ABSTRACT

American Pacific consist group of islands scattered across the vastness of the Pacific Ocean in the western and central region between Japan and Hawaii. The Commonwealth of Northern Marianas, Guam, Palau, Marshall Islands and Federated States of Micronesia are located in central and western pacific, whereas American Samoa is in further south. In recent years, PGRs playing important role towards agriculture development in the island groups through cell and tissue culture techniques mainly with the assistance received from the United States Department of Agriculture’s Cooperative Research, Extension and Education Service. Tissue culture was introduced in the region a decade ago and has contributed significantly in crop production and improvement. Main emphasis of the application has been in-vitro regeneration and multiplication of disease free and quality planting material of economically and culturally important crops such as root and tubers, fruits, vegetables and ornamentals. In-house protocol of regeneration and mass multiplication has been developed for the various crops using various growth regulators, media and cultural conditions. The protocols developed worked well in bananas, taro, sweet potato and other agriculture crops. Several new varieties introduced into the islands through regional institutions, propagated through tissue culture and have adapted well into the local soil and climate conditions. This paper summarizes the micropropagation of taro, sweet potato and banana with special reference to the Northern Mariana Islands.

INTRODUCTION

The Commonwealth of the Northern Mariana Islands (CNMI) is located north of Guam in the Western Pacific. Banana, sweet potato and taro are economically important food crops and highly valued for cultural, dietary, and agriculture production in the CNMI (Nakamoto et.al, 1996). Traditional planting methods using shoot apex are time consuming and labor intensive. High production costs due to increased labor and management, spread of soil-born diseases and pests, limited genetic diversity and shortage of quality planting material are the major constraints of agriculture production in the CNMI (Nandwani et al. 2007. Tissue culture is considered the most potential system to achieve the goals of producing quality and disease-free planting material (Taylor 1998). In 2006, Northern Marianas College’s Cooperative Research, Extension and Education Service (NMC-CREES) initiated tissue culture program for the production of disease-free and quality propagating materials. The objective of the program is to develop in-house protocol of in-vitro regeneration and multiplication of banana, taro and sweet potato, conduct variety trials and select high-yielding, drought, disease- and insect pest-tolerant varieties with good eating qualities. Micropropagation of selected cultivars through tissue culture conducted and distributed to the local farming community.
This review presents the procedure for the rapid, high frequency in-vitro regeneration of plantlets in banana, sweet potato and taro in the Northern Mariana Islands.

**MATERIALS AND METHODS**

Tissue culture plants of selected promising accessions of banana, taro and sweet potato were imported from the Centre for Pacific Crops and Trees (CePCT), Secretariat of the Pacific Community (SPC), Fiji, which is a regional institution for the distribution of germplasm in the Pacific. Upon receipt of package, plantlets were transferred to plastic bags with commercial potting mix (ACE Hardware, Saipan) and kept in the nursery (57% shade) for 2-3 weeks for hardening and acclimatization. Well hardened plants were transferred to the field at the As Perdido Agriculture Experiment Station, Saipan. Shoot-tip explants (apical buds) and nodal segments were collected from the tissue culture plants of three crops grown at the As Perdido Agriculture Experiment Station in Saipan. The explants were first washed twice with liquid soap and rinsed thoroughly with distilled water followed by surface sterilization using 0.1 % (W/V) mercuric chloride for 5-6 minutes. Decanting of mercuric chloride was followed by repeated washed (4-5 times, 3 minutes each) by autoclaved distilled water. The surface sterilized explants were cut into 1.5-2.0cm. The explants were inoculated on MS (Murashige and Skoog 1962) medium supplemented with various concentration and combination of growth regulators. Protocol of regeneration and multiplication was adopted from the published reports in banana, taro and sweet potato and developed in laboratory conditions. Growth regulators were obtained from Phytotechnology Laboratory, USA. MS liquid and semisolid medium supplemented with Benzyl Adenine (BA), Kinetin, Indole 3-Acetic Acid (IAA), 1-Naphthalene Acetic Acid (NAA) and Adenine Sulphate in range of concentrations. MS medium supplemented with BA, and Kinetin with adenine sulphate were used for multiplication and elongation of shoots. The regenerated shoots (25-30 mm) were excised and sub cultured on MS medium free of growth regulators and MS medium containing different concentration of 1-Indole Butyric Acid (IBA) were used for root induction. Sucrose was added in the media as carbon source at 30g/l. pH of media was adjusted to 5.8 with 1N NaoH and without Agar-agar (Phytotechnology laboratories, USA) was added 8g/l for semisolid media. Glass bottles (200ml) with polypropylene lids were used as culture vessel for initiation and multiplication of cultures. Each glass jars/bottles poured with 25ml media. All the media, glass-wars, forceps and knife were autoclaved at 121°C (20psi.) for 20 minutes. The cultures were incubated in a growth room under16 hrs photoperiod in 2500-3000 lux light intensity and 25°C temperature. At the end of the multiple shoot production cycle individual shootlets were excised and transferred to semisolid BMS containing IBA (1.0mg/l). Well-developed single plantlets removed from the culture vessel washed carefully in running tap water to remove the adhered agar in roots. Plantlets transferred to pots containing sterilized promix and soil (1:1) covered with polybags for hardening. Transferred plantlets were lightly irrigated and maintained under high humidity for a fortnight. Established plantlets were transplanted with polybags (9” x 5”) containing the same potting mix and weaned in greenhouse. Normal irrigation was carried until plantlets were distributed to the farmers.
RESULTS AND DISCUSSION

