PRODUCTION OF QUALITY PLANTING MATERIAL OF RARE MEDICINAL PLANTS OF COMMERCIAL IMPORTANCE

N Sasidharan
EM Muralidharan
KC Chacko

Kerala Forest Research Institute
An Institution of Kerala State Council for Science, Technology and Environment (KSCSTE)
Peechi-680 653, Thrissur
KFRI

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2010

Title of the project	:	KFRI/460/2004, Production of Quality Planting Material of Rare Medicinal Plants of Commercial Importance
Objectives	:	• Survey and establishment of germplasm collection of the five species of medicinal plants viz. *Trichosanthes cucumerina, Merremia turpethum, Salacia oblonga, Saraca asoca* and *Ipomoea mauritiana.* • Evaluation and selection of superior genotypes based on morphological characters and yield of the medicinal parts. • Standardisation of propagation methods. • Distribution of superior planting materials.
Name and address of Principal Investigator Co-Investigators	:	1. Dr N Sasidharan, FEBC Progamme Division 2. Dr E M Muralidharan, Forest Genetics & Biotechnology Progamme Division 3. Shri K C Chacko, Extension and Training Progamme Division Kerala Forest Research Institute, Peechi 680 653, Thrissur Dist., Kerala
Research Fellows	:	Shri Pramod Pathrose Shri V.K. Sreenivas
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Contents

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Abstract

The medicinal plants resource in the wild is getting depleted over the years largely due to the ever-increasing demand and unsustainable extraction practices. Due to scarcity, substitution is prevalent with related species. In order to meet the demand for medicinal plants, their cultivation is inevitable. Cultivation can ensure the availability of genuine materials besides reducing pressure on the resources in the wild. However, cultivation of medicinal plants has not taken up to meet the requirement. The farmers willing to take up cultivation of medicinal plants often face the problem of planting materials. In the present study, trials were conducted to standardize the production of planting materials though seeds, vegetative methods and micropropagation. Attempts were also made to standardize the cultivation practices and estimating the yield. In all, five commercially important medicinal plants which are in high demand viz., *Saraca asoca, Salacia oblonga, Ipomoea mauritiana, Merremia turpethum* and *Trichosanthes cucumerina* were selected for the study.

Extensive surveys were conducted for the collection of medicinal plants. Eighteen accessions of *Saraca asoca* were collected from forests as well as from homesteads. Six accessions of *Trichosanthes cucumerina*, five accessions of *Ipomoea mauritiana* and three accessions of *Merremia turpethum* were collected. Only two accessions of *Salacia oblonga* could be collected as it is very rare in Kerala. All the collections were planted in the medicinal plants garden of the Institute.

Trials on production of planting materials were carried out in all the five selected species. In the case of *Saraca asoca* cuttings, up

to 95 per cent rooting was obtained in mist chamber with the application of Indole-Butyric-Acid (IBA). Through this method, mass production of planting materials could be achieved within short period. Up to 90 per cent survival was registered for the rooted cuttings when field planted. *Merremia turpethum* and *Ipomoea mauritiana* also gave up to 80 per cent rooting. Although up to 70 per cent rooting was obtained for *Trichosanthes cucumerina,* survival of the rooted cuttings in the field was very poor. As the population of *Salacia oblonga* in Kerala is very sparse, enough seeds could not be collected for conducting germination trials. However, up to 80 per cent cuttings rooted in the macropropagation trials. The trials on vegetative propagation revealed that mass multiplication of planting materials could be conveniently done through rooting of cuttings for *Saraca asoca, Salacia oblonga, Merremia turpethum* and *Ipomoea mauritiana.*

Among the five species selected, three are short duration and two long duration crops. *Merremia turpethum* prefers moist localities. Marshy areas are ideal for better growth and yield. In the dry areas the plants get dried up in summer if there is no watering. *Trichosanthes cucumerina* can be harvested six months after planting and *Ipomoea mauritiana* after two years.

1. Introduction

Kerala has a rich tradition in Ayurveda and local health care practices from very ancient period. Apart from trained Ayurvedic Doctors from Institutions, there are a large number of registered as well as unregistered medical practitioners in the State. There are about 750 licensed Ayuvedic Medicine Manufacturing units and about 1000 unregistered units in Kerala. These medicine manufacturing units and practitioners of local healthcare systems use a substantial quantity of medicinal plants, collected mostly from wild.

The consumption of raw drugs by the Ayurvedic medicine manufacturing industry has been studied recently (Sasidharan and Muraleedharan, 2000, 2009). The study revealed that, 80 percent of the raw drugs are collected from the wild and only 20 per cent are obtained from cultivated sources, which include spices like ginger, turmeric, cardamom, pepper, etc. During the past three decades, there has been substantial increase in the production of herbal medicines, particularly Ayurvedic in India. This increased demand has led to over-exploitation from the wild. Due to the continuous extraction, the medicinal plant resource in the wild is getting depleted and several species have become rare or endangered. The medicinal plants habitats have been reduced due to alternate land use and increase in human population. Considering the increase in demand and decrease of raw drugs in wild, the requirement can be met only through cultivation. However, cultivation of medicinal plants was not been taken up by the farmers at the required level. In the past, medicinal plant resources were in plenty and requirement was limited. But, with the production of medicines on commercial basis, the scenario has changed. Nowadays, the requirement of much needed, rare raw drugs is met through

substitution. Substitution with related or unrelated raw drugs affects the quality of the prepared medicines.

