Bulletin of the Department of Plant Resources No. 36

# Abstract of Tissue Culture

Government of Nepal
Ministry of Forests and Soil Conservation
**Department of Plant Resources**
Thapathali, Kathmandu, Nepal
2013

Bulletin of the Department of Plant Resources No. 36

# Abstract of Tissue Culture

Government of Nepal
Ministry of Forests and Soil Conservation
**Department of Plant Resources**
Thapathali, Kathmandu, Nepal
2013

**Editor**

Ms. K. M. Rajkarnikar
Ms. S. Rajbahak
Mr. S. Panthi

**Sub editor**

Mr. H. Ray
Mr. P. Basnet

**Advisors**

Dr. A. N. Das
Ms. S. Upadhya



Department of Plant Resources
Government of Nepal
Thapathali, Kathmandu, Nepal

**Date of Publication (Revised)**

2013

Copies of Publication

750 only

**Price:** Rs……

**Cover Photo:** Incubation Room at Biotechnology Section, Thapathali

Printed in Nepal by
Super Printing Press
Kamal Pokhari, Kathmandu
Tel: 4410954

# Foreword

(Revised edition)

The present Bulletin (No. 36) is the revised and updated version, Abstract of Tissue Culture, Bulletin no. 14. In this edition there are 111 abstract of tissue culture of economically important medicinal, horticulture, ornamental and tree species.

Tissue culture, a new biotechnological tool, offers superior quality plant clone material free of disease in desired quantities. Since the establishment of Tissue Culture Laboratory in 1976 at Godawari, Lalitpur, different aspects of micro propagation, anther culture and microshoots rooting in non sterile sand have been carried out in a number of economic plant species. In 2000 A.D. Biotechnology laboratory was established in Department of Plant Resources, Thapathali. The main objective of the Biotechnology laboratory is to use the plant tissue culture technique for the mass propagation of economically important and endangered plant species, DNA Barcoding of high value crops species and virus testing.

The finding of research works have been published in different national and international journals. This bulletin consists of research abstract on tissue culture that has been conducted by Tissue Culture Laboratory, Godawari and Biotechnology Section, Thapathali. It is a compilation of abstract from 1983 to 2012.

I hope this effort of collection of research works and new revised edition of abstract will be useful for the research personnel who are engaged in the tissue culture research and biotechnology. I would like to thank Ms. K.M. Rajkarnikar, Scientific Officer, Ms. S. Rajbahak, Mr. Sishir Panthi, Assistant Scientific Officer, Mr. Haresh Ray, Assistant Botanist and Mr. Puskar Basnet for their efforts to compile the abstract and bring this revised volume for the publication.

July 2013

Dr. A. N. Das
Director General
Department of Plant Resources
Kathmandu, Nepal

# Introduction

Tissue culture is the development of new plants in an artificial nutrient medium under aseptic condition from explant. The explant is a small piece of plant used to start a culture. The explant can be very small pieces of plants, such as embryo, seed, stem, shoot tip, meristem, root tip, callus, single cell and pollen grain.

Plant tissue culture is an important aspect of biotechnology. It has great potential for rapid, large scale and true to type multiplication. The plants from tissue culture are generally clean and healthy. This technique has been applied to several crop species. This technique is of particular interest in case of highly heterozygous species which are virus infected and are in general vegetatively propagated.

Since 1963, *in vitro* culture has been widely used throughout the world. Mass propagation of some ornamentals is one of the most successful examples of commercialization of tissue culture technology. Multimillion dollar industries have been set up in the world to meet the demand for quality plants. Realizing the potential tissue culture technique protocol development for different species is being carried out in different laboratories.

Tissue Culture Laboratory under the Department of Plant Resources (DPR) was established in 1976 in Godawari. This laboratory has developed the techniques for micropropagation of economically important plants and elite trees as a incomes to produce best quality clone plants.

Micro shoots developed from tissue culture can be rooted in non sterile sand. For this the micro shoots in flask are acclimatized in glass house for one week and these are individually planted in sun dried clean sand. It is covered with polyethylene sheet to maintain humidity of 80% and temperature range of 25-30°C.

The advantage of the technique is to cut down the cost of production of tissue cultured plant. From this rooting technique, the cost of growth medium, incubation room, electricity and tissue culture manpower is lowered. This rooting technique can be easily carried out by nursery personnel for the mass production of micro propagated plants. This rooting technique in case of Nepal has a potential in the field of plant propagation.

Training on tissue culture laboratory technique and sand rooting of micro propagated shoots has been a regular program of this laboratory.

In Tissue Culture Laboratory, micro propagation methods for several plant species have been developed. These methods are simple, effective compared to the conventionally used *in vitro* cloning methods. In the conventional procedure four steps are involved.

1. Explant establishment,
2. Shoot multiplication,
3. Rooting of multiplied shoots in the medium and
4. Field establishment.

In the step 1, the explant isolation, surface sterilization, washing and establishment in the culture medium is done. In the step 2 the micro shoots are proliferated in a defined culture medium. In the step 3 micro shoots proliferated during incubation are transferred to a rooting medium within the flasks. In the step 4, the micro plants are transferred from the aseptic environment of the laboratory to the natural condition for field establishment.

The steps 3 and 4 are labor intensive and expensive such that the step 3 accounts 30-70% of production cost. And the step 4 encounters the severe problem of low percentage of survival of plants when the plants are transferred from the flask to the field.

In the method developed at Tissue Culture Laboratory, in the DPR instead of step 3 and 4 the micro shoots are directly rooted in sand under suitable day/night temperature regimes in the ordinary green house conditions. Such a procedure has reduced the production cost. It can be compared with the production cost of seedlings and cuttings.

Micropropagation protocols have been developed in following plant species by Department of Plant Resources.

**Trees:**

*Artocarpus lakoocha, Artocarpus heterophyllus, Dalbergia sisoo, Eucalyptus camaldulensis, Eucalyptus citriodora, Eucalyptus tereticornis, Ficus auriculata, Ficus carica, Ficus elastica, Ficus lacor, Ficus nemoralis, Ficus nerifolia, Ficus semicordata, Morus alba, Populous ciliate*

**Horticultural crops:**

*Brassica oleracea var. capitata, Citrus limon, Citrus sinensis, Fortunella* sp., *Musa* sp., *Poncirus trifoliate, Solanum tuberosum, Saccharum* sp., *Fragaria ananassa, Zinziber officinale.*

**Medicinal Plants:**

*Chrysanthemum cinerarifolium, Solanum laciniatum*

**Ornamental Plants:**

*Chrysanthemum morifolium, Chrysanthemum* sp., *Dianthus caryophyllus, Gerbera, Gladiolus, Lilium nepalensis, Lilium longiflorum, Rosa* sp., *Saintpaulia ionatha*

**Orchids:**

*Cymbidium giganteum, Cymbedium grandiflorum, Cymbidium longiflorum, Dendrobium densiflorum, Dendrobium fimbriatum, Vanda teris.*

**Bamboo:**

*Dendrocalamus hamiltonii, Dendrocalamus strictus.*

**Anther Culture:**

*Allium fistulosum, Nicotiana tabacum, Oryza sativa.*

New addition plants species

**Trees:**

*Elaeocarpus sphaericus, Daphne papyracea, Paulownia tomentosa, Azadiracha indica, Acacia auriculiformis, Santalum album*

**Horticultural crops:**

*Stevia rebaudiana, Citrus aurantifolia, Actinidia deliciosa, Solanum lacinatun*

**Medicinal Plants:**

*Piper longum, Withinia somnifera, Aloe vera, Swertia chirita, Amonum subulatum, Rauvolfia serpentina, Valeriana jatamansi, Rheum emodi, Cephaelis ipecacuanhe, Swertia ciliata, Neopicrorhiza scrophulariifolia*

**Ornamental Plants:**

*Spathiphyllum wallisii, Syngonium, Asiatic Lily, Primula obconica, Begonia tuberhybrida, Fuschia hybrida, Antirrhium majus, Streptocarpus* sp., *Gloxinia* sp., *Lisianthus* sp.

*Ryncostylis ritusa, Epidendron* sp., *Oncidium* sp., *Renenthera* sp., *Dendrobium moschatum, Dendrobium amoenum, Cymbidium aloifolium, Vanilla planifolia.*

**1. Regeneration of plantlets from leaf culture of *Solanum lacinatum* Ait.**

A. Karki and S. B. Rajbhandary

The leaf segments of *Solanum laciniatum* Ait. formed shoots when cultured *in vitro* on the basal medium of Murashige and Skoog (MS) supplemented with cytokinins. The proliferation potential of the culture was maintained for 5 years by regular subculture. The proliferated shoots were rooted in half concentration of MS without growth hormones. Then, these rooted seedlings were transferred in the pot and kept for 4 weeks in greenhouse before planting to the field.

**J. Inst. Sc. Tech. Vol. 6, 1983, T.U. Kathmandu, Nepal, pp. 35-40**

**2. Clonal propagation of *chrysanthemum cinerariaefolium* Vis. (Pyrethum) through tissue culture.**

A. Karki and S. B. Rajbhandary

The *in vitro* technique of shoot tip culture has been shown to produce many plants from a single shoot over relatively short period compared to the conventional propagation. The method involves excising shoot tip, sterilization and placing the bud in media for propagation of bud initiation and root induction. The stage involving the rooting of the shoot before transfer of the plantlets to the field establishment has been shown to be expensive due to the costly skilled manpower involved. An attempt has been made to show the need to eliminate the more expensive method of Pyrethrum mass clonal propagation and resort to the more cheap method involving transplanting the shoot directly to the beds for rooting.

**Pyrethrum post, 15 (4), pp118-121, 1984**

**3. Mass propagation of *Dendrobium fimbriatum* Hook. from seeding tips**

R. Niroula and S. B. Rajbhandary

The seedling tips of *Dendrobium fimbriatum* Hook. have been cultured in Murashige and skoog medium supplemented with cytokinins. From the explant, shoots were proliferated and and protocorms also developed. These shoots and protocorms when transferred in Vacin and Went medium grew into complete plantlets.

**J.Inst.Sc.Tech. Vol 8, 1985, T.U. Kathmandu, Nepal, pp. 7-10**

**4. Rooting in non sterile potting mix of *in vitro* potato and its field establishment**

A. Manandhar and S.B. Rajbhandary

*In vitro* proliferated shoots originated from shoot tips of potato (*Solanum*

*tuberosum* Linn.) cv. Cardinal was rooted readily in a non-sterile medium composed of sand and dried leaves mixed in equal proportion in volume. Apparently rooting was more efficient in January and February when the maximum and minimum temperature were 18°C, 21°C and 4°C, 6°C respectively. Rooted plants were successfully established in the field and grown to maturity with an average yield of 230 g and 18 tubers per plant.

**The Indian journal of Horticulture vol. 43, Nos. 3 and 4 September-Decmber, 1986, pp. 235-239**

## 5. *In vitro* propagation of *Dendrocalamus strictus* (Roxb.) Nees and its establishment in soil

R. Niraula and S. B. Rajbhandary

Creamy white callus was obtained aseptically from the shoot explants excised from the 2-3 weeks old *Dendrocalamus strictus* seedlings in medium supplemented with 5 mg/l 2,4-D. The creamy white callus developed into two types of calli, green chlorophyllous and white in the MS medium when 2, 4-D was reduced to 0.5 mg/l. From the chlorophyllous callus embroids originated which developed into plantlets. These plants were transferred to the sand followed by the establishment in the soil.

**Proceeding of Regional workshop on Tissue culture of Trop. Crop. Plants, Dhaka 1987, pp. 17-20**

## 6. Micropropagation of *Ficus Auriculata* Lour.

N. Amatya and S. B. Rajbhandary

Cotyledonary nodes obtained from the aseptic culture of *Ficus auriculata* were cultured in the MS medium (Murashige and Skoog 1962) supplemented with BAP 1.0 mg/l and NAA 0.01 mg/l to induce shoot formation. Multiple shoot thus formed were sub-cultured every eight weeks to produce more shoots. Rooting of microshoots occurred in a mixture of sand and dry leaf powder (2:1,v/v).Rooted plantets were established in the field.

