Ministry of Agriculture, Livestock and Irrigation

Department of Agriculture

Horticulture and Plant Biotechnology Division

Efficient Tissue Culture Protocol for Propagation of Elephant Foot Yam (*Amorphopallus sp.*)

Presented by

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Introduction

- Tropical tuber crop of south east Asian origin

Traditional

- Vegetable, low calories noodles and artificial meats
- Plant starch (good viscosity and stability) - food industry (**Moorthy *et al*., 1994**)

Medicinal

- **Starch (11-28%)**, sugar (0.7-1.7%), **protein (0.8-2.60%)**, fat (0.07-0.40%)mean energy value (236-566.70KJ/100g), potassium (327.83 mg/100 g), phosphorus (166.91mg/100 g), calcium (161.08 mg/100 g) and iron (3.43mg/100 g)

(Anuradha Singh, 2013)

- High glucomannan content in subterranean corms - reduce the serum cholesterol level (**Cescutti *et al*. 2002 and Ghani 2003**)
- Treatment of tumors, elephantiasis, inflammations, cough, asthma, vomiting, flatulence, colic, dyspepsia, constipation, fatigue and anemia **(Nair, 1993)**

Elephant Foot Yam Production in Southern Chin State

- Major cash crop for Chin farmers
- Produce 100% of households
- Chinese market for 85% of all Myanmar EFY chips
- EFY chips - 5000 MMK/viss
- Japan has permitted 240 metric tonnes of EFY imports from Myanmar Cambodia, Laos and Sri Lanka (white, yellow and red)
- Yellow and red EFYs are popular export items to China and Japan

Conventional Propagation Methods of EFY

True Seed

- One seed requires about five years to develop into a lager tuber
- Rarely kept or sown

Leaf bulbils

- Collect bulbils at the end of the wet season when the leaf has fallen to the ground
- First-year leaves may produce as few as six smallish bulbils
- After three years, a mature leaf may produce more than 20 bulbils and most favorable propagation material

Tuber

- Small tubers may be re-planted and harvested after one or two years
- Cut one large tuber into vertical wedges & Each wedge may develop into a harvestable tuber after one or two years
- Wedges will rot on the cut surface and fail to grow , Dry and dress the cut with fire ash to reduce the risk of failure

Major Production Constraints

- High incidence of **mosaic disease**
- Non availability of **quality planting materials**
- Cultivated immature yams from wild collection and to reach a suitable size of harvesting for **long time**
- Lack of sufficient seed material of **uniform size and dormancy**

Mosaic virus disease

Various size of tubers

Objectives

- To find out suitable explant source for *in vitro* propagation
- To develop an indirect regeneration protocol for disease free and high quality numerous planting material of EFY within a short period
- To increase agricultural production and income of growers and producers

Materials and Methods

- Sources - Chin state (Kanpetlet)
- Duration - (2019 November - 2020 November)
- Project area - **Plant Biotechnology Center, Palae Myothit, Mingaladon, Yangon**
- Explant types - **leaf, petiole, leaf bulbil, corm bud and floret**

(50 for each explants)




Sterilization of Explants

- Running tap water (30 min)
- Liquid detergent (15 min) & 0.05% (w/v) fungicide (15 min)
- 70% (w/v) Ethanol (1 min)
- 7% or 10% (w/v) NaOCl for 5 min (2 times)

- Wash 5-7 times with distilled water for each stage
- Dipping in absolute ethanol and flaming the unopened leaves covered by cataphyll

Five stages for *in vitro* propagation

Initiation stage (establishment of different types of explants)

Callus induction (Modified MS + BAP + NAA + 2,4-D)

Shoot regeneration (Modified MS + BAP + Citric acid + Adenine sulphate)

Root formation (MS, ½ MS, ¼ MS + NAA)

