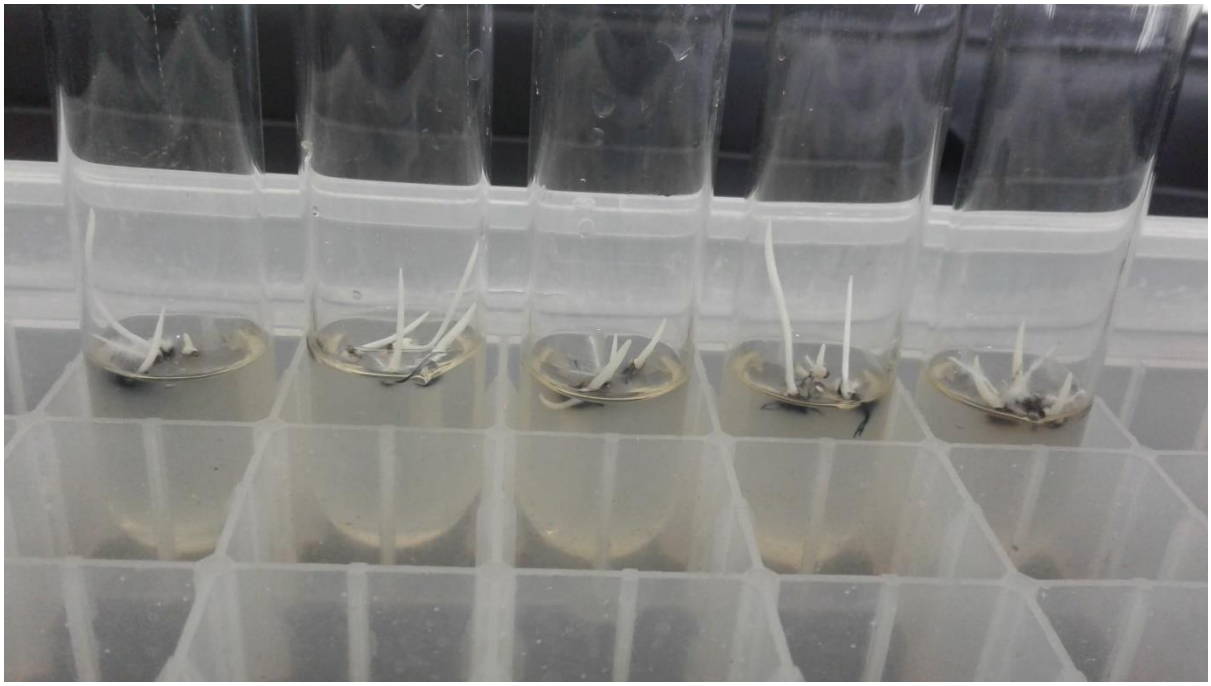
March 1, 2025

SOP13:

Standard Operating Procedure (SOP) for Embryo culture in Banana



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Standard Operating Procedure (SOP) for Banana Embryo Culture

Authors & Contributors

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1. Introduction

Banana/plantain breeding depends on successful controlled hybridization (or open pollinations) resulting in the formation of fertile seeds. However, most banana seeds fail to germinate. In vitro embryo culture, which involves the extraction and growth of embryos on suitable culture media for regeneration into plantlets is routinely done in breeding programs to maximize the recovery of progenies from hybridizations.

2. Purpose

The purpose of this SOP is to describe a series of activities conducted during the culture of excised embryos from banana seeds to obtain rooted plantlets ready for weaning.

3. Scope

This SOP depicts all embryo germination activities implemented within the IITA Musa breeding programme. The SOP covers seed sterilization, embryo extraction and culture, subculturing and rooting as well as electronic data entry.

4. Definition of terms (need to establish what terms to define)

Dormant or seed dormancy is a state where a seed remains inactive (fails to germinate) for a certain period under favorable conditions.

