

SOP12:

Standard Operating Procedure (SOP) for Yam hybridization



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Standard Operating Procedure for Yam Hybridization

1. Introduction

Hybridization is a key technique in yam breeding involving the transfer of pollen from male to female flowers to produce viable seeds with desired genetic combination. This process can be achieved through controlled manual pollination, ensuring specific crosses, or open natural pollination in polycross designs for broader genetic diversity. Effective hybridization requires a thorough understanding of yam floral biology, including flowering synchrony, pollen viability, and stigma receptivity, to maximize seed set and quality.

2. Purpose

To provide a standardized step-by-step procedure for yam hybridization, focusing on controlled pollination to generate full-sib progenies or unsupervised open pollination generating half-sib progenies for breeding programs, ensuring high success rates and reliable genetic outcomes. The SOP aims to ensure pollination activities are carried out with minimum errors and repeatability across the yam breeding networks.

3. Scope

This SOP covers processes and tasks 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
SOP	Standard Operating Procedures

Stagger Planting: This method is used to synchronize flowering windows between early and late flowering clones/genotypes to be involved in crosses. Staggered planting at intervals of a week or more is used to synchronize flowering time and duration among genotypes 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 Associate/Manager

- Ensures the availability of the crossing plans to the supervisor and technicians
- Monitors and ensure the overall success of the pollination operations including ensuring adherence to the crossing plan and appropriate data collection

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 and consumables require for pollination
- Collect pollination data
- Identify fields for crossing block establishment
- Monitor the overall operation with pollination

Pollinator: performs flower bagging, pollen collection, manual pollination, seed collection, seed processing, and storage. Record pollination data

6. *Procedure/Protocol*

6.1 Crossing Block Establishment

Select parents based on their breeding value or cross merit for a defined breeding goal. Consider flowering efficiency, flowering time, and trait complementarity while selecting parents. Three possible options could be used to establish a crossing block in yam breeding. These include

- i. Separate crossing block for controlled crossings
 - ii. Polycross mating design
 - iii. Pollination in trial plots
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- i. **Separate Crossing Block:** This involves planting male and female plants (parents) in separate fields. Staggering planting may be used here to synchronize flowering time and duration among genotypes.
 - ii. **Polycross Mating Design:** Fertile male and female yam genotypes of desired traits to be recombined are planted very close to each other in isolation from external pollen sources to minimize contamination (minimum 100 meters away from other yam fields). The females are planted in the center while the males are planted to encircle the females or vice-versa. Verify flowering synchrony among parents through historical data or staggered planting dates to enhance pollination success. Allow natural open pollination to occur mostly by insects within polycross block to produce half-sib progenies. This design is less expensive; however, the viable seeds obtained may be fewer than those 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

- Select a field site with well-drained, fertile soil and adequate rainfall or irrigation options

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.
- Label each plant (barcode) with a unique identifier and stake for vine support with bamboo or metal or plastic stakes or rope and staking must be up to 2m in height for high photosynthetic efficiency

- Field maintenance, such as weeding and insect pest control, is important across the growth stages, and especially at the early growth stage.
 - Yam crossing block field requires close monitoring to prevent the outbreak of insect pests that attack young leaves a few months after planting.
 - Weeding is done manually and with chemical only as pre-emergence application
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6.4 Flower Bagging

- The pollination bags 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 species 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 mature pollen (the anthers of matured pollens are creamy in colour, premature pollens are greenish, while old pollens are yellowish or brownish) from male parents.
- Immature and ripped pollen are not suitable for use in pollination.
- Pollens of best quality are usually collected at middle section of the spikes as the pollens from the distal or proximal parts may be premature or over-mature respectively
- Pollen is better collected in the morning before anthesis: opening of anthers for pollen shedding.
- Keep the collected pollen in a container bearing a lid for cover.
- Pollen collected should be used for pollination between 1-3 hours after collection.



Yam plant with male flower



Mature male flowers



Female flower

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, select female flowers at the receptive stage (slightly open, stigmas glistening), and open the male anthers using pollination pins or fine-tipped forceps to take out pollen grains and gently apply collected pollen to the stigma ensuring even coverage as quickly as possible. Remove immature or overmature female flower spikes from the inflorescence ready for pollination.
- An anther is enough for a female flower when the weather is cool; harsh weather makes pollen dries, in that case, more than one anther from a fresh open flower may be gently placed on the stigma
- Wear magnifying lens during pollination due to smallness of yam pollens
- Re-bag the pollinated flower immediately after pollination spikes again
- Label the pollinated spike with cross details (male and female parent IDs, date, number of flower pollinated and pollinator's identity etc).
- 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 the hardness of the seeds. Hard and well filled seeds 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 envelopes with family IDs using barcode labels for easy retrieval

7. Reference

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

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