The annual consumption of raw drugs with regard to plant/plant parts, roots and rhizomes constitutes 50 per cent of the total quantity. Collection of roots is by uprooting the plant which will lead to the death of the plants. According to Sasidharan and Muraleedharan (2009) the annual consumption of 230 important raw drugs in the State by the Ayurvedic drug industry is 20,517 tonnes. The study also revealed that there is absolute scarcity for as many as 30 species which are required in large quantities. Therefore, cultivation of these medicinal plants is essential to meet the demand.

Considering the increase in the demand for herbal medicines in the country and abroad, the Government of India has established the National Medicinal Plants Board (MPB) under Department of Ayurveda, Sidha, Unani and Homoeopathy (AYUSH) to formulate policies for the overall development of the Indian System of Medicines. The NMPB has drawn up various schemes for providing financial assistance to the cultivation, conservation and utilization of the medicinal plants resources of the country.

One of the problems faced by farmers is the lack of quality planting materials and the agro-techniques of medicinal plants. Therefore, the study was taken up for the production of quality planting materials of five commercially important rare medicinal plants which are in high demand. The plants selected for the present study are *Trichosanthes cucumerina* (Kaipan padavalam), *Salacia oblonga* (Ponkoranti) *Ipomoea mauritiana* (Palmuthaku), *Saraca asoca* (Ashokam) and *Merremia turpethum* (Thrikolpakonna).

2. Materials and Methods

2.1. Selected species and their profile

Ipomoea mauritiana Jacq., Coll. Bot. 4: 216. 1719.
I. paniculata R. Br., Prodr. 486. 1810.
I. digitata sensu Clarke in Hook. f., Fl. Brit. India 4: 202. 1883, non L., 1759.
Family: Convolvulaceae
Common name: Palmuthukku
Sanskrit name: Kshiravidari

Stout perennial climbers with tuberous roots, stem glabrous. Leaves to 15 x 15 cm, orbicular in outline, deeply lobed, lobes elliptic, acuminate; petiole to 12 cm long. Capsule 14 x 12 mm, ovoid, glabrous, 4-valved; seeds 7 x 5 mm, obtusely trigonous, densely covered with long cottony hairs. Fruits ripen during November-December.

The species is ingredient of 36 Ayurvedic preparations. The root is the medicinal part and annual consumption in Kerala is 165 tonnes.

Merremia turpethum (L.) Shah & Bhat, J. Bombay Nat. Hist. Soc. 74: 567. 1978.
Convolvulus turpethum L., Sp. Pl. 155.1753.
Ipomoea turpethum (L.) R. Br., Prodr. 485.1810.
Operculina turpethum (L.) Manso, Enum. Subst. Bras. 16.1836.
Family: Convolvulaceae
Common name: Thrikolppakonna, Kuzhalkonna
Sanskrit name: Trivrith

Stout twiners; young stem 3-winged, fleshy, latex milky. Leaves orbicular or broadly ovate, acuminate or acute at apex, cordate or hastate at base, pubescent beneath, margin coarsely

dentate, to 15 x 12 cm. Fruit a thin walled capsule, depressed-globose, subtended by the enlarged sepals, pedicels thickened; seeds usually 3 or 4, black, smooth, 4-5 mm across. Fruits ripen during November-January.

The species is ingredient of 28 Ayurvedic preparations. The annual consumption in Kerala is 65 tonnes.

Salacia oblonga Wall. ex Wight & Arn., Prodr. 106. 1834.

Family: Celastraceae

Common name: Ekanayakaveru

Sanskrit name: Vairi, Peethika

Stout climbers, branchlets densely lenticellate, lenticels elongate. Leaves to 21 x 8 cm, oblong, acute or obtuse at apex, acute at base, green when dry. Berry *ca.* 4 cm across, orange-red, smooth; seeds 2-8, angular, covered by mucilaginous aril. The fruits ripen during December-April.

The root is ingredient of 6 Ayurvedic preparations, particularly in the anti-diabetic drugs. The annual consumption of root in Kerala is 88 tonnes.

Saraca asoca (Roxb.) de Wilde, Blumea 15:393. 1968.

Jonesia asoca Roxb. in Asiat. Res. 4:365. 1799.

Saraca indica auct. non L.1769, sensu Bedd., Fl. Sylv. t.57.1870.

Family: Caesalpiniaceae

Common name: Asokam

Sanskrit name: Asokah

Small to medium sized trees, bark brownish black. Leaves to 25 cm long; leaflets 4-6 pairs, oblong-lanceolate, base oblique, to 18 x 5 cm. Pods to 15 x 2.5 cm; seeds ellipsoid-oblong, 3.5 cm long.