**Proceeding of an international workshop on application of biotechnology in forestry and horticulture, January 14-16, 1988, Tata Energy Research Institute, New Delhi, pp. 157-163**

## 7. Micropropagation of potato cultivars and their field performance

A. Manandhar, S. Rajbhandary, P. Joshi and S. B. Rajbhandary

Seven potato cultivars obtained as test tube plants were multiplied rapidly by using tissue culture method of propagation. The tissue culture derived seed tubers of these cultivars and the other 'Cardinal' cultivar were planted in the field to assess the growth yield and late blight resistance. In the spring

as well as in the autumn plantations in 1986, the two CIP cultivars I 853 and MS 91.18 were found resistant to the late blight where as the other CIP cultivars MS 82.60, MS 35.22, Br. 63.15 and Sangema showed various levels of late blight infection. Cardinal cultivar was found to be severely infected by the late blight.

**Proceeding of National Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal. pp. 135- 140**

## 8. *In vitro* propagation of *Poncirus trifoliata* (L.) Rafin (Trifoliate orange)

R. Niraula and S. B. Rajbhandary

In the culture of *Poncirus trifoliate*, a citrus root stock obtained from Japan, the shoot induction was caused in the Murashige and Skoog medium (1962) in the presence of benzyl amino purine (BAP) 1.0 mf/l and napthaleneacetic acid (NAA) 0.1 mg/l when cotyledonary node was used as explant. On subculture these shoots continued to proliferate in the basal medium supplemented with lower concentration of BAP 0.1 mg/l and NAA 0.1 mg/l.

Roots were produced in shoots when transferred on basal medium supplemented with NAA 0.1 mg/l.

**Proceeding of Nationl Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal, pp. 141-144**

## 9. Micropropagation of *Brassica oleracea* L.Var. capitata through cotyledonary node culture

N. Pradhan and S. B. Rajbhandary

Shoot proliferation of *Brassica oleracea* var. capitata (K.K. cross) was observed in Murasgige and Skoog (MS) medium supplemented with benzylaminopurine (BAP) 1.0 mg/l and Napthaleneacetic acid (NAA) 0.01 mg/l. When cotyledon was used as explant. These microshoots developed root on transfer to unsterile sand under ordinary greenhouse condition.

**Proceeding of Nationl Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal, pp. 309-312**

## 10. Plant tissue culture method of propagation and its potential

S. B. Rajbhandary

A brief description of tissue culture activities in the Herbarium and Botanical Laboratory, Godawary is discussed. Achievement with regard to development of fesible method of producing tissue culture plants are projected. As an example, the significance of using tissue culture seed potatoes in increasing

the yield by more than 100% is discussed. Need for improvement of agriculture, horticulture and forestry crops through the use of tissue culture technique is emphasized.

**Proceeding of Nationl Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal, pp. 315-317**

## 11. *In vitro* Anther culture of *Allium fistulosum* L.

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

Callus was initiated from anthers of *Allium fistulosum* L. in $N_6$ medium supplemented with 50 g/l of sucrose, 1 mg/l of 2,4-dichlophenoxyacetic acid (2,4-D) and 0.25 mg/l 0f 6-Furfuryl amino purine (Kinetin). The callus cultured on Murashige and Skoog (MS) medium plus 2% coconut milk formed green embryoids. The embryoids culture on MS supplemented with 5 mg/l Benzylaminopurine (BAP) formed shoots.

**Proceeding of Nationl Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal, pp. 318-321**

## 12. Meristem culture of *Cymbidium giganteum* Wall. ex Lindl.

M. Shrestha and S. B. Rajbhandary

Plant regeneration through meristem of *Cymbidium giganteum* Wall. ex Lindl. is describe. Shoot were initiate on Murashige and Skoog's medium (1962). MS supplemented with Benzyleamino purine (5 mg/l), Napthalene acetic acid (1 mg/l) and 10% coconut milk. The proliferation continued on transfer to pot containing tree fern fiber and survived in green house.

**Proceeding of Nationl Conference on Science and Technology April 24-29, 1988, Kathmandu, Nepal, pp. 345-349**

## 13. The *in vitro* proliferation of forest tree *Dalbergia sisoo* Roxb. ex DC

B. M. Suwal, A. Karki and S. B. Rajbhandary

Multiple shoots were induced on cotyledonary node culture of *Dalbergia sisoo* in the presence on Benzyleaminopurine (BAP) at 1 mg/l and napthaleneacetic acid (NAA) at 0.1mg/l. These shoots continued to proliferate at a sustained rate of 10-15 microshoots over two years of 8 weekly subculture in the basic medium, with the supplement of BAP at 0.25 mg/l. Such microshoots rooted readily in non-sterile sand beds with subsequent successful field establishment.

**Silva Genetica 37, 1 (1988) pp. 26-28**

## 14. *In vitro* propagation of *Dalbergia sisoo* Roxb. ex Dc

S. B. Rajbhandary

*In vitro* propagation from the cotyledonary node of *Dalbergia sisoo* seedlings has been successfully practiced. A field trail has shown that the resulting plants compare well with those raised by traditional methods.

**Banko Jankari Vo.2, No.1, Autumn 1988, pp. 31-33**

## 15. Micropropagtion of *Eucalyptus camaldulensis* A fast growing essential oil bearing tree

S. Gurung and S. B. Rajbhandary

In a trail plantation in Nepal, *Eucaluptus camaldulensis* exhibited heterozygosity, and individual trees with enhanced growth rate and higher essential oil content (2%) and cineole (86%) could be selected from a one year old plantation. The present study was carried out to develop micropropagation method that might find application in cloning of superior trees. Shoot tip and young leaves excised from six month old trees were used as explants. Shoot bud proliferation was achieved on a MS medium supplemented with 1000mg/l casein hydrolysate, 1.0 mg/l BAP and 0.1 mg/l NAA. A proliferation rate of 1 to 30 within every 10 weeks was maintained during repeated sub-culturing. Reduction of BAP concentration to 0.25 mg/l promoted shoots elongation. Elongated shoot (2-4 cm) readily rooted in non-sterile sand beds. Rooted plants developed to a height of 25 cm. in two months in polythene bags in the greenhouse. The *in vivo* rooting procedure used in the present study drastically reduced the cost of producing tissue culture trees, indicating thereby its possible commercial application.

**Tissue culture and Biotechnology of Medicinal and Aromatic Plants, 1989, CIMAP, Lucknow, India, pp. 17-21**

## 16. Propagation of Bamboo through tissue culture

R. Niroula and S. B. Rajbhandary

Callus from aseptically grown seedlings of *Dendrocalamus strictus* in Murashige and Skoog medium (MS) in the presence of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) at 5.0mg/l gave rise to plantlets when transferred to lower level of 2,4-D 0.5mg/l. The callus culture which had undergone over three years of regular subculture still exhibited plantlets formation in MS medium supplemented with 1.0 mg/l 6-Benzyleaminopurine (BAP) and 0.3 mg/l 2, 4-D. *In vitro* regenerated plants were field established.

**Seventh International congress of plant tissue and cell culture, Amsterdam Netherlands, 1990, pp. 120**

**17. Sand rooting of *in vitro* produced shoots of horticulture and forestry species**

S. B. Rajbhandary

Plant regeneration protocol from shoot tip and cotyledonary node culture via non-sterile rooting of the multiple shoots in the ordinary sand, a depart from the generally practiced *in vitro* rooting, is described. Field established plants via such procedure include horticulture crops (*Chrysanthemum* sp., *oxalis tuberose, poncirus trifoliate, solanum tuberrosum, pyrethrum, Ullucus tuberosus*) and forestry tree species (*Bischofia javanica, Dalbergia sissoo, Eucalyptus camaldulensis, Ficus auriculata, F.lacor, F. nemoralis and F. semicordata)*. A substantial reduction in production in production cost of tissue culture plants coupled with leser requirement of skilled labour following this non-sterile rooting procedure is projected to causes enormous increase in the commercial production of tissue culture plants and forestry tree species.

**18. Assessment of somaclonal variation in plants regeneration from petals of C*hrysanthemum morifolium* (Ramat. and Hemfl.)**

A. Karki

Plant regeneration and assessment of somaclonal variations were investigated. For the four genotypes of early charm varieties of C*hrysanthemum morifolium* were taken into consideration. Petal explants were cultured on MS medium (Murashige and skoog medium), 1962 supplemented with 10.0 mg/l kinetin and 2.0 mg/l 1-Naphthylacetic acid. Adventitious roots were initiated on MS medium containing 0.1 mg/l 3-Indoleacetic acid, after 15 days of transfer from the regeneration medium. Comparison between plants derived petal explants and parental were made for seven characters. Out of seven characters measured differences were observed in the number of petals and height of plants as compared to the parents.

**Seventh International Congress of plant Tissue and Cell culture, Amsterdam, Netherlands, 1990, pp. 157**

**19. Androgenesis in *Allium fistulosum* L.**

Hari K. Saiju, S. B. Rajbhandary and S. B. Malla

Anthers of Allium fistulosum were cultured on Chu (N6) medium supplemented with 2,4-Dichlorophenoxyaceticacid (2,4-D) and 6-Furfurylaminopurine (Kinetin) for the induction of callus. The callus subcultured on Murashige and Skoog medium (MS) with coconut milk formed green embroids. These embroids formed shoots in MS medium supplemented with 6-Benzyleaminopurine (BAP). These shoots formed roots in non-sterile sand.

**Seventh International Congress of Plant Tissue and Cell Culture, Amsterdam Netherland, 1990, pp. 190**

## 20. Plant propagation by tissue culture: A step to realise its potential

S. B. Rajbhandary

Although tissue culture means of Clonal propagation is visibly much faster than the traditional vegetative methods, its use in plant propagation industry is mainly confined to ornamental. The unrealized potential of tissue culture in propagation of other field crops and forestry trees is largely attributable to the methods currently used for producing tissue culture plants. The current practice of commercial production of tissue culture plants requires high labor and expensive controlled facilities for both (1) induction of culture flasks and (2) field establishment of plants when plants are moved from culture flasks to the outside environment, rendering the propagation by tissue culture for most crops uneconomica. A substantial reduction in the requirement of skilled labor and controlled facilities via sand rooting of multiple shoots of potatoes, *chrysanthemums*, a *citrus* rootstock, and some forest trees and moss rooting of banana shoots are discussed. The potential to extend the tissue culture technology from "Lab to Land" through training of nurserymen for establishing plants from multiple shoots of cultures is also discussed.

**International Congress on Genetic Engineering and Biotechnology April 15-20, 1991, (ICGEB), Nepal Biotechnology Association, pp. 10-11**

## 21. *In vitro* propagatuion of *Ficus lacor* Buch. Ham

N. Amatya and S. B. Rajbhandary

Shoot tip explants from standing trees were used for micropropagation of *Ficus lacor* Buch. Ham., a fodder tree. Shoot proliferation was obtained on Murashig and Skoog medium, supplemented with BAP at 1.0 mg/l, Kinetin at 1.5 mg/l, and 1.0 g/l casein hydrolysate. Shoot were multiplied by a factor of 20-30 (which remained unchanged) at each subculturing every 8-12 weeks for two years. The microshoots are rooted in sand in high humidity under a polythene cover. Rooted plants were readily established in soil, with no losses during the transfer from sand to soil.

**Banko Jankari Vol. 3, No.1 Summer 1991, pp. 17-20**

## 22. *In vitro* Androgenesis of *Allium fistulosum* L.

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

*Allum fistulosum* anther were cultivated on chu (N6) medium supplemented with 2, 4- dichlorophenoxyacetic acid and 6-furfurylaminopurine (Kinetin).

The callus developed from *in vitro* cultured anthers. The callus was then subcultured on Murashige and Skoog medium (MS) added with coconut milk for the formation of green embryo like structures. This green structure developed into shoots and leaves after subcultureing to MS medium supplemented with benzylaminopurine. These shoots fromed roots in non-sterile sand bed under high humidity.

**International Conference on Genetic Engeneering and Biotechnology April 15-20, 1991, (ICGEB), Nepal Biotcecnology Association, pp. 67**

## 23. Non sterile sand rooting of *Allium fistulosum* L. shoots obtained from anther culture

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

*Allium fistulosum* L. flowers were picked fresh from the green house grown plant. The flowers were washed with sterile distilled water. The anthers were removed aseptically and cultured on N6 medium (Chu 1978) supplimented with 1 mg/l 2, 4 – Dichlophenoxyaceticaacid (2, 4-D) and 6-Furfurylaminopurine (Kinetin) for the induction of callus. The callus was subcultured on Murashige and Skoog medium (MS) medium with 2% coconut milk for the formation of embryo-like structures. These green structures formed shoots in MS medium supplemented with Benzylaminopurine (BAP). These shoots were kept in non-sterile sand and covered with polythene sheet. After four weeks of subculture the anthers formed callus. These callus upon transfer to MS medium with coconut milk formed green embryo-like structure after eight weeks of incubation. These green structure formed shoots in MS medium supplemented with BAP in ten weeks. The shoots kept in non sterile sand formed roots after four weeks.

