# "DEVELOPMENT OF AN EFFICIENT PROTOCOL FOR *IN VITRO* CLONAL PROPAGATION AND DNA FINGERPRINTING ANALYSIS OF SELECTED ENDANGERED MEDICINAL PLANTS OF CONSERVATION CONCERN FROM SOUTH INDIA"

### FINAL REPORT

### Submitted to



# UNIVERSITY GRAND COMMISSION BAHADUR SHAH ZAFAR MARG

**NEW DELHI – 110 002** 

Submitted by

Dr. P. VENKATACHALAM Principal Investigator UGC – Major Research Project [F.No.37-297/2009 (SR)]



DEPARTMENT OF BIOTECHNOLOGY PERIYAR UNIVERSITY SALEM – 636 011 TAMIL NADU

### UNIVERSITY GRANTS COMMISSION BAHADUR SHAH ZAFAR MARG NEW DELHI – 110 002

## PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Name and Address of	
the Principal Investigator	: Dr.P.Venkatachalam Associate Professor Department of Biotechnology Periyar University, Salem-636 011, TN
2. Name and Address of the Institution	: Department of Biotechnology Periyar University, Salem-636 011, TN
3. UGC Approval No. and date	: F.No.37-297/2009 (SR) dt.01.02.2010
4. Date of Implementation	: 01/04/2010
5. Tenure of the Project	: 3 years (Extension 4 months requested)
6. Total Grant Allocated	: Rs. 13, 49,100/-
7. Total Grant Received	: Rs. 12,58,570/-
8. Final Expenditure	: Rs. 12, 49,783/-

9. Title of the Project : "Development of an efficient protocol for *in vitro* clonal propagation and DNA fingerprinting analysis of selected endangered medicinal plants of conservation concern from South India"

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### 10. Objectives of the Project

- a) To develop an efficient protocol for *in vitro* mass propagation of selected potential endangered medicinal plants
- b) To evolve a strategy for field establishment of in vitro raised plants
- c) To ensure the production of genetically identical plants by molecular marker studies
- d) To standardize an efficient DNA extraction method for different tissues collected from various genotypes
- e) To apply PCR based DNA fingerprinting techniques (RAPD, AFLP) for detection of genetic diversity as well as correct authentication and
- f) To identify species specific DNA marker, cloning and development of SCAR marker.



### 12. Achievements from the Project

- a. Received Best Paper-First Award to the Presented a paper entitled "Factors influencing rapid *in vitro* clonal propagation of *Gymnema sylvestrc* An important antidiabetic medicinal plant" by M. Thiyagarajan and P. Venkatachalam in the National Seminar on "Advances in Biotechnology and Biopharmaceutical Technology" held on 6 March, 2013 at Mother Terasa Women's University, Kodaikanal.
- b. Received Best Paper-First Award to the Presented a paper entitled "Evaluation of genetic fidelity of *in vitro* propagated natural sugar Killer plant (*Gymnema sylvestre*) using DNA-based marker" by M. Thiyagarajan and P. Venkatachalam in the National Seminar on "New Horizons in Biotechnology and Bionanomedicines" held on 24th September 2012 at Periyar University Salem.
- c. Received Young Scientist Award to the Presented a paper entitled "Evaluation of The Genetic Fidelity of *in vitro* Propagated Natural Sweetener Plant (*Stevia rebaudiana* Bert.) Using DNA-Based Markers by M. Thiyagarajan and P. Venkatachalam" in the National Conference on "Genomics and Genetic Engineering Strategies for Crop Improvement" held on February 27 and 28, 2012 at Periyar University Salem.

### 13. Summary of the Findings : Annexure I

14. Contribution to the Society : The traditional plant based medicines have now become quite popular in both developing and developed countries. The ever-increasing demand of herbal drugs is causing loss of precious biodiversity and also creating shortage of raw material. Whatever herb can be collected from plants growing naturally in the mountain or propagated through traditional methods is far from able to meet medicinal needs. With over-exploitation, medicinal wealth is rapidly lost, such that many valuable plants including *Alpinia officinarum*, *Costus speciosus*, *Curculigo orchioides*, *Gloriosa superba*, *Gymnema sylvestris*, and *Stevia rebaudiana* are threatened with extinction. Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted. In order to overcome this problem, a large scale *in vitro* production protocol has been developed for six important medicinal plant species for the continuous supply plant materials to meet the medicinal needs of the society.