Summary of the micropropagation in three crops banana, taro and sweet potato is given in Table 1. Islands’ favorable environment for the growth of microorganisms, contamination was a major hurdle in the establishment of aseptic cultures. Shoot tip explants of taro transferred on the medium begun to develop after 2-3 weeks’ culture. One or two buds regenerated from one shoot tip. After 4 months of culture, about 80% of isolated tips regenerated shoots and roots on the same medium (Fig. 1-3). Protocorm-like bodies were produced on MS medium with IAA (1.0 mg/l) and Kinetin (1.0 mg/l) without shoot formation. The protocol of regeneration from shoot tips of taro was adopted from the Matsumoto’s work conducted on dasheen taro (*Colocasia antiquorum* Schott). In sweet potato (*Ipomea batata*), a maximum of 85% explants were showed shoot regeneration on MS medium supplemented with BAP (1.0mg/l). By further increasing the concentrations of PGRs in MS liquid medium up to 5.0mg/l BA, percentage of shoot induction potential of explants were reduced from each explant. Shoot buds were initiated within 18 to 21 days from the date of inoculation of explants. The shoot buds attained a length of 29mm in 30 days incubation period in growth room. Further multiplication of *in vitro* shoots was achieved by repeated harvesting of micro shoots and re-culturing of same explants on fresh MS semi-solid medium containing 2.0mg/l BA (Fig. 4-6). Auxin in the medium promoted callus formation in the cultures. Spontaneous rooting was observed or on MS medium free of growth regulators in taro and sweet and sweet potato. The maximum percentage (95%) of rooting was achieved on basal MS medium in 75% shoots with callus formation. The *in vitro* plantlets were successfully subjected to hardening on small earthen pots containing a mixture of potting mix (ACE Hardware, Saipan) under relative humidity (60-70%) and at temperature (28ºC-32 ºC) for 21 days. At least 10% mortality rate observed in the plantlets transferred for the hardening and acclimatization. These plants were then transferred to the field conditions.

Micropropagation of banana (var. Cavendish) is shown in Fig. 6-9. Shoot-tip explants of banana were initially on stationary liquid medium supplemented with BA (2.5 mg/l) and Kinetin (2.5 mg/l) and NAA (0.1 mg/l). In a week, explants swelled a little, outer leaf sheaths and surface of explants turned brownish. Additional of activated charcoal (2.0 gm/l) was found suitable to overcome the problem of browning and phenolics. The curved superficial and overlapping leaf sheaths carefully exercise under a microscope to expose apical meristem (shoot apex). The apical meristem measuring 5mm in diameter was excised and transferred to semisolid MS medium of the original composition. About four weeks later, the explant swells up, turns into green showing morphogenic activity. Initially tiny, cream-greenish protuberances begin to appear which eventually developed in large shoot clusters with leafy structures in eight weeks. Induction of multiple shoots in var. dwarf Cavendish was observed under various combination of growth regulators (data not shown). Maximum 8-10 shoots could be achieved on MS containing NAA (0.1 mg/l) and Kinetin (2.5 mg/l). Addition of adenine sulphate (100mg/l) promoted shoot bud induction. Multiple shoots were subculture on fresh medium generated high numbers of populations in subsequent cycles of weeks each. Extending eight or nine subcultures showed vitrification and morphological variation in shoots. However, MS medium supplemented with IBA (1.0 mg/l) was found optimal for root formation. Rooting was induced within a week nearly 90% rooting achieved. In-vitro produced plantlets performed well in field conditions after hardening.
Micropropagation of taro (Chand et al., 1999), sweet potato (Cantliffe et al., 1992) and in various cultivars of banana through shoot-tip explants (Pandey et al., 1993; Sudhavani and Reddy, 1997) is well documented. In almost all cases, different combinations of cytokinin and auxin in various concentrations were reported for multiple shoot regeneration. The physiological state of explants, seasonal and cultivar difference are the reasons, perhaps that different workers have reported different media for plantlets regeneration in banana. Rooting in regenerated shoots has been achieved modified medium supplemented with or without an auxin. In present case spontaneous root formation in shoots was observed, however, basal MS medium containing IBA (1.0 mg/l) was found suitable for rooting.