The species is ingredient of 3 Ayurvedic preparations. The annual consumption in Kerala is 110 tonnes.

Trichosanthes cucumerina L., Sp. Pl. 1008. 1753.
T. anguina L., Sp. Pl. 1008. 1753.
Family: Cucurbitaceae
Common name: Snake gourd
Sanskrit name: Kattupadavalam

Annual climbers. Leaves to 17 cm across, 5-9-lobed; lobes obovate or ovate, acute, spineus serrate, densely villous and coarsely reticulate below; petiole 3.5 cm long. Berry 3-7.5 cm long, ovoid-fusiform, narrowed at both ends, green with white stripes, becomes orange-red or yellow when ripe. Seeds 12 x 6 mm, 5-10 per fruit, ellipsoid, compressed, rugulose, covered with reddish pulp. Fruit ripens during September-December.
The species is ingredient of 28 Ayurvedic preparations. The annual consumption in Kerala is 190 tonnes.

2.2. Survey and establishment of Germplasm collections

Extensive surveys were conducted for collection of the five selected medicinal plants in forests as well as non-forest areas. Eighteen accessions of *Saraca asoca* (both stem cuttings and seeds) were collected. Among the accessions, four are from forest areas such as Nelliyampathy (Nenmara Forest Division), Kuttiyadi (Kozhikkode Forest Division), Kallar (Shenduruny Wildlife Sanctuary) and Kollathirumedu (Vazhachal Forest Division). Except in Kuttiyadi, the populations of *Saraca* are sparse. The rest of the collections of *Saraca* were made from homesteads, parks and gardens in Thrissur district. Six accessions of *Trichosanthes cucumerina* were collected from Kottakkal Arya Vaidya Sala, Malappuram; Kerala Agricultural University, Thrissur; Kunnamkulam, Thrissur; Peechi, Thrissur and Payyannur, Kannur. Five accessions of *Ipomoea mauritiana* were collected from Vazhachal (Vazhachal Forest Division), Peechi (Peechi Wildlife Sanctuary), Begur (Wayanad Wildlife Sanctuary), Nenmara (Palakkad district) and Kalpetta (Wayanad district). Only two

accessions of *Salacia oblonga* could be collected. One from Vellanimala (Thrissur Forest Division) and the other from Kuttiyadi (Kozhikkode Forest Division). In addition, *Salacia fruticosa* (Vellanimala, Thrissur Forest Division) and *Salacia chinensis* (Nilambur Forest Division) were also collected. Three accessions of *Merremia turpethum* were collected from Feroke (Kozhikkode district), Nilambur (Nilambur Forest Division) and Aluva (Ernakulam district). The propagules collected were planted in the medicinal plants garden of the Institute.

2.3. Propagation studies

In the present study, propagation through seeds, micro and vegetative methods have been tried for standardizing the methodology for mass multiplication. An efficient, rapid and large scale *in vitro* multiplication protocol was developed for *Trichosanthes cucumerina.* Eight day old cotyledonary node without cotyledon was found to be most efficient explant with regard to multiple shooting (Kawale and Choudhary, 2009).

2.3.1. Saraca asoca

Seedlings

Ripe seeds were collected during the months of August-September. On an average 45-70 seeds weigh one kilogram. Seeds can be stored up to six months under low temperature (Chacko *et al.*, 2002). The seeds were dibbled directly in polythene bags of size 30 x 15 cm filled with potting mixture. Seeds were also sown in root trainers (size 10 x 5 cm) with potting mixture and compost. Necessary watering was done periodically. Seeds started germinating after one month and the germination completed by 50 days. A maximum of 80 per cent germination was obtained when seeds were dibbled in polythene bags and 91 per cent germination, in root trainers.

Macropropagation

Propagation through stem cuttings

Trials were carried out during the months of May-June, July-August, and September- October. Healthy stem cuttings of *Saraca asoca* were collected from natural populations in the forests, gardens and homesteads.

Two types of stem cuttings were used (mature cuttings: one to two-year-old and juvenile cuttings: less than one year old) with two nodes excised either at the node or internode with 2 to 4 leaves. The leaf blades were pruned more than half of their size with the help of a sharp scissors without causing any damage to the apical bud, so as to reduce the rate of transpiration. All the stem cuttings were treated with broad-spectrum systemic fungicide 1% Bavistin (Carbendazim 50% WP) solution for 15 min as prophylactic treatment. After treatment with fungicide, the basal portion of the cuttings was treated with various concentrations of IBA (Indole-butyric-acid) mixed with commercial purified talc using Mikro-dismembrator (supplied by B. Braun, Germany); and also with Rootex (Commercial grade root inducing powder). A set of stem cuttings without any hormone treatment was also maintained as control to compare the effect of IBA and Rootex treatments. All the prepared stem cuttings were planted in 10 cm X 5 cm root trainers filled with vermiculite (Supplied by Keltech Energies, Ltd, Bangalore). The cuttings were kept under intermittent mist inside the mist chamber. The temperature inside the mist chamber was maintained between 30 – 40°C and the relative humidity between 80 – 90 %. (The atmospheric temperature and the relative humidity during the experimental period are shown in the Table 1).