### 15. Whether any Ph.D. Enrolled/Produced

Out of the Project

: A full-time project fellow is working in this

Project and registered for Ph.D Degree at Periyar University, Salem-636011

# 16. No. of Publications out of the Project: 6 (Attached separately)

(Please Attach Re-Prints)

Principal Investigator)

Dr. P. Venkatachalam Principal Investigator UGC - Major Project Department of Biotechnology Periyar University Salem - 636 011. TN, India

(Registrar) 24.8-17 REGISTRAR PERIYAR UNIVERSITY SALEM-636 011.

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### Title of the Project:

Development of an efficient protocol for *in vitro* clonal propagation and DNA fingerprinting analysis of selected endangered medicinal plants of conservation concern from South India

### **Objectives of the proposed Project:**

- To develop an efficient protocol for *in vitro* mass propagation of selected potential endangered medicinal plants
- To evolve a strategy for field establishment of *in vitro* raised plants
- To ensure the production of genetically identical plants by molecular marker studies
- To standardize an efficient DNA extraction method for different tissues collected from various genotypes
- To apply PCR based DNA fingerprinting techniques (RAPD, AFLP) for detection of genetic diversity as well as correct authentication and
- To identify species specific DNA marker, cloning and development of SCAR marker.

### The present project is focused with the following objectives:

### **Experiment I**

- Large Scale in vitro propagation of selected endangered medicinal plants
- Collection of explants, seeds, rhizomes from different locations of Tamil Nadu
- Preparation and culture of various explants such as shoot tips, embryonic axes, rhizomes which are amenable to *in vitro* studies
- Standardization and identification of suitable media and growth regulators for shoot bud multiplication.
- Shoot regeneration via direct and indirect organogenesis and/or somatic embryogenesis.
- Multiplication and elongation of regenerated shoots by organogenesis
- Subculture of somatic embryos for maturation and germination
- After standardization of plant regeneration protocol, regenerants could be established in soil.

### **Experiment II**

## DNA fingerprinting analysis of selected endangered medicinal plants

- Collection of leaf samples from selected medicinal plants from different locations and transportation into Lab
- Identification of efficient protocol for good quality DNA isolation, Quantification and Standardization of PCR conditions for RAPD analysis
- RAPD-PCR will be carried out with several random 10-mer primers
- PCR amplicons will be analyzed on Agarose Gel Electrophoresis
- PCR amplified DNA fragments will be size fractionated and viewed under UV transilluminator
- DNA fingerprints generated by RAPD-PCR image will be documented
- DNA fingerprinting data will be analyzed using software for identification of genetic variability

# DEVELOPMENT OF AN EFFICIENT PROTOCOL FOR *IN VITRO* CLONAL PROPAGATION AND DNA FINGERPRINTING ANALYSIS OF SELECTED ENDANGERED MEDICINAL PLANTS OF CONSERVATION CONCERN FROM SOUTH INDIA

#### SUMMARY

The present project was focused to develop an efficient *in vitro* propagation protocol for large scale production and also to investigate the genetic diversity within the populations of six important medicinal plant species (*Alpinia officinarum Costus specious, Curculigo orchioides, Gloriosa superba, Gymnema sylvestre* and *Stevia rebaudiana*) by DNA fingerprinting analysis.

#### **Experiment I**

### In vitro propagation of selected endangered medicinal plants

*Alpinia officinarum* rhizome nodes were placed on MS medium augmented with various concentrations of BAP and KIN (1.0 - 5.0 mg/l) and/or different concentrations of BAP (1.0 - 5.0 mg/l) combination with 2.0 mg/l KIN for multiple shoot bud induction. Multiple shoot bud development (6.41 shoots/explant) was achieved on MS medium containing 3.0 mg/l BAP along with KIN (2.0 mg/l). In order to enhance shoot bud multiplication, MS medium supplemented with different concentrations of Silver nitrate (10-50  $\mu$ M/l). Maximum number of shoot bud induction (7.2 shoots/ explant) was achieved on MS medium containing 3.0 mg/l BAP + KIN (2.0 mg/l) + 20 $\mu$ M/l AgNO<sub>3</sub> combination. For root induction, elongated shoots were cultured on half-strength MS medium supplemented with different concentrations of NAA (0.5-2.0 mg/l). Highest frequency (85.1%) root induction was obtained on half-strength MS medium containing 0.5 mg/l NAA (7.25 roots/shoot). Regenerated plantlets were successfully established in the field condition with 95% survival rate.

