EFFECT OF LONG-TERM *IN VITRO* SUB-CULTURING ON QUALITY DEGENERATION OF SWEET POTATO VARIETIES

by

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INTRODUCTION

- Plant tissue culture is becoming an integral part of sweet potato seed systems through germplasm conservation, virus cleaning, varietal regeneration and rapid multiplication of clean foundation seeds.

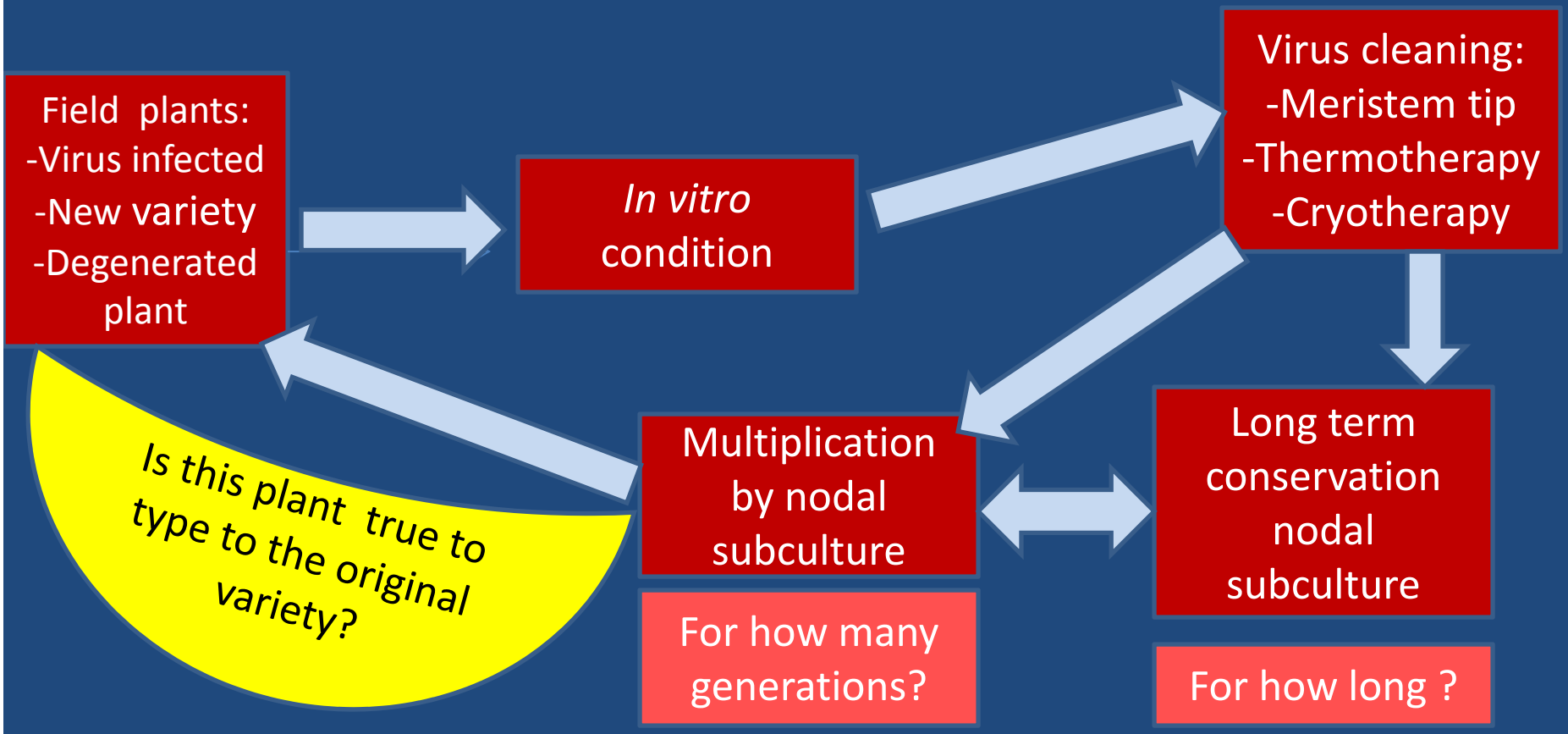

Figure 1. Model showing tissue culture role in sweet potato seed system

- Conservation and multiplication process required long term nodal sub culturing of a single line for many generations
- The assumption of nodal sub-culturing
  - multiplication of organised meristems, such as stem nodes are genetically stable
- The limitation
  - Factors such as increased sub-cultured generations and longer duration in vitro can cause variation
- Some variations are desired (enhanced rooting)
- Most variation are undesirable (off type, poor quality and varietal decline)