**Twenty third International Horticulture Congress, Florence, Italy, 1991**

## 24. *In vitro* culture of *Brassica oleracea* L. Var Capitata (K.K. Cross)

N. Pradhan and S. B. Rajbhandary

*Brasica oleracea* L. var. capitata is used as vegetable. The seeds were of F1 generation and were imported from Japan. Since tissue culture offers a possibility of obtaining a large number of Clonal plants, we have attempted of obtaining a large number of Clonal plants, we have attempted to clone the hybrid plants via organogenetic pathway. This paper deals with the formation of multiple shoots from hypocotyls culture with subsequent non sterile rooting in sand and field establishment.

Seeds were surface sterilized with 0.1 ppm mercuric chloride solution for 10 minutes and grown aseptically on MS medium (Murashige and Skoog, 1962). After 10 days of incubation hypocotyls explants were cultured on MS medium

containing different suppliments. After 10 to 12 weeks of explants culture, multiple shoot proliferation was obtained in MS medium supplemented with BAP 1.0 ppm and NAA 0.01 ppm. The number of shoots varied with the NAA concentration. In NAA 0.1 ppm and BAP 1.0 ppm the numbers of proliferated shoots varied from 10 to 15 whereas in NAA 0.01 ppm and 1.0 ppm it varied from 25 to 30.

Rooting was done by treating the excised microshoot pieces in 0.1 ppm indol acetic acid for 5 to 10 minutes. The treated shoots were rooted in non–sterile sand box. The sand boxes were covered with polythene sheets to maintain high humidity. Roots were visible in 10 to 12 days after transfer to sand. The rooted plants were successfully established in the field. Head formation was observed in more than 90 percent of the plants.So this method provides an excellent means of mass propagation of hybrid plants. The diret non-sterile rooting of multiple shoots apparently makes the production of tissue cultured plants cost effective.

**Cruciferae Newsletter, Nos. 14/15 June 1991, EUCARPIA**

## 25. Rooting of *in vitro* produced shoots in nonsterile sand, an inexpensive and efficient technique for enmasse micropropagation

S. B. Rajbhandary and Y. P. S. Bajaj

One of the reasons that limit the use of micropropagation, especially those of the forest tree species, is the high cost of production of *in vitro* plants (Chu 1989). In our protocol, multiple shoots from *in vitro* cultures are directly transferred to nonsterile sand beds to induce rooting, thus skipping the rooting of shoot in the culture medium. var 25 species including foret trees have been produced by this sand rooting technique. More than 10,000 trees regenerated by these procedures are undergoing field trials. It is believed that this technique can substantially reduce the cost of producing tissue cultured plants since *in vitro* rooting has been reported to account for 30-70% of micropropagation production costs. This non sterile sand method has the potential of enlarging the current world market of micropropagation of plants.

**Biotechnology in Agricuclture and Forestry, Vol. 17, High Tech and Micropropagation I (Ed. by Y.P.S.Bajaj), 1991, pp. 262-269**

## 26. *In vitro* propagation of *Citrus limon* L.

R. Niraula, S. Rajbahak and S. B. Rajbhandary

Multiple shoots were initiated on cotyledonary node culture of *Citrus limon* L. in Murashige and Skoog (MS)medium in the presence of Benzylaminopurine (BAP) 1 mg/l and Napthyl acetic acid (NAA) 0.1 mg/l

These shoots on subculture continued to proliferate in the same medium supplemented with lower concentration of BAP 0.5 mg/l and NAA 0.01 mg/l. The microshoots produced roots when transferred in non sterile sand and successfully established in the field.

**First National Botanical Conference, (August 11-12, 1992), Nepal Botanical Society, Kathmandy, Nepal**

## 27. Tissue culture of *Ficus elastic* Roxb.

M. Awal, N. Amatya and S. B. Rajbhandary

Shoot tip of different aged and different colored rubber plants have been used as explants. For the establishment and multiplication of shoot tips Murashige and Skoog medium supplemented with BAP 1 mg/l and Kinetin 1.5 mg/l and caseinhydrolysate 1 gm/l used. Microshoot of 8-10 weeks old was used for rooting in sand beds under polythene cover. Success of rooting was calculated 80 percent.

**First National Botanical Conference, (August 11-12, 1992), Nepal Botanical Society, Kathmandu, Nepal**

## 28. Micropropagation of *Ficus nemoralis* Wall.

N. Amatya and S. B. Rajbhandary

Induction of microshoots and field establishment of *Ficus nemoralis* has been described. Shoot tips of young plants about a height of 2ft. have been used as explants. Establishment of shoot tips and good induction of microshoots were found in Murashige and Skoog medium with BAP 1 mg/l, kinetin 1.5 mg/l and caseinhydrolisate 1gm/l. Microshoots were multiplied by factor of 20-25 at each subculture for rooting in sand beds under polythene cover. Sucrose of rooting was calculated 60 percentages.

**First National Botanical Conference, (August 11-12, 1992), Nepal Botanical Society, Kathmandu, Nepal**

## 29. Rooting of *in vitro* produced *Musa* cultivar willium hybrid plantlets on substrate moss (*Entodon* sp.)

A. Karki, P. S. Tuladhar and S. B. Rajbhandary

Shoot tips of 2mm size *Musa* cultivar William hybrid were culture on Murashige and Skoog medium supplemented with 5.0 mg/l Benzylaminopurine. The subcultured of the same concentration increased the number of shoots. The roots were initiated on Moss (*Entodon* sp.) after 6-8 days. The rooted plants were ready to transplant in soil within 4 weeks.

**Proceedings of First Botanical Conference, (August 11-12, 1992), Nepal Botanical Society, Kathmandu, Nepal. pp. 54-58**

## 30. Tissue culture of *Lilium longiflorum* for mass production

H. K. Saiju and S. B. Rajbhandary

*Lilium longiflorum* shoot tips were cultured in MS medium supplemented with NAA for the embryoid initiation. These embryoids were recultured in the medium supplemented with NAA and BAP for the formation of microshoots. The tips of microshoots were cut down and these shoot were subcultured in the shoot formation medium. The microshoots were transferred in the sand box for rooting. The establishment plants are growing in the field.

**Proceeding of First National Botanical Conference (August 11-12, 1992), Nepal Botanical Society, Kathmandu, Nepal, pp. 59-61**

## 31. Clonal propagation of forest tree *Ficus nerifolia* Sm. by tissue culture technique

N. Pradhan and S. B. Rajbhandary

Multiple shoot were regenerated from excised shoot tips (2-3 mm) of *Ficus nerifolia* Sm. (four years old plant) in MS medium supplemented with 1 mg/l BAP and 1.5 mg/l Kinetin. The microshoots were rooted in nonsterile sand and successfully established in the field.

**Proceeding of First National Botanical Conference (August 11-12, 1992). Nepal Botanical Society, Kathmandu, Nepal, pp. 67-70**

## 32. Regeneration of plants from leaf explant in orchid *Vanda teres* Lind

R. Niraula and S. B. Rajbhandary

Explants of *Vanda teres* Lind. were excised from aseptically grown seedling and cultured on Murashige and Skoog medium supplemented with auxine and cytokinin. Protocorm like bodies were developed from the explants. These prtocorm like bodies were transferred to a Vacin and Went and grew into complete plantlets.

**Role of Biotachnology in Agriculture 1992, Kathmandu, Nepal, pp. 103-107**

## 33. Generation of genetic variability by the *Chrysanthemum* tissue culture method

A. Karki

Plants regenerated through tissue culture were established for various explants sources such as leaf, pedicles, flowers, buds and petals. Plants from petal explants were phenotypically different when coupled with the parental plants. Upon analysis, these regenerated plants differed in relation to the height of the plant and flower morphology. So, it was found possible to regenerate genetic variability in *Chrysanthemum*.

**Role of Biotechnology in Agriculture 1992, Kathmandu, Nepal, pp. 109-114**

## 34. *In vitro* propagation of *Ficus nerifolium* Sm. through cotyledonary node culture

N. Pradhan

Shoot proliferation from cotyledonary node culture of *Ficus nerifolia* Sm. (Dudhilo) on Murashige and Skoog (MS) medium supplimentd with benzyle aminopurine (BAP) 1.0 mg/l, Kinetin (KN) 1.5 mg/l and adenine 10%. The microshoot produced roots when transferred to non-sterile sand and were successfully established in the field.

**Proceeding of the Regional Seminar on Fodder Trees, Forest Fodder and Leaf Litter, 27-28 December, 1992, Heatauda, pp. 16-20**

## 35. *In vitro* propagation of *Artocarpus lakoocha* Wall. ex Roxb

M. Kayastha

The multiple shoots of *Artocarpus lakoocha* (Badahar) were grown in Murashige and Skoog (MS) medium supplemented with 1.0mg/l Benzyleamino purine (BAP, 1.5 mg/l Kinetin (KN) and 100 ml/l coconut milk, when cotyledonary node was used as explant. The microshoots produced root when transferred to non sterile sand medium.

**Proceeding of the Regional Seminar on Fodder Trees, Forest Fodder and Leaf Litter, 27-28 December, 1992, Heatauda, pp. 30-34**

## 36. Clonal propagation of Forest Trees *Eucalyptus camaldulensis* L. from mature trees by tissue culture technique

N. Pradhan

Multiple shoots were initaiated from axiallary and terminal buds of 8 year old tree of *Eucalyptus camaldulensis* on Murashige and Skoog medium supplemented with Benzyle aminopurine (BSP) 1.0mg/l and Napththyle acetic acid (NAA) 0.1 mg/l. These shoots on subculture continued to proliferate in the same medium supplementd with lower concentration of BAP 0.25mg/l and coconut milk 10%.

The microshoots produced roots when transferred in non sterile sand. About ten thousand plants produced from sand rooting were distributed to different sectors of government office and to private parties for field establishment.

**First Regional Conference of Prospct of Biotechnolgy in Nepal. April11-13\1993, Birgunj, Nepal; Biotechnology and Biodiversity Society of Nepal, pp. 11**

## 37. Meristem culture of *Cymbidium grandiflorum* Griffith

M. Shrestha and S. B. Rajbhandary

Plants regenerated through meristem of *Cymbidium grandifolium* are described.

It was carried out on Murashige and Skoog medium (MS) supplemented with Benzyleaminopurine (2.2 mg/l), Napthelene acetic acid (1.8mg/l) and 10% coconut milk. The protocorms were subcultured on the same medium. Shoot developed from protocorm rooted in the MS basal medium without growth hormones and coconut milk. The plants on transfer to pot containing tree fern fiber survived in the green house.

**First Regional Conference of Prospct of Biotechnolgy in Nepal, April 11-13/1993, Birgunj, Nepal; Biotechnology and Biodiversity Society of Nepal, pp-11**

**38. Peroxidase isoenzyme study and statistical analysis of number of leaves, its length and number of flowers in anther derived plants and mother plants of *Allium fistulosum***

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

*In vitro* anther culture of *Allium fistulosum* L. in N6. Medium supplemented with kinetin and 2, 4-D produced diploid plants. These anther derived plants and mother plants were compared by the study of peroxidase-isoenzymes. Difference in band numbers observed in anther derived plants and mother plants.

Statistical analysis of number of leaves per plant, its length and numbers in anther derived plants and mother plants were carried out. The computed means were tested by Student's T-test. The test showed that the means were significant at both 5% and 1% levels for number of leaves and flowers. The means of length of leaves were significant at 5% level only.

The difference in peroxidase-isoenzyme and computed statistical data indicated that anther rived plants and mother plants have different genetic structure in these two groups of diploid plants. The anther derived plants have more uniform and homogenous data than in mother plant. The uniformity and homogeneity in character in anther derived plants indicated that they are diploid. The diplodization process might have occurred during the callus phase of androgenesis.

