could be maintained up to 1 year without sub-culture in 1/2 M S + 10 gl⁻¹ sucrose + 15 gl⁻¹ mannitol in screw capped culture tubes^{12, 13, 17, 22}.
9. **Hardening and field performance:** The rooted plants could be separated and transferred to soil with over 80% success. The field planted tissue cultured plants after 1 year, performed similar to that of control.
10. **Applications:** Protocols can be used for multiplication of disease-free planting material of elite genotypes. The slow growth method is ideal for *in vitro* conservation of the species.



In vitro multiplication of plantlets



Tissue cultured plants in pots

1. **Plant species:** GINGER (*Zingiber officinale* Rosc.).
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** Ginger is native of the tropical southern Asia and is now widely grown in India, China, Hawaii, Japan, Pakistan, West Indies, Africa and Australia.
4. **Accessions in the germplasm:** 450
5. **Uses:** It is used in sweet and savoury cooking, curry powder, spice blends and in medicine as a digestive aid. Ginger tea helps to improve the circulation and eases travel sickness. Gingerol and shogaol are the major constituents of ginger essential oil.
6. **Explants for micropropagation:** Vegetative buds, immature inflorescences and rhizome bits for clonal propagation. Vegetative buds, ovary, leaf and anther tissues for plant regeneration via callus phase and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media :** Surface sterilisation with 0.1% HgCl_2 , incubation at $22 \pm 2^\circ\text{C}$ with 14 h photoperiod at 3000 lux.
 - a) MS + 1 mg l^{-1} NAA for multiple shoots and *in vitro* rooting.
 - b) MS + 2 mg l^{-1} 2,4-D for callus induction.
 - c) MS + 10 mg l^{-1} BA + 0.2 mg l^{-1} 2,4-D for conversion of floral buds to plantlets and production of somatic embryos.
 - d) $\frac{1}{2}$ MS + 10 g l^{-1} sucrose + 10 g l^{-1} mannitol for *in vitro* conservation by slow growth.
8. ***In vitro* responses:** Ginger could be micropropagated from both vegetative buds and rhizome explants on MS medium + 1 mg l^{-1} NAA. Callus could be induced from different explants in MS+2 mg l^{-1} 2, 4-D. Plant regeneration *via* callus derived from leaf, ovary, vegetative buds and anther was obtained on MS + 10 mg l^{-1} BA + 0.2 mg l^{-1} 2,4-D. Conversion of floral buds into vegetative buds was induced on the same medium. Ginger plantlets could be stored *in vitro* upto 1 year in $\frac{1}{2}$ MS + 10 g l^{-1} sucrose + 10 g l^{-1} mannitol in screw capped tubes^{2,10,11,12,13,16,17,20,22}.



Micropropagated plantlets

9. **Hardening and field performance:** *In vitro* rooted plantlets were established with 90% success, when transplanted into a mixture of garden soil : sand and vermiculite (1:1:1) and kept in humid chamber for 20 days. Field evaluation of tissue cultured plants indicated that it takes three crop seasons for the micropropagated plants to develop rhizomes of normal size, hence they cannot be used directly for commercial planting.
10. **Applications:** *In vitro* techniques can be used for *in vitro* pollination and seed set. Somaclonal variation can be an important source of variability to evolve high quality lines and lines resistant to rhizome rot. The slow growth method can be utilised for germplasm conservation. Somatic embryos encapsulated in sodium alginate, (synthetic seeds) are of immense use in germplasm exchange and conservation.

1. **Plant species:** MANGO GINGER (*Curcuma amada* Roxb.).
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** Mango ginger occurs wild in parts of Bengal, Konkan and Madras and is native of Malay archipelago, Siam and India¹.
4. **Accessions in the germplasm:** 6
5. **Uses:** Rhizome of mango ginger is considered as carminative and stomachic and is also used for contusions and sprains. It is used in food as spice because of its typical mango like flavour. The plant is also important for its essential oil content, which has antifungal properties^{1,20}.
6. **Explants for micropropagation:** Vegetative buds, rhizome explants with buds for micropropagation and plant regeneration through callus and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14 h photoperiod of 3000 lux.
 - a) MS + 1mg l⁻¹ BA + 0.5 mg l⁻¹ NAA for micropropagation.
 - b) 1/2 MS + 10 gl⁻¹ sucrose + 10 gl⁻¹ mannitol for *in vitro* conservation by slow growth.
8. ***In vitro* responses:** Multiple shoots and rooting could be induced from vegetative buds as well as rhizome explants on MS with 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA. The plantlets could be maintained up to 1 year without subculture on 1/2 MS + 10 gl⁻¹ sucrose + 10gl⁻¹ mannitol in screw capped culture tubes^{2,15,20}.
9. **Hardening and field performance:** The plantlets could be established in soil with over 80% success, when transferred to a mixture of garden soil, sand and perlite (1:1:1) and kept in humid chamber for about 20 days. It takes three crop seasons for micropropagated plants to produce normal sized rhizomes hence cannot be used directly for commercial planting.
10. **Applications:** These protocols can be used for production of disease free planting material and *in vitro* storage of germplasm.