- Therefore it is important to develop early detection techniques of variation in tissue culture as part of quality assurance
- Sweet potato is sub-cultured under *in vitro* condition for longer period but no information available about the effect of long term sub-culturing on the quality of plantlets

Objective

- To study the effect of long-term *in vitro* sub-culturing on the plantlet quality of sweet potato varieties
- To develop techniques for early detection of off-type plantlets

MATERIALS AND METHODS

Plant material: Varieties Monate, Mokone and Ndou

Sub-culturing media: MS salt (4.43 g/l), sucrose (30g/l), gelrite (2g/l), pH 5.6-5.8,

Culture environment: Temperature (25±2 ^{0} C), photoperiod (16/8 day/ night)

Table 1. The subculture generation of Monate, Mokone and Ndou

No	Variety	Subculture generation
1	Monate	32
2	Mokone	23
3	Ndou	12

The sub-culture was done every 30 day after culturing

Types of assessment conducted

(a) Days to root and shoot organogenesis

(b) *In vitro* growth performance

(c) Leaf stomata density

using negative nail varnish replicas (Sampson, 1961)

(d) Morphological assessment after two months of outside acclimatization

based on the CIP/AVRDC/IBPGR (1991) sweet potato descriptors list

(d) Molecular characterization with SSRs

DNA extraction was based on Doyle and Doyle (1987)

The PCR cycling conditions were based on Butler *et al.* (1999).

Table 2 . Characteristics of the five primers used for SSR

Primer Name	Sequence 5'⟷3'	Annealing Temp. °C	Run time	Expected size[bp][a]
IB-242 Forw.	GCG GAA CGG ACG AGA AAA	57	2h00	135
IB-242 Rev.	ATG GCA GAG TGA AAA TGG AAC A			
IB-318 Forw.	AGA ACG CAT GGG CAT TGA	59	1h20	150
IB-318 Rev	CCC ACC GTG TAA GGA AAT CA			
IB-255F Forw	CGT CCA TGC TAA AGG TGT CAA	60	2h40	242
IB-255F Rev.	ATA GGG GAT TGT GCG TAA TTT G			
IB-248 Forw.	GAG AGG CCA TTG AAG AGG AA	60	2h30	171
IB-248 Rev.	AAG GAC CAC CGT AAA TCC AA			
IB-255 Forw.	T TGG GCA TTC TCA TAT TTT GCT	60	1h10	161
IB-255 Rev.	GCC ACT CCA ACA GCA CAT AA			

The five primers were developed and tested by Butler *et al.* (1999)

RESULTS AND DISCUSSION

a) Root formation:

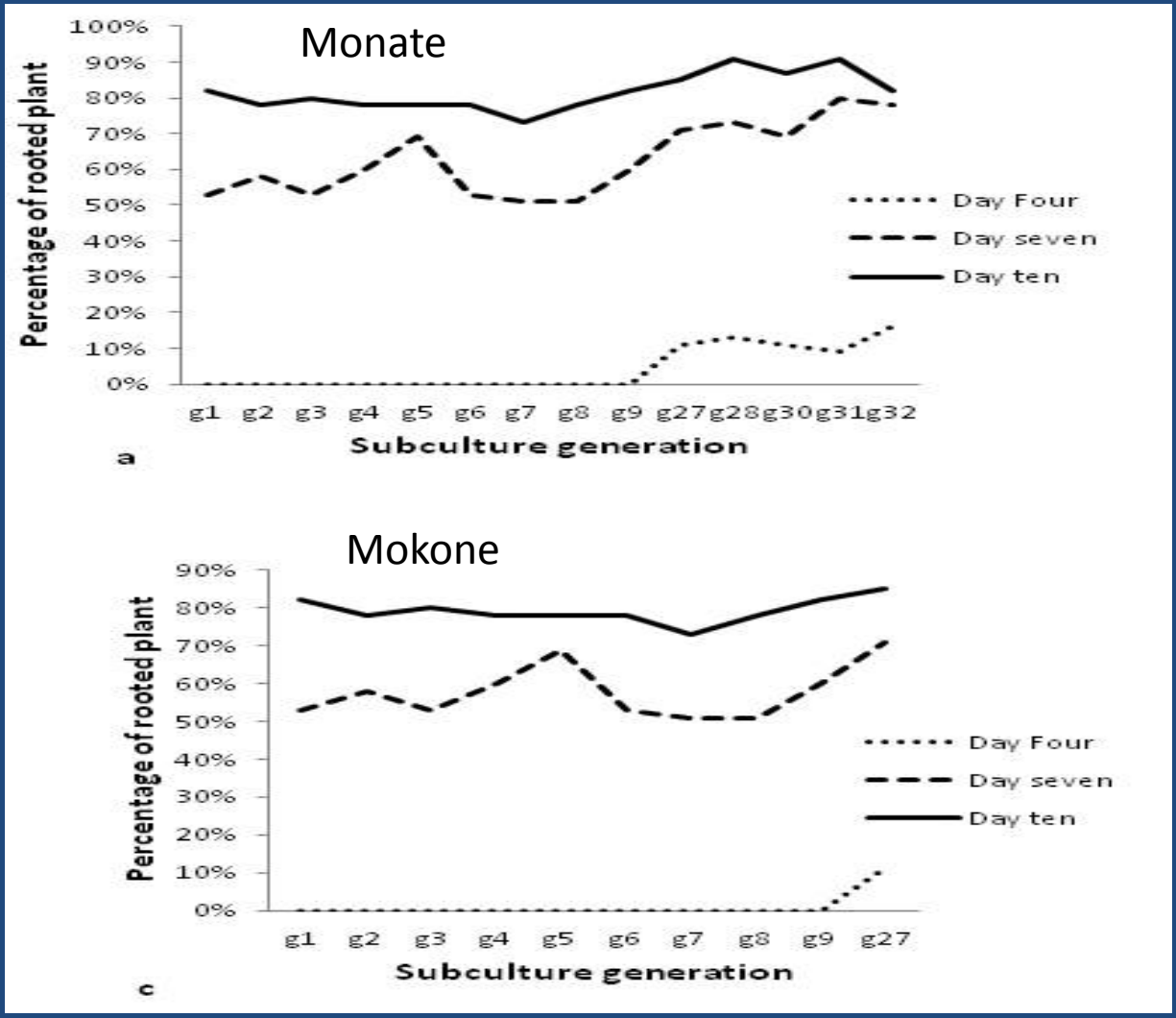

Figure 2. Root formation (%) of Monate and Mokone

- Monate: Early root formation observed after 27th generation (16-21 %)
- Mokone: early root formation observed after 21st generation
- The reason for this change in early root formation is unknown

b) Shoot formation:

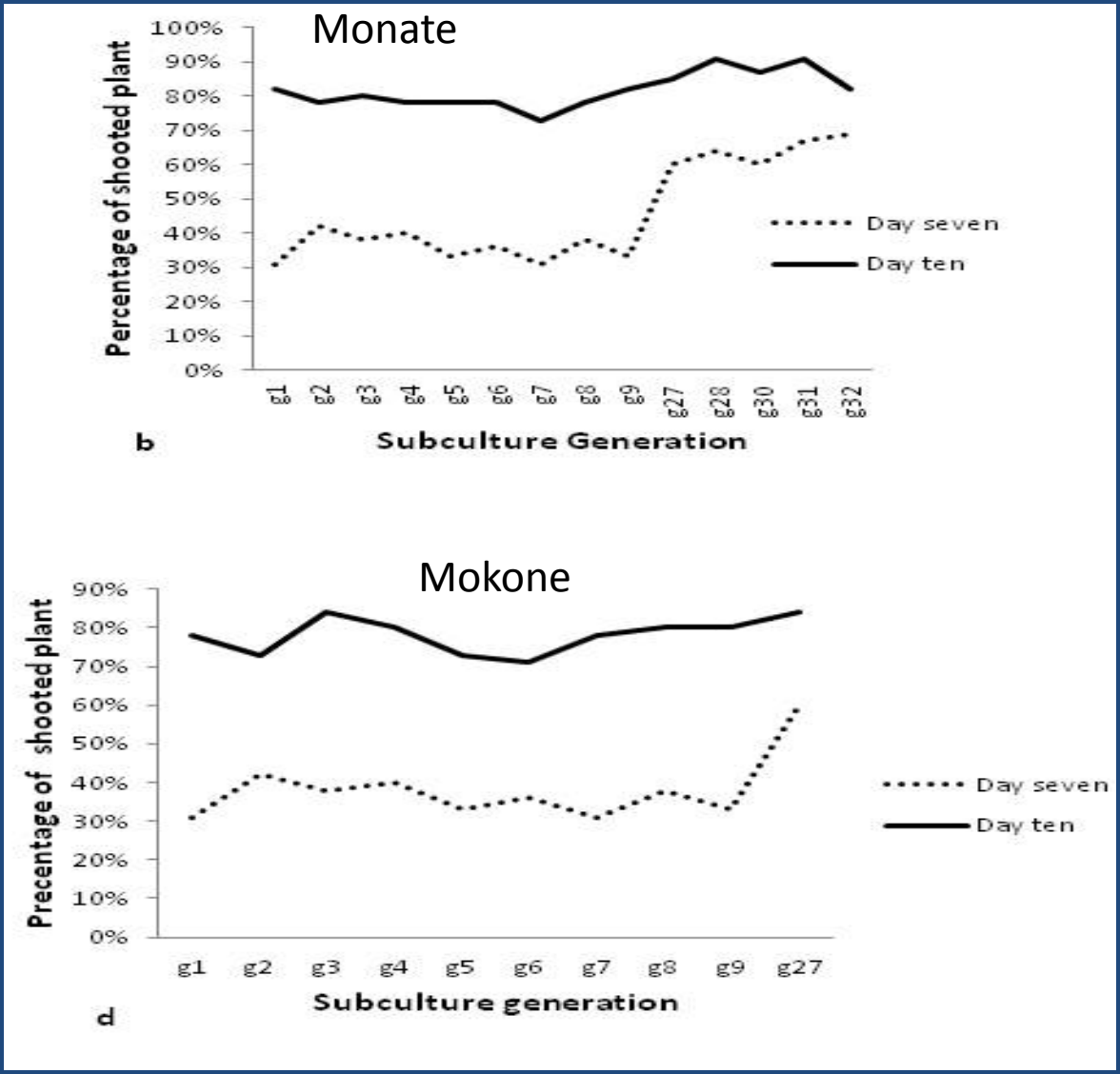

Figure 3. Shoot formation (%) of Monate and Mokone

- Shoot formation started on the 7th day for all subculture generations
- Higher subculture lead to higher shoot formation percentage
- Highest shoot regeneration achieved on the 10th day
- Earlier organogenesis will shorten the period of production

c) Changes in leaf stomata density of sub-cultured generations:

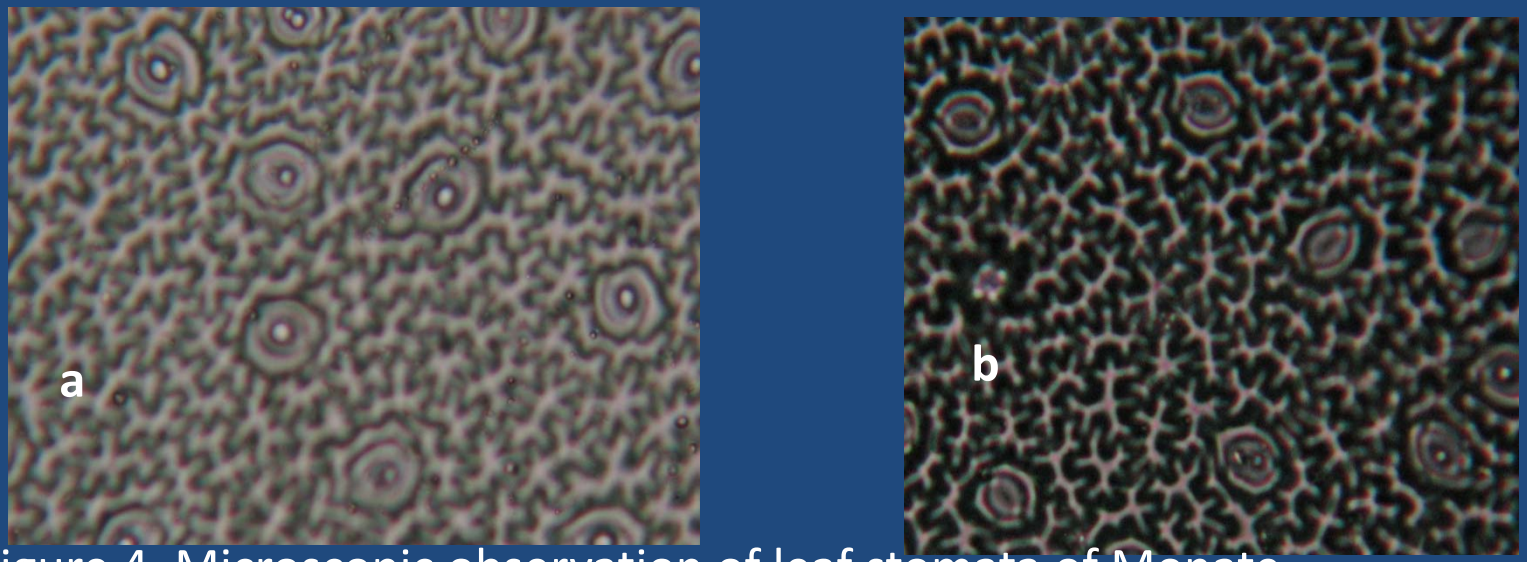

Figure 4. Microscopic observation of leaf stomata of Monate.
a) 2nd generation lower leaf, b) 29th generation lower leaf

- Significant difference in nr of stomata/mm^2 but no specific trend: no increase or decrease of stomata nr during successive sub culturing

  - Adaptation to higher humidity should increase stomata density

High stomata density → Higher transpiration → Low plantlet quality

d)Morphological characterization of sub-cultured generations during acclimatization:

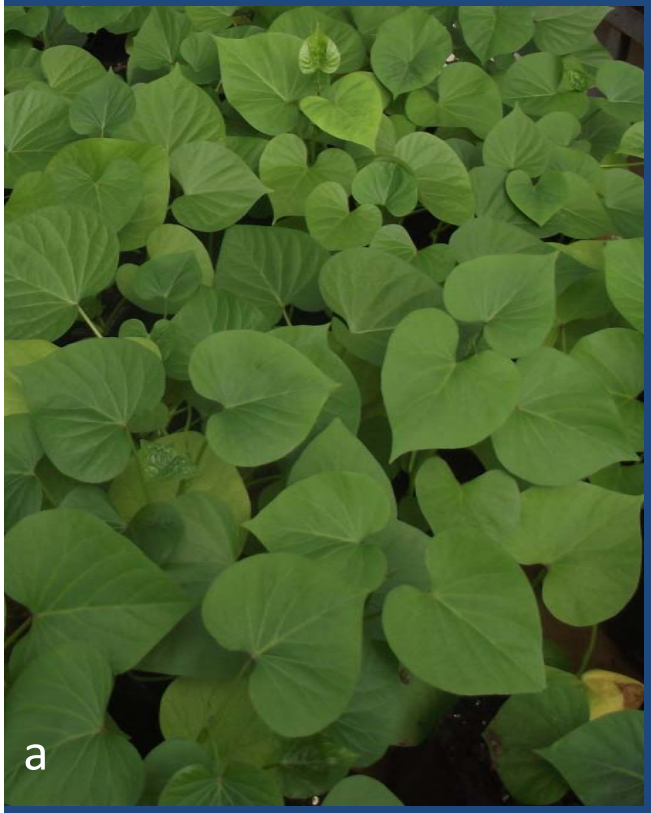

Figure 5. a) acclimatization b) Leaf wrinkling , c) light green leaf, d) flower, e) In vitro Monate generation 5 and 27 looking the same

- During acclimatization all plants had same growth
- Leaf wrinkling was mostly on young leaf and disappeared gradually
- Flower formation was un even

Table 4. The most dominant and variant type of six morphological characteristics of Monate during acclimatization.