Table 1. Meteorological data during the investigation period (May-October)

Months	Temperature		Relative humidity	
	Maximum	Minimum	Maximum	Minimum
May	37	23	100	41
June	32	22	100	60
July	34	22	100	56
August	33	21	100	52
September	35	22	100	60
October	44	22	100	41

The number of days taken for initial sprouting was recorded and the cuttings showing symptoms of withering were removed. On confirming the cuttings for adequate rooting, the humidity and temperature were gradually reduced to avoid algal attack. Minimum irrigation was given so as to keep the rooting medium just moist. On completion of two months, the well-rooted cuttings were transplanted into polybags filled with potting mixture (sand, soil and farm yard manure (FYM) in the ratio 1:1:1) and kept under shade for hardening for about two weeks.

Propagation through air layering

Air layering was carried out on branches of approximately twenty-five year old Asoka trees as well as on one-year-old seedlings in the KFRI medicinal plant garden.

A small strip of bark (2.5 – 3.0 cm) was carefully girdled out using a sharp knife from branches, which were slightly thicker than a pencil. Layers were treated either with 5000 ppm (500 mg of the chemical mixed with 100g of purified talc) of IBA or Rootex during the month of September. About 5 mg of IBA (5000 ppm) and Rootex were applied to the upper portion of the girdled region. A handful of vermiculite mixed well with 1 % Bavistin

solution was placed around the girdled area and wrapped with a transparent polythene sheet so that the girdled surfaces were completely covered tied tight on both ends. A set of layers without any hormone treatment was also maintained to compare the effect of IBA and Rootex treatments.

2.3.2. Trichosanthes cucumerina

Seedlings

Seeds were washed to remove the pulp and dried before sowing. They were sown in nursery beds, rectangular plastic trays and dibbled in polythene bags filled with potting mixture. Seeds started germinating from the fourth day and completed by seventh day. On an average 85 per cent germination was obtained. A total of 2000 seedlings were raised.

Fruits of *T. cucumerina*

Seed germination in nursery bed

Seed germination in tray

Seed germination in polythene bags

Macropropagation

For macropropagation, cuttings with 1 or 2 leaves were prepared. The cuttings were treated with Bavistin solution (0.5%) in water for 30 mins. This was followed by hormone treatment with IBA 3000 ppm in talc by dip method. The relative humidity of the ambient was 80% and temperature 32°c. The treated cuttings were planted in Vermiculite filled root trainers kept in mist propagation chamber.

Micropropagation

Tissue culture was carried out with nodal and shoot tip explants. Explants were taken from seedlings germinated *in vitro*. Seeds were first treated with 70 % alcohol for 1 minute followed by a treatment under a laminar flow bench with 0.1 % mercuric chloride solution for 10 minutes, rinsed with three changes of sterile distilled water and inoculated on a simple semi-solid medium consisting of Murashige and Skoog's minerals and vitamins supplemented with sucrose 2 % (w/v). The germinated seedlings were maintained in light until 3-5 leaves had formed. The nodal region of about 1-2 cm was excised from the seedlings to serve as explants. Leaves and internodes were also excised for induction of callus.

Shoot induction from nodal explants was attempted on MS basal media supplemented with various levels (0.5, 1.0, 2.0 and 3.0 mg/l) of the cytokinin BAP. To induce callusing MS basal media supplemented with various levels of the auxins 2,4-D and NAA (viz. 1, 2 and 3 mg/l was used.

2.3.3. Ipomoea mauritiana

Seedlings

Seeds were sown in nursery beds and rectangular plastic trays filled with garden soil. Seeds germinated between 5th and 9th days. In both the cases up to 85% germination was obtained.

Macropropagation

Cuttings with 1 or 2 leaves were prepared and treated with Bavistin solution (0.5%) in water for 30 mins. Fungicide treatment was followed by treatment with IBA 6000 ppm in talc by dip method. The propagation experiments were conducted at a relative humidity of 80% and temperature 32°C.

Cutting after 2 months

After one month the rooted cuttings were transplanted in to polythene bags, filled with garden soil, sand and FYM. The poly potted plants were transferred to hardening chamber.

Micropropagation

Seeds were germinated on hormone-free basal media after surface sterilization as described above for *Trichosanthes cucumerina.*

2.3.4. Salacia oblonga

Seedlings

Seeds were washed to remove the fleshy aril and dried before soil. Germination trials on seed germination could not be conducted for want of enough seeds. Seeds are usually sown in polybags filled with potting mixture. Germination takes place in 20-30 days after sowing. Seedlings raised in polybags can be planted in the field after 2-3 months (Oommen *et al.*, 2000).

