Banana / plantain micropropagation – principles and practice

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Plan

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- Why banana micropropagation?
- Principle of micropropagation
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  - ✓ Explant preparation and sterilization
  - ✓ Culture initiation
  - ✓ Multiplication
  - ✓ Rooting
  - ✓ Acclimatization/ hardening (stage 1 and 2)
- Common challenges
- Conclusion and outlook
- Recommended reading

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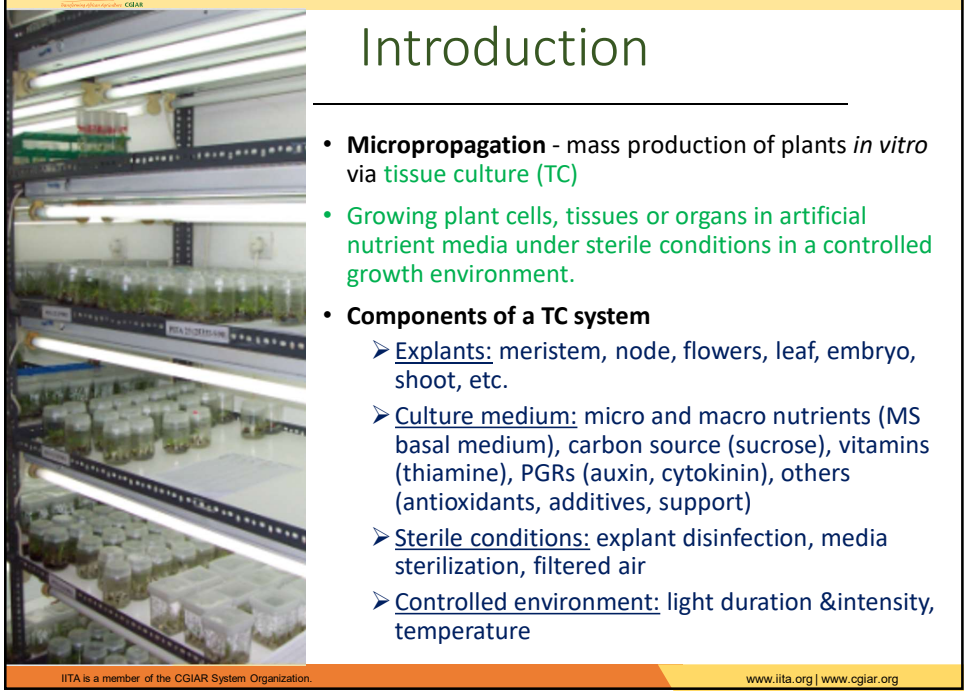
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Introduction
• Micropropagation - mass production of plants *in vitro* via tissue culture (TC)
• Growing plant cells, tissues or organs in artificial nutrient media under sterile conditions in a controlled growth environment.
• Components of a TC system
➢ Explants: meristem, node, flowers, leaf, embryo, shoot, etc.
➢ Culture medium: micro and macro nutrients (MS basal medium), carbon source (sucrose), vitamins (thiamine), PGRs (auxin, cytokinin), others (antioxidants, additives, support)
➢ Sterile conditions: explant disinfection, media sterilization, filtered air
➢ Controlled environment: light duration &intensity, temperature
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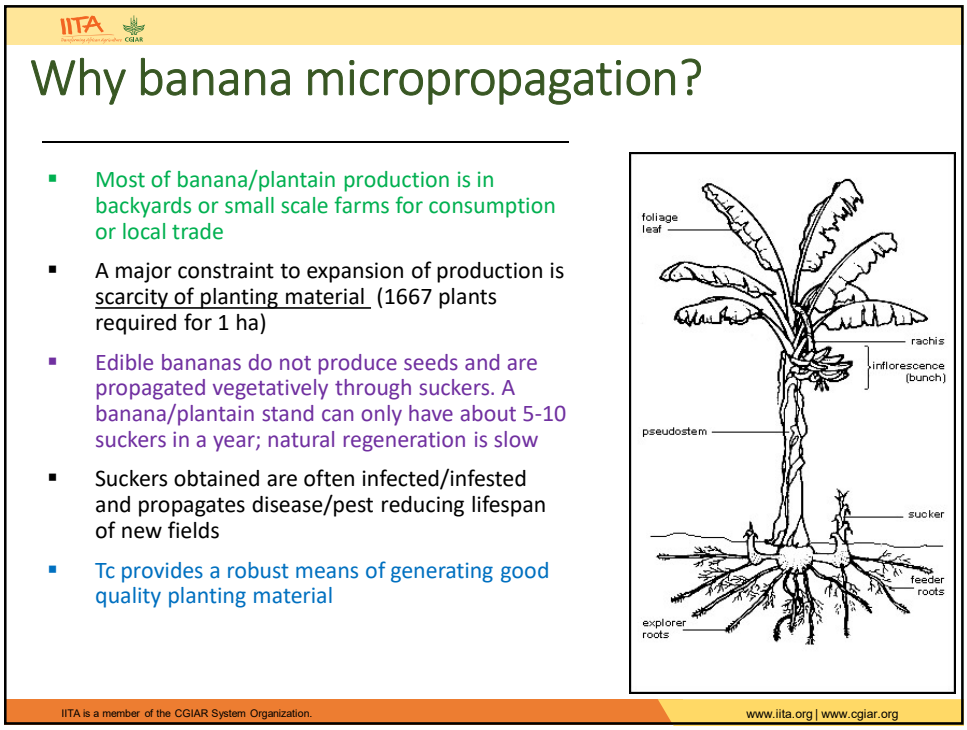
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Why banana micropropagation?
▪ Most of banana/plantain production is in backyards or small scale farms for consumption or local trade
▪ A major constraint to expansion of production is scarcity of planting material (1667 plants required for 1 ha)
▪ Edible bananas do not produce seeds and are propagated vegetatively through suckers. A banana/plantain stand can only have about 5-10 suckers in a year; natural regeneration is slow
▪ Suckers obtained are often infected/infested and propagates disease/pest reducing lifespan of new fields
▪ Tc provides a robust means of generating good quality planting material
foliage leaf
rachis
inflorescence (bunch)
pseudostem
sucker
feeder roots
explorer roots
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Conventional suckers