Hardening stage

- Modified MS media - half concentration of **NH_4NO_3** and **KNO_3 (Srinidhi *et al.*, 2008)**
- **All the media were adjusted to pH 5.8, solidified with 0.7% agar and sterilized by autoclaving at 121°C for 20 min**

Statistical analysis

❖ The frequency of callus induction, regeneration and root formation was calculated as follows:

Callus induction frequency (%)	= number of explant producing callus / number of explant plated	× 100
Regeneration frequency (%)	= number of plants recovered / number of callus plated	× 100
Root formation frequency (%)	= number of plants recovered / number of callus plated	× 100

- Factorial complete randomized design (5 treatments × 3 replications)
- ANOVA (Analysis of Variance) & 5% level with (LSD) using Statistix 8.0 software
- Two factors - **Explant types** (leaf**,** petiole, leaf bulbil, corm bud and floret)
  - **Media combination**

Media Combination

Shoot Regeneration				
No.	Media Combination	BAP (mg/l)	Citric Acid (mg/l)	Adenine Sulphate (g/l)
1.	MSYS1	1	1	0.05
2.	MSYS2	2	1	0.05
3.	MSYS3	3	1	0.05
4.	MSYS4	4	1	0.05
5.	MSYS5	5	1	0.05

Root Formation		
No	Media Combination	
1.	MSYR1	Full MS
2.	MSYR2	Full MS+ NAA (1mg/l)
3.	MSYR3	½ MS+ NAA (1 mg/l)
4.	MSYR4	¼ MS + NAA (1 mg/l)

Results and Discussion

Callus Induction

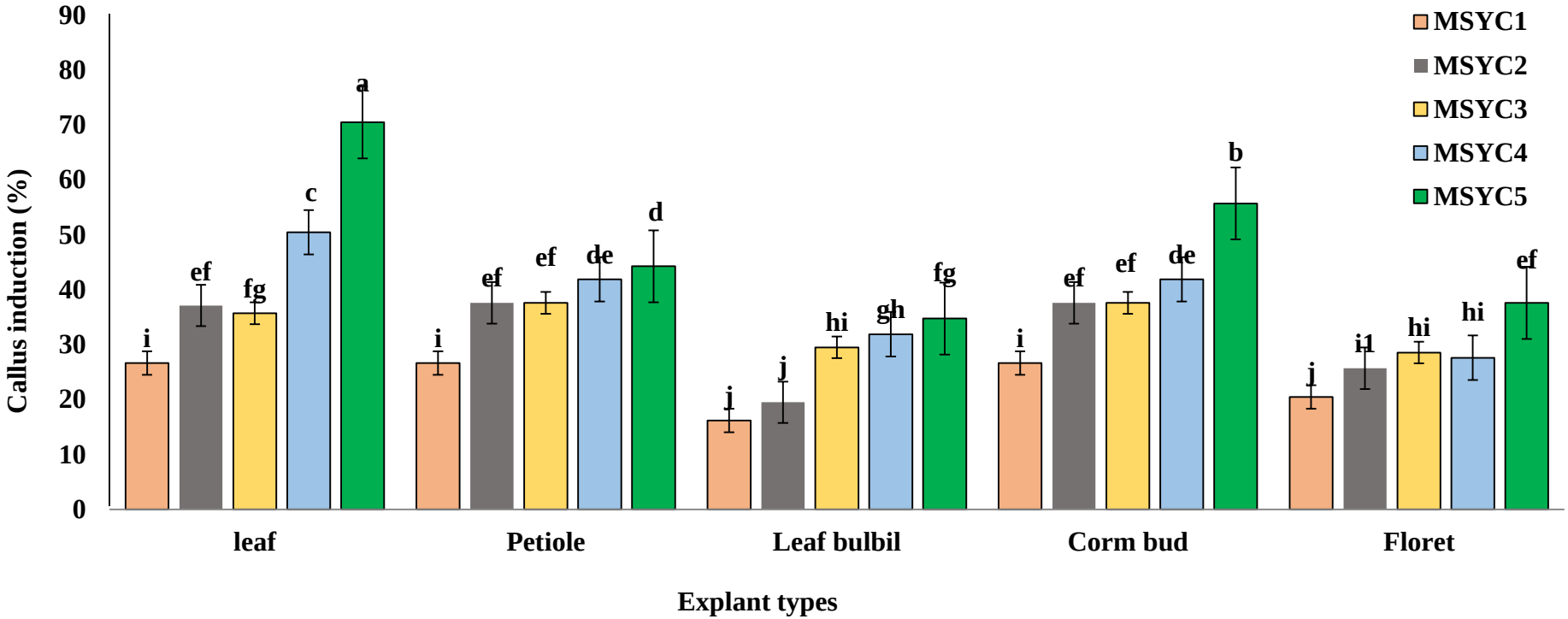
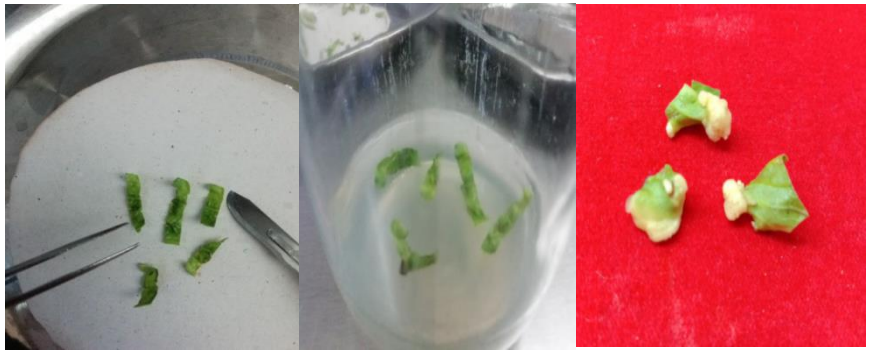


