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Standard Operating Procedure (SOP) for Field Establishment of Banana crosses

Authors & Contributors

V. Akech*, R. Swennen(R.Swennen@cgiar.org), A. Brown(A.Brown@cgiar.org)

*Correspondence: V.Akech@cgiar.org

1. Introduction

The hybrids developed from the different cross combinations undergo a series of trials starting with the early evaluation trial (EET), in which only two replications per genotype are planted and evaluated for traits such as black sigatoka resistance, bunch size, fruit parthenocarpy, and dwarfness for over two production cycles. This activity is under stage 3: crossing and screening; during the Matooke product development process as shown in the figure 1. The purpose of EET is to quickly select for promising hybrids to advance for further evaluations in preliminary yield trials (PYT).

Stages & Gates	Stage 0		Stage 1		Stage 2		Stage 3		Stage 4		Stage 5		Stage 6		Stage 7
Stage Title	**Product Design**		**Trait Discovery**		**Trait Deployment**		**Crossing & screening**		**Early Testing**		**Late Testing**		**Pre-commercial Testing and Product Registration**		**Product Introduction**
Description in banana	Market research, Crop Strategy Review, Product Profile Review		Evaluation of germplasm sources, trait validation, inheritance and molecular discovery		Introgression of trait into improved diploid and/or tetraploid parents		Product development, seed production, clonal multiplication		Clone development from selected EET, small plot testing with 2-5 clones/genotype		Selected clones in replicated multi-site representing TPE (yield/resistance stability)		National Performance Trials and On-Farm trials		Official release and product launch
Short name in banana	Product profile		Prebreeding		Parent development		Generating EET (Early evaluation trial)		PYT: Preliminary Yield Trial		AYT: advanced yield trial (multilocational testing)		By TARI, NARO, etc		By TARI, NARO, etc

Figure 1 Showing the stage and gates during the Matooke product development process.

2. Purpose

The purpose of this SOP is to describe a series of activities carried out when weaned banana plantlets are received from the tissue culture (TC) laboratory and cared for in the nursery until they are established in the field for evaluation. However, some of these procedures also apply to plants that go through the tissue culture process for various purposes.

3. Scope

The SOPs apply to all breeding activities implemented under the IITA banana breeding programme in Namulonge, Uganda in collaboration with the National Banana Programme of NARO, Uganda. It aims to reach the broader banana breeding community in Uganda and for use as a blue-print in other banana breeding programmes in Africa.

4. Definition of terms

- **Hybrids:** Varieties of banana plants generated after crossing two different varieties.
- **Ploidy level:** This is the number of sets of chromosomes in a cell or an organism. For banana, genotypes could be diploids (2x), triploid (3x) and tetraploid (4x)

5. Roles and Responsibilities

All staff involved in implementing breeding activities in the banana breeding programme at IITA-Uganda must use the SOP manual. No alteration should be made to the procedures unless approved exceptionally by the programme leaders. The SOP manual will be revised once a year for possible updates. The list of individuals responsible for each section of the SOP in the breeding activities are listed below.

Senior banana breeder: Responsible for managing and overseeing all trials and data production conducted by the banana breeding programmes of IITA in East Africa

Research associate/Data Curator: Responsible for the creation of trials and all aspects of the trial data once it is established. This includes trial creation, its management and data collection, and checks on the implementation of defined protocols on sites ensuring that meta data for all trials is accurately recorded, has no outliers, is complete and

uploaded in Musabase. Provides end-user support and training on the usage of Musabase and digital tools.

Field technicians: Perform field tasks such as such as performing the hand pollination, harvesting, seed extraction, and field data collection or in field management practices as defined in the trial protocols using digital tools defined in the protocol for capturing, storing, transmitting, and ensuring quality of data within defined time periods.

Laboratory technicians: Perform laboratory tasks such as embryo rescue, culturing and subsequent sub-culturing and eventual weaning of plantlets in tissue culture laboratory. They also ensure tissue culture activities are recorded with the ODK App and uploaded on and uploaded to the Banana Tracking Tool (BTracT). Molecular laboratory technicians perform tasks like ploidy analysis for hybrids and DNA extraction for quality control and other breeding related needs.

Data technician: Ensure the quality and accuracy of collected data follows the protocol of each trial. Ensure that collected data are daily backed up, devise and implement efficient and secure procedures for data handling and sharing with the concerned scientific team. Work with the database curators to organize and maintain data in the correct formats for upload and storage on Musabase. Keep good custody and maintenance of data collection gadgets and apps, identify, justify, and report any need for updating, upgrading, or replacing the data collection gadgets. Assist with reports and data extraction when needed.