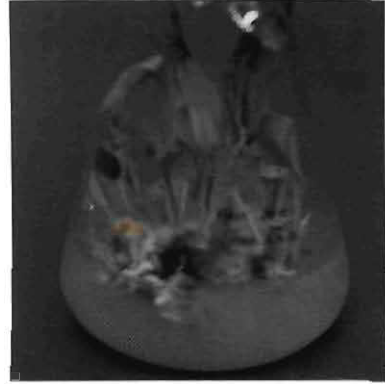


In vitro multiplication of plantlets



Tissue cultured plants in pots

1. **Plant species:** **TURMERIC** (*Curcuma longa* L. syn. *C. domestica* Val.).
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** Turmeric is native to Indo Malayan region and is cultivated in India, Indonesia, China, Bangladesh, South America and Caribbean Islands²¹.
4. **Accessions in the germplasm:** 700
5. **Uses:** Turmeric is used as a condiment and is an essential ingredient of curry powder for flavouring South Asian dishes. It is taken as a tonic, remedy for liver problems and to treat skin diseases. Curcumin from turmeric is also a traditional textile dye. In paste form, it is applied as a beauty mask and in face creams^{3,4}. Volatile oil consists of turmerones and tertiary alcohols^{1,3,4,21}.
6. **Explants for micropropagation:** Vegetative buds and rhizome explants with axillary buds for clonal propagation and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2° C with 14 h photoperiod at 3000 lux.
 - a) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA for micropropagation and regeneration from callus.
 - b) 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for slow growth.
8. ***In vitro* responses:** Multiple shoots (8-10) could be induced from vegetative buds as well as rhizome explants of turmeric on MS medium with 1 mg l⁻¹ BA and 0.5 mg l⁻¹ NAA in 40 days of culture. Production of callus and regeneration of plantlets from callus were also noticed in the same medium. Plantlets could be maintained up to one year without subculture in 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in screw capped culture tubes^{2,12,13,17,22}.
9. **Hardening and field performance:** The plantlets could be established in soil with over 80% success, when planted out in a mixture of garden soil, sand and vermiculite (1:1:1) and kept in humid chamber for about



In vitro production of plantlets



Tissue cultured plants in pots

20 days. It takes three crop seasons for micropropagated plants to produce normal sized rhizomes hence cannot be used directly for commercial planting.

10. **Applications:** Micropropagation techniques can be used for clonal multiplication of elite genotypes. Somaclonal variation can be used for selecting better genotypes. The slow growth method can be used for *in vitro* conservation of germplasm. It was reported that high curcumin types were isolated from tissue cultured plantlets of turmeric.

1. **Plant species:** KASTURI TURMERIC (*Curcuma aromatica* Salisb.)
2. **Family :** Zingiberaceae.
3. **Origin and distribution:** Kasturi turmeric occurs wild throughout India and is cultivation chiefly in Bengal and Travancore¹.
4. **Accessions in the germplasm:** 15
5. **Uses:** Rhizomes are used as a substitute for turmeric. They are externally applied in combination with astringents and aromatics, to bruises and sprains. Rhizome possesses a camphoraceous odour.
6. **Explants for micropropagation:** Vegetative buds and rhizome bits with axillary buds for clonal propagation and vegetative buds for *in vitro* conservation.
7. **Culture media and condition:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14 h photoperiod under 3000 lux.
 - a) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA for multiplication and rooting
 - b) 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for *in vitro* conservation by slow growth.
8. **In vitro responses:** Multiple shoots and rooting could be induced from vegetative buds as well as rhizome buds on MS medium with 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA. The cultures could be maintained up to 1 year, without subculture in 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in screw capped culture tubes^{2,13,17,22}.
9. **Hardening and field performance:** The plantlets could be established in soil with over 80% success, when planted out into a mixture of garden soil, sand and vermiculite (1:1:1) and kept in humid chamber for about 20 days. It takes three crop seasons for micro propagated plants to produce normal sized rhizomes.
10. **Applications:** This technique could be used for production of disease free planting material of elite genotypes. The slow growth method can be used for *in vitro* conservation of germplasm.

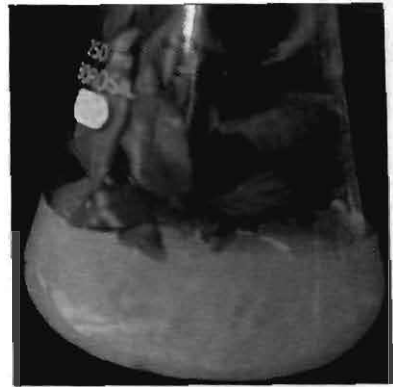


Production of multiple shoots



Tissue cultured plants in pots

1. **Plant species:** GALANGAL (*Kaempferia galanga* L.)
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** It is distributed in the tropics and sub tropics of Asia and Africa. It is found throughout the plains of India and is cultivated for its aromatic rhizomes in Kerala¹.
4. **Accessions in the germplasm:** 15
5. **Uses:** The herb is used in flavouring rice. Rhizomes and leaves are employed as perfumes and also possess a series of medicinal properties i.e., as stimulant, expectorant, carminative and diuretic. n-pentadecane and ethyl p-methoxycinnamate are reported in the oil.
6. **Explants for micropropagation** Vegetative buds, rhizome bits with axillary buds for micropropagation and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14 h photoperiod of 3000 lux.
 - a) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA for micropropagation.
 - b) 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for *in vitro* conservation by slow growth.
8. ***In vitro* responses:** Multiple shoots (5-10) with good root system from rhizome or vegetative bud explants could be induced in about 90 days of culture on MS medium supplemented with 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA. Cultures could be maintained under slow growth conditions in 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in screw capped culture tubes, upto a period of 14 months without subculture^{2,12,13,17,22}.
9. **Hardening and field performance:** The plantlets could be established in soil with over 90% success, when planted out into a mixture of garden soil, sand and vermiculite (1:1:1) and kept in humid chamber for about 15 days. It takes three crop seasons for formation of normal sized rhizomes in tissue



In vitro production of plantlets



Rhizome formation in tissue cultured plants

cultured plants and only after this they can be used for commercial cultivation.

10. **Applications:** This protocol can be used for production and multiplication of disease free planting material. The slow growth method is suitable for *in vitro* conservation of the species.