- Low multiplication ratio, 5-10 suckers in 12 months
- Often infected planting material
- Non-uniform planting material; staggered harvests - greater labour requirements
- Slower field establishment with lower yields
- Limited availability of planting material

TC plants

- High multiplication ratio, up to 1000 plants in 12 months
- Disease-free planting material
- Uniform planting material; synchronized harvests - less labour requirements
- Fast field establishment with higher yields
- Planting material obtained all year round

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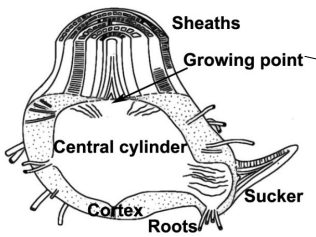
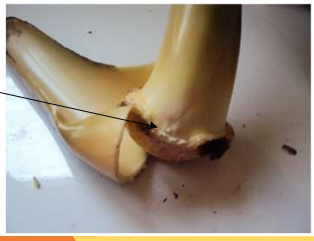
Principle of banana micropropagation

- Stimulation of multiple bud/shoot formation from apical and lateral meristems (growing points) by use of high levels of cytokinin
- A well developed plant has a basal corm with clumps of meristems of different ages / developmental stages
  - *Meristem culture* - shoot meristem tip <1mm (require microscope)
    - useful for virus cleaning, high mortality, slow establishment
  - *Shoot tip culture* - shoot tip meristem + >3 leaf primordia
    - Fast establishment – plants from apical and lateral meristems
  - Others; tip of immature floral bud





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Banana micropropagation facility

Components of a basic tissue culture unit

- Media preparation room
- Washing room
- Transfer / inoculation room
- Growth room / culture room
- Chemical storage room
- Screen house / shade house with 50% shade