**Fifteenth International Botanical Congress, Tokyo, Japan, August 28-September 3, 1993, pp. 537**

**39. *In vitro* culture of shoot tips from mature trees of *Ficus carica* Linn.for mass production**

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

The shoot tips from mature tree of *Ficus carica* were *in vitro* cultured in MS medium supplemented with NAA (0.01mg/l) and 10% coconut milk (CM). The established shoot tips were subcultured in MS medium supplemented

with BAP (0.5mg/l) and 10% CM. Six microshoots developed from each shoot tip after fifth subculture. These microshoots developed roots in non sterile sand within two weeks. In this way production of mature trees has been possible for field plantation.

**Fifteenth International Botanical Congress, Tokyo, Japan, August 28-September 3, 1993, pp. 539**

## 40. Rooting of *in vitro* produced ginger (*Zingiber officinale*) Rosc. plantlets on substrate moss (*Entodon* sp.)

A. Karki

*In vitro* culture of ginger (*Zingeber officinale*) was achieved by using buds (2 mm) size from rhizome on Murashige and Skoog medium (1962) supplemented with 1.0 mg/l 6-benzylaminopurine and 1.5 mg/l kinetin. The subcultured of *in vitro* shoot in fresh Murashige and Skoog medium of the same concentration increased the number of shoot. The roots were initiated on Moss (*Entodon* sp.) After 10-15 days. Rooted plants were successfully transferrd to soil in the green house.

**National conference of Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 13**

## 41. Anther culture of Rice (*Oryza sati*va L. var. Taichung 176)

H. K. Saiju, S.B. Rajbhandary and S. B. Malla

The young panicles of *Oriza sativa* var. Taichung 176 were refrigerated. After 17 days of cold treatment the panicles were dipped in sterilized water. Then the anthers were cultured in Gamborg medium supplemented with BAP and NAA (0.5mg/l) and 2, 4-D (1 mg/l). The anthers developed callus after 32 days. The callus was subcultured in Gamborg medium supplemented with NAA and Kinetin (1 mg/l). The callus formed green embryonic structure which developed green leafly shoots and roots.

**National Conference of Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 13**

## 42. Micropropagation of *Dianthus caryophyllus* L. (Carnation)

M. Awal, M. Shrestha and S. B. Rajbhandary

Multiple shoots were obtained from shoot tip culture of carnation(*Dianthus caryophullus*) on a define Murashige and Skoog medium supplimented with Benzylaminopurine (BAP) 1.0mg/l and Indole 3 Acetic acid (IAA) 0.01 mg/l. Cultured resumed growth after 6-weeks storage. Multiple shoots were formed with in 6-8 weeks. After repeated subculture on the fresh

medium, it produced more than 50-60 healthly shoots from a single shoot tip. Microshoots of 12-16 weeks seperated and used for rooting in sand box compared to the normal flowers.

**National Conference of Biotechnology, April 29-30, 1993I, Nepal Biotechnology Association, pp. 25**

## 43. Clonal propagation of *Dendrobium densiflorum* Linn. through shoot meristem culture

M. Shrestha and S. B. Rajbhandary

Clonal propagation of *Dendrobium densiflorum* Lindl. using shoot tip was successfully achieved. Protocorms were initiated from shoot tip explant in Murashige and Skoog's medium (MS) supplemented with 15% coconut milk, Benzylaminopurine (BAP) 2.5 mg/l, Napthalene acetic acid (NAA) 1 mg/l and Caseinhydrolysate 1g/l. These protocorms were cultured in MS medium with BAP 0.25 mg/l, NAA 0.01 mg/l, Adenine Sulphate 20 mg/l and coconut milk 10%.These shoots were transfered to Vacin and Went's (VW) control medium for rooting. The rooted shoots were transferred to community plots containing tree fibers and established well in the green house.

**National Confrence on Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association. pp. 25.**

## 44. *In vitro* propagation of *Artocarpus heterophyllus* Lam.

M. Kayastha

The multiple shoots of Artocarpus heterophyllus Lam. were initiated in the MS medium supplemented with 1.0 mg/l BAP and 0.01 mg/l NAA when cotyledonary node was used as explant. The roots were produced when the microshoots were transferred in non sterile sand.

**National Confrence on Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 26**

## 45. Sand rooting of *Citrus limon* L. shoots propagation through tissue culture

R. Niroula and S. Rajbahak

Cotyledonary node of *Citrus limon* L. when cultured in Murashige and Skoog medium in the presence of Benzylaminopurine (BAP) 1.0mg/l and Napthalene acetic acid (NAA)0.1 mg/l initiated shoots. These shoots proliferated on subculture in the same medium supplemented with lower concentration of BAP 0.5 mg/l and NAA 0.01 mg/l. The microshoots produced roots in non sterile sand and the rooted plantlets established successfully in the field.

**National Conference of Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 26**

### 46. Tissue culture of *Ficus semicordata* Buch.-Ham. ex Sm. for mass production

N. Amatya and S. B. Rajbhandary

Cotyledonary nodes developed at aseptic condition have been used as explants established and their good induction was found in Murashige and Skoog's medium with BAP 0.5 mg/l and caseinacidhydrolysate 1 g/l. Frequency of multiuplication rate was found to be from 15-20 at each subculture. Microshoots of 8-10 weeks old were used for rooting in non-sterile sand under humid condition. The rooted plants were ready to transplant in soil with in 4 weeks.

**National Conference of Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 27**

### 47. Clonal propagation of forest tree *Eucalyptus citriodora* Hook. F. from mature tree by tissue culture

N. Pradhan

Few shoots were induced fron nodal segments of 12 year old trees of Eucalyptus citriodora Hook F. on Murashige and Skoog's basal medium supplemented with BAP 1.0 mg/l and coconut water 10%. These shoots were subcultured in basal medium supplemented with lower concentration of BAP 0.25mg/l andcoconut water 10% for proliferation. The microshoots produced root on transfer to non-sterile sand.

**National Conference of Biotechnology, (April 29-30, 1993), Nepal Biotechnology Association, pp. 27**

### 48. Effect of growth regulatora on *in vitro* propagation of *Citrus limon* Burm.f., *Citrus sinensis*, (L.) *Fortunella* sp. and *Poncirus trifoliate*. (L.)

R. Niroula

Tissue culture has proved to be a useful method for Citrus cro*p* improvement and propagation. Cotyledonary tissues of *Citrus limin, C. sinensis, Fortunela* sp. and *Poncirus trifoliate* were cultured on Murashige and Skoog's (1962) medium (MS) with benzyleaminopurine (BAP) 1.0 mg/l and Napthalene acetic acid (NAA) 0.1 mg/l. The salt mixture of MS medium has proved satisfactory and the requirement of growth regulating substances for the initial stage was common, although the combination of growth regulator varies in different genera with the system and mode of shoot multiplication.

**National workshop on Plant Growth Regulators (PGRs) and Biofertilizers (BFs) Kathmandu, Nepal, May 24-26, 1994**

## 49. Tissue culture of Sugarcane (*Saccharum* L. sp.) for mass production

P. S. Tuladhar and S. B. Rajbhandary

Meristem and meristimatic tissue of any parts of sugarcane were excised under aseptic condition and cultured on MS medium (Murashige and Skoog 1962) supplemented with 2.0mg/l 2, 4-D and 15% coconut milk for callus initiation. These calli were recultured in the medium supplemented with 15 mg/l kinetin and 2.0mg/l IBA for the formation of plantlets (Shoot). The plantlets were ready to transplant in nonsterile sand for rooting. The rooted plantlets were transplanted in soil within 4-6 weeks.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-2**

## 50. *In vitro* culture of *Gladiolus*. psittacinus Hook.f.

K. M. Rajkarnikar and S. B. Rajbhandary

The paper describe the initiation of shoot buds from axillary corm buds of *Gladiolus* in Murashige and Skoog medium (1962) supplimented with 1.0mg/l of BAP (Benzyleaminopurine) and 0.02mg/l of NAA (Napthaleneacitic acid). The initiated buds produced micro-shoots when cultured in MS medium supplemented with 1.0mg/l of Kinetin. The micro-shoots were rooted in non-sterile sand. The rooted shoots produced cormlets in soil with in 2 to 3 months.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-2**

## 51. Clonal multiplication of *Cymbidium longiflorum* D. Don by shoot tip culture

M. Shrestha and S. B. Rajbhandary

Clinal multiplication of *Cymbidium longiflorum* D.Don was achieved with shoot apical meristems culture *in vitro*. Protocorms like bodies were initiated within six weeks after cultureing on Murashioge and Skoog medium (1962) supplemented with Benzylaminopureine (2mg/l), 1.0mg/l Napthalenescetic acid, 10% coconut milk and 3% sucrose. Rapid multiplication of shoots occurred in MS medium containing 1.0mg/l BAP, 1.5mg/l kinetine and 10mg/l Adenine sulfate. The proliferation continued on subculturing on same medium. Rooting occurred oin MS basal medium without growth hormones and coconut milk. Establishment of the mericlone plants in community pots was 80% successful.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-21**

## 52. *In vitro* propagation of *Rose* sp. for mass production

M. Awal and S. B. Rajbhandary

The shoot tips (2-3mm) of rose were cultured in Murashige and Skoog medium supplemented with benzylaminopurine (BAP) 1.0mg/l and Napthaleneacetic acid (NAA) 0.01mg/l. The microshoots were recultured in the medium supplemented with BAP (1.0mg/l), Kinetin (1.5mg/l) and coconut milk (10%). The microshoots were transferred in non-sterile sand for rooting and successfully established in the field.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-45**

## 53. Rapid clonal multiplication from mature tree of *Populus ciliate* Wall. ex. Royal. through tissue culture

A. Karki and S. B. Rajbhandary

Shoot tips (2-4 mm) were taken from the sprouts of mature tree of *Populous ciliate*. The shoots were cultured in MS liquid medium supplemented with BAP 5.0 mg/l and NAA 0.1 mg/l, over a filter paper bridge in order to avoid browning of shoot explant. After 4-6 weeks green shoot were transferred in solid medium, supplemented with BAP 0.5 mg/l, NAA 0.2 mg/l, adenine sulfate 20mg/l and 10% coconut milk. On average 20-25 multiple shoots were observed within 10-12 weeks. These shoots were sub-cultured again in the same medium in every 4-6 weeks interval. These microshoots were excised from the flask and rooted in sand beds. After 15-20 days roots were developed in sand which were then transferred in the pots.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-47**

## 54. *In vitro* propagation of *Eucalyptus tereticornis* Sm. from mature tree

N. Pradhan

Few shoots were initiated from the nodal segments of eight year old mature tree of *E.tereticornis* on Murashige and Skoog medium supplemented with BAP 1.0 mg/l, kinetin 0.1 mg/l and coconut milk 10%. These shoots in turn proliferate in mass when subcultured on MS medium supplemented with low concentration of BAP 0.25 mg/l and coconut milk 10%. Sand medium was used for rooting and rooted plants were transferred to field for establishment.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-48**

## 55. *In vitro* propagation of *Citrus sinensis* (L.) (Junar)

R. Niroula

The shoots were regenerated from the cotyledonary node of *Citrus sinensis* when cultured in Murashige and Skoog medium in the presence of Benzylaminopurine (BAP) 1.0 mg/l and Napthaleneacetic acid (NAA) 0.1mg/l. The shoots were increased in number when the microshoots were subcultured in the same medium supplemented with lower concentration of BAP and NAA and addition of coconut milk. The microshoots produced roots when transferred in sand and the rooted plants established in the field.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-48**

## 56. Tissue culture of *Lilium nepalensis*

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

Young nodal sections of *Lilium nepalensis* were *in vitro* cultured in Murashige and Skoog medium supplemented with Napthaleneacetic acid (NAA). The microshoots developed from nodal sections were subcultured in NAA, Benzylaminopurine (BAP), coconut milk and casein hydrolysate for induction of multiple shoots. The microshoots rooted in non sterile sand were established in the field.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-53**

## 57. *Nicotiana tabacum* L. plant from anther culture

H. K. Saiju, S. B. Rajbhandary and S. B. Malla

Nicotiana tabacum L. anthers were cultured in Murashige and Skoog (MS) medium supplemented with napthaleneacetic acid (NAA) and benzyleaminopurine (BAP). Some anther formed shoots directly whereas other callus only. The callus formed shoots after transferred to MS medium supplemented with BAP. The microshoots were transplanted to non-sterile sand for the formation of roots. The rooted plants were transferred to field. The plants derived directly from anthers were haploid whereas callus derived plants were diploid.

**Second National Conference on Science and Technology, (June 8-11, 1994), RONAST, Kathmandu, Nepal, BIO-54**

## 58. *In vitro* propagation of *Gloxinia* sp. by leaves culture

S. Rajbahak

The leaf explant is used for initial material for the propagation of *Gloxinia* sp.

