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Standard Operating Procedure for Yam Multiplication in Semi-Autotrophic Hydroponics

Authors & Contributors

Olugboyega Pelemo o.pelemo@cgiar.org

Ryo Matsumoto R.Matsumoto@cgiar.org

Agre Paterne A.paterne@cgiar.org

Alex Edemodu E.edemodu@cgiar.org

Amele Asrat A.Amele@cgiar.org

1. Introduction

Semi-Autotrophic Hydroponics (SAH) is a robust technique for efficiently and rapidly multiplying clonally propagated crops. It is a low-cost and licensed method for high ratio propagation to meet the breeding scheme and breeder seed needs. A sustainable out-scaling of this technology and its commercialization is critical to IITA's mission and vision. Seed Yam Tubers (SYT) is required in quality and quantity for the Fastrack of the breeding scheme and commercial seed production to close the critical seed gap in the yam production system. Rapid yam multiplication technique: Semi Autotrophic Hydroponics is one of the high-ratio propagation techniques (HrPT) with the potential for sustainable quality and quantity of SYT

2. Purpose

The multiplication rate of yam is still relatively low and challenging especially in the breeding scheme. This SOP provides highlights of the procedure for yam multiplication using SAH. A multiplication tool with potential for rapid multiplication of breeder seeds and seedlings from botanical seeds.

3. Scope

This standard operating procedure (SOP) covers the procedures for yam seedling culture in the laboratory and the transplanting of same to the field for the production of seed yam tubers

4. Definition of terms

GRC	Genetic Resources Centre
HrPT	High ratio Propagation Technology
IITA	International Institute of Tropical Agriculture
LED	Light-Emitting Diode
MAP	Months After Planting
NARES	National Agricultural Research and Extension Services
NPK	Nitrogen Phosphorus and Potassium
SAH	Semi-Autotrophic Hydroponics
SYT	Seed Yam Tubers
TDa	Tropical Dioscorea alata
TDr	Tropical Dioscorea rotundata

SAH	Semi Autotrophic Hydroponics
SOP	Standard Operating Procedures
YM	Yam

5. Roles and Responsibilities

Crop Lead/Scientist:

Overall supervision of the SAH laboratory and activities, including fund sourcing and partnering with the NARES for the out scaling of the Technique for the enhanced breeding scheme and multiplication of released varieties for the diffusion of improved yam varieties among various end-users.

1.0 **Field/ Seed System Manager:**

1.1 Assist the scientist with trial management and supervision of laboratory and field staff

1.2 Ensure availability of consumables and ensure the functionality of the SAH facilities

1.3 Ensure and monitor the establishment of plant culture and maintenance

1.4 Ensure the availability of all logistics required for all activities Lab and field activities

1.5 Prepare activities schedule/plan for timely execution of seed multiplication activities

1.6 High-ratio propagation of virus-free yam seedlings for seed yam tuber production and re-introduction into Tissue Culture

1.7 Reduce breeding cycle and scale testing scheme to accelerate yam breeding gain

1.8 Acclimatization of in vitro plantlets for onward transplanting into the field

1.9 Efficient viral and microbial inoculation, viral indexing and adequate symptom expression and data capture

1.10 Germplasm exchange and the establishment of seed yam value chain

2.0 Supervisor/Technician:

2.1 Coordinate the establishment of the field plants and management of same in the screen house and under field condition

2.2 Coordinate the field maintenance: watering, fertiliser application, weeding and staking.

3.0 **Laboratory Technician:**

3.1 Culturing of plants in the laboratory

3.2 Maintenance of plants in the laboratory

3.3 Substrate and nutrient preparation.

3.4 Harvesting of tubers from the senesced plants in the SAH boxes and the transplanted materials on the field.

6. Procedure/Protocols

SAH Laboratory procedures

6.1 Standard workplace safety procedure of IITA applies to all staff working in the SAH Facility. For details, refer to IITA Employee Health and Safety Handbook.

6.2 All Staff working in the SAH facility must wear an apron or lab coat.

6.3 Strictly follow the safety instructions for the use of reagents and tools.

6.4 Use chemicals, reagents and tools specified for SAH work. Do not store or use chemicals, reagents and tools not necessary for SAH propagation work.

6.5 Extra care should be taken to avoid accidental ingestion or reagent exposure.

6.6 Food consumption is prohibited in the SAH laboratory facility.

6.7 Hands should be washed and disinfected with Tween-20 and or 70% ethanol severally when coming in and leaving the laboratory.

In the cutting room, laboratory fittings and equipment such as chairs, tables and surfaces should be disinfected daily with ethanol. At the same time, plants can only be sprayed with Tween-20/insecticide if the infection is noticed.

6.2 Selection of yam varieties for SAH propagation

Start-up sources must be in vitro plants authenticated for true-to-type and virus-free status. A certificate/health statement should accompany the first set of materials of the start-up source. Before introducing them in SAH, the manager or supervisor should request tissue culture plantlets of selected varieties at most minuscule a month.

Sourcing and collection of Tissue culture plantlets

Reference No.

No of varieties

Name of varieties

Number of tubes/varieties

Age of plantlets after the last subculture

Name of requestor

Genotyping status confirmed (tick) Yes/No

Healthy status confirmed: ……. (tick) Yes/ No

Name and signature of collector

Name and signature of Genetic Resource Centre provider

Date of collection

Any information about plantlet

Authorization for use in SAH

Propagation

Name: …….

Signature……...

Date…….

6.3 **General Procedures**

6.3.1 Rooms should be entirely free from dust and possible entry of insects and rodents. Every little outlet, such as beneath doors, should be sealed

6.3.2 Growth room should constantly maintain the following conditions

6.3.3 Temperature: Minimum temperature of 22ºC and a maximum of 28ºC had been found suitable for yam and 25±2°C in the growth chamber. In contrast, a temperature of 20±2 °C in the cutting area is recommended.

6.3.4 Light: 18-watt LED Light at two tubes per plate is recommended for plants in the growth room

6.3.5 Photoperiod of 12−14 hours' light and 12−10 hours' dark is recommended.

6.3.6 Adequate circulation of air by using an electric fan.

6.3.7 Symptomatic plantlets in boxes should be discarded

6.3.8 Disinfect/fumigate the laboratory once or twice a year

6.3.9 There should be an alarm system and surveillance camera to alert the lab supervisor in case of any emergency or mishap.

6.4 Cutting Procedure

6.4.1 Prepare the cutting Procedure by cleaning the table surface with Tween-20.

6.4.2 Place plantlets on several layers of paper towel in a stack on the table surface before cutting.

6.4.3 Remove the top paper towels before cutting the plantlets from the subsequent boxes to avoid the transfer of any infection that comes with that box to the next box.

6.4.4 Spray the paper towels with Tween-20 solution and keep them wet while cutting the plantlets to avoid dehydration.

6.4.5 Start propagation or multiplication using clean, tested, fresh and vigorous *in vitro* plantlets from meristem culture.

6.4.6 Do not plant *in vitro* plantlets with media, ratoon plantlets from the base, and leaf

stalk with root in the media.

6.4.7 Handle *in vitro* plantlets gently and with great care because of their delicate nature, as they are likely to be injured.

6.4.8 Cut SAH plants leaving at least one leaf with a node and a shoot on both mother and daughter plantlets.

6.4.9 When cutting *in vitro* plantlets, cut them into 2-3 segments if possible. Plant all segments and ensure the roots are completely buried in the substrate.

6.4.10 Care should be taken not to plant cuttings inversely. To do this, make the portion above the node shorter than below the node.

6.4.11 After making a small hole and planting the cuttings, press the substrate against the cuttings gently.

6.4.12 Ensure the bottom node is inside the substrate but do not plant too deep, but the substrate must cover the node.

6.5 Field Activities

6.5.1 Direct field transplanting:

6.5.1.1 Grown-up plants with at least three leaves are selected for transplanting.

6.5.1.2. Construct a 1.5-meter-tall shade over the area to be planted

6.5.1.3 Select actively growing plants with up to 4 leaves/nodes and carefully transplant same to

the field

6.5.1.4 Irrigate and Trellised the plants immediately after transplanting.

6.5.1.5 Make spot application of NPK: 15 15 15 at 5 g per plant at 2 weeks after transplanting. 7.1.8 Keep weed-free for up to 4MAP.

6.6 Indirect transplanting (No shade)

6.6.1. Secure crates or trays with multiple cells

6.6.2. Fill same with sterilized topsoil and place them on a raised platform

6.6.3. Carefully remove seedlings with at least two leaves/nodes from the SAH boxes and transplant one seedling per cell in the crate.

6.6.4. Fertigate for at least three weeks and observe new root, vine, and leaf formation for establishment.

6.65. Select actively growing plants and transplant same to the field

6.6.6. Irrigate and trellised the plants immediately after transplanting.

6.6.7 Make spot application of NPK: 15 15 15 at 5 g per plant for 2-4 weeks after transplanting

6.6.8 Keep weed-free for up to 4 MAP.

6.6.9 Plant density of a minimum of 40,000 and a maximum of 60,000 per hectare is recommended

7. References

Pelemo, O. S., De Koeyer, D., Matsumoto, R., Agre, P., Asiedu, R. and Asfaw, A. 2019. Semi-Autotrophic Hydroponics: A robust technique for accelerated basic seed yam production IITA. March 2019

Annexe: Forms/Templates to be used for monitoring and data collection

8. Appendix