6. Procedure/Protocols

6.1 Required tools

- Musabase

This is the global banana breeding database and a data management tool in support of breeding. Use this system to generate the crossing plan as stipulated in the breeder's wish list of parents and priority order. It can be accessed at https://musabase.org/.

- Banana Tracking Tool (BTracT)

The banana tracking system for crosses, seeds and plantlets accessed at http://btract.sgn.cornell.edu/btract/.

- Android device

- Plant ID field labels

Product OL125LP - Weatherproof Polyester Laser - 4" x 2" Shipping Labels purchased from 4" x 2" Shipping Labels - Weatherproof Polyester Laser - OL125LP (onlinelabels.com).

- Zebra mobile printer
- Zebra ZQ520 series.
- Zebra desktop printer
- Zebra ZT411 industrial printer.
- Zebra designer software
- Zebra designer Professional version 3.5.
- Cross ID field labels
- SC-TTL - Revolutionary Self-Contained Thermal Transfer Labels. 2.0mil Polyester with a resin ribbon (240 per roll), 3.375" wide, .75" core, 2.25" OD.
- Cross ID lab and nursery labels
  PP White self-adhesive film Plain labels special layout 3 Across 33 x35 mm packed in rolls of 5,000 pcs on 76 mm Core.
- Computer with internet access.
- Potting soil
- Sterile forest soil or loam soil, dry manure and saw dust mixed in ratio of 4:2:1 respectively.
- Transparent 100 ml volume plastic ice cream cups with holes in the bottom, used as weaning cups.
- Black polyethene potting bags (21.8 cm x 26.4 cm) with holes in the bottom.
- Rigid white plastic plant labels, 12.cm length by 1.4cm width.

6.2 Nursery handling of seedlings

6.2.1 Weaning

- Rooted plantlets are received in baby jars (barcode labelled) from the TC laboratory as seen in figure 2a.
- Fill the weaning cups with potting soil.

- Re-print the bar code labels shown on the baby jars (number of reprinted bar code labels = number of plantlets to be transferred, usually four).
- Place the bar code labels on the rigid white plastic tags and stick them into the cup that will contain the plantlets as seen in figure 2b and c.
- In the morning, transfer the plantlets from the baby jars into the plastic cups containing potting soil evenly placing four plantlets per cup and water them.
- Scan plantlet bar code ID to record date of weaning, number of weaned plantlets and status of weaned plants e.g. 'contaminated' and take pictures of the status, using the ODK app.
- Do this for all plantlets\genotypes weaned.
- Place the cups containing plantlets on plastic trays and put them in a humidity chamber standing under a shade as in figure 2d.
- Keep mist spraying the plantlets and chamber to maintain a high humidity to prevent drying out of the plantlets.
- Leave the plantlets in the humidity chamber for 3-8 weeks until fully weaned. Fully weaned plants should have at least 3 - 4 fully open green standing leaves.

Figure 2. Barcode labelled baby jars with plantlets (a), plants in weaning cups (b and c) and plantlets in humidity chamber.

6.2.2 Transfer to the screen house

The screen house for banana plants is a structure covered in insect screening material (netted material) with a shade roof to provide environmental modification and protection against pests and severe weather conditions. The nursery at Namulonge is fitted with an automatic irrigation and misting system as seen in the figure 3 below.

Figure 3. Screen house at IITA-Namulonge

- Fill to 3\4 capacity of the black polyethene potting bags with potting soil.
- In the morning transfer one plantlet with its bar code label/tag in each potting bag and gently push the roots into the soil so it firmly stands in the middle of the potting bag, taking care not to damage the roots and plant.
- Scan plantlet bar code ID with ODK app to record date of potting, number potted, status and photo of status of potted plant into BTracT.

- Transfer the potted plants into the nursery, placing them in an orderly manner systematically (Figure 4).

Figure 4. Potted tissue culture plants in the nursery

- Water them after transfer until water passes through the bottom holes regardless of the irrigation schedule of the nursery.
- Set the automatic irrigation and misting system in the nursery to twice a day (morning and evening) for ten minutes each run during the dry season and only once a day during the rainy season The automatic settings are programmed as desired on the irrigation systems control board in the figure 5 and a manual provided by the manufacturers Rain Bird Agri-products on how to do this is available at the station.