Figure 1. Effect of PGRs on callusing response in different explants of EFY

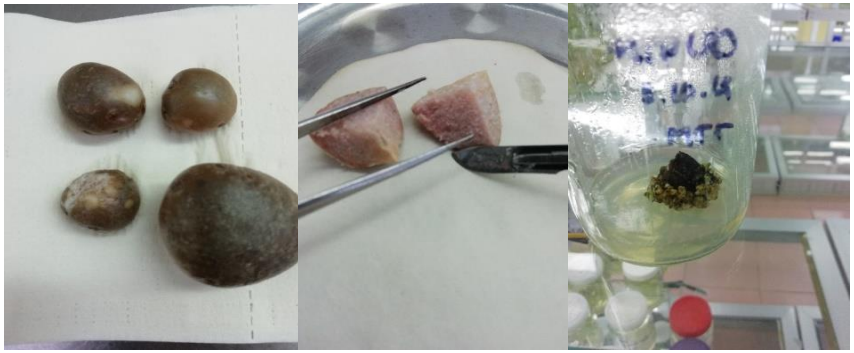
***** a-j within a column having the same letters are not significantly different at 5% level**

- 2,4-D is one of the most effective auxin for callus induction in sweet potato. **(Oggema, J.N, 2017)**
- A combination of 3 PGRs (BAP, NAA and 2,4-D) increase callusing % in petiole and leaf explants of EFY (**Kamala and Makesh kumar, 2014**)