*Costus specious* rhizome and pseudostem explants were cultured on MS medium supplemented with different concentrations of BAP and KIN (1.0 - 5.0 mg/l) alone and/or in combination with 1.0 mg/l KIN for multiple shoot bud induction. Maximum number of shoot buds was noticed on MS medium supplemented with 2.0 mg/l BAP in combination with 1.0 mg/l KIN (6.8 shoots/explant). Well developed roots(>2cm length) were placed on half-strength MS medium augmented with different concentrations of IBA (0.5 -2.0 mg/l). Maximum number of roots was noticed on 1.0 mg/l IBA and more than 70% of the plantlets were survived.

For *in vitro* plant regeneration, rhizome segments of *Curculio orchioides* were cultured on MS medium supplemented with different concentrations of BAP and KIN (1.0- 5.0 mg/l), while for somatic embryogenesis, rhizome explants were cultured on MS medium fortified with different concentrations of 2,4- D (1.0 - 3.0 mg/l). The highest frequency of shoot bud proliferation (80.9%) was observed on MS medium containing 3.0 mg/l BAP. Maximum percent of somatic embryogenesis (87.5%) with 25.1 embryos/explant was noticed on MS medium supplemented with 2.0 mg/l 2,4-D. The somatic embryos were cultured on MS medium fortified with different concentrations BAP and KIN (1.0 - 3.0 mg/l) for maturation. Highest percent (97.5  $0 \pm 1.0$ ) of embryo germination was observed on MS medium supplemented with 2.0 mg/l BAP. Also, the effect of different concentrations of silver nitrate and carbohydrate on maturation of somatic embryos was evaluated. The rooted plantlets were successfully hardened in plastic cups containing sand: soil in the ratio 1:2 and subsequently established in the greenhouse. After two weeks, plants were successfully transferred to shade and gradually acclimated in the field, where about 75 % of the plants were survived.

Gloriosa superba tubers were placed vertically on MS medium supplemented with different concentrations of BAP (1.0-4.0 mg/l) alone and various concentrations of BAP (1.0-3.0 mg/l) in combination with 0.5 mg/l KIN for shoot bud induction. Highest frequency of shoot bud proliferation (87.3%) was observed on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l KIN combination. In another experiment surface sterilized shoot tip and internodes explants were cultured on MS medium supplemented with different concentrations of BAP and KIN (0.5-3.0 mg/l) alone and/or different concentrations of BAP (0.5-3.0 mg/l) in combination with 0.5 mg/l KIN for multiple shoot bud induction. After two weeks, the internode explants were failed to induction of multiplication shoot bud but the shoot tip explants, the shoot bud development was observed. Highest percent of shoot bud induction was observed on MS medium augmented with 2.0 mg/l BAP + 0.5 mg/l KIN combination. The regenerated shoot buds were cultured on half- strength MS medium supplemented with different concentrations of IBA and NAA (0.5 -2.0 mg/l) alone for root induction. Among the different concentrations of auxin tested, the root induction was noticed at 1.0 mg/l IBA. The rooted plantlets with expanded leaves were successfully transferred into plastic cups containing sand and soil in the ratio of 1:2 and subsequently they were transferred to the field conditions and the survival rate noticed was 50%.

Mature axillary node explants of *Gymnema sylvestre* were cultured on MS medium supplemented with different concentrations of BAP and KIN (0.5-3.0 mg/l) for shoot bud induction. In order to enhance the shoot bud multiplication, regenerated shoot buds were further subcultured onto MS medium fortified with different concentrations of BAP (0.5-3.0 mg/l) in