Embryo culture: is the sterile growth of an embryo in vitro with the goal of obtaining a viable plant.

Germination: Germination can be described as the development and emergence of essential plant structures including the radicle and plumule.

Hybrids: Plants or offspring which are a result of crossing two parental genotypes.

Cracking: It is the opening of a seed to expose the embryo.

Laminar flow hood: An enclosed workplace used to create a contamination free work environment through filter sterilization.

Micropylar plug: is a minute opening in the integument of an ovule of a seed.

Banana breeding Tracking Tool (BTtracT): It is an open-source system used to track information in banana breeding

5. Roles and Responsibilities

Tissue culture expert: Responsible for managing and overseeing all tissue culture laboratory activities conducted by the banana breeding programme in IITA and collaborations with public and private sector labs in Africa.

Research associate/ supervisor, Tissue culture laboratory: Responsible for managing and overseeing all laboratory activities, quality control, report writing, stock taking, training and demonstration/exhibition.
Laboratory research technician: Perform laboratory tasks such as embryo and shoor tip explant preparation, aseptic transfers and culture management, proper labelling including use of BTracT and data entry.
Laboratory attendant/assistant: Responsible for cleaning of the instruments and the laboratory

6. Procedure/Protocols

6.1 Required tools and reagents

- Sterile glass jars/vials
- Sodium hypochlorite (household bleach)
- Absolute ethanol
- Measuring cylinder
- Sterile distilled water
- Refrigerator
- Timer
- Laminar Flow hood
- Alcohol burner or spirit lamp
- Glass bead sterilizer
- Blunt tip forceps
- Scalpels with No. 11 blades
- Sterile paper
- Embryo culture medium in tubes (refer to SoP for banana tissue culture media preparation)
- Proliferation medium in tubes or jars (refer to SoP for banana tissue culture media preparation)
- Dark cupboard (25-27°C)
- Lighted growth room (25-27°C, 12-16 hours photoperiod).

6.2 Embryo culture procedure

- Seeds are received in an envelope with details comprising cross ID number, number of seeds, male and female parents, date of pollination, date of harvest and date of extraction.
- Record all the information on the seed envelope in the embryo rescue book/datasheet.

6.3 Sterilization of banana seeds

- Transport seeds in sterile containers labelled accordingly with cross number to laminar flow hood and sterilize as follows.
    - Soak in Ethanol (95%) for 3 minutes
    - Soak in Sodium hypochlorite (20% household bleach) + 0.2% tween 20 for 20 mins
    - Rinse 3 times in sterile distilled (SD) water

- Soak seeds in SD water for three days and keep in a refrigerator (4ºC) and then repeat the sterilization as above (Luyiga *et al.*, 2022).
- Rinse 3 times with SD water and proceed with embryo extraction.

6.4 Embryo Extraction and Culture

- Place seeds on sterile paper in the laminar flow hood.
- Grip seed firmly with a blunt tip forcep such that the micropylar plug faces up.
- Using a scalpel blade no 11, cut at one end (1) as shown (Figure 1). Note that the embryo is located just beneath the micropylar plug and avoid cutting close to it.