Use of PGRS in commercial farming is very limited in the American Pacific, however, various plant growth regulators being used through tissue culture programs at various USDA Land Grant institutions (Table 2). Various reports on micropropagation in agriculture crops are available from the Republic of the Marshall Islands (Nandwani et al., 2003), Federated States of Micronesia (Josekutty et al., 2001, 2003), Republic of Palau (Del Rosario, 2003), CNMI (Nandwani, 2009), Guam (Marutani, unpublished) and American Samoa (Fred Brooks, unpublished). Advantages of tissue culture is well-recognized such as plantlets are: i) uniform planting material, ii) clean and free of diseases, iii) planting material can be generated in large volumes for any planting season and farm size and iv) high yielding and quality corms (Morishita 1988). The in house protocols developed in the laboratory enables production of several plants from single explants by repeated re-culture of same explants on fresh medium to make available micro-shoots for rooting. The technique outlined in this communication being gainfully employed for pathogen-free and large-scale production of banana, taro and sweet potato plants in local and introduced varieties in the CNMI. The protocols reported in present paper are highly reproducible and has been successful for the propagation, conservation and also for improvement of the crops.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Explant</th>
<th>Medium</th>
<th>Growth Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana (Musa sp)</td>
<td>Shoot-tip</td>
<td>MS + Kinetin (2.0mg/l) + NAA (0.1mg/l)</td>
<td>Plantlets formation</td>
</tr>
<tr>
<td>Taro (Colocasia esculenta)</td>
<td>Shoot-tips (apical and axillary buds)</td>
<td>MS + IAA (1.0mg/l) + Kinetin (1.0mg/l)</td>
<td>Plantlets formation</td>
</tr>
<tr>
<td>Sweet potato (Ipomea batata)</td>
<td>Shoot-tips (apical buds), Nodal segments</td>
<td>MS + BA (1.0mg/l)</td>
<td>Callus, Plantlets formation</td>
</tr>
</tbody>
</table>
Table 2: Tissue Culture Program at the USDA Land Grant Institutions in the American Pacific

<table>
<thead>
<tr>
<th>Institution*</th>
<th>Crops</th>
<th>Program Initiated (Year**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Northern Marianas College, Saipan, Northern Mariana Islands</td>
<td>Banana, Taro, Sweet potato</td>
<td>2006</td>
</tr>
<tr>
<td>2. College of Micronesia, Pohnpei, Federated States of Micronesia</td>
<td>Banana, Taro, Sweet potato, Citrus, Citrus</td>
<td>1997-98</td>
</tr>
<tr>
<td>3. College of the Marshall Islands, Majuro, Republic of the Marshall Islands</td>
<td>Banana, Taro, Sweet potato, Breadfruit</td>
<td>1999</td>
</tr>
<tr>
<td>4. Palau Community College, Koror, Republic of Palau</td>
<td>Banana, Taro, Sweet potato, Yam, Cassava</td>
<td>2002-03</td>
</tr>
<tr>
<td>5. American Samoa Community College, American Samoa</td>
<td>Taro, Sweet potato</td>
<td>2003-04</td>
</tr>
<tr>
<td>6. College of Agriculture and Natural Sciences, University of Guam, Guam</td>
<td>Orchids, Vegetables, Banana, Taro, Sweet potato</td>
<td>2000-01</td>
</tr>
</tbody>
</table>

*University of Hawaii is not included
** Tentative in some cases

LITERATURE CITED


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Micropropagation of economically important crops of the Northern Mariana Islands

Fig. 1: Taro explant
Fig. 2: Shoot bud induction and elongation
Fig. 3: Rooting
Fig. 4: Shoot bud induction
Sweet potato
Fig. 5: Rooting on basal MS medium
Fig. 6: Hardening and Acclimatization (2 weeks)
Fig. 7: Shoot bud induction in banana
Fig. 8: Multiplication & Rooting
Fig. 9: Well-hardened plants