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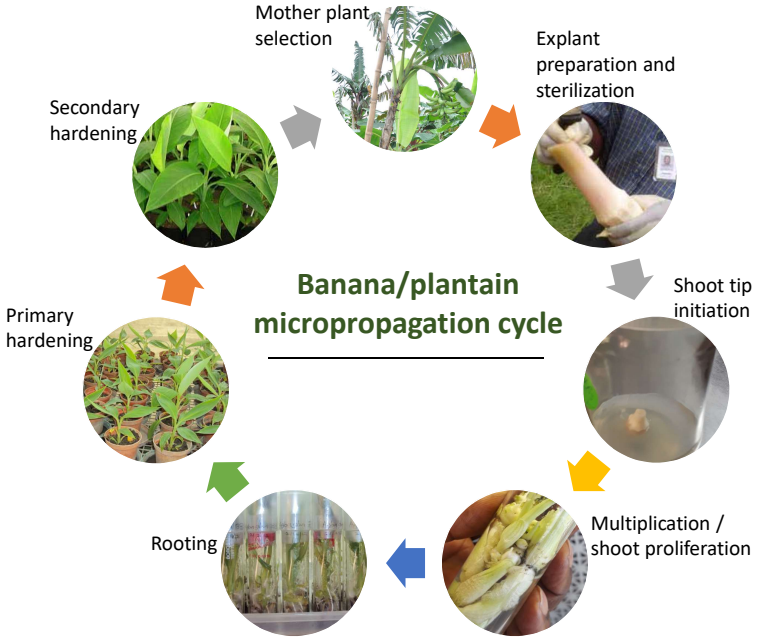

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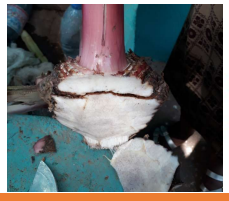
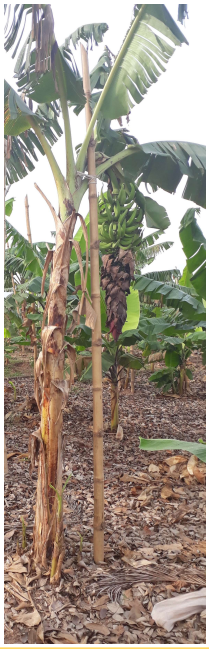
Mother plant selection

- Identify healthy, high yielding, vigorous true-to-type mother plant of desired variety (preferably after bunch emergence)
- Extract pre-flowered suckers (sword or peepers) and trim of roots and debris
- Avoid damaging as cracks are entry points for micro organisms (source of contamination)
- Wash off soil abundantly and convey labelled material to lab explant preparation area





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Explant preparation and sterilization

- leaf sheaths and basal corm tissue are removed and trimmed to a smaller piece enclosing the shoot apex
- Trimmed tissue portions containing active meristems are washed abundantly and surface sterilized
- one or two step sterilization process (alcohol, 10-15% bleach solution etc.)

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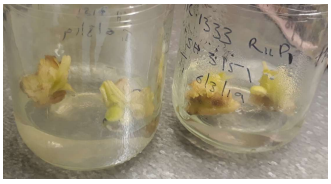
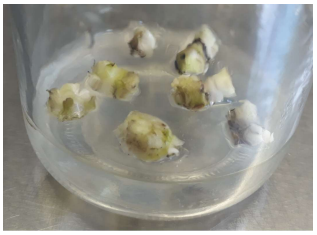
Culture initiation

- Shoot tips are aseptically extracted and placed on multiplication medium (MS + BAP)
- Bacterial and fungal indexing of initial explant stocks
- Initiated cultures are incubated in a well aerated culture room with 12-14 hrs photoperiod and 28±2°C temperature
- Explants are aseptically transferred to fresh media at 4-6 weeks interval (or less depending on tissue blackening)
- During transfers outer blackened tissue are removed and shoot tips are dissected at meristem point to stimulate lateral shoot formation



Multiplication

- Once apparent, shoot/bud clusters are dissected, separated into groups of 2-4 buds, trimmed and sub-cultured on fresh media at 3-4 weeks intervals
- # of buds/shoots increase exponentially through repeated sub culturing
- Multiplication ratio is variable and influenced by genotype, hormone concentration and level of subculture
- Level of subculture is restricted to 6-8 to avoid somaclonal variation (off types)



Rooting

- Start when sufficient culture stocks are established to meet production targets
- Individual well formed shoots with 3 or more leaves are separated, trimmed, and cultured on rooting/regeneration medium (auxin NAA or IBA, activated charcoal)
- After 4-6 weeks in culture, regenerated/rooted plants are ready for planting out/acclimatization (well developed root system and at least 4 leaves) in screenhouse
- Some labs deliver plants to clients at this stage