combination with 0.5 mg/l of NAA/IBA/IAA/KIN. The highest frequency (84.22%) of multiple shoot bud regeneration with maximum number of shoots (14.20 shoots/explant) was noticed on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l KIN combination. In another experiment, *in vitro* derived shoot buds were cultured on different concentrations of GA<sub>3</sub> (0.5-2.0 mg/l) and various concentrations of BAP (0.5-3.0 mg/l) in combination with KIN (0.5 mg/l) and GA<sub>3</sub> (1.0 mg/l) for shoot bud multiplications as well as elongation. The effect of different concentrations of silver nitrate and carbohydrates for multiple shoot bud induction was also studied. For large scale plant production, *in vitro* derived axillary buds were cultured on MS medium fortified with BAP (1.0 mg/l) + KIN (0.5 mg/l)+ GA<sub>3</sub>(1.0 mg/l) combination, in which about 418.72 shoots/explant were obtained after five subcultures on the same media composition. Elongated shoots (>2 cm) were dissected out from the *in vitro* proliferated shoot clumps and were cultured on half-strength MS medium containing different concentrations of various auxins (IAA, IBA and NAA) (0.5-2.0 mg/l) for root induction. Half-strength MS medium supplemented with 2.0 mg/l IBA was found to be best for maximum rooting response and regenerated plantlets were successfully established in the field condition with 85% survival rate.

Nodal explants of Stevia rebaudiana collected from the field were cultured on MS basal medium fortified with different concentrations of BAP (0.5-3.0 mg/l) and KIN (0.5-3.0 mg/l) individually for shoot bud induction. In vitro derived nodal buds were cultured on MS medium supplemented with different concentrations (0.5-3.0 mg/l) of BAP and KIN for multiple shoot bud regeneration. In the second experiment, in vitro derived shoot buds were placed on MS medium supplemented with different concentrations of BAP (0.5-3.0 mg/l) in combination with 0.5 mg/l IAA or IBA or NAA for shoot bud multiplication. The highest frequency (94.50%) of multiple shoot regeneration with maximum number of shoots (15.69 shoots/explant) was noticed on MS medium supplemented with 1.0 mg/l BAP. In addition to that, the effect of different concentrations of silver nitrate and carbohydrate on multiple shoot bud induction was examined. For large scale plant production, in vitro derived nodal bud explants were cultured on MS medium fortified with 1.0 mg/l BAP, in which about 123 shoots/explant were obtained after three subcultures on the same media composition. Elongated shoots (>2 cm) dissected out from the in vitro proliferated shoot clumps were cultured on half-strength MS medium containing different concentrations of NAA (0.1-0.5 mg/l) and/or MS medium fortified with various concentrations (0.5-2.0 mg/l) of auxins (NAA, IAA and IBA) for root induction. Highest frequency of rooting (96%) was noticed on half-strength MS medium augmented with 0.4 mg/l

NAA and subsequently they were transferred to the field conditions and the survival rate noticed was 65.8%.

The protocol described herein can be applied for commercial scale plant production as well as conservation of these endangered medicinal plants from the possible extinction in the near future.

#### **Experiment II**

# <u>Genetic diversity analysis of 6 selected endangered medicinal plants by DNA</u> <u>fingerprinting</u>

Genetic diversity studies, the genomic DNA from eight accessions of *Alpina officinarum* plant species (L1 to L8) was isolated and quantified. Out of hundred primers only eight RAPD primers (OPA08, OPA15, OPA16, OPB01, OPB10, OPC05, OPC16 and OPC20), produced clear and reproducible polymorphic DNA fragments from eight accessions of *Alpina officinarum*. The selected primers produced 124 bands and 108 bands were showed polymorphisms. The levels of polymorphism were found to be higher.

DNA fingerprinting analysis of *Costus speciosus*, hundred oligo-nucleotide primers were used to amplify the genomic DNA from seven accessions. Out of Hundred primers only nine RAPD primers (OPA10, OPA16, OPB01, OPC05, OPC15, OPC16, OPC18, OPC19 and OPC20), produced clear and reproducible polymorphic DNA fragments among the eight accessions of *Costus specious* plant species. The selected primers produced 143 bands and 139 bands were showed polymorphisms. A dendrogram was constructed using Hierarchical joining method of cluster analysis separated all the 7 accessions into two clusters at 0.89 similarity coefficient.

For genetic diversity studies of *Curculico orchiodes* initially hundred oligo-nucleotide primers were used to amplify the genomic DNA from five accessions (L1 to L5). Out of hundred primers only eight RAPD primers (OPA02, OPA04, OPA05, OPA07, OPB04, OPC09, OPC16 and OPC19), produced clear and reproducible polymorphic DNA fragments among the five accessions of *Curculigo orchiodes*. The selected primers produced 91 bands and 90 bands were showed polymorphisms.