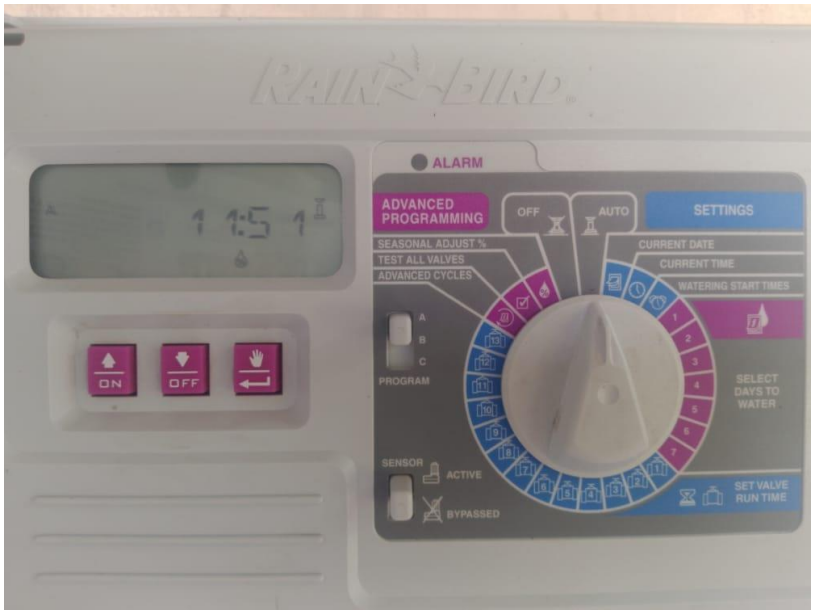

Figure 5. Control panel of the automatic irrigation and misting system at IITA-Sendusu

6.2.3 Hardening

Hardening is done to promote plant survival in the field after the transition from the protected screen house to the fluctuating outside environment in the field. It is done for one week prior to planting in the field.

- Remove the plants from the screen house and place them under shade.
- Scan plantlet bar code ID to record date of hardening, number hardened, status and photo of status of hardened plant.
- Water the plants once a day for seven days.

6.3 Ploidy analysis through flow cytometry

This is to establish the ploidy level of the hybrids so that hybrids of the same ploidy level are planted together and evaluated with the reference to their ploidy levels. Leaf samples

are taken while the plants are still in the nursery. The most accurate and user friendly method is the flow cytometry. A complete standard operating protocol on the ploidy analysis process is under development.

6.4 Field establishment of hybrids

6.4.1 Field preparation

- Choose land that is relatively flat for efficient cultivation and management.
- Clear the land of bushes by hand slashing.
- Plough to break the hard surface and open it up with a tractor mounted with plough discs.
- Harrow 3-7 days after ploughing using a tractor mounted with a harrow discs that deeply cut to loosen up and mix the soil thoroughly.
- Measure and field mark to spacing of 3 metres (between plants) x 3 metres (between rows) for triploid and tetraploid hybrids, giving a plant density of 1,111 plants/ha.
- Measure and field mark to spacing of 2 metres (between plants) x 3 metres (between rows) for diploid hybrids, giving a plant density of 1,666 plants/ha.
- Dig or drill planting holes measuring 60 cm wide and 60 cm deep.
- Separate the top soil from the bottom soil while digging the planting holes, to be used at planting.

6.4.2 Trial design

In early evaluation trials (EETs), a completely randomized design (CRD) is used with two replications per hybrid making sure the replicates are not next to each other. This is ideal because of the large number of genotypes/hybrids to be evaluated, large space requirements for banana plants and limited number of replicates per genotype. For

example in season two 2021 (April - September 2021), and in Season one 2022 (October 2021- March 2022), 613 hybrids and 533 hybrids were planted in EETs with two replications per hybrid, respectively.

6.4.3 MusaBase to design or add trials

MusaBase is used to design new trials but also existing trials can be added. Detailed step-by-step instructions on how to create or add a trial in MusaBase are available at: https://solgenomics.github.io/sgn/03_managing_breeding_data/03_07.html#adding-trials. Only MusaBase users with user status of 'submitter' can create or add trials. The general steps are as per below.

- The accessions in the trial to be added must already exist in the database. If the hybrids were recorded by BTracT, they already exist in the database and there is no need to add them.
- Click on the 'Manage' tool and choose 'field trials'.
- There are two options, 'Upload Existing Trial(s)' or 'Design New Trial' as seen in the figure 6 below.

Figure 6. How to design new or upload an existing trial on MusaBase.

- Choose according to the nature of the trial.
- For each option, there is a workflow 1-7 to follow (Figure 7 and 8). Fill in the information required with each step. Only after correctly providing the right information at each step can you proceed to the next step.