Leaves were surface sterilized with 0.1% mercuric chloride for 5 minutes and rinsed with sterilized water for few time and inoculated aseptically on Murashige and Skoog medium supplemented with 1.0 mg/l Benzyleaminopurine 0.01 mg/l Napthemlene acetic acid, 100 mg/l adenine sulphate. The embroids were initiated from leaf explant and subcultured in the same medium for multiplication. 90% of rooted plants were observed within 7-10 days after transplanting the microshoots in the non sterile sand. These plants were successfully established in the field.

**Third National Conference on Science and Technology, organized by Nepal Academy of Science and Technology, March 8 -11, 1999, Kathmandu, pp. 66**

## 59. *In vivo* rooting of *in vitro* produced microshoots of *Lilium longiflorum*

H. K. Saiju, S. B. Rajbhandary, S. B. Malla

*Lilium longiflorum* young nodal cutting were *in vitro* culture in MS medium supplemented with 0.1 mg/l NAA, for embroieds formation. These embroids developed microshoots in the same medium added with 0.01 mg/l NAA, 0.5mg/l BAP, 1.0 gm/l casein hydrolysate and 10% coconut milk. They were further subcultured in MS medium for multiple microshoots formation

These microshoots were acclimatized for ten days in green house condition and planted in sand in a box. It was covered with polythene hood to maintained 80% humidity and 25°C temperature.The roots developed in two weeks. They were kept in the same box for two more weeks to developed more roots. Then these plants were transferred to soil. They produced normal flowers. *In vivo* rooting of *in vitro* produced microshoots has eased the micropropagation of plants technically and reduced the production cost of elite clone plants.

**Seventh Internation Congree of Plant Tissue and Cell Culture, Firenze, Italy, June 12-17, 1994, pp. 37**

## 60. Propagation of Bamboo-*Dendrocalamus hamiltonii*

R. Niroula

Multiple shoots were obtained from the seeds of *Dendrocalamus hamiltonii* which were cultured in Murashige and Skoog medium supplemented with BAP 5 mg/l. The further proliferation of the shoots occurred when these shoots were sub-cultured in the same medium with lower concentration of BAP and coconut milk. The micro shoot rooted in non sterile sand and the rooted plantlets established in the field.

**Seventh Internation Congree of Plant Tissue and Cell Culture, Firenze, Italy, June 12-17, 1994. pp. 39**

## 61. Anther culture of *Allium fistulosum* L.

H. K. Saiju, S. B. Rajbhandary, S. B. Malla

Anther of *Allium fistulosum* were culture on N6 medium supplemented with 2,4-Dichlorophenoacetic acid (2,4-D) and 6-furfuryl amino purine (Kinetin).The callus developed from cultured anthers. The callus was then subcultured on Murashige and Skoog's (MS) medium with coconut milk for the formation of green embryo-like structure. These developed shoots after transfer to MS medium supplemented with Benzylaminopurine (BAP) formed roots after transferring to non sterile sand.

**Phytomorphology, 44(1and2): 139-142, 1994**

## 62. Micropropagation of Mulberry (*Morus alba* L.) for large scale production

H. K. Saiju, S. B. Rajbhandary, S. B. Malla

The shoot tips from mature mulberry (*Morus alba* L.) were washed with teepol in running tap water. Then these tips were srerilized in HgCl2 (0.1%) for 5 minutes and washed thoroughly in distilled water. The outer covering of the tips were removed and the innermost shoot tip was cultured in Murashige and Skoog's (MS) medium with 0.01 mg/l NAA and 0.25 mg/l BAP for four weeks. The established shoot tips were subcultured in the same medium with 0.5 mg/l BAP for six weeks. Subculturing of microshoots was done every six weeks. Ten microshoots developed in sixth subcultuere. These microshoots developed roots in non-sterile sand with two weeks. In this way large scale production of mulberry has been possible.

**Proceeding of Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 149-153**

## 63. Mass propagation of *Gerbera* Bolus. plants through tissue culture

R. Niroula

The shoot tips of *Gerbera* plants cultured on Murashige and Skoog's (1962) medium (MS) containing organic constituents of MS, BAP 1.0 mg/l and NAA 0.1 mg/l. After eight weeks of culture 204 shoots developed from the explants. Shoots of 1-2 cm in length were subcultured in the same medium except the NAA was reduced to 0.01 mg/l, 10% coconut milk and 4% sucrose. After fourth subculture 30-40 shoots developed. Shoots which were 3 cm or more length were transplanted in sand bed. Roots developed in about two to three weeks. 80% of roots were found and the rooted plants survived easily.

**Proceeding of Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 154-157**

## 64. Large scale propagation of Strawberry through Tissue culture

A. Karki and S. B. Rajbhandary

Test tube containing *in vitro* plants of strawberry was received from Japan. *In vitro* explants were cultured in Murashige and Skoog's medium (1962) supplemented with 6-Benzylaminopurine 1.0 mg/l and Kinetin 1.5 mg/l with 10% coconut milk. 60-80 multiple shoots were formed after 10-12 weeks. These shoots were subcultured every 4 weeks interval in same medium. Rooting of microshoots was observed after 8-10 days in sand. Rooted plantlets were established in the filed.

**Proceeding of Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 158-161**

## 65. *In vitro* propagation of *Saintpaulia ionantha* Wendl.

M. Kayastha, M. Shrestha, S. Rajbahak and S. B. Rajbhandary

The present paper deals with the initiation of embroids of *Saintpaulia ionantha* Wendl. from, leaf,petiole and pedicel section of mature plants on Murashige and Skoog medium supplemented with 0.5 mg/l Benzyleaminopurine 0.01 mg/l Napthemlene acetic acid,100 mg/l adenine sulphate and 10% coconut milk. The embroids were subcultured in the same medium for multiplication. 90% of rooted plants were observed within 7-10 days after transplanting the microshoots in the non sterile sand. These plants were successfully established in the field.

**Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 32**

## 66. Tissue culture of *Dendrobium fimbriatum* Hook. for mass production

K. M. Rajkarnikar and R. Niroula

Protocorms of *Dendrobium fimbriatum* Hook. were initiated from shoot tip explant in the Murashige and Skoog's medium (1952) supplemented with 5 mg/l of BAP (Benzylaminopurine), 1 mg/l of NAA (Napthaleneacetic acid) and 10% coconut milk.Protocorms were subcultured in MS medium with 1.0 mg/l of BAP,1 mg/l of NAA and 10% coconut milk for multiple shoot production. Roots were produced in shoos when transferred on MS medium with 0.5 mg/l of NAA.

**Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 34**

## 67. *In vitro* germination of orchids

M. Shrestha and S. B. Rajbhandary

The native and exotic orchids have been successfully propagated *in vitro* from

seed. These are total 36 species of native as well as exotic orchids that have been germinated in different culture media namely Burgef f, Knodson's c, Vacin and Went, Thompson, Murashige and Skoog and N3f. The establishment of orchids in community pot in the green house has been successful.

**Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 35**

### 68. Clonal propagation of *Chrysanthemum morifolium* (Ramat. and Hemfl.) through tissue culture

U. Kharel and A. Karki

Shoot tips (2-4 mm) of *Chryanthemum morifolium* varieties "Giant fishtail violet" were cultured on Murashige and Skoog (MS) medium supplemented with Benzyleaminopurine BAP 1.0 mg/l and Napthaleneacetic acid NAA 0.01 mg/l. Microshoots were successfully rooted in sand and grown into normal plants. The plants showed no abnormalities in flower morphology and colour.

**Second National Botanical Conference, December 23, 1994, Kathmandu, Nepal, pp. 35-36**

### 69. Rooting of micropropagation of *Artocarpus heterophyllus* Lam. on non-sterile sand as a potentially cost-effective means of mass propagation

M. Kayastha

Cotyledonary nodes of *Artocarpus heterophyllus* Lam. when cultured in Murashige and Skoog (MS) supplemented with 5.0 mg/l Benzyleaminopurine (BAP) initiated shoots. These shoots were subcultured in the same medium supplemented with 1.0 mg/l BAP and 0.01 mg/l naphthalene acetic acid (NAA) for multiplication.The roots initiated on non sterile sand after five weeks but did not survive well on transplantation to the field.

**Banko Jankari, Vol. 5, No. 1, March 1995**

### 70. Tissue culture of *Ficus carica* L. and rooting of microshoots in sand

H. K. Saiju, S. B. Malla and S. B. Rajbhandary

Tissue culture of a mature fig tree (Ficus carica L.) has been successfully carried out. The shoot tips (explants) from mature trees were removed and washed in running tap water for one hour. These shoot tips were sterilized with 0.1% mercuric chloride solution for five minutes and washed thoroughly in distilled water. The innermost shoot tip was removed and cultured in Murashige and Skoog (MS) medium supplemented with 0.01 mg/l with pH 5.8 before autoclaving. It was incubated at 25 C and 3 lilolux light supplied by florescent tubes (Philips) for sixteen hours/ day. The established shoot tips were kept in

the same medium for 6 more weeks. It was subcultured in MS medium supplemented with 0.5 mg/l 6-Benzyleaminopurine (BAP) and 10% coconut milk at an interval of every eight weeks. Six to eight multiple microshoots devlop after the fifth subculture.

The flasks with microshoots were kept in room temperature for two weeks before rooting for acclimatization, hardening and change to autotrophic mode of nutrition.

Rooting of these microshoots was done in non sterile sand. Sand was cleaned thoroughly by washing several times in water and sun dried for two days. The sand was wetted evenly with 10% water in a box. The microshoots were cut into one inch long pieces and dipped in 1 mg/l NAA for five minutes. They were planted in sand and covered with polythene sheet. The box temperature was mainted at 30°C, 80% humidity and 8-25 kilolux sun –light intensity. The roots developed with in 3 weeks period. They were left in the same box for two more weeks to develop healthly roots. In this way micropropagation of fig tree has been possible for field plantation.

**IUFRO Twentith World Congress, 6-12 August 1995. Tampere, Finland, pp. 59-60, Poster 99**

## 71. Rooting of *Populus ciliata* explants on non-sterile sand as a potentially cost effect means of mass propagation

A. Karki

Shoot-tip explants from the cutting of mature *Populus ciliate* were cultured over a filter paper bridge in Murashige and Skoog (MS) liquid medium and supplemented with 5mg/l Benzylaminopurine (BAP) and 1 mg/l Napthelene acetic acid (NAA). This avoids browning of explants. The latter were subcultured in MS solid medium with 0.5/l BAP and 0.2mg/l NAA and 10% coconut milk. After 10 to 12 weeks the shoots were rooted in non sterile sand. This obviates the need for transplantation in the laboratory and the associated costs that would be incurred.

**Banko Janakari, Vol.5, No. 3, September 1995, pp. 138-139**

## 72. Pilot scale production of Nepal cardamon (*Amomum subulatum* Roxb. cultivar Dambersay) through tissue culture

A. Karki and H. K. Saiju

Nepal Cardamom (*Amomum subulatum* Roxb. cultivar Dambersay) is a popular cash crop in Nepal. The multiple shoots were regenerated from this cultivar when young shoots tips were used as explants for tissue culture in Murashige and skoog's medium supplemented with 6-Benzyleaminopurine

(BAP) 1.0mg/l and Naphthalene acetic acid (NAA) 0.1mg/l. In the moss 90% micro shoots developed healthy roots. The plants were successfully established in the field.

**Bulletin of Plant Resources No. 22, July 2003, pp. 5**

## 73. Micro propagation of *Dendrobium fimbriatum* Hook.

K. M. Rajkarnikar

*Dendrobium fimbriatum* belongs to family Orchidaceae. It occurs as an epiphytic herb from tropical to temperate region. Protocorms of *Dendrobium fimbriatum* were initiated from shoot tip explant in the MS (Murashige and skoog's, 1962) medium fortified with 5mg/l BAP (Benzylaminopurine), 1mg/l NAA (Napththalene acetic acid) and 10% coconut milk. Protocorms were subcultured in MS medium with 1mg/l BAP, 1mg/l NAA and 10% coconut milk for multiple shoots and protocorms production.