1. **Plant species:** *Kaempferia rotunda* L.
2. **Family:** Zingiberaceae.
3. **Origin and distribution:** It is possibly a native of Indo-China and is cultivated widely in South East Asia. It is distributed throughout India¹.
4. **Accessions in the germplasm:** 5
5. **Uses:** Rhizomes are used in cosmetics, tubers are used as a dye. It is also used as a local application for tumors, swellings and wounds. It is stomachic and is given in gastric complaints. On distillation, rhizomes yield oil that contains cineol and probably methyl chavicol¹.
6. **Explants for micropropagation:** Vegetative buds and rhizome bits with axillary buds, for multiplication and vegetative buds for *in vitro* conservation.
7. **Culture conditions and media :** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with a 14 h photoperiod under 3000 lux.
 - a) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA for micropropagation.
 - b) 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for *in vitro* conservation by slow growth.
8. **In vitro responses:** Rhizome and vegetative bud explants produced multiple shoots (5-10) with good root system in about 90 days of culture on MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ NAA. The cultures could be maintained upto one year without sub-culture in 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in screw capped culture tubes^{2,12,13,17,22}.
9. **Hardening and field performance:** The plantlets could be established in soil with over 90% success, when transplanted into a mixture of garden soil, sand and vermiculite (1:1:1) and kept in humid chamber for about 20 days. Tissue cultured plants require three crop seasons to develop normal sized rhizomes.
10. **Applications:** Production and multiplication of disease free planting material and somaclones for exploiting somaclonal variation. The slow growth method is ideal for *in vitro* conservation of the species.



Production of multiple shoots



Tissue cultured plants in pots

1. **Plant species: VANILLA** (*Vanilla fragrans* (Salisb.) Ames syn. *V. planifolia* Andrews).
2. **Family:** Orchidaceae.
3. **Origin and distribution:** It is indigenous to South East Mexico, Gautemala and parts of Central America. It is cultivated in other parts of tropics especially in the Malagasy Republic, Reunion and the Comoro Islands^{9,21}.
4. **Accessions in the germplasm:** 110
5. **Uses:** Vanilla, the fully grown fruit of *V. fragrans*, harvested before it is fully ripe, fermented and cured, is an important flavouring material and spice. It is used extensively in confectionery, perfumery and to a small extent in medicine.
6. **Explants for micropropagation:** Seeds, shoot tips and nodal segments for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22± 2°C at 14 h photoperiod under 3000 lux.
 - a) MS + Kin (0.5 mg l⁻¹) for *in vitro* seed germination.
 - b) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for multiplication and micropropagation.
 - c) 1/2 MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol for *in vitro* conservation by slow growth.
8. **In vitro responses:** Vanilla seeds could be germinated on MS medium supplemented with 0.5 mg l⁻¹ Kin, which later developed into minute protocorms and multiplied subsequently to plantlets in the same media. Conversion of root meristems to shoots was also induced. Individual shoots produced multiple shoots in MS medium with 1 mg l⁻¹ BA and 0.5 mg l⁻¹ IBA. Vanilla shoot tip cultures could be stored *in vitro* under slow growth conditions, upto 1 year in MS + 10 g l⁻¹ sucrose + 10 g l⁻¹ mannitol in sealed culture vessels^{7,17,22}.
9. **Hardening and field performance:** Plantlets could be established in soil with 90% success, when planted out in a mixture of garden soil, sand and vermiculite (1:1:1)



In vitro multiplication of plantlets



Seed culture of vanilla

and kept in humid chamber for 15 days. The seedling progenies showed morphological variation.

10. **Applications:** These *in vitro* techniques could be used for germination of seeds, rapid multiplication for production of disease free planting material. *In vitro* germination of seeds can be used to build up a population of varying progenies for crop improvement programmes.

1. **Plant Species: CINNAMON** (*Cinnamomum zeylanicum* Blume, syn. *C. verum* Bercht & Presl.)
2. **Family:** Lauraceae.
3. **Origin and distribution:** Native to Srilanka, produced in Seychelles and Malagasy Republic. In India it is grown on the west coasts^{1,3}
4. **Accessions in the germplasm :** 330
5. **Uses :** Cinnamon bark is the most popular spice. It is aromatic, astringent, stimulant and carminative. Bark Oil contains cinnamaldehyde, eugenol, benzaldehyde etc and is extensively used for flavouring confectionery, in pharmaceuticals etc. Cinnamon leaf oil equals clove oil in eugenol content²¹.
6. **Explants for micropropagation :** Shoot tip and nodal segments for multiplication and rooting; cotyledonary explants for somatic embryogenesis.
7. **Culture conditions and media** Surface sterilisation with 0.1% HgCl₂ incubation at 22±2°C with 14 h photoperiod under 3000 lux
 - (a) WPM + 3mg l⁻¹ BA + 1mg l⁻¹ Kin for multiple shoots.
 - (b) WPM + 2g l⁻¹ activated charcoal for rooting.
 - (c) WPM + 3mg l⁻¹ BA + 1mg l⁻¹ Kin for somatic embryogenesis.
8. **In vitro responses:** Multiple shoots could be induced from shoot tips and nodal segments in WPM + BA + Kin. Rooting could be induced in WPM supplemented with activated charcoal. The miniature *in vitro* developed shoots and somatic embryos were encapsulated in sodium alginate to make synthetic seeds which germinated in *in vitro* conditions after three months of storage^{6,22,25}.
9. **Hardening and field performance:** Rooted plantlets were transferred to sterilised coir dust in cups inside incubation room for 2 weeks and then transferred to bags with sand, garden soil and perlite (1:1:1) in nursery.



Induction of multiple shoots



Tissue cultured plants in polybags

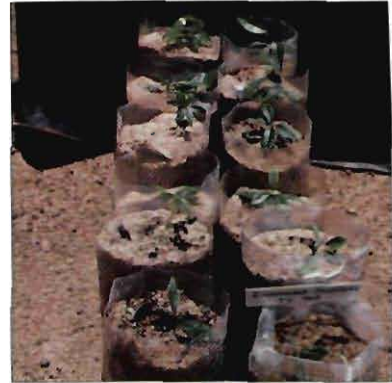
10. Applications :

Rapid clonal multiplication of identified 'elite' lines. Synthetic seeds are ideal for germplasm exchange.