Macropropagation

Cuttings with 2-4 leaves were prepared as described earlier. The cuttings were treated with Bavistin solution (0.5%) in water for 30 mins. Fungicide treatment was followed by treatment with different concentrations of IBA 6000 in talc by dip method. The propagation experiments were conducted at a relative humidity of 80% and temperature 32°C. Treated cuttings were planted in Vermiculate filled root-trainers and were kept in the mist chamber.

After 4 weeks, the well-rooted cuttings were transplanted into polythene bags filled with garden soil, sand and FYM. The potted plants were transferred into the hardening chamber.

Micropropagation

Seeds after surface sterilization were germinated *in vitro* on simple MS medium to serve as explant source. Leaves, internodes and nodes were excised and inoculated into a wide range of media to induce shoots. Nodes from mature plants growing in the garden were also used as explants.

A range of media supplemented with different levels of BAP was used to induce shoot development in nodal explants.

2.3.5. Merremia turpethum

Seedlings

Seeds were sown in nursery beds and in rectangular plastic trays filled with garden soil. The seeds germinated in 4 to 7 days, and 80-85% germination obtained.

M. turpethum mature fruits

M. turpethum seeds

Macropropagation

Cuttings with 1 or 2 leaves were prepared as usual. Prophylactic treatment was given with Bavistin solution (0.5%) in water for 30 mins. This was followed by hormone treatment with IBA 3000 ppm in talc by dip method. The ambient humidity was 80% and temperature, 32°C. Treated cuttings were planted in Vermiculite filled root trainers kept in mist propagation chamber.

After 4 weeks the rooted cuttings were transplanted in to polythene bags, filled with garden soil, sand and FYM. The poly-potted plants were transferred to the hardening chamber.

Micropropagation

Seeds were germinated *in vitro* on simple MS medium to serve as explants source. Leaves, internodes and nodes were excised and inoculated into wide range of media to induce shoots.

2.4. Field Trials

2.4.1. Trichosanthes cucumerina

Pits of 60 cm diameter and 30-45 cm depth were dug at an espacement of 2 m. Well-rotted FYM was mixed with top soil in the pit and 4 to 5 pre-soaked seeds were sown per pit. After two weeks, the unhealthy plants were removed, retaining 3 plants per pit.

Trichosanthes in pot

As an alternative method, large pots were used to propagate the pre-soaked seeds. About 4 to 5 seeds were sown per pot. Well-rotted FYM mixed with top soil was used as potting medium.

During the initial stages of growth, the seedlings were irrigated at an interval of 3-4 days. Watering was done on alternate days during flowering and fruiting periods. 'Pandals' were erected for the trailing *Trichosanthes.* Weeding and raking of the soil were done at the time of manuring.

The important pest attacking the *Trichosanthes cucumerina is* fruit flies, mostly attacking the fruits.

Important diseases are downy mildew and mosaic. Bavistin is applied to check these diseases.

Field planted *T. cucumerina*

The vegetative growth of plants raised from seedlings was more vigorous than that of the rooted cuttings. In the rooted cuttings the size of the fruits was more or less uniform and smaller than that of the plants raised from seedlings. However, the number of fruits produced was more in the plants raised from rooted

cuttings. Though 70 percent rooting was obtained for the cuttings treated with IBA, the survival was only 10 per cent when planted in the field. With respect to growth and survival of the plants in the field, plants raised from seeds performed well than the rooted cuttings. Therefore, further production of rooted cuttings was discontinued. In the case of *Trichosanthes cucumerina*, seeds are the best planting material. The variation in the size of fruits was due to the crossing between *T. cucumerina* and *T. anguina*, which is widely cultivated as a vegetable.

2.4.2. Merremia turpethum

Well established rooted cuttings of *Merremia turpethum* and seedlings were planted at spacing of 1 m in pits of 60 cm diameter and 30-45 cm depth. FYM was applied as basal dose.

Plants at initial stages of growth were irrigated at an interval of 3-4 days. Irrigation was done on alternate days during flowering and fruiting periods.

'Pandals' were erected for the trailing *Merremia*. Weeding and raking of the soil were conducted at intervals.

No serious pest or disease noticed.

Field trial experiment stage III

2.4.3. Ipomoea mauritiana

Well established rooted cuttings and seedlings of *Ipomoea mauritiana* were used as planting material. The rooted cuttings were planted in raised mounds at an espacement of 1 m. FYM was applied as basal dosage.

Pest attacked plants

During the initial stages of growth the plants were irrigated at an interval of 3-4 days. During flowering and fruiting periods they were irrigated on alternate days.

Field planted *Ipomoea mauritiana*

Support 'Pandals' were erected for the trailing *Ipomoea*. Weeding and raking of the soil were done at the time of fertilizer application.


Eucromia polymena

A caterpillar of the moth (*Eucromia polymena*) was found feeding the leaves. Against the pest, Ecalex and Roger were applied for its control.

Bavistin is applied to check the fungal and leaf spot diseases.

