OPTIMIZATION OF TISSUE CULTURE PROTOCOLS FOR COST-EFFECTIVE PRODUCTION OF DRACAENA, BAMBOO, AND SUCCULENT PLANTS

Master’s Defense
Lee C. Chaille
Spring 2011
Outline

❖ Introduction

❖ Research Project Goals
  ➢ Cost-effective production system
    ■ Dracaena
      ■ Methods
      ■ Results
      ■ Commercial Application
  ➢ Optimization of Tissue Culture (TC) protocol
    ■ Bamboo
      ■ Methods
      ■ Results
      ■ Commercial Application
  ➢ Innovation/Added value products
    ■ Succulent Plants/ Echeveria
      ■ Methods
      ■ Results
      ■ Commercial Application

❖ Conclusion
Introduction

- Model Production/Successful TC labs
Introduction

- Application of Commercial Tissue Culture (TC):
  - Cloning allows for aseptic propagation of genetically identical plants

- US Tissue Culture laboratory limitation:
  - High cost of production
Research Project Goals

How to create a Cost-effective TC system? (Globally Competitive)...

1. Decrease cost of production
   - Streamline production towards automation (Bioreactor)

2. Optimized protocol development
   - Embryogenesis vs. Organogensis (TC Pathways)

3. Innovation in Business Modeling
   - Liner and added value specialty TC products
COST-EFFECTIVE TC SYSTEM
Why Cost-Effective Production?

- In European labs, labor accounts for 60-70% of expenses required for in-vitro plantlet production (FAO 2002)
- Conventional micropropagation via the organogenesis pathway requires continual sub-culturing and requires skilled technicians for explant grading (Ibaraki & Kurata, 2001)
Research Approach

- Temporary Immersion Bioreactor
  - Reduction of production costs by 10x (coffee)
  - Improved Development of Somatic embryo’s (rubber tree)
  - Production cost decreased as more vigorous plants were produced in a shorter time (eucalyptus)

http://www.vitropic.fr/rita
Model Plant for Study...

- *Dracaena deremensis* ‘Lisa’
Production Trialing

- 3-shoot multiplying explants were segregated into semi-solid and bioreactor systems.
- MS basal medium supplemented with 3% sucrose, 2.0 mg/L Kinetin (Kn), and 0.2 mg/L Indole-3-Acetic Acid (IAA) for eight weeks.
- At 4 week intervals explants were sub-cultured
## Cost Comparison: 2 Production Systems

<table>
<thead>
<tr>
<th>Estimated Cost of Production</th>
<th>8-week production cycle (2 transfers)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
<td>RITA Bioreactor</td>
</tr>
<tr>
<td>Explants (3-shoot clusters)</td>
<td>40</td>
</tr>
<tr>
<td>Total media (liters)</td>
<td>2</td>
</tr>
<tr>
<td>Total media costs</td>
<td>$1.80</td>
</tr>
<tr>
<td>Transfer time (minute/explant)</td>
<td>1.4</td>
</tr>
<tr>
<td>Technician wage (per hour)</td>
<td>$12.00</td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td></td>
</tr>
<tr>
<td>Multiplication rate</td>
<td>3.1</td>
</tr>
<tr>
<td>Total shoot production</td>
<td>368</td>
</tr>
<tr>
<td><strong>Cost of Production</strong></td>
<td></td>
</tr>
<tr>
<td>Total cost of production</td>
<td>$24.19</td>
</tr>
<tr>
<td>Cost per shoot</td>
<td>$0.07</td>
</tr>
</tbody>
</table>
Application: New Dracaena Varieties

- ‘Waikiki’
- ‘Golden Lotus’
- ‘Haka’
- ‘Ohe’
- ‘Ruth Luka’
OPTIMIZATION OF TC PROTOCOL
Why Optimized Protocol?

- Commercial tissue culture laboratories often follow strategic culturing recipes that are applicable to multiple genera of plants.
- When developing a protocol, lower rates of explant viability or vigor, may be experienced throughout production (Shirin and Rana 2007).
- Published protocols are often difficult to repeat.
Research Approach

- Problem Solving via a two-pronged approach: Organogenesis vs. Embryogenesis
- Step by step development of media for each production phase
- Establish flow charts for repeatable TC protocols
- Track cost of production by monitoring inputs and compare explant growth for TC pathways
Model Plant for Study…

- *Bambusa ventricosa ‘Buddah Belly bamboo’*
Protocol Development: (Organogenesis)

- Production Obstacles:
  - Phenolics
  - Tissue Browning
  - Vitrification
  - Decreased Vigor
  - Insufficient rooting

- Critical pathway bottleneck
  - Pathway is jammed
Protocol Development: (Organogenesis)

- Research Approach:
  - Basal Salts
    - M&S/Lloyd& McCown/Anderson
  - Plant Growth Regulators
    - Multiplication/Rooting
  - Additives
    - Activated Charcoal/ Ascorbic Acid
  - Bioreactor System vs. Semi-solid
  - Transfer Frequency

- 4-Step sub-optimal Protocol

<table>
<thead>
<tr>
<th>PGRs (mg l⁻¹)</th>
<th>Bud Elongation</th>
<th>Multiplication</th>
<th>Root Induction</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>3.0</td>
<td>2.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td>2.0</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>IBA</td>
<td>4.0</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 2 3 4
Protocol Development: (Embyrogenesis)

- Production Obstacles:
  - Limited literature
  - Callus browning
  - Embryogenic competency
  - Practical inexperience
Protocol Development: (Embyrogenesis)

- Research Approach:
  - Basal Salts
    - M&S/Lloyd&McCown/DKW
  - Plant Growth Regulators
    - 2,4-D Concentration/Cytokinin:Auxin
  - Light
    - Full/Diffused/Darkness
  - Culture Assessment
    - Embryogenic Callus

- 4-Step optimized protocol

<table>
<thead>
<tr>
<th>PGRs (mg l⁻¹)</th>
<th>Bud Elongation</th>
<th>Callus Induction</th>
<th>Callus Proliferation</th>
<th>Embryo Maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td></td>
<td>3.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>BAP</td>
<td>3.0</td>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>IBA</td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Kinetin</td>
<td></td>
<td>2.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

1 2 3 4
Embryogenesis Production Flow Chart

Bud Elongation - week 4

Callus Induction - week 12

Callus Proliferation - week 30

Embryo Maturation - week 36

Shoot Elongation - week 40

In-Vitro Finishing - week 44

Acclimatization - week 50
## Production Comparison: 2 pathways

<table>
<thead>
<tr>
<th>Estimated Cost of Production</th>
<th>Inputs</th>
<th>12-month production cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial explants</strong></td>
<td>60</td>
<td>190</td>
</tr>
<tr>
<td><strong>Transfer time (minute/explant)</strong></td>
<td>0.70</td>
<td>1.50</td>
</tr>
<tr>
<td><strong>Subcultures</strong></td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td><strong>Technician wage (per hour)</strong></td>
<td>$12.00</td>
<td>$12.00</td>
</tr>
<tr>
<td><strong>Total media (liters)</strong></td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td><strong>Media cost (per liter)</strong></td>
<td>$1.55</td>
<td>$1.50</td>
</tr>
</tbody>
</table>

### Clonal Production

| Explant viability | 55%   | 17%   |
| Rooted plantlets  | 33    | 32    |

### Cost of Production

<table>
<thead>
<tr>
<th>Cost of Production</th>
<th>Subculture labor</th>
<th>Media</th>
<th>Cost per plantlet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$134.40</td>
<td>$18.60</td>
<td>$4.64</td>
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<tr>
<td></td>
<td>$1482.00</td>
<td>$57.00</td>
<td>$48.09</td>
</tr>
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</table>
Applications: Optimized Protocol

- Cost-effective production utilizing the embryogenesis pathway with a bioreactor system to further reduce costs
- Production of bamboo species via embryogenesis pathway for:
  - Biomass/Timber/Windbreak/Ornamental/Pharmaceutical/Edible/CO2 sequestration..
  - New agribusiness industry in Hawaii
INNOVATION IN BUSINESS MODELING
Why Innovation in Business Modeling?

- TC labs in India produce over 200 million TC plants per year. Over 90% of which are exported (Shukla 2010).
- Estimates of market growth exceed 20% per year (Shukla 2010).
- US model TC companies offer only two products (Liners and stage III microcuttings).
- Purchases may be limited to brokered sales and bulk shipments.
Research Approach

- Produce added value specialty TC products
- Market high end products with attractive pots and new rotations of TC plants
- Market low maintenance hybrid succulents
Model Plant for Study...

- *Echeveria gibbiflora*
Innovation in Business Modeling

- Specialty added value TC products:
  - Inexpensive, decorative clay pots collected in China
  - Attractive succulent plants
  - Acclimatization and green house production
  - Dry-run marketing and sales at UH
Successful Dry-Run?

<table>
<thead>
<tr>
<th>Input Costs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>150 plants</td>
<td>$150</td>
</tr>
<tr>
<td>48 Pots</td>
<td>$75</td>
</tr>
<tr>
<td>½ bag Pro-mix</td>
<td>$26</td>
</tr>
<tr>
<td>10 Liner trays</td>
<td>$13</td>
</tr>
<tr>
<td>total</td>
<td>$264</td>
</tr>
</tbody>
</table>

- **Business Profitability**
  - Gross = $420
  - Gross – Costs = Net ($156)
  - Net/Costs = %Profit
  - Net ($156)/Costs ($264) = %profit
  - 59% Profit

- **Sales goals achieved in one 5 hour day**
Application: Innovation in Business Modeling

- Opportunity for trend setting companies specializing in product presentation as well as added value marketing for TC plants
- Industry niche focusing on attracting new potential market with selective TC plants
- Merging of different industries for common goal (clay pots and small plants in unique arrangement)
Conclusion

- Cost-effective production was exhibited with the utilization of the RITA bioreactor by decreasing costs by $\frac{1}{2}$ in *Dracaena deremensis*.
- Cost of production per plantlet was reduced by 10x when explant development was achieved by protocol optimization via the embryogenesis pathway in *Bambusa ventricosa*.
- A profit margin of 59% was achieved by utilizing an innovative business model in which decorative clay pots in conjunction with TC succulent plants were sold at UH
References


Acknowledgements!

Advisors and Professors:
Dr. Kheng Cheah
Dr. Kent Kobayashi
Dr. Joe DeFrank
Dr. Tessie Amore
Dr. Michael Tanabe

Fellow Grad Students:
Peter Toves
Peter Wiggins
Scott Lukas

Office Ladies:
Shirley & Susan

My Family & My Sweet Pea

Thank You!