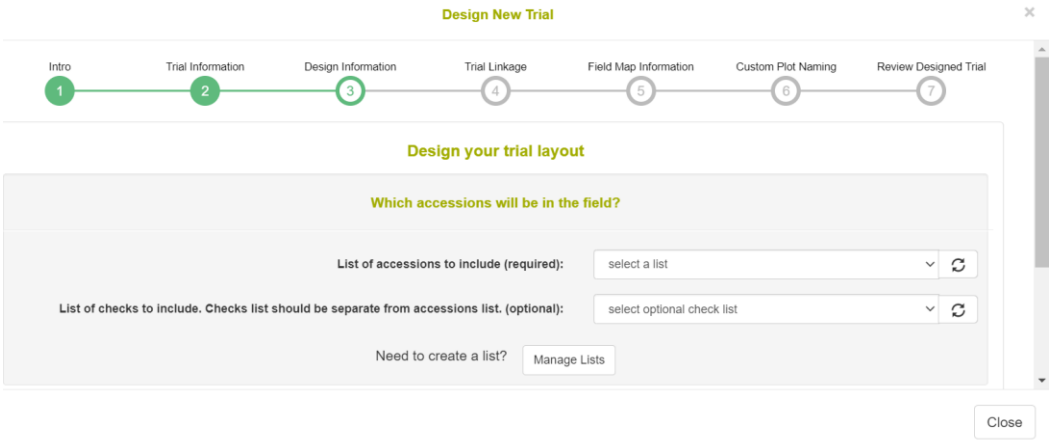

Figure 7. Workflow on how to design a new trial in MusaBase.

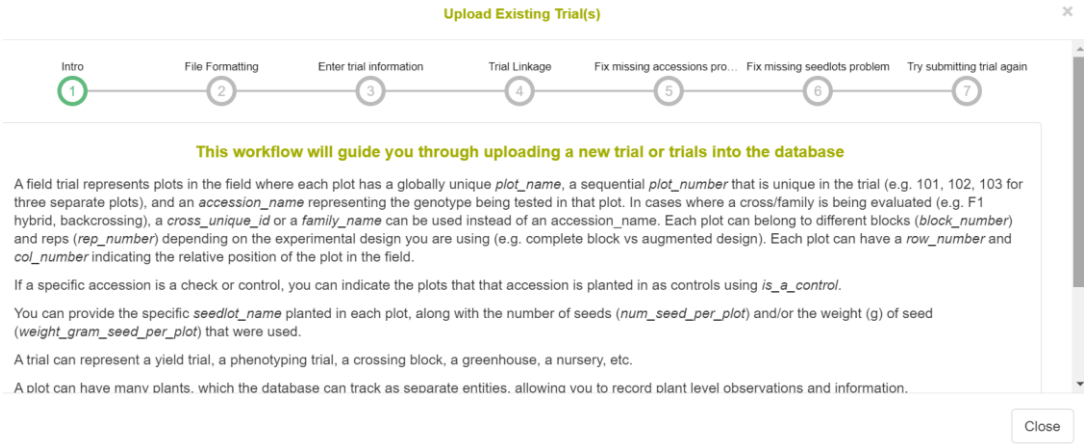

Figure 8. Workflow on how to upload an existing trial in MusaBase.

- When uploading an already existing trial (layout in a spreadsheet), be sure to use the required format by MusaBase (Figure 9).

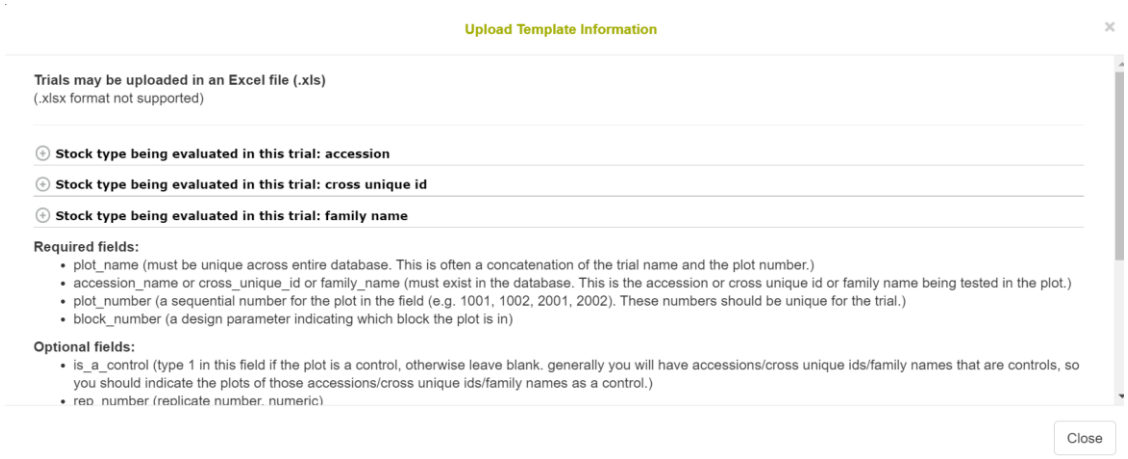

Figure 9. Template and required headers for formatting existing trial layout spreadsheet.