In a preliminary screening hundred oligo-nucleotide primers were used to amplify the genomic DNA from nine accessions of *Gloriosa superba* plant species (L1 to L9) for genetic diversity studies. Out of hundred primers only seven RAPD primers (OPB01, OPB07, OPB10, OPC06, OPC09, OPC16 and OPC20), produced clear and reproducible polymorphic DNA fragments among the nine accessions of *Gloriosa superba* plant species. The selected primers produced 72 bands and 58 bands were showed polymorphisms.

For DNA fingerprinting studies of *Gymnema sylvestre*, initially hundred oligo-nucleotide primers were used to amplify the genomic DNA from seven accessions (L1 to L7). Out of hundred primers only ten RAPD primers (OPA04, OPA10, OPA13, OPB15, OPB17, OPB18, OPC06, OPC09, OPC16 and OPC20), produced clear and reproducible polymorphic DNA fragments among the seven accessions of *Gymnema sylvestre* plant species. The selected primers produced 91 bands and 56 bands were showed polymorphisms.

Initial screening, hundred oligo-nucleotide primers were used to amplify the genomic DNA from three accessions of *Stevia rebaudiana* plant species (L1 to L3) for DNA fingerprinting studies. Out of hundred primers only nine RAPD primers (OPA10, OPA13, OPA16, OPB01, OPB07, OPC09, OPC15, OPC16 and OPC19), produced clear and reproducible DNA fragments among the seven accessions of *Stevia rebaudiana* plant species. The selected primers produced 111 bands and 90 bands were showed polymorphisms.

The results of these present studies provide a scientific basis for system classification, overall genetic resource management, variety selection, and selection of areas for cultivating of the six important medicinal plants (*Alpinia officinarum, Costus specious, Curculigo orchiodes, Gloriosa superba, Gymnema sylvestre* and *Stevia rebaudiana*). These findings therefore may be highly beneficial for protecting genetic resources and promoting sustainable use of the selected commercially important medicinal plants.

#### **Publications**

- Thiyagarajan, M. and Venkatachalam, P. 2010. Rapid and large scale *in vitro* propagation of *Stevia rebaudiana* (Bert): An anti-diabetic medicinal plant. Plant Cell Biotech. Mol. Biol. 11:31-39. (NASS Rating: 3.2)
- Thiyagarajan, M. and Venaktachalam, P. 2012. Large scale *in vitro* propagation of *Stevia rebaudiana* (Bert) for commercial application: pharmaceutically important and antidiabetic medicinal herb. Indus. Crops Prod. 37:111–117 (Impact factor: 2.84).
- 3. Thiyagarajan, M. and Venaktachalam, P. 2012. Evaluation of the genetic fidelity of *in vitro* propagated natural sweetener plant (*Stevia rebaudiana* Bert.) using DNA-based markers. Plant Cell Biotech. Mol. Biol. **13**(3-4):99-104. (NASS Rating: 3.2)
- P. Venkatachalam, N. Ezhili and M. Thiyagarajan, 2012. *In vitro* shoot multiplication of *Gloriosa superba* L. – An important anticancer medicinal herb. International Conference on Biotechnology, Biological and Biosystems Engineering (ICBBBE'2012) December 18-19, 2012 Phuket (Thailand).
- Thiyagarajan, M. and Venaktachalam, P. 2013. A Reproducible and High Frequency Plant Regeneration from Axillary Node Explants of *Gymnema sylvestre* (Gurmur) - An Important Antidiabetic Endangered Medicinal Plant. Indus. Crops Prod. 50:517-524 (Impact factor: 2.84).
- Kayalvilzhi, T., Thiyagarajan, M. and Venkatachalam, P. 2013. High frequency of plant regeneration of *Alpinia officinarum* using rhizome explants. Plant Cell Biotech. Mol. Biol. (Accepted). (NASS Rating: 3.2)

