TRAINING COURSE IN VITRO CLONAL PROPAGATION OF TROPICAL PLANTS

The aim of this training is to provide the techniques of plant tissue culture to mass Clonal propagation of tropical plants, with special emphasis in the scaling up of production with commercial purposes.

By the end of this course the trainees will have the basis for implementing and management of a commercial plant tissue culture facility (Biofactory).

The students will receive at the beginning of the training, a CD with the conferences and presentations, a list of reference materials and the PDFs of key articles and general information.

1. Objectives of the Technical Cooperation Program

   1.1. Theoretical and practical training of English-speaking Caribbean countries specialists, in the techniques of tissue culture.
   1.2. Identification and following up of projects of main importance (two proposals)

2. Contents (see annex 1)

3. Profile of participants

   i. Agricultural engineers, degree in biology, biochemistry, microbiology or related field, with basic knowledge of anatomy, genetics and plant physiology.
      a. The candidates should have at least some basic experience of work on plant tissue culture
      b. The candidates and the institution their represent, must have a genuine interest in the field and will continue their training and use the acquired knowledge and experience to work in propagation laboratories and contribute, through biotechnology, to solve agricultural problems in the region.
   ii. After receiving the proposals made by the Ministers of Agriculture of the countries included in the call, with their respective CVs, the definite acceptance will be informed with a survey regarding the main interests, crops, etc.
   iii. According to the profile of the participants and the results of the survey, the program of the training course will be customized in consequence.
4. Planned Activities

i. Course in English-speaking Caribbean country to train specialists in the

ii. theory and practice of commercial multiplication in vitro of tropical species

iii. of interest.

iv. On the Job training of selected participants in the Biofactory of CICY.

v. Technical assistance for Capacity Building in the selected laboratories.

5. Duration

**Stage I:**
- Compilation of the list of materials that are needed for course delivery
- Check protocol for transferring samples and supplies.

**Stage II:**
Visit to Trinidad and Tobago and Jamaica to:
- Browse conditions and costs to develop the theoretical and practical course and logistical feasibility.
- Identify technical assistance for Capacity Building of the visited laboratories.
- Comparison of conditions and costs for course development in Trinidad & Tobago, Jamaica or in the Scientific Research Centre of Yucatan (CICY).

**Stage III:**
- Preparation of a manual that will form the basis of the course, and specialized multimedia video (all in English). 6 weeks

**Stage IV:**
- Theoretical and practical course. 2 weeks (10 days actually).

**Stage V:**
- On the job training of selected participants and development programs of technical assistance to the Caribbean by the instructors. 6 weeks for in the job training and four weeks for the country assistance (two weeks per country)

**Stage VI:**
- Evaluation report and process results. 4 weeks
6. **Evaluation**
   Learning assessment method considered in the program.
   • A theoretical course evaluation will be conducted
   • Each participant must submit a proposal of interest to his country, to be presented the final day of the training course (June 26th)

7. **Proposed Implementation Schedule**
   i. Preparation of the proposed course in English and its corresponding call.
   ii. (March-April 2015)
   iii. Preliminary exploratory visit at least 2 countries in the region to identify specific demands on the issue and potential and willingness to accommodate
   iv. the theoretical and practical course. (April 2015)
   vi. Preparation of documents and biological materials for the course and call,
   vii. proposals and selection of participants. (April-May 2015)
   viii. Theoretical and practical training course. (June 2015)
   ix. Advising selected laboratories in the Caribbean (April-September 2015)
   x. Final evaluation of the project. October 2015

8. **Important Dates**
   8.1. The participants should arrive on Sunday June 14th, 2015.
   8.2. The main theoretical course: June 15th - June 20th
   8.3. Conclusion of theory and all practical activities: June 22th - June 25th
   8.4. Evaluation: June 26th

9. **Logistics**
   i. The course will be held in University of West Indies, St. Augustine Campus,
   ii. Port of Spain, Trinidad and Tobago.
   iii. The professors and students will be accommodated on the University inn of
   iv. St. Augustine Campus, in respective accommodations facilities.
   v. The National Focal Point for the course will be
   vi. Dr. Isaac Bekele. Dean of the Faculty for Food and Agriculture, UWI, St.
   vii. Augustine Campus. Phone: +1 868 371 9818 ext. 82112. Email:
   viii. isaac.bekele@sta.uwi.edu
10. Other

i. The course participants will be 22 people
   a. 13 people per CARICOM English speaking countries (one each)
   b. 2 extras for Ministry of Agriculture of Trinidad & Tobago as Host country
   c. 1 Tobago
   d. 1 CARDI
   e. 1 Bahamas Agriculture and Marine Science Institute
   f. 1 Center of Biotechnology, UWI, Mona Campus, Jamaica
   g. 2 UWI, St Augustine Campus, Trinidad & Tobago
   h. 1 Coconut Industry Board, Jamaica

ii. From CICY will participate 4 instructors (3 professors and 1 technician during the practical)
Annex 1. Detailed Contents of the course
PART I THE FUNDAMENTALS OF TISSUE CULTURE (10h)

1.1-Introduction
• What is tissue culture?
• Brief history
• Basic biological principles
• Structure of plant tissues
• Cell differentiation
• Totipotency

1.2-The basic procedures of Tissue Culture
• Culturing under aseptic conditions
• The laboratory
• Sterilization
• The air flow cabinet
• Selection of the explant
• Leaves
• Stems
• Apical meristems
• Microspores and anthers
• Seed embryos
• Disinfestation procedures
• Composition of the culture media
• Macronutrients
• Micronutrients
• Vitamins
• Growth regulators
• Gelling agents
• The five stages of tissue culture according to Murashige and Debergh
  • Stage 1: Initiation
  • Stage 2: Multiplication
  • Stage 3 Elongation and Rooting
  • Stage 4: Transfer to soil and acclimatization
  • Stage 0: Pre-conditioning of stock plants
• Culture systems
• Semi-solid
• Liquid
• Temporary immersion
• Culture pathways
• Dedifferentiation
• Callus culture
- Single cell (suspension) culture
- Organogenesis
- Meristem culture
- Adventitious shoot formation
- Embryogenesis
- Anther and pollen culture

1.3. Organogenesis
- Ground, competent and differentiated states
- Wounding
- Caulogenesis
- Rhyzogenesis

1.4. Somatic embryogenesis
- The origin and structure of seed embryos
- Somatic embryos
- Single cell origin
- Direct embryogenesis
- Indirect embryogenesis
- Secondary embryogenesis
- Embryo development

1.5. The effect of environmental factors on cultured cells and tissues
- Light
- Temperature
- pH
- Humidity

1.6. The use of growth regulators, their antagonists and inhibitors in tissue culture
- Auxins
- Cytokinins
- Gibberellins
- Ethylene
- Abscisic acid
- Other

1.7. Microbial contamination of cultures
- Methods to control microbial growth in culture
- Antibiotics
- PPM
- Vitrofural
- How to deal with endophytic bacteria

1.8. Other problems
- Genetic instability (Somaclonal variation)
- Hyperhydricity (vitrification)
• Ontogenic age
• Recalcitrance

1.9.- **General applications of tissue culture**
• Micropropagation
• Virus elimination
• Thermotherapy
• Meristem micrografting
• Genetic improvement
• Clonal propagation of elite materials
• Induction of new genetic variability
• Genetic rescue
• Germplasm conservation
• Slow growth
• Low temperature methods

1.10.- **Applications to some economically important crops**
• Industrial crops
• Fruit crops
• Roots and tubers
• Trees
• Agaves
• Ornamentals

**PART II THE APPLICATION OF TISSUE CULTURE FOR LARGE SCALE MICROPROPAGATION OF HIGH QUALITY MATERIALS (10 H)**

1. Scaling production and development of commercial laboratories (Biofactory)
   1.1. Basics of the Scaling up
   1.2. Plant Tissue Culture Scaling up
   1.3. Layouts
   1.4. Production flow
2. Good Manufacturing Practices (GMP)
   2.1. Principles
   2.2. GMP in a Commercial Tissue culture facility
   2.3. Integral system of assurance and quality control
   2.4. Standard Operating Procedures (SOPs)
3. Monitoring and traceability
4. Temporary immersion bioreactors
5. Acclimation (hardening)
   5.1. Principles
   5.2. Layouts
   5.3. GMP
6. Economy of scaling up

PART III PRACTICAL ACTIVITIES (20h)

Study plant (banana)
3.1.-Selection of elite healthy mother plants and their care and preparation
3.2.-Preparation of culture media and sterilization
3.3.-Working under aseptic conditions in laminar flow cabinets
3.4.-Getting explants
3.5.-Implantation
3.6.-Multiplication
3.7.-Rooting
3.8.-Removing plants from culture vessels and pass to ex vitro phase
3.9.-Greenhouse Acclimation and shade house.
3.10.-Packing and shipping

PART IV LITERATURE SEARCH AND SMALL PROJECT (20h)