- On completion of all required steps with the required information, upload the trial. If all necessary information and format is correct, you will get a message as seen in figure 10.

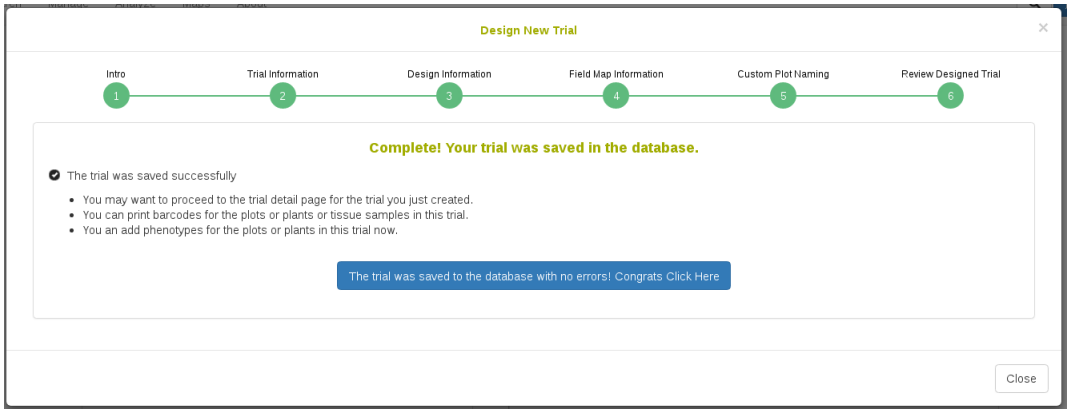

Figure 10. Confirmation of successful trial upload on MusaBase.

6.4.4 Creating and printing field labels

Detailed step-by-step process of how to create and print bar code labels is available at http://solgenomics.github.io/sgn/03_managing_breeding_data/03_12.html

The general steps followed are:

- Open the manage tool on MusaBase.
- Click on label designer.
- Select the trial you want to create labels for.
- Create a new design or load a saved design. The saved design for field labels used at Sendusu is named ‘‘Sendusu labels’’ in the database. This design has been customized to fit the bar code label papers mentioned in section 3.6.1.4.
- Download the pdf file of all barcodes of the trial.
- Print the bar codes using a laser jet office desktop printer.
- Stick the bar codes on 0.5 metre long PVC pieces, flattened at the area of bar code placement for easier QR code reading as seen in figure 11.

Figure 11. Bar code label stuck on PVC piece

6.4.5 Planting Early Evaluation Trials (EET)

- Choose healthy and strong looking plants with a girth of 5.0 cm and above for planting. We use girth and not height because we need plants that will not break during transportation and planting exercises.
- Mix earlier separated topsoil with 20 kg of well dried farmyard manure (cow dung is commonly used).

- Place the tissue culture plantlet in the middle of the planting hole and add the soil-manure mix until plantlet is firmly standing.
- Place mulch around the plant (ring mulching) to conserve soil moisture and control weeds. Be careful that the mulch does not touch the plant.
- Label your plants by placing the PVC pipe in front of the plant.

6.4.6 Field maintenance

- Mulch the fields with dry grass to a thickness of about 10 cm to control weeds and conserve moisture.
- Control weeds by manual removal of weeds using hand hoes only because the plants are still young and fragile for herbicide use. Later when the plants are six months old and bigger (about 100 cm tall) you can use herbicides (50 ml of 2, 4-D amine salt 720g/l and 150ml of Agrosate - 480g/L glyphosate, mixed in 15 litres of water).
- De-trash the fields by cutting off the broken, dead and dry leaves to keep the fields clean and reduce sigatoka disease inoculum.
- Routinely scout for banana Xanthomonas wilt (BXW) infected plants and destroy them by cutting, collecting them in one place and burning them. Disinfect all tools used by dipping them in 3.5% sodium hypochlorite (household bleach) for 30 seconds to prevent spread of the disease from one plant to another.
- Remove the male buds to control banana Xanthomonas wilt (BXW).
- De-sucker after taking cycle 1 data for number of suckers at flowering. Remove excess suckers to maintain only three plants; mother, daughter and granddaughter plants (MDG) per mat.

- Always make sure the plant ID labels are well placed on the right plants and replace any damaged bar codes.

7. References

8. Appendix