IITA Transforming African Agriculture CGIAR	**Crop:** Cassava **Function:** Hybridization and crossing block management	**SOP #**	IITA-CS-SOP01
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STANDARD OPERATING PROCEDURE (SOP) FOR HYBRIDIZATION AND CROSSING BLOCK MANAGEMENT IN CASSAVA

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1. Introduction:

Genetic variation is the basis of selection in plant breeding. Hybridization is the process of making controlled crosses between selected genotypes to provide genetic variation for stage 1 of plant breeding. This diversity created in the genetic resources provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics.

2. Purpose

This document describes the step-by-step procedure of crossing block establishment, management, and hybridization in a cassava breeding programme.

3. Scope

This document describes the hybridization procedure used by the IITA Cassava Breeding Programme in Ibadan, Ikenne and Ubiaja and can be used by other Cassava Breeding Programmes.

4. Definition of terms

DOP: Date of Pollination

CrossID (BagID): pre-printed unique identifiers that will be assigned to a cross in the field while pollinating.

5. Roles and Responsibilities

Staff	**Roles and Responsibilities**

Breeder	Supervises the hybridization plan
Data Manager	provides digital tools and barcodes for data collection
Hybridization Coordinator	Serves as a link between breeders and the hybridization team
Hybridization Group	The group contains the leader and three other members. They carry out the pollination activities such as identifying parents, bagging, pollen collection, data collection, andcrossing ,etc.

6.1 Procedure/Protocols for crossing block establishment and management

- Breeder Plan: Identify parents based on breeding goals and objectives
- Select a field to establish a crossing block (Index site selection SOP) and mark out at and 1m inter-row spacing and 0.8m intra-row spacing.
- Prepare 100 – 150 healthy cuttings of selected clones to use as progenitors
- Tag the plants with barcode labels containing parent information
- For weed control, refer to standard operating procedures for weed control and management

6.2 Procedure/Protocols for hybridization

- Flag the parents using ribbons of different colours based on target product profile. Barcode labels are tagged on both parents
- Monitor and identify mature male and female flowers

Mature male flower

Mature female flower

- Bag mature female flowers and collect mature male flowers in a pollination bag or petri-dish. Both activities should be carried out in the morning.

Bagging of female flowers

- Label the male flowers appropriately using sticky barcode labels and store in a cool dry place
- Prior to pollination in the afternoon when female flowers are expected to open, prepare pollination tags containing barcode labels that carry information on the bag ID, male parent information, date of pollination, number of female flowers pollinated and tie them to plants using the rope attached to the tag
- Open the female flowers bagged earlier in the morning.
- Take the selected male flower from the pollination bag

- Dust the pollen from the male flower on the stigma of the already opened female flower.
- Cut off immature female flowers to avoid mix-up of pollinated and un-pollinated flowers

Deposition of pollen on the stigma

- Scan the barcode using intercross App to retrieve parental information.
- Enter number of flowers pollinated into intercross App.

Scanning of barcode label

- Completely cover the pollinated flowers with clean and sterile pollination bags to avoid contamination. Pollination bags are oven- or sun-dried for 1-2 days to sterilize after each use.

Covered pollinated flowers

- Unbag the pollinated flowers 72 hours (3 days) after pollination
- Start fruit set scoring 3 weeks after pollination.
- Use net bags to cover mature fruits to prevent seed loss. Allow fruits to dry and shatter into the net bags
- Harvest mature fruits 45-90 days after pollination.

Mature fruit

- Harvest mature fruits and process the seeds at the seed processing centre.

Collection of fruits

- Separate seeds from the shell after shattering

Seeds after shelling

- Sort,count and pack the seed in a barcoded seed envelope, package and store in a cool dry place

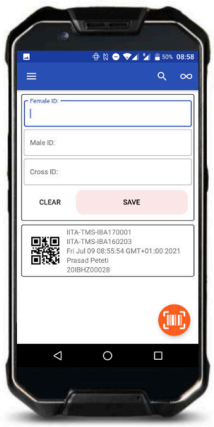
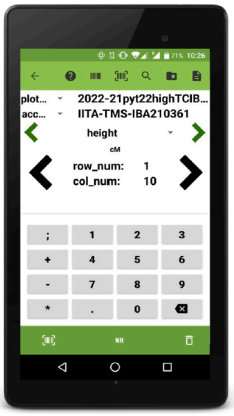
7. **References**

1. Hahn, S.K., Howland, A.K. and Terry, E.R.1977. Cassava breeding at IITA, Proceedings of the Third Symposium of the International Society for Tropical Root Crops, IITA, Ibadan, Nigeria, 2-9 December, 1973. Ibadan, Nigeria: IITA, (p. 4-10).
2. I.I.T.A. 1990, Cassava in Tropica Africa: A reference manual International Institute of Tropical Agriculture. 184 pp
3. Intercross - Apps on Google Play
4. Cassavabase Manual on Crossing management

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

Tools and Supplies

Intercross App for data collection	 https://play.google.com/store/apps/details?id=org.phenoapps.intercross&hl=en&gl=US
Female barcode label that will be tagged to the plant	
Male barcode label that will be stuck to the container or bag while collecting the pollen	
Cross Barcode that will be assigned to the cross for data collection	

Phones/Tablets with barcode scanner and power bank	 
Pollination bags / Fruit set bags	
Pencil (3D)	
Pencil sharpener	

Eraser	
Seed envelopes	
Ribbons (different colors)	

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Step by step pollination activities by timeline

Steps	Timeline	Activities
1	Previous days	Identifying the potential parents. This can be done with the help of cassavabase and analytical tools
2	Previous days	Looking for availability of potential parents and flagging using barcode labels
3	Day 1 (morning)	(i) Bagging (cloth bag type) of mature female flowers and (ii) Collection of mature pollen is done in the morning hours (from 8:00 am). Also, emasculation is carried out where necessary.
4	Day 1 (afternoon)	Pollination is carefully done while all needful information is recorded on a tablet or mobile device (female genotype x male, number of flowers and date and initials).
5	Day 1 (afternoon)	Tieing of pollination tags should be done. -> Enter the details into crossing tool in cassavabase.
6	Day 4	Remove pollination bags after 72 hours. Tie red ribbon around pollinated flowers for identification.
7	Month 1	(Score for fruits set 3-4 weeks after pollination using the fieldbook app. -> Data collected is exported to cassavabase
8	Month 1	Fruit bagging (net bag type) to avoid seed loss due to shattering
9	Month 2-3	Harvesting: Net bags containing all mature fruits (usually shattered) and seeds are collected with the cross label.
10	Month 2-3	Seed shelling, sorting counting and packaging. -> Data collected is exported to cassavabase
11	Month 2-3	Store seeds in a cool dry place
12	Later	Check for seed viability before planting particularly when direct seeding is to be done

13	Later	The complete process can be tracked in cassavabase using the crossing tool

Flow chart of pollination process.
A) Identification of female flower; B) Covering of female flower, C) Pollination; D) Bagging to avoid contamination; E) Netting to avoid shattering of fruit.

B (7:30- 11:00 am)

C (1:30 pm)

A (3MAP)

E (1 month after pollination)

←

D (72 h after pollination)