1. **Plant species:** CAMPHOR (*Cinnamomum camphora* Nees & Ebern.)
2. **Family:** Lauraceae.
3. **Origin and distribution:** Native to eastern China, Japan and Formosa. Grows best between 1220 to 1828 m. Introduced to Java, Malaysia, Philippines, Indonesia, East and South Africa, Trinidad, Southern USA and India¹.
4. **Accessions in the germplasm:** 2
5. **Uses:** Major components from leaves are camphor, camphor oil containing d- pinene, dipentene, cineol, terpineol and caryophyllene. Camphor is applied to sprains, inflammations, rheumatic joints. It is also used to calm convulsions, epileptic attacks, as carminative, cardiac and respiratory stimulant. It is used as an antiseptic, antispasmodial, antiasthmatic, antipruritic and counter irritant. It is also used to repel insects and moths as well as used as flavouring agent¹.
6. **Explants for micropropagation:** Shoot tip and nodal segments, for multiplication and rooting.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2° C with 14 h photoperiod under 3000 lux.
 - a) WPM + 3 mg l⁻¹ BA+ 1 mg l⁻¹ Kin for multiple shoots.
 - b) WPM + 2 g l⁻¹ charcoal for *in vitro* rooting.
8. **In vitro responses:** Multiple shoots (10-12) could be induced from nodal segments in WPM with 3 mg l⁻¹ BA and 1 mg l⁻¹ Kin. These shoots developed a good root system when cultured on hormone free WPM supplemented with activated charcoal (2 g l⁻¹). The miniature *in vitro* developed shoots were encapsulated in sodium alginate to make synthetic seeds which germinated in *in vitro* conditions after three months of storage^{13,22,25}.
9. **Hardening and field performance:** *In vitro* rooted plantlets were planted out in to sterile coir dust in thermocol cups and kept



Induction of multiple shoots



Tissue cultured plants in polybags

in mini humid chambers in incubation room for 40-60 days. Later, they were transferred to polybags containing soil, perlite and sand (1:1:3) and kept in the nursery.

10. **Applications:** Rapid multiplication of elite genotypes and production of disease free planting material. Synthetic seeds are ideal for germplasm exchange.

1. **Plant species** : CHINESE CASSIA (*Cinnamomum cassia* Blume. syn. *C. aromatica*)
2. **Family**: Lauraceae
3. **Origin and distribution** : Cultivated in provinces of China. Trees growing at high altitudes (180-300 m) yield better quality bark with higher volatile oil content^{1,21}.
4. **Accessions in the germplasm**: 27
5. **Uses** : The volatile oils are used in culinary preparations, perfumery, cosmetics as well as in pharmaceutical preparations. Cinnamaldehyde is the main constituent of cassia oil.
6. **Explants for micropropagation**: Shoot tips and nodal segments for multiplication and rooting.
7. **Culture conditions and media** : Surface sterilisation with 0.1% $HgCl_2$, incubation at $22 \pm 2^\circ C$ with 14h photoperiod under 3000 lux
 - a) WPM + 3 $mg\ l^{-1}$ BA + 1 $mg\ l^{-1}$ Kin for multiple shoot induction.
 - b) WPM + 0.5 $mg\ l^{-1}$ IBA + 0.5 $mg\ l^{-1}$ NAA + activated charcoal (2 $g\ l^{-1}$) for rooting.
8. **In vitro responses** : Multiple shoots upto 10 could be produced in medium with BA and Kin, from mature explants. Roots were induced when a combination of IBA and NAA were used²².
9. **Hardening and field performance**: Rooted plantlets were transferred to sterilised coir dust in cups inside incubation room for 2 weeks and then transferred to bags with sand, garden soil and perlite (1:1:1) in nursery.
10. **Applications**: Rapid multiplication of available germplasm to meet the demand for quality planting material.



Induction of multiple shoots



In vitro rooted plantlet

1. **Plant species:** THYME (*Thymus vulgaris* L.)
2. **Family:** Lamiaceae.
3. **Origin and distribution:** Native to Southern Europe from Spain to Italy. Commonly cultivated in mild temperate (France, Germany & Greece) and sub tropical climate of Central Europe, Southern USSR, Morocco, Asiatic Turkey, Syria, Israel, North India etc. The western temperate Himalayas may be ideal for commercial cultivation of this crop in India¹.
4. **Accessions in the germplasm:** 1
5. **Uses:** Thyme fresh or dried is extensively used as a culinary herb. Thyme oil is used in flavoring liquors and in food industry. It is valued as an antiseptic and fumigating herb. Red thyme oil is germicidal and is used in disinfectant preparations; its chief constituent being thymol, linalool, linalyl acetate³.
6. **Explants for micropropagation:** Seedlings and shoot tips, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2° C with a 14h photoperiod under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for *in vitro* seed germination and multiplication.
 - b) MS without growth regulators for further multiplication and rooting.
 - c) 1/2 MS + 20 g l⁻¹ sucrose for induction of slow growth.
8. ***In vitro* responses:** Multiple shoots with good root system could be produced in growth regulator free MS medium^{21,22}. The rate of multiplication is high and a tenfold increase in the biomass could be obtained in 30 days of culture. Thyme cultures could be stored in 1/2 MS supplemented with 20 g l⁻¹ sucrose upto twelve months without subculture, in sealed culture vessels^{17,22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success



In vitro multiplication of thyme



In vitro conservation of thyme

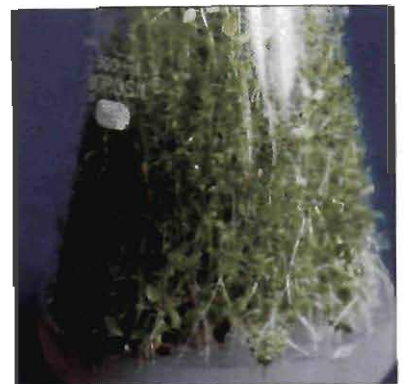
when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.