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Acclimatization (primary hardening)

- Carried out in a screen house with about 50% shade which provides a suitable microclimate
- Rooted plantlets removed from tubes and washed in potable water to remove agar
- Planted in sterile substrate (peat, sawdust, soil, cocoa husks etc.) in 5-10cm wide plastic or net pots, or at high density in large nursery trays.
- Potted plants incubated in humid environments (mist chambers or plastic covers on pots) for 2-3 weeks, survival usually >90%
- Some labs distribute primary hardened plantlets to clients

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Acclimatization (secondary hardening)

- Carried out in screenhouse or shade house with 50% shade
- Primary hardened plants are transferred to larger 15cm nursery bags containing pasteurized top soil or appropriate soil mixtures
- Plants are hardened for 8-12 weeks after which they can be planted out in the field (30 cm height with 4-5 open leaves)
- Efficient nursery management (regular watering and foliar spray for insect control) is essential for planting material quality and survival
- Variations (off types) discarded if observed



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Planning production

- Projected number of plants from a shoot tip assuming a multiplication ratio of 1:4 per subculture cycle of 4-6 weeks

Up to 10,000 plants from a single shoot tip in 10 months

Duration	Stage (number of plants)
Week 0	• Initiation (1 explant/shoot tip)
Week 4-6	• Transfer 1 (2 explants)
Week 8-10	• Transfer 2 (4 explants)
Week 12-14	• Sub culture 1 (16 explants)
Week 16-18	• Sub culture 2 (64 explants)
Week 20-22	• Sub culture 3 (256 explants)
Week 24-26	• Sub culture 4, + rooting (1024 explants)
Week 28-30	• Sub culture 5, + rooting (4096 explants)
Week 32-34	• Rooting (16,384 explants)
Week 36-42	• Hardening (approx. 10,000 plants)

- estimates based on ideal scenario.. but good basis for planning production

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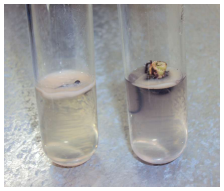
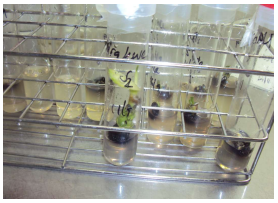
Common challenges

- Contamination (bacteria and fungi)
  - good aseptic techniques; identify and autoclave contaminated cultures
  - bacterial indexing of initial ex-plant to eliminate endogenic bacteria
- Mis-identification of clones and mixtures
  - obtain mother plants from credible sources
  - proper labelling and tracking at each stage,
- Poor establishment & low multiplication rates
  - media optimization for specific cultivar/cultivar groups
  - efficient cutting techniques to target and save meristematic points





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Conclusion and outlook

- Tc is a 'sophisticated' technology requires initial investment for infrastructure, technical knowledge and skills
- Technique readily available and widely applied for banana
- Used by research labs (component of breeding programs), germplasm repositories for conservation/distribution, but also by commercial labs for mass production of commercial varieties
- Techniques need to be optimized for varieties of interest to get optimum multiplication
- Most commercial labs rely on cost saving substitutes for tc lab supplies and consumables without compromising quality, increasing profit margins
- TC labs in Nigeria?? Possible opportunity for investment

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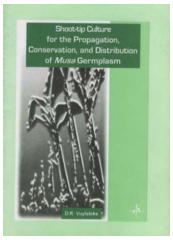
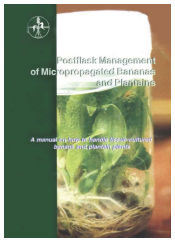
Recommended reading



http://www.iita.org/wp-content/uploads/2016/05/Shoot-tip-Culture-for-the-Propagation-Conservation-and-Distribution-of-Musa-Germplasm.pdf



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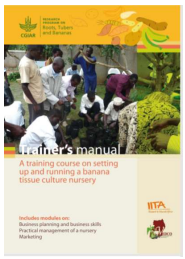

http://biblio.iita.org/documents/U13ManLuleTrainersmanualNothomNodev.pdf-581ca5135bec2761a67929f3168aba19.pdf

https://www-pub.iaea.org/MTCD/Publications/PDF/te_1384_web.pdf

… and several published articles on banana/plantain micropropagation available on http://www.musalit.org/

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