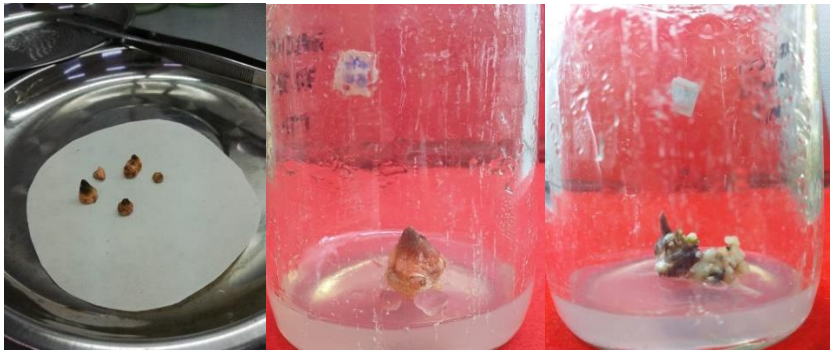


(a) Young leaf

(b) Petiole



(c) Leaf bulbil



(d) Corm bud

(e) Floret

Figure 2. *In vitro* Callus induction response of different explant in EFY

Shoot Regeneration

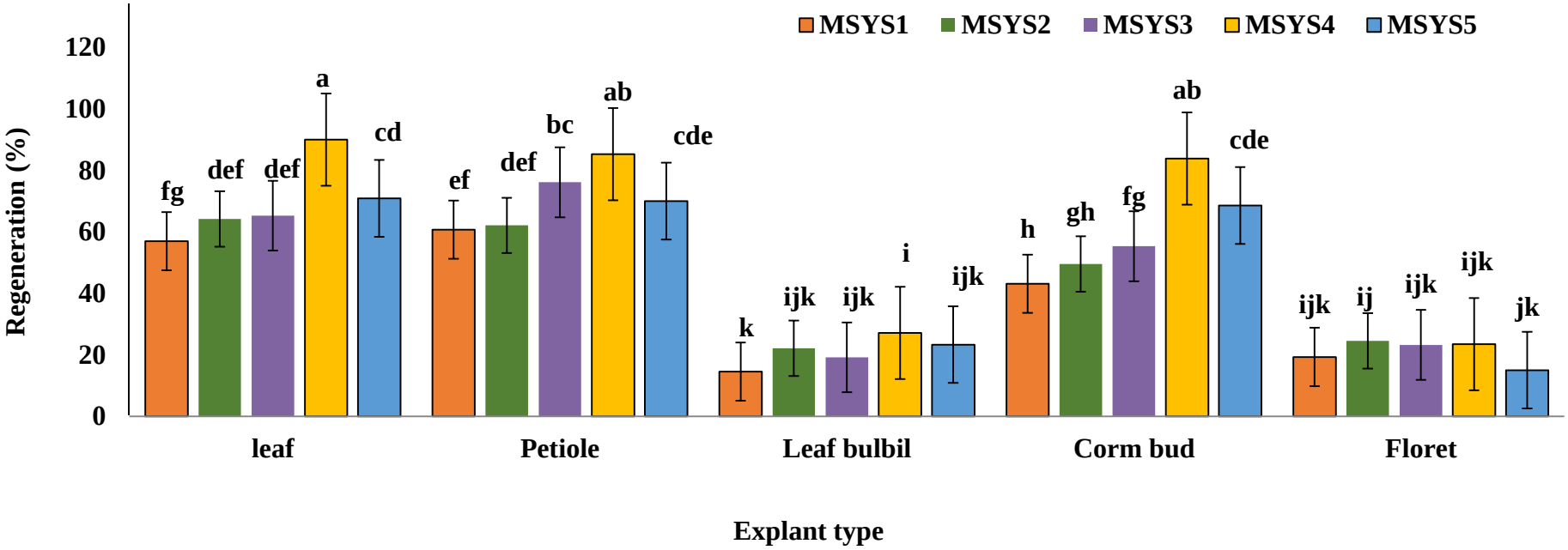


Figure 4. Effect of PGRs (BAP) on *in vitro* shoot regeneration of EFY from callus

*** a-k within a column having the same letters are not significantly different at 5% level

- Use of adenine sulphate in combination with cytokinins (BAP) achieve enhanced shoot multiplication from *in vitro* induced callus in potato. (**Alok K.S *et al.*,2017**)