10. **Applications:** These protocols can be used for clonal multiplication of desirable genotypes, production of biomass for extraction of essential oils and *in vitro* conservation of genetic resources.

1. **Plant species:** PEPPERMINT (*Mentha piperita* L.).
2. **Family:** Lamiaceae.
3. **Origin and Distribution :** Native to Europe. Cultivated in temperate regions of Europe, Asia, North America and Australia. It is grown in Indian gardens and cultivated in Kashmir, Nilgiris, Mysore, Dehradun etc.
4. **Accessions in germplasm:** 1
5. **Uses:** The herb is a source of the peppermint oil extensively used for flavouring and in pharmacy. Major constituent is menthol. The pleasant aroma is mainly due to the presence of menthofuran³.
6. **Explants for micropropagation :** Shoot tips and nodal explants, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media :** Surface sterilisation with 0.1% HgCl₂, incubation at 22 ± 2°C with a 14h photoperiod under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for seed germination.
 - b) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for micropropagation.
 - c) 1/2 MS + 20 g l⁻¹ sucrose for induction of slow growth.
8. **In vitro responses :** Multiple shoots upto 30 and healthy roots were produced in MS + 1.0 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA. Cultures could be stored in 1/2 MS supplemented with 20 g l⁻¹ sucrose, in sealed culture tubes, upto twelve months^{17,22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.
10. **Applications:** The method can be used for multiplication of clones, their maintenance *in vitro* and for biomass production.



Peppermint

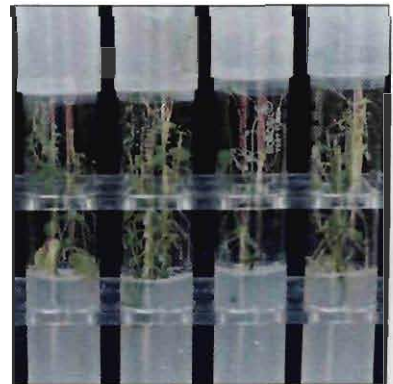


In vitro multiplication of peppermint

1. **Plant species:** SPEARMINT (*Mentha spicata* L.).
2. **Family:** Lamiaceae.
3. **Origin and distribution:** Native to China, mint was introduced to Japan. Now grown commercially in Brazil, Argentina, South Africa and Korea². In India, it is grown to some extent at altitude of 260 m.
4. **Accessions in the germplasm :** 2
5. **Uses:** It is used as a home remedy against coughs and colds. Leaves are used for flavouring culinary preparations. Major constituent is L-carvone. It is used as a stimulant, carminative and antispasmodic⁴.
6. **Explants for micropropagation:** Seedlings and shoot tips, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with a 14h photoperiod under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for seed germination.
 - b) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for micropropagation.
 - c) 1/2 MS + 20 g l⁻¹ sucrose for induction of slow growth
8. ***In vitro* responses:** Multiple shoots, upto 30 with good root system were produced in MS supplemented with IBA and BA. Roots were produced in the same medium as well as in medium devoid of any growth regulators. Cultures could be stored in 1/2 MS supplemented with 20 g l⁻¹ sucrose, in sealed culture vessels upto six months^{17,22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.



In vitro multiplication of spearmint



In vitro conservation of spearmint

10. **Applications:** The protocols developed are ideal for multiplication of planting materials and production of biomass and *in vitro* conservation.

1. **Plant species : MARJORAM** (*Marjorana hortensis* Moench.) Sweet Marjoram
2. **Family** : Lamiaceae.
3. **Origin and distribution**: Native to Southern Europe and Asia Minor, marjoram is grown in Indian gardens particularly in hill stations.
4. **Accessions in the germplasm**: 1
5. **Uses**: Sweet marjoram is characterised by a strong spicy and pleasant odour. Leaves are used fresh or dried as a condiment for seasoning. It is carminative, expectorant and tonic. Leaves and seeds are astringent. Volatile consists of cavacrol, eugenol, chavicol, α - linalool etc.
6. **Explant for micropropagation**: Seedlings and shoot segments for micropropagation and shoot tips for *in vitro* conservation.
7. **Culture conditions and media** :Surface sterilisation with 0.1% $HgCl_2$ incubation at $22\pm 2^\circ C$ with 14h photoperiod under 3000 lux.
 - a) MS + 0.5 $mg\ l^{-1}$ kin for seed germination and multiplication.
 - b) MS without growth regulators for further multiplication.
 - c) $1/2$ MS + 20 $g\ l^{-1}$ sucrose for induction of slow growth.
8. **In vitro responses**: Shoots at the rate of 1 : 50 could be multiplied and induced to root on MS medium without growth regulators. Marjoram cultures could be stored up to three months on $1/2$ MS supplemented with 20 $g\ l^{-1}$ sucrose in sealed culture vessels ^{17,22,21}.
9. **Hardening and field performance**: Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.



Marjoram



In vitro multiplication of plantlets

10. Applications:

Clonal multiplication of desirable genotypes, production of biomass and conservation of genetic resources of marjoram could be done using the above protocols.