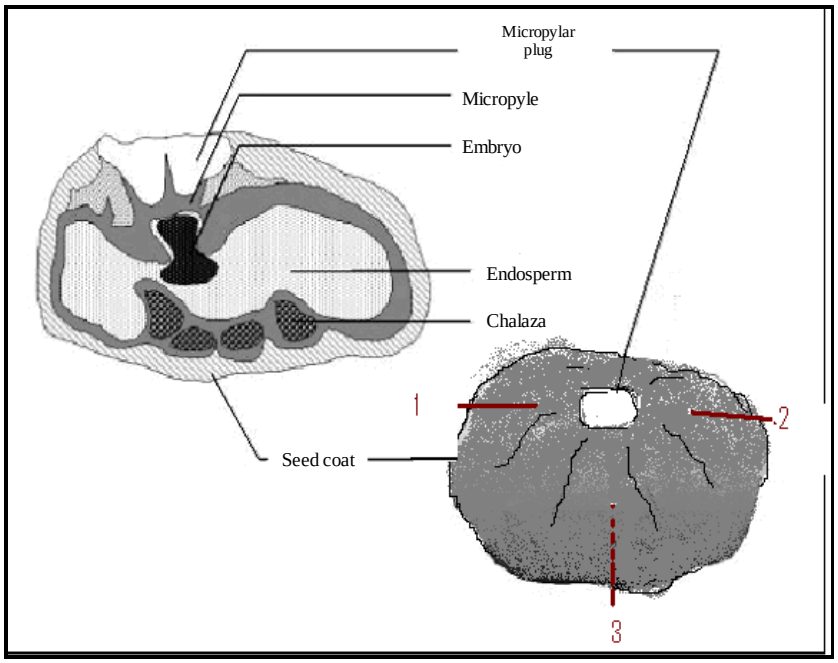


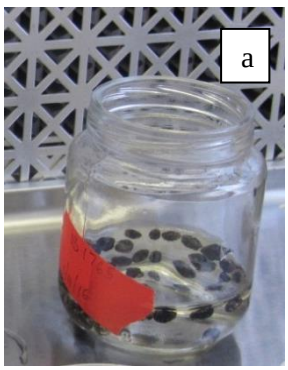
Figure 1 Longitudinal cut of a banana seed showing location of the embryo and three incisions

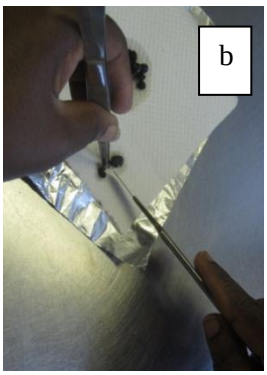
- Rotate the seed and make another cut at the opposite end (2) such that the seed coat separates, and the embryo becomes accessible. If not, a third incision (3) can be made.
- Gently pick out the embryo by tipping it with the blunt edge of the scalpel blade.
- Place embryos on the surface of sterile paper.
- Dip the tip of a sterile forcep in culture medium to make it moist, pick up the embryo by touching with the moist tip of forcep and place on embryo culture medium. Avoid culturing colored (likely dead) or callous (malformed) embryos as these will not germinate. Avoid leaving several embryos on cutting paper for a long time before transfer and make sure forceps are cold before picking embryos.
- Label tubes with cross ID number and date.
- Enter the number of cultured embryos into BTtracT by clicking on the web form which displays a range of activities in tissue culture lab. Selection is performed according to activity done, such as embryo rescue, germination, subculture, rooting, weaning 1 and contamination. Upon clicking on any of the activities above, for example embryo rescue, proceed by scanning the barcode of the

embryo (barcode of the cross ID), date of embryo rescue, number of good seeds and then submit. The zebra printer system has a software which is installed onto the computer, where barcodes are printed from.

- The barcodes are put on their respective tubes, then the embryos are taken to the dark cupboard at 25^{o}C for incubation.
- Check on a weekly basis to select germinated embryos for a maximum incubation period of 2 months.
- Pick out germinated embryos and transfer to proliferation media containing 4.5mg/l BAP in culture tubes.
- Enter the number of germinated embryos into the BTtracT system to generate identification numbers, each germinated embryo should be assigned a number i.e. 1, 2, 3, etc. (offspring from same cross ID). Print barcode labels and place each onto a tube containing a germinated embryo, then transfer to the lighted area.
- Continue sub-culturing after every 3-4 weeks and incubate in the lighted area to obtain the number of plantlets desired from a single embryo. During each subculture, endeavor to enter the activity into the BTtracT.
- Identify cultures with plants ready for rooting (at least 3 leaves and a well-formed pseudo stem 1.5-3 cm long).
- Two to three leaves per genotype are cutoff and wrapped in a piece of aluminium foil labelled with a barcode and provided for ploidy analysis. The pseudo stem is trimmed to about 1-2cm and placed on rooting medium.
- After obtaining ploidy results in BTtracT, barcodes are printed with both identification number and the ploidy level and placed on respective tubes.
- After 3 weeks, the plants with roots are entered into BTtracT as weaning 1 and sent to the nursery for weaning.