Morphology	Dominant	Variant
Mature leaf color	Yellow green (73%)	GWPA (27%)
Immature leaf color	GWPA (73%)	YG (27%)
Abaxial leaf vine pig.	MRPP (60%)	PSBMR (0.13%), PSSV (27%)
Vine pigmentation	GWPN (73%)	G (27%)
Vine internode length	VS (73%)	Short (27%)
Petiole pigmentation	GPCS (60-100%*)	GPCL (0-40%*)

- Leaf color and vine pigmentation were the most unstable characters

e) Cluster analysis based on morphological characters

- Plantlets of different generation grouped in same group
- No relation between subculture generation and morphological variability
- In all varieties the semi-partial R-square value was less than 0.5,
- Mokone showed relatively higher semi partial R-square value of 0.3
- Therefore, observed morphological variation were not a result of long-term sub culturing

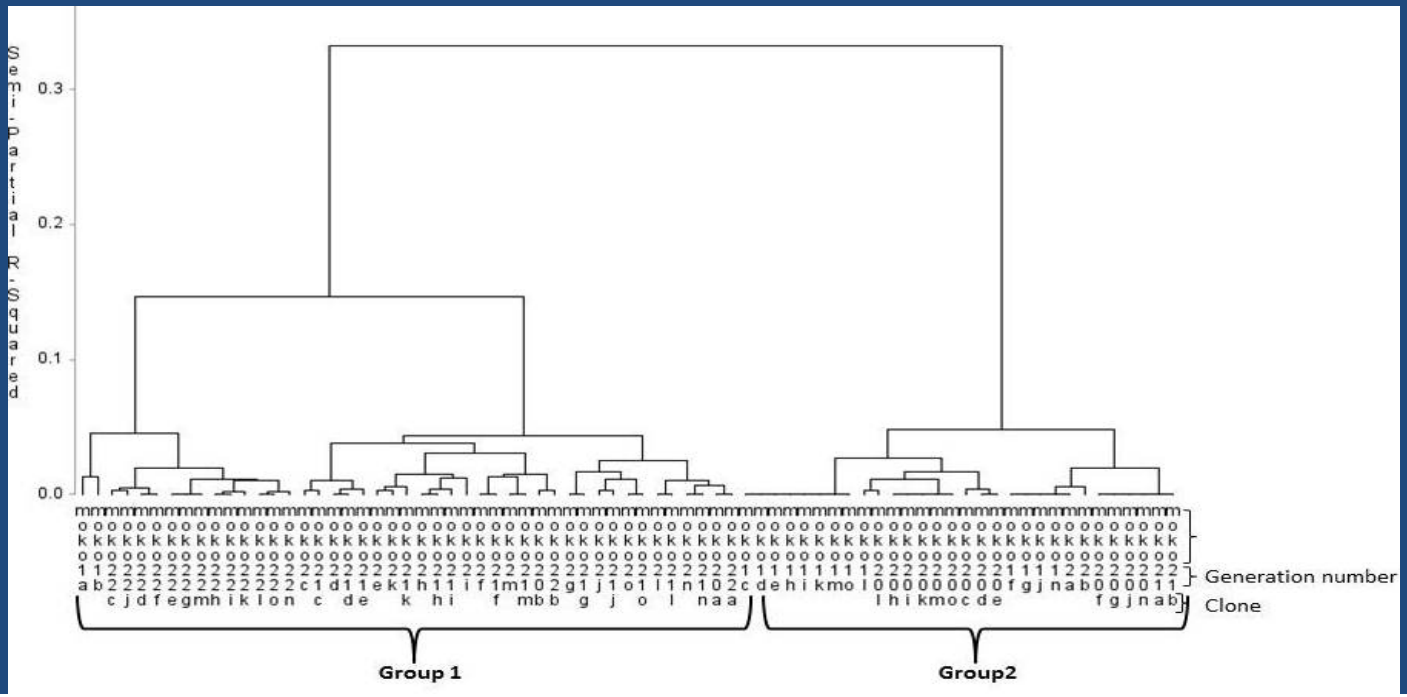

Figure 5. Cluster analysis of plantlets from different subculture generation of Mokone based on morphological characteristics

However, are these morphological variations genuine and heritable?

f) Identification of genetic purity through the use of simple sequence repeats (SSR)