Root Formation

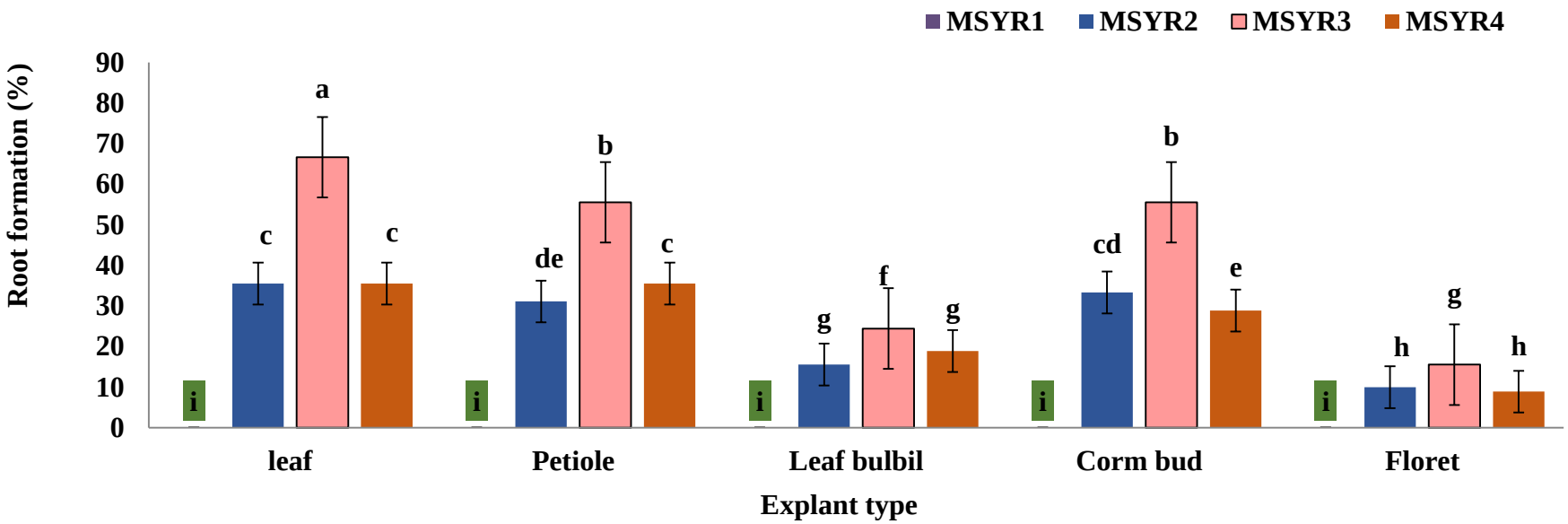


Figure 5. Effect of MS media and auxin (NAA) on rooting of EFY *in vitro* conditions

*** a-i within a column having the same letters are not significantly different at 5% level

- *Amorphophallus konjac*, NAA proved to be efficient in root induction **(Zhao et al., 2012)**, culturing shoots of *D.rotundata* (yam) on MS media with 0.5 mg/l NAA increase root formation (**Sahar *et al.*, 2017**)

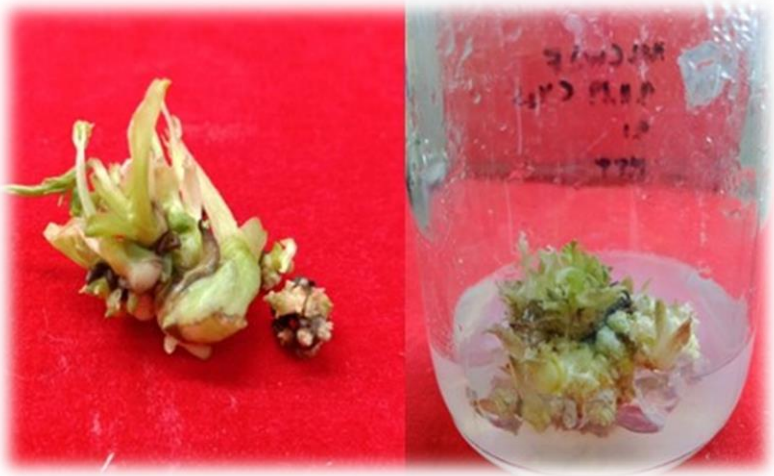
Table : Analysis of Variance for the effects of culture media and explants