2.5. Distribution of planting materials

Fifteen thousand rooted stem cuttings were distributed to the Kerala Forest Department, Kerala State Medicinal Plants Board, and Police academy, Thrissur, NGOs and the Public on request. Further, 8000 propagules of *Saraca* raised during the project period were also distributed. About fifty rooted cuttings of *Saraca asoca* from different accessions were planted in the Institute Campus as part of establishing the Germplasm collections. Five hundred and sixty rooted cuttings of *Salacia oblonga* were given to the Botanical Gardens of Colleges, schools and to the public. One thousand five hundred gram seeds of *Trichosanthes cucumerina* were also distributed to the farmers. Among the planting materials raised during the project period *Saraca asoca* and *Trichosanthes cucumerina* were the species with most demanded from the farmers and public.

3. Results and Discussions

Apart from regeneration through seeds, propagation from vegetative parts such as stems, roots, rhizomes, bulbils or leaves is common in several plants. Propagules raised by vegetative methods retain their genetic constitution of their parent plants. In order to maintain

the genotype, many horticultural plants are propagated largely through vegetative methods. Further, hybrid crop plants produced through breeding are propagated by vegetative methods in order to overcome their failure in fruit setting or sterility. In forestry, vegetative propagation has been practiced to produce planting stock of various trees. Vegetative methods are adopted in species having irregular fruiting, poor seed setting, low germination percentage, etc.

Grafting and layering are the common vegetative propagation methods adopted for tree species. These methods have been standardized for horticultural crops such as mango, cashew, rubber, etc. Micropropagation, the clonal propagation using tissue culture, has also been standardized and protocols developed for several spices and ornamental plants. The success rate of tissue culture of the tree species has been relatively few. Propagation through rooted cuttings has been standardized and protocols developed for species such as *Eucalyptus* (Campinhos and Ikemori, 1980; Surendran, 1987), *Dipterocarpus* (Srivastava and Maggil, 1981) and *Gmelina arborea* (Florido, 1978). Trials conducted on tree species (Nanda, 1970; Amatya, 1982) indicate that optimum conditions vary with species and also depend on the age of the cuttings. Hence, standardization of growth regulating hormones, dosage and best season for induction of rooting are to be standardized. Among the five medicinal species taken up for the study, mass multiplication has been not been previously attempted.

3.1. Macropropagation

3.1.1. Saraca asoca

Sprouting of buds from the upper nodes was observed after 15 days in juvenile stem cuttings whereas in mature stem cuttings it took 20 – 25 days. Cuttings were found to develop callus from which young adventitious roots emerged and established well. The

optimum hormone concentration for the maximum rooting in stem cuttings are given in the Table 2.

In the stem cuttings planted in the month of May, root induction started just after 16 days of planting and 20 days in cuttings planted in the month of September. However, it took 26 days after planting for the initiation of rooting in the cuttings planted in the month of July. In all these cuttings the number of roots formed is 3 – 5 per cutting.

The maximum percentage of rooting, i.e., 96 % was recorded in mature stem cuttings and 94% of rooting in the juvenile stem cuttings which were planted in the month of May. This followed by 88 % of rooting response in the mature stem cuttings and 83% of rooting success in the juvenile stem cuttings, planted in the month of September. A maximum of 85 % rooting response was observed in the mature stem cuttings and 81% of rooting was obtained in juvenile cuttings planted in the month of July.

The mature stem cuttings treated with 1000 ppm of IBA gave the maximum rooting percentage (96 %) in cuttings excised at the node while in cuttings excised at the internode gave maximum rooting percentage (85 %) with 5000 ppm of IBA. The juvenile cuttings treated with 1000 ppm of IBA gave maximum rooting percentage (92%) in cuttings excised at the node while cuttings excised at the internode gave maximum 94 % with 5000 ppm of IBA.

Cuttings used as controls gave a maximum of 24 % of rooting in mature nodal cuttings and 20 % of rooting in mature internodal cuttings. Juvenile nodal as well as internodal cuttings did not show any response and eventually they perished. Cuttings treated with Rootex gave a maximum of 65 % of rooting in mature nodal cuttings and 58 % of rooting in mature internodal cuttings whereas juvenile stem cuttings excised at nodal region gave 62 % of rooting and juvenile cuttings excised at internode gave 48 % which were planted in the month of May.

Table 2. Optimum concentration of hormones for maximum rooting in stem cuttings

Different treatments given			Rooting percentage		
Hormone	Concentration	Type of stem cutting	May-June	July-August	September-October
IBA	1000 ppm	Mature node	96	85	88
	3000 ppm		92	83	86
	5000 ppm		90	81	80
	1000 ppm	Mature internode	83	72	81
	3000 ppm		85	75	83
	5000 ppm		90	78	85
	1000 ppm	Juvenile node	92	70	86
	3000 ppm		90	75	84
	5000 ppm		86	72	80
	1000 ppm	Juvenile internode	84	70	72
	3000 ppm		90	74	76
	5000 ppm		94	81	80
Control		Mature node	24	18	20
		Mature internode	20	13	20
Rootex		Mature node	65	52	62
		Mature internode	58	43	51

In stem cutting propagation, the roots produced are said to be adventitious since they do not emerge from pre-existing root system of a plant. This is distinctly a polar phenomenon. The adventitious roots are formed preferentially at the basal end of the cuttings irrespective of their orientation. These polar regeneration patterns are indicative of the asymmetric distribution of the plant hormone auxin. (Singh *et. al.*, 2005).