#### Papers presented in Conferences/Seminars

- Presented a paper entitled "Factors influencing rapid *in vitro* clonal propagation of *Gymnema* sylvestre – An important antidiabetic medicinal plant" by M. Thiyagarajan and P. Venkatachalam in the National Seminar on "Advances in Biotechnology and Biopharmaceutical Technology" held on 6 March, 2013 at Mother Terasa Women's University, Kodaikanal.
- Presented a poster entitled "In vitro plant regeneration from rhizome explants of Costus speciosus (Koen)- An important anti-diabetic medicinal plant" Thiyagarajan, M. and Venkatachalam, P. in the National conference on "Phytomedicine" held on 4<sup>th</sup> and 5<sup>th</sup> October 2012 at the Bharathiar University, Coimbatore.
- Presented a poster entitled "Evaluation of genetic fidelity of *in vitro* propagated natural sugar killer plant (*Gymnema sylvestre*) using DNA-based marker" Thiyagarajan, M. and Venkatachalam, P. in the National Seminar in "New Horizons in Biotechnology and Bionanomedicines" held on 24<sup>th</sup> September 2012, Periyar University, Salem.
- Presented a poster entitled "Application of Plant Tissue Culture for conservation of endangered antidiabetic medicinal plant- *Gymnema sylvestre*" by M.Thiyagarajan and P. Venkatachalam in 12<sup>th</sup> Tamil Science congress held on 23-25 August 2012, Periyar University, Salem.
- 5. Presented a paper entitled "Evaluation of the genetic fidelity of *in vitro* propagated natural sweetener plant (*Stevia rebaudiana* Bert.) using DNA-based markers" by M. Thiyagarajan

and Venkatachalam, P. in the National Conference on "Genomics and Genetic Engineering Strategies for Crop Improvement" held on February 27 and 28, 2012 at Periyar University Salem.

- 6. Presented a poster entitled "Micropropogation of *Alpinia officinarum*: an anti-cancer medicinal plant" by Kayalvizhi, T., Thiyagarajan, M. and Venkatachalam, P. in the National Conference on "Genomics and Genetic Engineering Strategies for Crop Improvement" held on February 27 and 28, 2012 at Periyar University Salem.
- 7. Presented a poster entitled "In vitro propagation of Costus speciosus- An important antidiabetic medicinal herb" Thiyagarajan, M. and Venkatachalam, P. in the National Conference on "Genomics and Genetic Engineering Strategies for Crop Improvement" held on February 27 and 28, 2012 at Periyar University Salem.
- Presented a poster entitled "Green synthesis of silver nanoparticles using leaf extracts of *Gymnema sylvestre* medicinal plant and evaluation of their antibacterial activity" Thiyagarajan, M. and Venkatachalam, P. in the National Conference on "Advanced Nanomaterials" held on February 6-7, 2012 at Periyar University Salem.
- Presented a paper entitled "In vitro shoot bud multiplication from tuber explants of Gloriosa superba L. – An anticancer medicinal herb" Kayalvizhi, T., Thiyagarajan, M., and Venkatachalam, P. in the 3<sup>rd</sup> International Conference on Medicinal Plants and Herbal Medicines Held on 19 to 21 December 2011 at Colombo, Sri Lanka.
- Presented a paper entitled "High frequency plantlet regeneration from axillary node cultures of *Gymnema sylvestre* R.Br. –An important anti-diabetic medicinal plant" by Thiyagarajan, M., Kayalvizhi, T. and Venkatachalam, P. in the 3<sup>rd</sup> International Conference on Medicinal Plants and Herbal Medicines Held on 19 to 21 December 2011 at Colombo, Sri Lanka.
- Presented a paper entitled "In vitro callus induction and shoot regeneration in Stevia rebaudiana (Bert) "- an important anti-diabetic medicinal plants" by Thiyagarajan, M. and Venkatachalam, P. in the American Society of Plant Biologists held on February 26, 2011 at California State University, Long Beach.
- Presented a paper entitled "In vitro plant regeneration from rizhiome explants of Alpinia officinarum an anticancer medicinal plants" Kayalvilizhi, T., Thiyagarajan, M and Venkatachalam, P. in the American Society of Plant Biologists held on February 26, 2011 at California State University, Long Beach.
- 13. Presented a paper entitled "An efficient protocol for *in vitro* shoot multiplication and plant regeneration from rhizome of kali musli (*Curculigo orchioides* Gaertn.): An endangered anticancer herb" By Kayalvizhi, T. Thiyagarajan, M. and Venkatachalam, P. in the 2<sup>nd</sup> International Seminar on seminar on "Medicinal Plants and Herbal Products" held on 27-29 December 2010 at Sri Venaktaswara University, Tirupati.
- 14. Presented a Research paper entitled "Rapid and large scale *in vitro* propagation of *Stevia rebaudiana* (Bert): An antidiabetic medicinal plant." by Thiyagarajan, M. and Venkatachalam, P. in the National Conference on Herbal Medicine held on 8<sup>th</sup> & 9<sup>th</sup> September 2010 at Bharathiar University, Coimbatore.