Source (Callus Induction)	DF	MS	F value
Media	4	486.65	**144.6****
Explants	4	333.74	**99.16****
Media * Explant	16	29.27	**8.70****
Error	48	3.37	
CV %	**3.37**		
LSD $^{0.05}$	**3.01**		

Source (Shoot Regeneration)	DF	MS	F value
Media	4	495.43	**29.44****
Explants	4	3670.8	**218.12****
Media * Explant	16	65.12	**3.87****
Error	48	16.83	
CV %	**9.29**		
LSD $^{0.05}$	**6.73**		

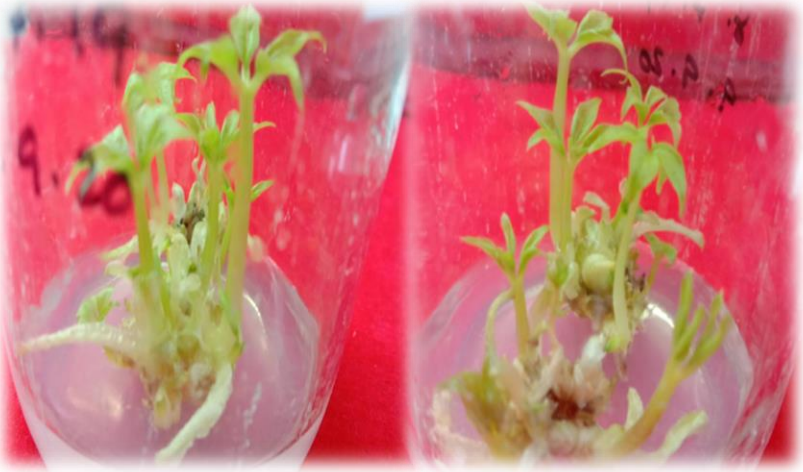
Source (Root Formation)	DF	MS	F value
Media	3	4582.21	**1791.3****
Explants	4	663.9	**259.53****
Media * Explant	12	98.44	**38.48****
Error	38	2.56	
CV %	**6.4**		
LSD $^{0.05}$	**2.64**		

ANOVA using the Statistix 8.0 **Significant at P < 0.001 level



(a) Shoot Induction

(b) Shoot Multiplication



(c) Shoot Elongation

(d) Root Formation

Figure 6. *In vitro* Shoot regeneration and root formation from Leaf explant of EFY

Hardening

- Remove well rooted plantlets and treat with 0.2% atonic for 5 min
- Transplant on potting mixture (soil, sand, burnt paddy husk, 1:1:1 in the net house)

Harden plant kept in the net house

Conclusions

Explant type

- Leaf is suitable explant material for production and preservation

Media combination

Callus induction

- **Modified MS + BAP (0.5 mg/l) + NAA (0.5mg/l) + 2,4-D (0.5mg/l)**

Shoot regeneration

- **Modified MS + BAP (4.0 mg/l) + Citric acid (1mg/l) + adenine sulphate (0.05g/l)**

Root formation

- **½ MS + NAA (1.0 mg/l)**

Future Trust

- **Numerous plants will be produced all the year round and is more independent of seasonal changes**
- **The lost of plants can be minimized when transfer to natural environment and reduced production cost**
- **Providing the useful tools of tissue culture technology for mass propagation and conservation of Elephant Foot Yam**

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Thank You for your Attention!