In air layering both IBA (5000 ppm) and Rootex treated layers showed 100 percentage rooting response. However, the number of roots and the root length were higher in IBA treatment. In the IBA treated layers a mean number of 10 – 14 roots per layer with an average length of 8.5 cm were observed after 60 days of layering while in the Rootex treated layers the number of primary roots was 2 - 4 with a mean length of 6.5 cm. Root initiation was observed after 18 days of layering in IBA treated layers whereas in Rootex treated layers root initiation started only after 30 days of layering. In controls, root initiation started only after 45 days of layering. There were also qualitative differences between Rootex and IBA treated layers. A mass of hardened callus surrounding the upper part of the girdled portion was observed in Rootex treated layers (this was also presented in controls), which was absent or not prominent in IBA treated layers. The roots formed in Rootex treated layers were much larger but very brittle than the IBA treated layers. The quick and higher rooting observed in the study may be due to the juvenility of the plant as well as due to the warm humid condition of the late–monsoon season during the experiment. The layered plants have established well in the nursery. Table 3 shows the details of callus induction and root initiation in air layers during the experimental period (September-October).

Studies on the process of adventitious root formation in air layered stem branches revealed that the removal of bark, cambium and phloem layers around the circumference of the shoot prevents carbohydrates and photosynthetic flow down along the trunk, resulting in the accumulation of these substances at the upper part of the girdled site. However, the leafy portion of the girdled branch received continuous supply of water and mineral nutrients through the xylem and therefore, remains alive. The presence of carbohydrates and photosynthates especially auxin at the girdled site causes formation of root primordia to develop into roots (Jose and Pandurangan, 2006).

Table 3: Responses in callusing and rooting in Air layers (September–October)

Treatment	Types of layers	Callus initiation (Days)	Root induction (Days)	Number of roots per layer (cm)	Avg. length of roots (cm)	Rooting %
IBA 5000 PPM	Mature branch	15 - 18	20 - 23	10 - 14	8.5	100
	Seedling	13 – 16	18 - 21			
Rootex	Mature branch	28 - 32	34 - 38	2 - 4	6.4	100
	Seedling	27 - 30	32 - 36			
Control	Mature branch	40 - 42	45 - 48	5 - 8	7.2	80
	Seedling	38 - 42	42 - 46			

3.1.2. Trichosanthes cucumerina


Root formation

Root initiation started at 15th day of planting and a maximum number of roots at an average of 3-4 per cuttings formed within 20 days. However, shoot initiation started after 18th day of planting.

After 4 weeks, the rooted cuttings were transplanted into polythene bags, filled with garden soil, sand and FYM. The poly potted plants were transferred to the hardening chamber.

About 70 percent rooting success was obtained. But it was very difficult to establish the rooted cuttings in the hardening chamber. A total of 3000 cuttings were rooted.

3.1.3. Ipomoea mauritiana

Planting was done in Vermiculite filled root trainers kept in mist propagation chamber.


Shoot formation

Root formation: Root initiation started after 15 days of planting and a maximum number of roots at an average rate of 3-4 per cuttings formed within 20 days. Shoot initiation started on 18th day of planting and new healthy shoot obtained within 25 days. About 60% rooting success obtained.


Cutting after 3 months

3.1.4 Salacia oblonga


Root formation after one year

It took around 45 days to initiate the rooting at an average of 4-5 roots.

Sprouting of buds from the upper nodes started after one month in the case of seedlings, where as in mature nodes, it took more than 45 days.

At 6000 ppm 80% rooting obtained in *Salacia oblonga*. Cuttings used as control without any hormone treatment did not show any rooting response. Table 4 shows the effect of different concentration of hormone on rooting.

The rooted cuttings transferred into poly-bags registered 60% survival.

Table 4. Effect of different concentration of rooting hormone on *Salacia oblonga*

Type of stem cuttings	IBA (mg/l)	Rooting %	Average no. of roots per cutting
Mature, node	5000	70	2
Mature, node	6000	80	4
Mature, inter-node	5000	70	2
Mature, inter-node	6000	80	4
Seedling, node	5000	60	2
Seedling, node	6000	65	3
Seedling, inter-node	5000	60	2
Seedling, inter-node	6000	65	3
Control, node	0	00	0
Control, inter-node	0	00	0

3.1.5. Merremia turpethum

Root formation: Root initiation started after 18 days of planting and a maximum number of roots at an average rate of 2-3 per cuttings formed within 20 days. Shoot initiation started after 20th day of planting. About 65 % rooting success is obtained.

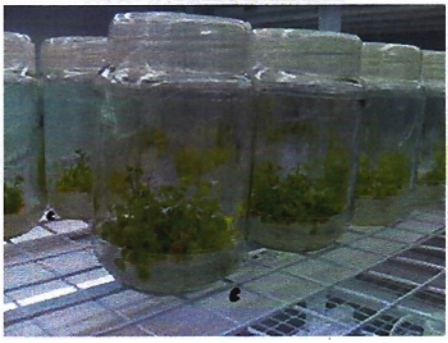
3.2. Micropropagation

3.2.1. Trichosanthes cucumerina

Shoot development with multiple shoot formation was observed from nodal explants on all media supplemented with BAP.

Sprouting of axillary buds in nodal explants

Multiple shoot formation



Multiplying cultures of T. cucumerina

Elongated shoots of T. cucumerina

Best results were obtained with MS supplemented with 1.75 mg/l BAP and 0.1 mg/l NAA where up to 10 multiple shoots were formed per passage. Some amount of callusing was formed at the base of the shoot clusters. Shoots could be rooted in hormone-free basal media or rooted ex vitro in the mist chamber in vermiculite. Plantlets were hardened for 2-3 weeks and later

transferred to soil in polybags and maintained in the nursery. Plantlets were prone to damping off if watering was not regulated carefully.

3.2.2. Ipomoea mauritiana

Multiple shoot formation was obtained from nodes on a media containing BAP (1-3 mg/l) with or without NAA (0.1 or 0.5 mg/l). When transferred to a liquid medium and maintained without agitation, the shoots elongated and leaves developed and occasionally roots also developed.

Attempts to induce rooting in shoots with auxins (NAA and IBA (0.5, 1.0 and 3.0 mg/l) failed to produce consistent results.

Induction of Multiple shoots in nodal explants of *I. mauritiana*

Shoot development in liquid media

3.2.3. Salacia oblonga

In *Salacia oblonga* the seed germination and growth of seedlings was poor in culture. Since seeds were difficult to obtain further experiments could not be undertaken. Lack of fresh shoot growth

from the seedlings in culture was a major hurdle in obtaining sufficient explants for shoot multiplication experiments. Mature nodal explants also gave no results.

3.2.4. Merremia turpethum

In *Merremia turpethum* nodal explants failed to establish in culture due to high contamination. Leaves gave rise to callus on medium containing 2,4-D (1, 2, 3 mg/L). Further morphogenesis was, however not obtained when different combinations of hormones were tested.

3.3. Yield

The average yield per plant was determined for *Ipomoea mauritiana, Trichosanthes cucumerina* and *Merremia turpethum* by harvesting. *Trichosanthes cucumerina* planted in April was harvested in the month of November, when the plants started drying. The tubers of *Ipomoea mauritiana* were harvested in the month of January. *Merremia turpethum* was harvested in the month of December.

The fresh weight of *Ipomoea mauritiana* tuber ranged from 106-210 g. The dry weight of *Trichosanthes cucumerina* (whole plant) ranged from 100-180 g. The dry weight of *Merremia turpethum* (stem with root) was between 80-140 gm. Thus at an average yield of 158 g per plant, 1580 kg fresh tuber of *Ipomoea mauritiana* can be obtained when planted at spacing of 1 m. With an average yield of 140 g dry weight for *Trichosanthes cucumerina,* from one hectare 1400 kg plants can be obtained. In the case of *Merremia turpethum,* the average yield from one hectare is 1100 kg, when planted at a spacing of 1 m.

Tuberous roots *Ipomoea mauritiana*

Dried plants of *Trichosanthes cucumerina*

Roots of *Merremia turpethum*

4. Conclusion

The trials on rooting of cuttings of *Saraca asoca* using growth regulator hormone Indole Butryic Acid (IBA) were very successful. This method is very convenient for mass production of planting materials of *Saraca* within a short period. Cuttings collected from homesteads gave higher percentage of rooting than the cuttings collected from forest areas. This indicates that the cuttings are to be fresh for better rooting. The rooted cuttings can be planted in the field after six months. Germination trials of seeds of *Salacia*

oblonga could not be undertaken for want of enough seeds. The population of this species in Kerala is extremely sparse due to over-exploitation. The rooting of cuttings of *Salacia oblonga* gave up to 80 per cent when treated with 6000 ppm IBA. Rooting of cuttings of *Merremia turpethum*, *Ipomoea mauritiana* and *Trichoanthes cucumerina* also gave very good percentage of rooting.

There is not much difference in the growth and yield of vegetative propagated and seed raised plants of *Merremia turpethum* and *Ipomoea mauritiana*. The survival and growth of *Trichosanthes cucumerina* propagules raised from rooted cuttings and tissue cultured plants were poor compared to the seed raised plants. For *Trichosanthes cucumerina*, seeds are the best planting materials. The size of fruits showed much variation in the seed raised plants of *Trichosanthes cucumerina*, may be due to natural hybridization with *T. anguina* which is widely cultivated in Peechi and neighboring areas. The trials showed that *Saraca asoca* and *Salacia oblonga* which are rare and seed production is sparse, can be mass multiplied within a period six months through rooted cuttings.

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