**Bulletin of Plant Resources No. 22, July 2003, pp. 8**

## 74. Micropropagation of Cactus, *Mammillaria carnea* Zucc. ex Pfeiff.

K. M. Rajkarnikar, G. D. Bhatt

The conventional propagation method of cacti are slow and inadequate. In this present paper the micropropagation of cactus; *M. carnea* is attempted for clonal multiplication. The microbulbs were initiated in MS medium supplemented with 1mg/l BAP and 0.1 mg/l NAA. The new microbulbs were initiated from areoles portion of explants. These microbulbs were transferred to non sterile sand and MS medium with 0.5mg/l NAA for rooting. The microbulbs produced roots after 15 to 20 days in sand and in medium; it takes 20 to 25 days after transfer.

**Bulletin of Plant Resources No. 26, July 2005, pp. 1**

## 75. *In vitro* multiplication of *Vanilla planifolia* Andrews using axillary bud explants

S. Rajbahak, A. Karki and H. K. Saiju

*Vanilla*, a climbing, branching and terrestrial orchid is being tested for its Clonal propagation through tissue culture. The axillary buds (2-3 cm in length) were collected from mother plants. The buds were washed in running tap water with few drops of liquid detergent (Teepol) for half an hour. Then the axillary buds were sterilized with 0.1% mercuric chloride solution for 8 to 10 minutes and rinsed with sterilized distilled water for four times. The tip of the axillary bud about 0.5-1.0 cm long were cut off aseptically and cultured in MS medium (Murashige and Skoog medium 1962) supplemented with

1.0 mg/l Benzyl amino purine, 1.5mg/l Kinetin along with 10% coconut water. The culture flasks were kept in incubation room under the fluorescent light with 3000 lux and 25±2°C room temperature. Two to three new shoots were developed after 4-6 weeks of culture. The micro shoots were sub cultured in every 6-8 weeks in the same concentration of growth hormone along with coconut water. The mature shoots were transferred in cocopit or moss for rooting.

**Bulletin of Plant Resources No. 26, July 2005, pp. 3**

## 76. Micropropagation of *Neopicrorhiza scrophulariifolia* (Pennell) Hong.

K. M. Rajkarnikar and G. D. Bhatt

A protocol for the *in-vitro* multiplication of *Neopicrorhiza scrophulariifolia* was developed by using shoot tip explants. The regeneration of microshoots from explant was found to be best in 0.8% agar solidified MS medium (Murashige and Skoog's, 1962) supplemented with 0.5 mg/l BAP, 0.01 mg/l NAA and 10% coconut water. The microshoots regenerated roots on MS medium with 0.5mg/l or with 0.1mg/l NAA. The experiment is still in investigation for successful establishment of plantlets in the field.

**Bulletin of Plant Resources No. 27, June 2006, pp. 9**

## 77. Micropropagation of *Bergenia ciliate* (Haw.) Sternb. through leaf culture

A. Karki and S. Rajbahak

The leaf explants of *Bergenia ciliata* (Haw.) Sternb. were cultured in Murashige and Skoog medium on filter paper bridge supplemented with BAP (1.0 mg/l) and NAA 0.1mg/l. After 8-12 weeks of culture the leaf explants were transferred from liquid to MS solid medium supplemented with BAP 0.5 mg/l and NAA 0.1 mg/l addition of Adenine sulphate 100 mg/l.The shoot proliferation was observed after 6-8 weeks.The micro shoot were regularly subcultured in the same medium at intervals of 6 weeks for maintenance. The micro shoots were rooted in sand. After rooting micro shoots they were transferred in polybags.

**Bulletin of Plant Resources No. 29, July 2007, pp. 35**

## 78. Micropropagation of *Rhyncostylis retusa* Bl. from seeds

K. M. Rajkarnikar

The seeds of *Rhyncostylis retusa* Bl. were cultured on Murashige and Skoog's medium (MS medium, 1962) without growth hormone and supplemented with 1mg/l casein hydrolysate, 30gm/l sucrose and 8gm/l agar. The Protocorms like bodies (Plbs) were obtained from 6-8 weeks old culture. The

Plbs were again subcultured on hormone free MS medium for further growth. Some of Plbs were subcultured on Ms medium supplemented with 1mg/l Benzyl amino purine, 1.5 mg/l Kinetin and 10% coconut milk for further multiplication of plbs and seedlings. The seedlings were transferred in moss for their establishment, collected from Godawary surroundings.

**Bulletin of Plant Resources No. 29, July 2007, pp. 38**

## 79. *In vitro* culture of *Piper longum* Linn.

K. M. Rajkarnikar and G. D. Bhatt

*Piper longum* belong to family piperaceae. It is creeping aromatic herb. It is important plant in aurvedic medicine. Knowing its importance, *in vitro* clonal propagation of *Piper longum* is attempted for rapid multiplication using shoot tip explants. The explants regenerated micro shoots along with few callus and embryoids on the MS medium supplemented with 1mg/l BAP and 0.1mg/l NAA. The NAA concentration of the same medium was lowered to 0.01 mg/l and added 10% coconut milk for subculture. For rooting, these regenerated microshoots were transferred to non sterile sand, after acclimatization. They initiated roots within 15 to 20 days and transferred to polybags for further growth and for field transfer.

**Bulletin of Plant Resources No. 29, June 2008 pp. 76**

## 80. Micropropagation of *Spathiphyllum wallisii* Hort.

K. M. Rajkarnikar and G. D. Bhatt

*Spathiphyllum wallisii* Hort. is a beautiful indoor plant because of its glossy pleasant foliage and its white arum and nodes were used as explants. After sterilization, aseptically these explants were cultured to 0.8% agar gelled Murashige and Skoog 1962 medium supplemented with different concentration of Benzyl Amino purine and Napththalene acetic Acid. The pH of the medium was adjusted to 5.8 before autoclave. Among these concentrations, the medium supplemented with 1mg/l BAP and 0.1mg/l NAA was found to be the best hormone combination for multiplication of microshoots. After acclimatization, the multiplied microshoots were transferred in non sterile sands for induction of roots in green house. About 90% of microshoots were rooted in non sterile sand within 15-20 days. These plantlets were grew well and flourished normally.

**Bulletin Plant Resources No. 29, April 2009, pp. 93**

## 81. Clonal propagation of *Stevia rebaudiana* Bertoni through shoot tips culture and antibacterial assay of its leaf extracts

A. Karki, S. Rajbahak and S. K. Sah

*Stevia* is a genus of about 240 species of herbs and shrubs in the sunflower family Asteraceae. Shoot primodia, which were able to propagate vegetatively with a very high rate and to redifferentiate easily to new plants were induced from shoot tips of *Stevia rebaudiana* on Murashige and Skoog agar media supplemented with 1.0 mg/l Benzyl amino purine and 0.1 mg/l Naphthalene acetic acid. The multiple shoots originated from a number of adventitious buds formed on the margin of the leaf. Multiple shoots were obtained by repeating the subculture in a same medium. The mature culture bottles were acclimatized for a week and transferred the micro shoots in non sterile sand for rooting. The rooted plants were then transplanted in polybag.

With an objective of understanding the antimicrobial potential of *Stevia rebaudiana*, chemical extracts from its leaves were subjected to bacterial assay using six solvents(petroleum ether, cyclo-hexane, chloroform, water, acetone and ethanol.) against six different clinically important bacterial (*Escherichia coli, Bacillus subtilis, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*) isolates. 250µg/l of petroleum ether extract (minimum inhibitory concentration) was found sufficient enough to inhibit the growth of test microorganism *E.coli* completely in petriplates (By plate dilution method). Among the test microorganism *S. aureus* exhibited highest range of susceptibility against four extracts namely, water, petroleum ether, cyclo-hexane and chloroform. Water extract of *Stevia* leaf showed activity against *B.subtilis* and *S. aureus* only. Petroleum ether gave the highest zone of inhibition against the entire tested microorganism. Highest antibacterial index ($A_bI$; 11mm) was obtained for petroleum ether whereas the lowest antibacterial index ($A_bI$;2.88mm) was obtained from water extract.

**Bulletin of Plant Resources No. 29, April 2009, pp. 86**

## 82. Micropropagation of *Withinia somnifera* (L.) Dunal from germinating seeds

N. Chapagain, S. Rajbahak and S. K. Sah

An efficient protocol was developed for large scale propagation using seed as explant in *Withanai somnifera*. Shoot multiplication was achieved *in vitro* from shoot tips of aseptically germinating seedling of *Withinia somnifera* L. using Murasshige and Skoog's agar media supplemented with 1.0mg/l Benzyl amino purine (BAP) and 0.1mg/l Naphthalene acetic acid (NAA). The multiple shoots were obtained by repeating the subculture in the same

medium with addition of 50 mg/l adenine sulphate. The mature culture bottles were acclimatized for a week and then the micro shoots were transferred in non sterile sand for rooting. The rooted plants were then transplanted in polybags.

**Bulletin of Plant Resources No. 29, April 2009, pp. 86**

## 83. *In vitro* propagation of *Dendrobium amoenum* Wall. ex Lindl. from seed culture

K. M. Rajkarnikar

The seeds of *Dendrobium amoenum* were cultured on agar gelled MS (Murashige and Skoog) medium supplemented with or without 10% coconut milk, 1% casein hydrolysate and 3% sucrose. The protocorm like bodies (PLBs) obtained from three weeks old culture were subcultured on fresh MS medium for seedling development. Very few PLBs were subcultured on MS medium with different hormone concentration for further multiplication of microshoots. The medium supplemented with 1.0mg/l BAP, 1.5mg/l kinetin and 10% coconut milk and 1.0mg/ BAP, 1.0mg/l NAA and 10% coconut milk were most suitable for multiplication of PLBs and for healthy seedling growth. The microshoots thus obtained were transferred on hormone free medium to regenerate roots and for further growth. Thus, obtained seedlings were washed thoroughly by water and transferred in mosses for further growth of seedling.

**Bulletin of Plant Resources No. 32, 2010, pp. 90**

## 84. Propagation of *Cymbidium aloifolium* (L.) Sw. *in vitro* by Seeds

K. M. Rajkarnikar

Plant tissue culture is a promising alternate method for conserving and multiplying the plants. The present study deals with *in vitro* germination of *Cymbidium aloifolium* seeds and their subsequent development into seedlings for conservation and multiplication. *Cymbidium aloifolium* seeds were cultured on solidified MS medium supplemented with 0.1% casein hydrolysate and 3% sucrose. The protocorm like bodies (PMB) were obtained from eight weeks old cultures. These PLBs were subculture again and again (1 to 2 times) on fresh medium to obtain 10 to 12 cm long normal and healthy seedling. Some PLBs were subculture on MS medium supplemented with different combination of Benzylaminopurine (BAP) and Napthaleneaceticacid (NAA) for further proliferation of micro shoots and protocorm. Among two tested combination of BAP and NAA, the combination of 2mg/l BAP, 1.5mg/l NAA and 10% coconut milk was best for further multiplication of PLBs and micro

shoots. These regenerated seedlings were acclimatized and transferred to moss substrate for further growth. The obtained result showed that *in vitro* growing of *Cymbidium aloifolium* seeds is a reliable method for the seedling growth and multiplication of its seedlings.

**Bulletin of Plant Resources No. 33, 2011, pp. 27**

## 85. *In vitro* shoots tips culture of *Aloe vera* Mill.

S. Rajbahak, N. Chapagain, J. Shrestha and P. Basnet

*Aloe vera* Mill. is an important medicinal plant and used worldwide in medicine and cosmetic industry. Micropropagation method for elite selection of *Aloe vera* by axillaries branching method using shoots tip as explants was standardized. Shoots tips cultures initiated on MS medium containing the growth regulators BAP and NAA. Maximum shoot proliferation was achieved on medium containing BAP 2.0mg/l with NAA 1.0 mg/l. the mature micro shoots were kept in greenhouse for acclimatization. Micro shoots were transfer in sand for rooting. The roots were initiated after two weeks of transplantation. Sand rooted plants were transferred to soil and they showed 85% survival. The regenerated plants were morphological similar to mother plants.

**Bulletin of Department of Plant Resources No. 34, 2012, pp. 78**

## 86. *In vitro* study of *Citrus aurantifolia* Swingle

R. Basnet and B. Pant

Multiple shoots were induced from nodal explants of *Citrus aurantifolia* (Vernacular name kagati) on Murashige and Skoog (MS) medium supplemented with 1 ppm BAP (Benzylaminopurine) and 0.5 ppm NAA (Napthalene acetic acid). Explants were taken from *in vitro* germinated plantlet on hormone free MS medium. Roots were developed from ½ MS strength medium supplemented with 1.5 ppm IBA (Indole butyric acid). These plants were transferred into ½ MS + IBA 1.0ppm by cutting its roots apices. These plants were acclimatized on non sterile sand and soil (1:1) by maintaining moisture and light. Fungicide (bavistine) 0.1% was sprayed uniformly.

**Bulletin of Department of Plant Resources No. 34, 2012, pp. 101**

## 87. *In virto* propagation of Cardamom (*Amomum subulatum* Roxb.)

A. Karki and H. K. Saiju

Cardamom was introduced and has been in use since immemorial in Nepal. However people were ignorant in the early days about its commercial value which off late has slowly begun to attract people's attention. More and more

people are becoming increasingly aware of its commercial value and being drawn into its cultivation. This has contributed tremendously in increasing the social, economic status of the farmers. The crop being environment friendly as well as exportable commodity can leave a very fruitful result for the people and the nation as a whole. The cardamom is very useful in agro forestry inter cropping and also helps in nature conservation as well as in medicine formulation.

In present paper the protocol of the two cultivars of Ramsai and Golsai were developed for large scale productionof plantlets round the year by *in vitro* culture. The shoot tips (1-2mm) were exised from the mother plants and cultured in Murashige and Skoog solid media (1962) supplemented with 1.0 mg/l 6-BenzylAmino Purine and α Naptheleneaceticacid 0.1mg/l. Multiple shoots with roots were produced by repeated sub-cultured in same concentration of liquid media. Rooted plants were successfully placed in the field.

**Proceeding of Nepal Japan Symposium on conservation and utilization of Himalayan medicinal Resources, Nov 6-11, 2000, Kathmandu, Nepal, pp. 224**

## 88. *In vitro* propagation of *Elaeocarpus sphaericus* (Gaertn) K. Schum.

P. Joshi, K. M. Rajkarnikar and H. K Saiju

Rudraksha (*Elaeocarpus sphaericus* (Gaertn) K. Schum.) belonging to the family Elaeocarpaceae is the important religious and medicinal plant. Because of its high valued fruit there is an urgent need to produce it in mass scale by *in vitro* propagation technique. The choice of explant as nodal cuttings (from 2 to 5 yrs. plant) in liquid MS medium was observed appropriate. Otherwise there was a great possibility of necrotic exudation of phenolic compound. The established green explants were then inoculated in MS medium with the combination of different concentration of BAP and NAA as well as MS medium with different concentration of BAP alone. It was also tried in MS medium with less ammonium nitrate (300 mg/l). The MS medium supplemented with BAP 0.5 mg/l and NAA 0.01 mg/l as well as MS medium with BAP 0.25 mg/l were observed good for proliferation of microshoots. The proliferated microshoots when sub cultured in MS medium with less ammonium nitrate (300 mg/l) supplemented with BAP 0.25 mg/l at the interval of 3 to 4 months, it showed good result of proliferation with no browning of microshoots. The microshoots develop roots successfully in non-sterile sand within 15 to 20 days in house condition.

**Proceeding of Nepal Japan Symposium on conservation and utilization of Himalayan medicinal Resources, Nov-6-11, 2000, Kathmandu, Nepal, pp. 227**

## 89. Micropropagation of the Nepalese Medicinal plant *Swertia chirata* (Wall.)

M. Kayastha

*Swertia chirata*, the Nepalese medicinally valuable plant that is used in treatment of different treatment ailments by different ethenic groups, is being tested for its mass propation through tissue culture technique.The shoot tips of two months old plant were cultured in Murashige and skoog's (MS) medium supplemented with 1mg/l Benzylaminopurine (BAP) and 0.01mg/l Napththaleneaceticacid (NAA) for multiplication. Ten to fifteen microshoots were developed after 4th subculture. These microshoots were transferred in non sterile sand for rooting. The roots were developed within two to three weeks. The rooted plants have been established successfully in the field.

**Proceeding of Nepal – Japan Symposium on conservation and Utilization of Himalayan Medicinal Resources November 6-11, 2000, Kathmandu, Nepal, pp. 230**

## 90. *In vitro* culture of *Rauvolfia serpentina* L. Benth. ex Kurz.

K. M. Rajkarnikar, H. K Sainju and G. D. Bhatta

*Rauvolfia serpentia* is a most important plant in aurvedic system of medicine. It is used for the treatment of hypertension and tranquillizing agent. It belongs to family Apocynaceae. It is endangered from natural habitat due excessive collection of roots. It is ban for expert outside the country under the Forest Act, 1993 in Nepal. So tissue culture method had been developed for its germplasm conservation and rapid clonal multiplication. The shoot tips were used as explants. The shoot tips were cultured in Murashige and skoog's (MS) medium supplemented with different concentration of Benzyl Amino Purine (BAP) and Napththalene acetic Acid (NAA) for multiplication. The MS medium supplemented with 1mg/l BAP and 0.1mg/l NAA was found to be a best medium. 12-16 microshoots were developed after 7th subculture from individual shoot. The developed microshoots were transferred in non sterile sand for rooting. The 90% of microshoots develope roots within one to two weeks. The rooted plants have been established successfully in the field.

**Proceeding of Nepal – Japan Symposium on conservation and Utilization of Himalayan Medicinal Resources November 6-11, 2000 Kathmandu, Nepal, pp. 232**

## 91. Micropropagation of *Valeriana jatamansi* Jones

R. Niroula and H. K.Saiju

Plants within theValerianaceae have long tradition in herbal medicine. *Valeriana wallichi, V. officinalis* and *Nardostachys jatamansii* have been widely

used in India, China and Nepal. *Valeriana jatamans*i (Sugandhawal), is a in perennial herb, ban for expert outside the country as raw material under the Forest Act, 1993 in Nepal. In order to propagate it in mass scale, tissue culture has been successfully carried out of this medicinal plant. In order to propagate the multiple shoots were regenerated from young shoot tips as an explants cultured in Murashige and Skoog medium supplemented with of Benzyl Amino Purine (BAP) 1mg/l and Napththalene acetic Acid (NAA)0.1mg/l. 90 of microshoots rooted in non sterile sand and these plants were established successfully in the field.

**Proceeding of Nepal – Japan Symposium on conservation and Utilization of Himalayan Medicinal Resources November 6-11, 2000 Kathmandu, Nepal, pp. 235**

## 92. Micropropagation of *Rheum emodi* Wall

R. Noroula

*Rhum emodi* Wall, a widely used medicinal plant in the verge of extinction because of its wide collection. In order to conserve its germplasm and to cultivate it in mass scale, attempts have been made to micropropagate this medicinal plant. The shoots were regenerated from aseptically germinated seedlings of *R. emodi* Wall as an explant cultured in Murashige and Skoog medium supplemented with Benzylaminopurine (BAP) 1mg/l and Napthylaceticacid (NAA) 0.1mg/l, The roots were initiated when the micro shoots were transferred in non sterile sand

**Proceeding of Third National Conference on Science and technology, March 8-11, 1999, pp. 1403**

## 93. Micropropagation of *Syngonium* 'CV' plants

S. Shrestha and A. Karki

Shoot calli inducd on MS medium containing low concentration of (NAA) 0.01 mg/l- 0.1 mg/l in combination with 1 mg/l (BAP) Benzylaminopurine were found to be component for shoot regeneration. Upon transfer of regenerated shoots on 1.0 mg/l BAP and 0.5 mg/l with Kinetin liquid medium, an increase in multiple shoots was observed. Those shoots increased in multiple frequency ratio. Such multiple shoots were readily rooted in non sterile sand and subsequently established successfully in the field.These results suggest that methodology can be used on a commercial scale for micropropagation of Syngonium from axillary buds.

**Proceeding of Third National Conference on Science and technology, March 8-11, 1999, pp. 1442**

## 94. Tissue Culture of Banana and its field plantation

A. Karki, S. Rajbahak and H. K. Saiju

Banana is one of the most popular fruits in Nepal. In Nepalese context this fruit plays quite an important role not only for its consumption value but for both cultural and religious values. Both the plant and the fruit are used in special occasion, especially for worshipping of the deities.

For tissue culture the suckers were collected from healthy mother plants of banana.The inner most shoot tips were taken out aseptically and cultured on Murashige and Skoog medium (1962) supplemented with 5.0 mg/l 6-Benzylaminopurine and 10% coconut milk.The cultured flasks were kept in incubation room under the fluorescent light with 3000 lux for sixteen-hour and room temperature 25$^{o}$ C.The shoot tip was swollen within six weeks of culture. The swollen shoot was cut vertically into two pieces and subcultured in the new fresh medium with same concentration of growth hormone and coconut milk. The microshoots were subcultured in interval of six week in the same concentration of nutrient medium.

The flasks with mature multiple shoots were brought out to ordinary room condition for acclimatization. After two weeks, the microshoots were taken out of the flasks and cleaned with water thoroughly. The microshoots were transplanted in clean sand in propagator for root development. The root developed within four weeks. The rooted microplants were transplanted in soil in polythene bag. One foot tall banana plants with six leaves were distributed. Upto now ten thousand banana plants were distributed for field plantation in different districts of Nepal.

**Proceeding of Fourth National Conference on Science and technology, pp. 1028**

## 95. Micropropagation of *Daphne papyracea*. Wallich ex. Steudel through shoot tip culture

K. M. Rajkarnikar

*Daphane papyracea* Wallich ex. Steudel belong to family Thymalaceae. *Daphne* species are locally known as Lokta and Kagajpate. This is the economically important plant for making Nepali paper from its barks. Conventionally, its multiplication is difficult because viability time of seeds are very short, cutting and layering are also difficult to established, take long time to initiate the roots and survival percentage of cutting shoots are low. Therefore, tissue culture of *Daphne papyracea* is attemted by using shoot tips. The shoot tips of *Daphne papyracea* are collected from naturally growing mature plant around Godawari area. These shoots after sterilization, aseptically cultured in MS

(Murashige and Skoog 1962) medium with different concentration of hormones (BAP, NAA, 2, 4-D). Knowing best concentration i.e. MS medium supplemented with 1mg/l BAP, 0.01 mg/l NAA, 50mg/l Adenine sulphate and 10% coconut milk, the regenerated microshoots were subcultured in same medium for multiplication at interval of ten to twelve weeks. After acclimatization, the multiplied shoots were transferred to non sterile sands for induction of roots in green house and some shoots were subcultured in MS medium supplemented with 0.5 mg/l NAA for initiation of roots.

**The fifth National Conference on Science and Technology, organized by Nepal Academy of Science and Technology, Nov. 10-12, 2008, Kathmandu, pp. 194**

## 96. *In vitro* Propagation of *Dendrobium amoenum* Wall. ex Lindl. from shoot tip culture

K. M. Rajkarnikar

The plants, *Dendrobium amoenum,* were collected from Dhumpus area of Kaski district and planted in green house of National Herbarium and Plant Laboratory, Godawari. It is epiphytic orchid having both horticultural and medicinal value. The sprouted shoots in these plants were used as explants.The shoot tips after sterilization were cultured on agar gelled MS medium supplemented with different concentration of Benzylaminopurine, Kinetin and Napthatene Acetic Acid. The medium is also fortified with 1% casein hydrolysate, 3% sucrose. The protocorm like bodies (PLBs) and few microshoots were obtained from six weeks old shoot tips culture. The medium with BAP 1mg/l and NAA (0.01-0.1mg/l) and medium with BAP 1mg/l and kinetin 1.5mg/l are best for culture establishment. These regenerated microshoots and PLBs were subcultured on MS medium with different hormone concentration for further multiplication of microshoots and PLBs. The medium supplemented with 1mg/l BAP, 1.5 mg/l Kinetin and 10% coconut milk and 1mg/l BAP, 1mg/l NAA and 10% coconut milk were seems to be most suitable for multiplication of PLBs and healthy microshoots regeneration. The microshoots thus obtained were transferred on the medium with 0.5mg/l NAA to regenerate roots and for further growth. Thus obtained seedlings were washed thoroughly by water and transferred in mosses for further growth of seedlings.This protocol could be helpful for production and conservation of this species.

**The sixth National Conference on Science and Technology, organized by Nepal Academy of Science and Technology, Sep. 25-27, 2012, Kathmandu, pp. 101**

## 97. Clonal propagation of *Paulownia tomentosa* (Thunb) Steud. for commercial production

S. Rajbahak, N. Chapagain, J. Shrestha and P. Basnet

Nodal explants were used for clonal propagation of *Paulownia tomentosa* by manipulating the cytokinin and auxin. Shoot bud proliferation was achieved from nodal explants derived from green house plant of *Paulownia tomentosa* on MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA. The number of shoot proliferation was enhancing by changing concentration of growth hormone BAP. The mature culture bottles were acclimatized for a week. *In vitro* grown shoot could successfully rooted *ex - vitro* in non sterile sand. The rooted plants were then transferred in polybags.

**The Sixth National Conference on Science and Technology, organized by Nepal Academy of Science and Technology, Sep. 25-27 2012, Kathmandu, pp. 115**

## 98. Micropropagation of Kiwi plant

K. M. Rajkarnikar and G. D. Bhatta

Kiwi plant (*Actinidia chinensis*) is belonging to family Actinidiaceae. It is dioeciously perennial climber. Nowadays, it has become a commercial fruit and is one of the most expensive fruit in the world. Its fruit contains vitamin 'C' at a concentration of 20-60 times higher than that in apple. The seedlings are highly heterozygous. If propagated through seeds, about 70% offsprings are male. So the tissue culture is appropriate method for propagation of required amount of male and female.

Shoots tip were taken from 2 to 3 yrs old plant and cultured on Murashige and Skoog (MS) medium supplemented with different concentration of BAP (Benzyl amino purine) NAA (Napththalene acetic acid). The shoot buds were initiated after two weeks of culture in MS medium with 2.5 mg/l BAP and 0.1 mg/l NAA. The microshoots develop, when the shoot buds were subcultured in MS medium with low concentration of BAP and NAA. Three to four centimeters micro shoots formed roots in non sterile sand. The roots were initiated after 15-20 days. The rooted plants were successfully established in soil.

**International Conference on Himalayan biodiversity, (ICHB-2003), Feb. 26-28, 2003, organized by Himalayan Resources Institute (HIRI), Biodiversity Research Group (BRG), Central Department of Zoology, TU, The Ecological Association of Nepal (ECOAN) and Nepal Biotechnology Association (NBA), pp. 49**

## 99. Micropropagation of *Azadirachta indica* A.Juss.

K. M. Rajkarnikar and G. D. Bhatt

*Azadirachta indica* A.Juss, the most valuable medicinal and economic plant, was selected for its micropropagation. Among the node and leaf explants of the tree, the node explants responded in MS medium supplemented with 0.25mg/l BAP, 0.01mg/l NAA, 0.5 mg/l charcoal and 50 mg/l Adenine Sulphate as most suitable for the regeneration of multiple shoots. *In vitro* raised microshoots developed roots in MS medium fortified with 0.5 mg/l NAA and 0.5 gm/l charcoal.

**Research on Plant Tissue Culture, Department of Plant Resources, Bulletin No. 24, (2004), pp. 4**

## 100. Micropropagation of *Cephaelis ipecacuanha* A.Rich.

K. M. Rajkarnikar, M. Kayastha and G. D. Bhatt

The shoot tips of *Cephaelis ipecacuanha* were used as initial explants. *In vitro* multiple microshoots were obtained from *in vitro* regenerated node segments in MS medium supplemented with 2mg/l BAP and 0.1mg/l NAA. These microshoots produced roots in non sterile sand within 2-3 weeks. The regenerated plants were successfully grown in soil in the green house.

**Research on Plant Tissue Culture, Department of Plant Resources, Bulletin No. 24, (2004), pp. 7**

## 101. Micropropagation of *Swertia ciliate* Buch-Ham.

K. M. Rajkarnikar, G. D. Bhatt and M. K. Adhikari

The explants (1-2mm shoot tips and leaves) of *Swertia ciliata* were cultured in MS medium supplemented with 1.0 mg/l BAP and 0.01mg/l NAA for multiplication of microshoots. The developed microshoots were transferred in non sterile sands for rooting. The roots initiated within 2-3 weeks of transplantation. The rooted plants were established successfully in field.

**Research on Plant Tissue Culture, Department of Plant Resources, Bulletin No. 24, (2004), pp. 10**

## 102. *In vitro* propagation of Hybrid of Asiatic Lily

G. D. Bhatt, K. M. Rajkarnikar and M. K. Adhikari

The MS medium supplemented with 0.5 mg/l of Benzylaminopurine (BAP) and 0.1mg/l of Napthaleneacetic acid (NAA) developed plantlets with in 5 to 6 weeks from nodal explants of Hybrid of Asiatic Lily. The established shoots tips were subcultured in the same medium for four times. 8-10

microshoots were developed in the 3rd subculture. These microshoots rooted in non sterile sand within 3 to 4 weeks.

**Research on Plant Tissue Culture, Department of Plant Resources, Bulletin No. 24, (2004), pp. 13**

## 103. Micropropagation of *Primula obconica* Hance.

K. M. Rajkarnikar, M. Kayastha and G. D. Bhatt

The multiple shoots were regenerated from the shoot tips (2-3mm) of *Primula obconica*, an ornamental plant, in MS medium supplemented with 1mg/l BAP and 0.1mg/l NAA. Eighty to ninety percent of plantlets produced roots in non sterile sand after 2-3 weeks. These rooted plants flourished well. No any morphological variation was noted when compared to mother stock.

**Research on Plant Tissue Culture, Department of Plant Resources, Bulletin no. 24, (2004), pp. 16**

## 104. *In vitro* propagation of *Acacia auriculiformis* A. Cunn. ex Benth.

A. Karki and R. Niroula

*Acacia auriculiformis,* an Australian species is an important agro-forestry tree. It thrives in harsh sites on poor soil. This species is valued for good fuel woods and makes good charcoal production. It is an important species for avenue plantation and reforestation programme. In Nepal, the species was introduced in 1950 and has planted in different parts of the country. It is suitable for Terai region and inner Terai region. In the present paper, revised a method for large scale production of plantlets all the year around *in vitro* culture method. The *in vitro* propagation of *A. auriculiformis* seedling were investigated. The shoot tips from 12-16 days old seedlings were excised and cultured in Murashige and Skoog's medium (1962) supplemented with different concentrationof 6 Benzylamino purine and Napthylene Acetic acid. 25-30 multiple shoots were proliferated in MS medium supplemented with BAP 0.5 mg/l and NAA 0.1mg/ l after fourth sub culture. The micro shoots 3-4 cm long were excised and rooted in non sterile sands. The roots were initiated after 15-21 days. The rooted plants were successfully established in soil.

**Proceeding of the Eighth International workshop of Bio-Refor, Kathmandu, Nepal, November 28-December 2, 1999, Kathmandu, Nepal**

## 105. Propagation of *Santalum album* L. through tissue culture

N. Pradhan and H. K. Saiju

Regeneration of microplants were observed from the excised shoot tips

(2-3mm) from five year old plant of Santalum album L. Green callus were initiated in Murashige and Skoog (MS) medium supplemented with Benzylamino purine (BAP), 1.0 mg/l and Napthalene acetic acid (NAA) 0.1 mg/l. Microplants were regenerated in MS medium supplemented with BAP 1.0 mg/l and Kinetin 1.5 mg/l and coconut milk 10%. Roots were observed in non-sterile sand. The rooted plants were successfully planted in the field.

**Proceeding of the Eighth International workshop of Bio-Refor, Kathmandu, Nepal, November 28-December 2, 1999, Kathmandu, Nepal**

## 106. Application of Tissue culture for plantation in Nepal

H. K. Saiju

Tissue culture is an important aspect of biotechnology. It has a great potential for rapid, large-scale production of clean and healthy plants. Since its establishment in 1976 tissue culture laboratories, Godawari, has developed micro propagation technique protocol for 90 economically important plant species for forestry, horticulture, medicinal, ornamental uses and agriculture. Hundreds of micro propagated plants have been under field performance trial. In Nepal, four private tissue culture factories are producing plants for domestic and foreign markets.

**Proceeding of the Seventh International workshop of Bio-Refor, Manila, Philippines November 3-5, 1998, pp. 54-56**

## 107. Micropropagation of *Begonia tuberhybrida* Voss

K. M. Rajkarnikar and G. D. Bhatta

Begonia belongs to family Bignoniaceae. These species are distributed throughout the tropical and the subtropical region from central and south America, Asia and Africa. Over 2000 species have been recorded (Inoue, 1983), most of them are perennial herb with rhizomatous or tubers (Willis, 1973).They are succulent plants with different colour flowers. They are important as ornamental plants through the world. Production by conventional method were inadequate to meet increasing demand of Begonias plants. Micropropagation of *Begonia tuberhybrida* of different colours had been performed to get large amount of healthy plants and homogenous plants.

Aseptically, the sterilized leaf and petiole explants were cultured in Murashige and Skoog medium (M S medium) supplemented with different concentration of BAP and NAA. After 6-8 weeks, shoot buds were initiated from upper surface of leaf explants. The initiated shoot buds were subcultured in the MS medium with 0.5mg/l BAP and 0.01mg/l NAA for shoot elongation and

multiplication at an interval of 4-6 weeks. The microshoots rooted in non sterile sands within 8-12 days after transferred in sands. The rooted plants successfully grew up and flowered as in normal plants.

**First International Florigulture Trade Fair 2001 Souvenir Published by Floriculture Floricuture Association of Nepal, Pg. 17**

## 108. Micropropagation of *Fuschia hybrida*

P. Joshi and K. M. Rajkarnikar

The genus *Fuschia* is belonging to family Onagraceae. *Fuschia* are ornamental and popular flowering plants. They are multiplied vegetatively from cuttings. Micropropagation is the potential means for mass production to reduce both cost and time factor. Nodal cutting from two year old plant were taken as explants. After sterilization, aseptically these explants were cultured in MS medium supplemented with different concentration of BAP and NAA. Among different hormone concentration, 1.0 mg/l BAP and 1.0 mg/l NAA was found to be the best concentration for multiplication of best quality micro shoots. These micro shoots were first acclimatized and transferred to non sterile sand for the induction of roots. The micro shoots initiated roots after 15 to 20 days.

**Floriculture Trade Fair (2004), Souvneir, Floriculture Association Nepal (FAN), pp. 7**

## 109. Micropropagation of *Antirrhium majus.* L

S. Rajbahak

Antirrhium (*Antirrhium majus*) is belonging to family Scrophulariaceae is commonly known as Snapdragon. It is a perennial plant but treated as an annual or biennial. Snapdragon is commonly grown for garden display and for cut flower. Seed were surface sterilized with 0.1% mercuric chloride and followed by rinsing with sterilized distilled water for few times. The sterilized seeds were cultured in MS basal media solidify with 0.8% agar powder and pH as adjusted to 5.8 before autoclaving and incubated under 16 hour light/ dark cycle. at 25±2$^{o}$C. The seds start to germinate after 7 days. A single flask contains 25-30 numbers of plants. The matured culture bottles were acclimatized in green house for 7-10 days. The shoots were transferred on non sterilized sand. The roots initiation takes place after 7-10 days.

**Floriculture Trade Fair-2004, Souvneir, Floriculture Association Nepal (FAN), pp. 9**

## 110. Mass propagation of *Streptocarpus* sp. through leaves culture

S. Rajbahak

Leaves were surface sterilized with 0.1% mercuric chloride for 5 minutes and rinsed with sterilized water for few time and inoculated aseptically in filter

paper bridge containing MS media supplemented with 1.0 mg/l BAP and 0.01 mg/l NAA. Embroids were produce fron the cut surface of the leaf explant. The embroids were transferred onto the solid MS media for multiplication. 90% of rooted plants were observed within 7-10 days after transplanting the microshoots in the non sterile sand. These plants were successfully established in the field and flowering as mother plants.

**Floriculture Trade Fair-2004, Souvneir, Floriculture Association Nepal (FAN), pp. 9**

## 111. Micropropagation of *Lisianthus* sp.

K. M. Rajkarnikar

*Lisianthus* sp. belongs to family Gentianaceae is commonly known as Eustoma. Regeneration of microplants was observed from the excised shoot tips (2-3mm) from germinating seedling. Microshoots were initiated in Murashige and Skoog (MS) medium supplemented with Benzylamino purine (BAP), 1.0 mg/l and Napthalene acetic acid (NAA) 0.1 mg/l. Roots were observed in non-sterile sand. The rooted plants were successfully planted in the pot. The plants were grown normally and flowered.

**Floriculture Trade Fair-2004, Souvneir, Floriculture Association Nepal (FAN), pp. 11**