1. **Plant species:** OREGANO (*Origanum vulgare* L.).
2. **Family:** Lamiaceae.
3. **Origin and distribution:** Also known as wild Marjoram, it is found in the temperate Himalayas from Kashmir to Sikkim at altitudes of 1500- 3600 m.
4. **Accessions in the germplasm:** 1
5. **Uses:** It possesses an aromatic, thyme like flavour and its leaves were used to flavour food, beer etc. before hops were introduced in brewing industry. The oil has an aromatic spicy odour and is carminative, stomachic, diuretic. Oil contains dl-pinene, dipentene, linalool, bi and tricyclic sesquiterpene and palmitic acid³.
6. **Explants for micropropagation:** Seed and shoot segments, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14h photoperiod under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for *in vitro* seed germination.
 - b) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for micropropagation.
 - c) 1/2 MS + 20 gl⁻¹ sucrose for induction of slow growth.
8. **In vitro response:** The shoots proliferated at the rate of 1 : 30, and could be rooted easily in MS with IBA and BA supplemented medium. Cultures could be stored in 1/2 MS supplemented with 20 gl⁻¹ sucrose, in sealed culture vessels^{17,22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.



Oregano

*In vitro* production of plantlets

10. **Applications:** These protocols are ideal for clonal multiplication of desirable genotypes, production of biomass and conservation of oregano genetic resources.

1. **Plant species:** SAGE (*Salvia officinalis* L.).
2. **Family :** Lamiaceae.
3. **Origin and distribution :** Native to South Europe. In India, sage is cultivated in the valleys of Kashmir, Himachal Pradesh and Uttar Pradesh, as a spice and for medicinal purpose.

4. **Accessions in the germplasm:** 1

5. **Uses :** Dried sage is used to flavour meat and meat products. Quality of oil is based on thujone and borneol.

6. **Explants for micropropagation:** Seedlings and shoot tips for multiplication, rooting and plant regeneration of callus.

7. **Culture conditions and media:** Surface sterilisation with 0.1% $HgCl_2$, incubation at $22 \pm 2^\circ C$ with a 14h photoperiod under 3000 lux

a) MS + 0.5 mg l^{-1} Kin for seed germination

b) MS + 1 mg l^{-1} BA + 0.5 mg l^{-1} IBA for multiplication, rooting and plant regeneration from callus.

c) MS + 20 g l^{-1} sucrose + 10 g l^{-1} mannitol for induction of slow growth.

8. **In vitro responses :** Multiple shoots were induced from seedlings as well as nodal segments on MS medium supplemented with IBA (0.5 mg l^{-1}) and BA (1 mg l^{-1}). Plant regeneration was also induced from callus cultures of sage on the medium of same composition. shoot cultures could be stored upto six months in MS medium supplemented with sucrose and mannitol in sealed culture tubes^{22,24}.

9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil,



In vitro multiplication of sage



Plant regeneration from callus

sand and vermiculite in equal proportions with 85% humidity for 20 days.

10. **Applications:** For multiplication of planting material and possible biomass production for extraction of volatile oils.

1. **Plant species:** LAVENDER (*Lavandula angustifolia* M.) Syn. *L. officinalis* Chaix.
2. **Family :** Lamiaceae.
3. **Origin and distribution:** Lavender is a native of the mountainous districts of Southern Europe. It is cultivated extensively in Southern France⁴.
4. **Accessions in the germplasm:** 1
5. **Uses:** Dried flowers are used in sachets, potpourie and for flavouring. Flowers and oil are used in moth repellent preparations and in perfumes. Lavender oil possesses carminative and stimulative properties, and is chemically constituted of Linalool and linalyl acetate.
6. **Explant for micropropagation:** Seedlings and shoot explants, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% $HgCl_2$, incubation at $22\pm 2^\circ C$ with a photoperiod of 14h under 3000 lux.
 - a) MS + 0.5 $mg\ l^{-1}$ Kin for seed germination.
 - b) MS + 1 $mg\ l^{-1}$ BA and 0.5 $mg\ l^{-1}$ IBA for micropropagation and plant regeneration from callus.
 - c) MS + 20 $g\ l^{-1}$ sucrose + 10 $g\ l^{-1}$ mannitol for induction of slow growth.
8. ***In vitro* responses:** The shoots multiplied and produced many (20-30) shoots which could be rooted easily on MS medium supplemented with IBA and BA. Regeneration of plantlets from callus was also induced in the same medium. Shoot cultures could be stored upto eight months in MS medium supplemented with sucrose and mannitol in sealed culture tubes^{22,24}.
9. **Hardening and field performance:**



In vitro multiplication of lavender

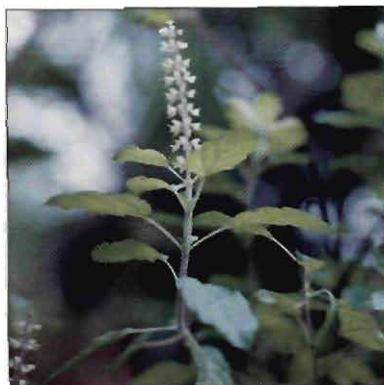


Plant regeneration from callus

Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.

10. **Applications:** For multiplication of planting material and possible biomass increase for extraction of volatile oils.

1. **Plant species** : SACRED BASIL (*Ocimum sanctum* L.).
2. **Family**: Lamiaceae.
3. **Origin and distribution**: Found throughout India upto an elevation of 1800 m. in the Himalayas and in Andaman and Nicobar Islands¹.
4. **Accessions in the germplasm**: 7
5. **Uses**: Used in indigenous medicine and soaps. Oil from leaves is reported to have antibacterial and insecticidal properties. The juice from leaves possess diaphoretic, antiperiodic and stimulating properties. Leaves are used as condiment in salads and soups. Basil oil is rich in linalool and methyl cinnamate and ocimene.
6. **Explant for micropropagation**: Seedlings, shoot and nodal explants, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media**: Surface sterilisation with 0.1% HgCl₂, incubation at 22 ±2° C with a photoperiod of 14 h under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for *in vitro* seed germination.
 - b) MS + 1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for micropropagation and multiplication.
 - c) 1/2 MS + 20 g l⁻¹ sucrose for *in vitro* conservation.
8. **In vitro responses**: *Ocimum* seeds germinated *in vitro* in MS supplemented with 0.5 mg l⁻¹ Kinetin. Further multiplication of the shoot segments (10-20) was also obtained in the medium with 1 mg l⁻¹ BA and 0.5 mg l⁻¹ IBA. Cultures could be stored in 1/2 M S supplemented with 20 g l⁻¹ sucrose in sealed culture vessels^{17,22,24}.
9. **Hardening and field performance**: Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.
10. **Applications**: For multiplication of planting material and possible biomass production for extraction of volatile oils.