Illustration of steps in embryo culture





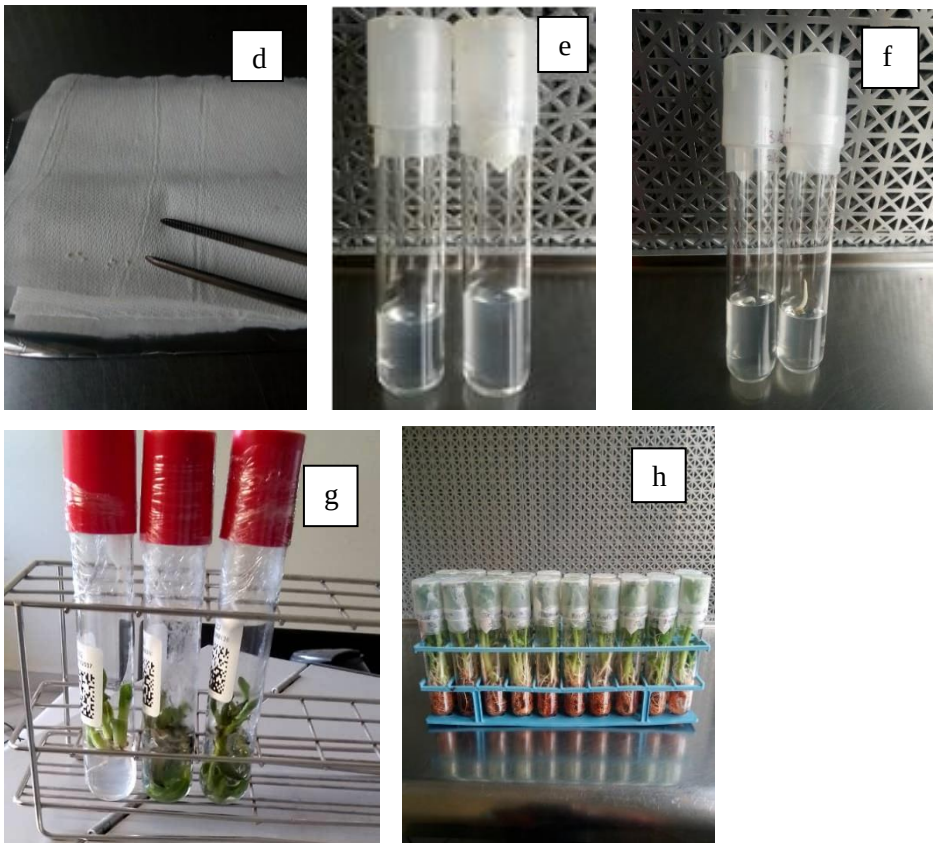


Figure 2: a) labelled seed disinfection b) Extraction of seed with forceps and scalpel blade c) Disected seeds d) Extracted embryo. e) Embryo placed on media. f) germinated embryo from dark cuboard. g) proliferated plantlets h) Rooted seedlings from embryo.

6.5 Using BTtracT for data recording

- Use the link https://btract.sgn.cornell.edu/btract or ona icon to generate a form for entering information.
- Click on the site station as depicted in figure 2.

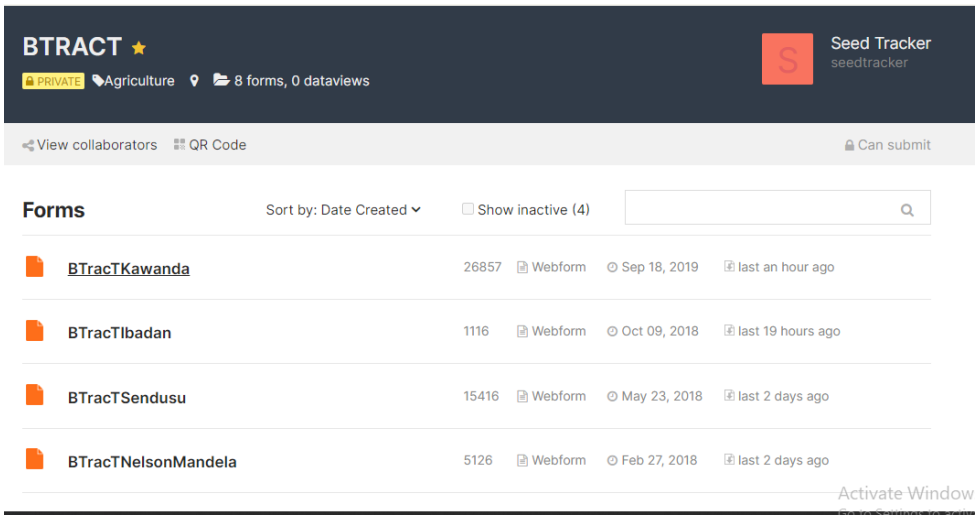


Figure 3: BTtracT site stations on webform

- Import of the webform is done as in figure 3

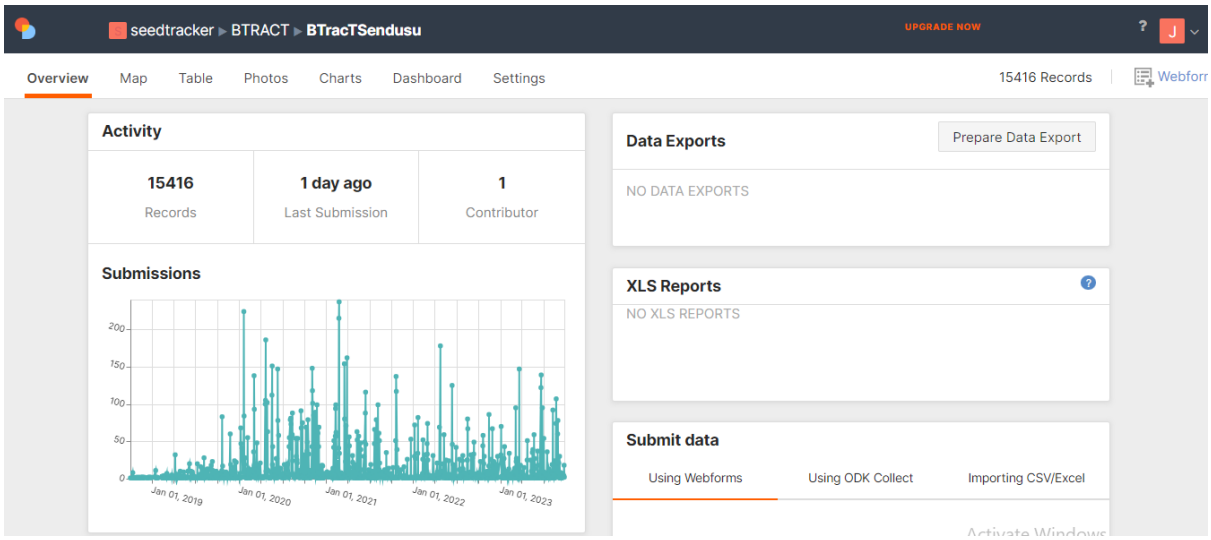


Figure 4: Bract displaying where a webform is located at the right-hand corner.

The steps are as follows.

- The webform should be updated (previous information from pollination must have been entered).
- Select 'