Moving tissue culture plantlets and hardening protocols

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Introduction

Main problems facing sweetpotato farmers

- ❑ Scarcity of healthy planting material (Marando Bora)
- ❑ Pests and diseases damaging the crop and reducing root yield

Sourcing of planting material from own fields

- Results in delayed planting /sprouts regenerate after the start of the rains / need at least a month of growth before harvesting

- Characterized with high sweetpotato virus disease infections due to repeated planting of the same material season after season

What is tissue culture ?

- To clean particular plants of viral and other infections and to quickly multiply these plants as cleaned stock
- The process involves the following :

  -Cleaning of the mother plant (virus elimination) – use of thermotherapy chamber/growth chamber- temperature 30-40 ºc; humidity 80%; light intensity 10,000 lux for 16hrs- 21 days

  -Sourcing of the ex-plants from the cleaned mother plant (preferably the meristem)

What is tissue culture ?

-Initiation of the ex-plant in MS media

-Generation of a few plantlets

- Testing the plantlets for virus, if found negative inoculate single nodes in the MS media for mass multiplication

What is hardening ?

- Nursing of the plantlets for final transfer to the field beds
- High humidity inside the culture vessels- keeps the cuticles less developed; stomata does not operate properly, sometimes misinformed or totally absent
- It is necessary to aid plantlets to initiate development of cuticles, stomata/root functions before transferring to the field growth complex environment

What was the task?

- To help farmers obtain clean planting material at the right time of planting
- CIP in collaboration with LZARDI/ KEPHIS/GTIL cleaned planting material of selected varieties
- CRS and its affiliated community development implementing partners were to do further multiplication and dissemination to farmers

Record of delivery of plantlets from Nairobi to Maruku and percent mortality during hardening

Lot	# of plantlets	Estimated % mortality	Comments
1	300	< 5	KEPHIS and CIP initiated hardening at Maruku
2	20,000	10	KEPHIS and CIP participated
3	15,600	40	KEPHIS and CIP participated. Mataya and Kiegea >98% loss, Kabode 60%, Ejumula 10%
4	3,500	> 60	KEPHIS and CIP only delivered in Mwanza. Kiegea 96% loss

What were the major challenges

- Slow multiplication rate of some varieties in the growth media
- High rate of contamination- due to high number of plantlets to be cultured within a short notice
- Bulky containers/boxes for packing the materials
- Long distance for shipment- 1500 km from Nairobi to Bukoba

What happened? Who should answer ?

SASHA
Sweetpotato Action for Security and Health in Africa

Different container shapes and labeling

Good results: How did we do it?

SASHA
Sweetpotato Action for Security and Health in Africa

Arrived when dead and could not perform well when potted

Emerging questions

- What could have happened at the different phases of delivery that resulted into the varying levels of mortality?
- How would we minimize on percent mortality ?
- How best can we share this experience of handling sweetpotato plantlets with other partners in the region ?
- Was it possible to organize for a technicians' meeting (those who directly participated in the development, transfer and hardening of the Sweetpotato plantlets) to provide the answers

Documenting the experiences- Draft manual

- “Hardening sweetpotato plantlets: A handbook for systematic management of sweetpotato tissue cultures from the laboratory to the primary field beds in East and Central Africa”

Documenting the experiences

- This draft manual - result of collaborative enthusiastic technicians from CIP, KEPHIS and LZARDI who actively succeeded in managing the chain of stages for in vitro sweetpotato materials up to the establishment of ex-vitro primary beds that subsequently bolstered wide supply of clean planting material to farmers

Fodder :>50%

Concept of hardening in this document

- Process of gradually accustoming plants to changing environmental conditions of relative humidity, temperature, light, moisture and medium nutrition besides changing levels of contamination during a series of the different stages along the course of culturing to weaning.

Theme of the document

- "Minimizing mortality of sweetpotato plantlets during systematic phases of culturing and gradual weaning to ambient growth environment".

Main focus of the document

- Relative manipulations of the key environmental factors of percent relative humidity, the intensity of heat, intensity of illumination,
- The type and amount of agar and medium used at the sequential phases including lab, glass house, in transit, pre-transplant and final screen houses
- and then field bed to cause a nodal bud successfully grow into a plant under ambient natural environmental growth conditions.
- Along the continuum of development and systematic movement, the principles of cost-effectiveness are consciously observed without compromising the quality of the outcome.
- The document has two parts- Part one (handling of mother plants/explants to minimize contamination; Part two (pre-transplant to screen house; hardening in the screen house; transfer of the hardened plantlets to the field beds; packaging; transportation,

Where are we with the document and what is the way forward?

- The document has been internally reviewed by the main authors
- The document to be circulated to external reviewers for their inputs- December 2011/January 2012
- Meeting to review the final document before publication- March/April 2012

Net tunnels

- Height: 1.2 -1.6 m in the middle
- Length up to 5m not more otherwise stability is compromised –plant distance between rows 20cm = 5 rows per m

Thank you