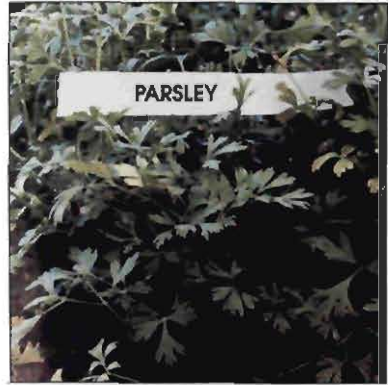


Ocimum sanctum



In vitro production of plantlets

1. **Plant species:** PARSLEY (*Petroselinum crispum* Mill.).
2. **Family:** Apiaceae.
3. **Origin and distribution:** It is a native of the Mediterranean region and is widely cultivated in Europe. In India, it grows better at high altitudes and is the only representative of the genus^{1,3,4}.
4. **Accessions in the germplasm:** 1
5. **Uses:** Fresh leaves are used for garnishing and seasoning. The leaves are employed to make tea with anti-scorbutic properties since it is a rich source of vitamin C. Dried leaves and roots are used as condiments, the herb possesses diuretic, carminative, choleric and antipyretic properties. Parsley oil is used for flavouring food products, and contains apiol and α -pinene.
6. **Explant for micropropagation:** Seedlings and nodal explants, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% Hg Cl₂, incubation at 22 ± 2°C with a photoperiod of 14h under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for seed germination.
 - b) MS + 1.0 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA for multiplication and rooting.
 - c) MS + 20 g l⁻¹ sucrose for induction of slow growth.
8. **In vitro responses:** Multiple shoots (up to 30) were produced with good root system in MS medium supplemented with 0.5 mg l⁻¹ IBA + 1.0 mg l⁻¹ BA. Shoot cultures could be conserved upto six months in MS medium supplemented with 20 g l⁻¹ sucrose in sealed culture tubes^{22,24}.



Parsley

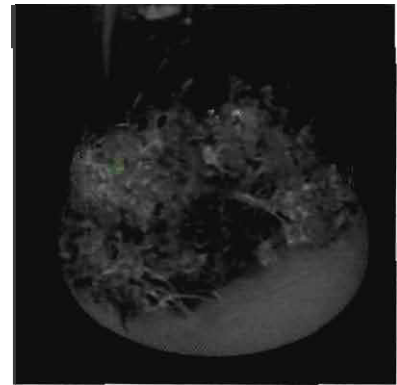
*In vitro* multiplication of parsley

9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 40% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.
10. **Applications:** This protocol can be used for clonal multiplication of desirable genotypes, production of biomass and maintenance of the genus in *in vitro* conditions.

1. **Plant species:** CELERY (*Apium graveolens* L.).
2. **Family:** Apiaceae.
3. **Origin and distribution:** Native to Southern Europe, celery is grown in Scandinavia to North Africa and in northern parts of America. It is cultivated in northern parts of India^{1,3}.
4. **Accessions in the germplasm:** 1
5. **Uses:** The essential oil is recommended against rheumatism. Celery is believed to be a tonic against asthma, treating liver diseases, bronchitis and fever. Celery seeds are used in food industry as a flavouring agent. Principle constituents of volatile oil are d-limonene, β - selinene.
6. **Explants for micropropagation:** Stem cuttings, seedlings for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% $HgCl_2$, incubation at $22 \pm 2^\circ C$ with 14 h photoperiod under 3000 lux.
 - a) MS + 0.5 $mg\ l^{-1}$ Kin for *in vitro* seed germination.
 - b) MS + 0.5 $mg\ l^{-1}$ IBA for multiplication.
 - c) $\frac{1}{2}$ MS + 20 $g\ l^{-1}$ sucrose for induction of slow growth.
8. ***In vitro* responses:** Celery seeds germinated *in vitro* in MS with 0.5 $mg\ l^{-1}$ Kin. Segments from the shoot and node produced multiple shoots (upto 30) in MS + 0.5 $mg\ l^{-1}$ IBA with good root system. Shoot cultures could be stored upto five months in $\frac{1}{2}$ MS supplemented with 20 $g\ l^{-1}$ sucrose in sealed culture vessel^{22,24}.



In vitro multiplication of celery



In vitro multiplication of biomass

9. **Hardening and field performance :** Micropropagated plants were successfully established in green house with 50% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.
10. **Applications:** This protocol can be used for multiplication of planting materials and biomass production.

