		SOP #	IITA-YM-SOP12
![IITA CGIAR logo]	**Crop: Yam** **Function: Yam hybridization**	**Revision #**	IITA-YM-SOP12-01
		Implementation Date	22-08-22
Page #	1 of 8	**Last Reviewed Date**	22-08-22
SOP Owner	Lead Breeder (Amale Asrat)	**Approval Date**	22-08-22

<u>**Standard Operating Procedure for Yam Crossing**</u>

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1. *Introduction*

Hybridization in yam is a process of transferring of male pollens to female flowers to generate viable seeds with desired genetic recombination of the parents. Hybridization process begins with pollination which can be done by controlled manual pollination or open natural pollination. This process requires proper understanding of the floral biology of yam.

2. *Purpose*

This is a step-by-step procedure of yam hybridization using conventional pollination techniques. This is to ensure that pollination activities are carried out using the protocol in this SOP, for uniformity, minimization of errors and repeatability in yam hybridization across the yam community of practice (YCoP).

3.   Scope

This SOP will explain yam pollination process. It will cover activities from crossing block establishment and maintenance, set preparation, hybridization activities including bagging, pollination, seed extraction, seed storage, sowing, nursery management, tuber harvesting and selection.

4.   Definition of terms

IITA   International Institute of Tropical Agriculture

TDa   Tropical Dioscorea alata

TDr   Tropical Dioscorea rotundata

GRC   Genetic Resources Centre

NARES   National Agricultural Research and Extension Services

SOP   Standard Operating Procedures

YCoP   Yam Community of Practice

Stagger Planting: This method is used to synchronize flowering windows between early and late variety to be involved in crosses. Staggered planting at interval of a week or more is used to synchronize flowering period and duration among genotypes with wide flowering time and duration for successful pollination

5.   Roles and Responsibilities

Crop Lead/Scientist:
- Select parents to be crossed
- Prepare crossing plan
- Monitor the overall operation with pollination

- Direct the families and progenies to be generated per season

Research Supervisor:

- Extract flower sex and flowering intensity data of parents identified for crossing and make available for the breeder / scientist
- Arrange plant materials of parents identified or nominated for crossing
- Prepare action plan for crossing operation
- Upload crossing plan in YamBase and yamcross
- Arrange equipment's and consumables require for pollination
- Collect pollination data
- Identify fields for crossing block establishment
- Monitor the overall operation with pollination

6. Procedure/Protocol

6.1 Crossing Block Establishment

Yam breeding unit has three methods of crossing block establishment to facilitate successful hybridization.
These include

  i. Conventional crossing block
 ii. Polycross mating design
iii. Pollination in trial plots

  i. **Conventional Crossing Block method:** This involves planting of male and female plants in separate fields. Staggering planting may be used here to synchronize flowering period and duration among genotypes.

ii. **Polycross Mating Design**: Male and female plants of desired traits to be recombined are planted very close to each other. The females are planted in the centre while the male is planted to encircle the female or vice-versa. Pollination is allowed to occur naturally between the parents. This design is less expensive; however, viable seeds obtained may be fewer than what is obtainable in controlled pollination

iii. **Pollination in Trial Plots**: Crosses can be generated among parents with preferred traits planted in trials, starting with intermediate breeding stages. There is more unintended open pollination in this kind of field due to proximity among male and female parents.

6.2 **Field preparation**

6.3 Sett Preparation and Field Maintenance in Yam Crossing Block
- A Sett weight for crossing block establishment should be at least 200g to obtain vigorous plant for high flowering intensity.
- Individual staking with bamboo or rope is required and this must be up to 2m in height for high photosynthetic efficiency
- Field maintenance such as weeding, and insect pest control is important across the stages and especially at the early growth stage.
- Yam crossing block field requires close monitoring to prevent outbreak of insect pest which usually attack yam young leaves few months after planting.
- Weeding is done manually and with chemical only as pre-emergence application

6.4 Flower Bagging

- Bag should be made of white cotton materials fine enough to prevent rodents and unwanted pollens.
- spikes of the female flower to be pollinated should be covered with bags within 2-3 weeks after flower initiation, before the flowers are opened
- The length of pollination bags to be used range from 10 to 50 cm depending on the specie and the length of yam spikes,
- Carefully insert spikes in the appropriate pollination bags
- Clip the mouth-end of bags to the base/stalk of the spike.

6.5 Pollen collection
- Collect only matured pollens (the anthers of matured pollens are creamy in colour, premature pollens are greenish while old pollens are yellowish or brownish).
- Immature and over ripped pollens are not suitable for use in pollination.
- Pollens are better collected in the morning before anthesis: opening of anthers for pollen shedding.
- Keep the collected pollens in a container bearing lid for cover.
- Pollens collected should be used for pollination between 1-3 hours after collection.

Yam plant with male flower

Matured anthers

6.6 Pollination Process

- Create crossing plan using yam cross on yambase website
- Observe flower initiation in female plants
- Bagging is done to control pollination at about 2-3 weeks after flower initiation to prevent unwanted pollination in a particular female parent, bagging is not required in open pollination
- Pollination is between 4-7 days after bagging
- During pollination, remove the bags, open the male anthers using pollination pins to take out pollen grains
- Drop the pollens grain on the female stigma as quickly as possible
- A pollen grain is enough for an inflorescence when weather is cool, harsh weather makes pollen dries, in that case, more than one pollen grains may be dropped on the stigma
- Wear magnifying lens during pollination due to smallness of yam pollens
- Bag the spikes again
- Label the pollinated spike with the date, time number of flower pollinated and pollinator's identity.
- Take fruit count at minimum of 2 weeks after pollination and record it accordingly
- Remove pollination bags from the plants after fruit set/count
- When the plant begins to senesce, cover the developed fruit with net bags to prevent the fruits from being shattered away.

- When the fruits turned from green to yellow colour, harvest with their individual identity from the field then airdry under shade until they are turned brown and shatter easily in the net bags

Data to be collected during pollination activities

Family ID

Date of bagging

Number of spikes

number of flowers per spike

Number of flowers pollinated per spike

Date of pollination

Time of pollination

Pollinator ID

Date of fruit count

Date of fruit harvest

Date of seed extraction

Number of viable seed

Number of non-viable seed

6.7 Seed Extraction
- Extract the seeds carefully by placing a tray inside bigger net bags
- Sort viable and non-viable seeds separately by feeling hardness of the seeds. Hard and well filled seed are viable while the non-filled seeds are unviable.
- Count viable and non-viable seeds and record accordingly
- Keep the viable seeds in a white envelope and seal

- Label the envelope with family identity, Seed processing date and the number of seeds
- Arrange and envelop the extracted seeds per family, keep in airtight container
- Label the envelops with family Ids using barcode labels for easy retrieval

7. Reference

Title & Version History

Institute: IITA

Crop Code: Ym

Function:

SOP Owner: Lead Breeder

SOP #: 012

Revision #: 01

Implementation Date: June 2022

Last Reviewed/Update Date: June 2022

Approval Date: June 2022

SOP Number: IITA-YM-012-01

8. *Annex: Forms/Templates to be used for monitoring and data collection*