There were no polymorphysms among different generations of the same variety

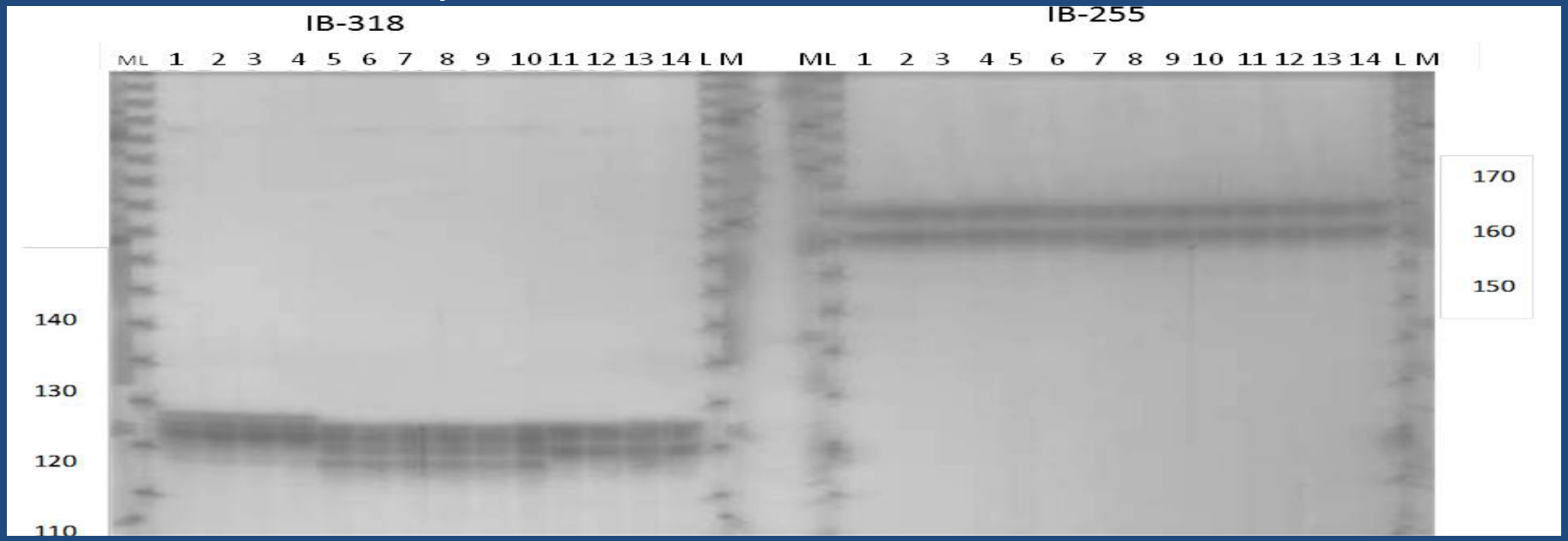

Figure 6. Gel electrophoresis patterns for simple sequence repeats (SSR) primers IB-318 and IB-255 of the different subculture generations of Mokone, Monate and Ndou (1 & 2 - Mokone (4th Gen.); 3 & 4 - Mokone (25th Gen.); 5 & 6 - Monate (4th Gen.); 7 & 8 - Monate (11th Gen.); 9 & 10 - Monate (33th Gen.); 11 & 12 - Ndou (4th Gen.); 13 & 14 - Ndou (13th Gen.).

Summary

- Long-term sub-culturing shortened days to *in vitro* root formation
- No growth problem was observed during acclimatization (stunted, survival…..)
- Leaf anatomy, stomata density and shape of plantlets was unaffected by long term sub-culturing
- Plantlet in the same variety showed change in morphology such as leaf color, vine pigmentation, leaf wrinkling and flowering %.
- However, the morphological changes were not related with long term sub-culturing, it appeared randomly in every generation.
- No allelic polymorphism was detected between morphologically deferent plantlets during SSR analysis

CONCLUSIONS

- Long term nodal sub-culturing did not cause quality degeneration in sweet potato plantlets
- Changes in leaf colour, leaf wrinkling and vine pigmentation are not reliable characters for early detection of off-types in sweet potato micro-propagation
- A combination of different techniques is necessary for detection of changes in plantlets
- Successive nodal sub-culturing can be used in large scale sweet potato seed propagation programs and in germplasm conservation

Thank You