1. **Plant species:** ANISE (*Pimpinella anisum* L.).
2. **Family:** Apiaceae.
3. **Origin and distribution:** It is commercially cultivated in the Southern republics of the former USSR, in Turkey, Spain, France and India. It is native to eastern Mediterranean region and is cultivated in Europe and North Africa. In India it is grown to a small extent as a culinary herb^{1,3,4}.
4. **Accessions in the germplasm:** 1
5. **Uses:** In Europe it is used in preparation of cakes and biscuits. Seeds are used for flavouring food, confectionery etc. In India anise water is used as a cologne. Alcoholic extract of aniseed possesses fungicidal activity. Chief constituent of anise oil is anethole.
6. **Explant for micropropagation:** Seedlings and stem cuttings for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% $HgCl_2$, incubation at $22 \pm 2^\circ C$ with 14h photoperiod under 3000 lux.
 - a) MS + 0.5 $mg\ l^{-1}$ Kin for *in vitro* seed germination.
 - b) MS + 0.5 $mg\ l^{-1}$ IBA + 1 $mg\ l^{-1}$ BA for multiplication, rooting and plant regeneration from callus.
 - c) $\frac{1}{2}$ MS + 20 $g\ l^{-1}$ sucrose for induction of slow growth.
8. ***In vitro* responses:** Anise seeds germinated *in vitro* in MS with kinetin (0.5 $mg\ l^{-1}$). Segments from the shoots produced multiple shoots in MS with IBA and BA. Regeneration of plantlets from callus was also induced in the same medium. Cultures could be stored in $\frac{1}{2}$ MS supplemented with 20 $g\ l^{-1}$ sucrose in sealed culture vessels^{17,22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.



In vitro multiplication of anise



Plant regeneration from callus

10. **Applications:** The protocols can be used for multiplication of planting material, biomass production and *in vitro* conservation.

1. **Plant species:** DILL (*Anethum graveolens* L - European Dill).
2. **Family:** Apiaceae
3. **Origin and distribution:** Dill is native to Southern Russia and the Mediterranean. The main producer of dill are Poland, Russia, Turkey and the UK. In India it is cultivated in Jammu and Kashmir^{1,3}.
4. **Accessions in the germplasm:** 1
5. **Uses:** Dill is believed to have soothing effect on the digestive system. Both seeds and leaves are used by nursing mothers to stimulate milk. It is also used in pickles and confectionery. Essential oil contains high proportion of terpenes; limonene and carvone. d-phellandrene is responsible for odour flavour.
6. **Explant for micropropagation:** Seedlings and shoot tips, for multiplication and shoot tips for *in vitro* conservation.
7. **Culture conditions and media:** Surface sterilisation with 0.1% HgCl₂, incubation at 22±2°C with 14h photoperiod under 3000 lux.
 - a) MS + 0.5 mg l⁻¹ Kin for seed germination and micropropagation.
 - b) MS + 2.0 mg l⁻¹ 2,4-D for callus induction.
 - c) MS basal medium for plant regeneration.
 - d) 1/2 MS + 20 g l⁻¹ sucrose for induction of slow growth.
8. ***In vitro* responses:** Shoots multiplied at the rate of 1:7 in MS medium supplemented with kinetin(0.5 mg l⁻¹). These shoots rooted in hormone free medium. Callus to be induced in MS + 2.0 mg l⁻¹ 2,4-D and plant regeneration was induced in growth regulator free MS medium. Shoot cultures could be stored upto four months in 1/2 MS medium supplemented with 20 g l⁻¹ sucrose in sealed culture tube^{22,24}.



In vitro multiplication of dill



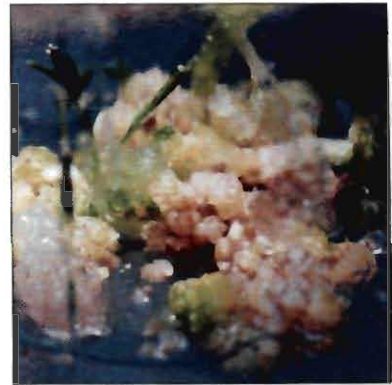
Plant regeneration from callus

9. **Hardening and field performance:** Micropropagated plants were successfully established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.
10. **Applications:** The protocols can be used for production and multiplication of planting material and biomass production.

1. **Plant species:** FENNEL (*Foeniculum vulgare* Mill.)
2. **Family:** Apiaceae.
3. **Origin and distribution:** It is native to Southern Europe and Asia. Fennel is cultivated as a garden or homeyard crop throughout India^{1,3}.
4. **Accessions in the germplasm:** 1
5. **Uses:** Fennel is a pot-herb and is now cultivated in certain pockets of India. Widely used spice for culinary purposes as food flavourant and in medicine as carminative. Both the vegetative parts as well as seeds are used. The main constituent of the oil is anethole and is used as a flavouring agent and in medicine.
6. **Explant for micropropagation:** Seedlings, shoots and nodal explants for micropropagation and shoot tips for *in vitro* conservation.
7. **Culture conditions and media :** Surface sterilisation with 0.1% HgCl₂ incubation at 22±2°C with a photoperiod of 14h under 3000 lux
 - a) MS + 0.5 mg l⁻¹ Kin for seed germination.
 - b) MS +0.5 mg l⁻¹ IBA + 1.0 mg l⁻¹ BA for micropropagation and plant regeneration from callus.
 - c) 1/2 MS + 20 g l⁻¹ sucrose for induction of slow growth.
8. ***In vitro* responses:** Fennel seeds germinated *in vitro* in MS supplemented with Kin (0.5 mg l⁻¹) developed multiple shoots and roots in MS with 1.0 mg l⁻¹ BA and 0.5 mg l⁻¹ IBA. Plants could be successfully regenerated from callus cultures of fennel on the medium of same composition. Shoot cultures could be stored upto four months in MS medium with 20 g l⁻¹ sucrose in sealed culture vessel^{22,24}.
9. **Hardening and field performance:** Micropropagated plants were successfully



In vitro multiplication of fennel



Plant regeneration from callus

established in green house with 60% success when planted out in a mixture of garden soil, sand and vermiculite in equal proportions with 85% humidity for 20 days.

10. **Applications:** The protocols can be used for multiplication of planting material and possible biomass production for extraction of volatile oil and exploiting somaclonal variation for crop improvement.

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Back cover

1. Plant regeneration in vanilla
2. *In vitro* development of fruit in ginger
3. Conversion of floral buds into vegetative buds in ginger
4. Micropropagated plants of various spices established in polybags
5. Field evaluation of ginger somaclones
6. A view of the tissue culture laboratory

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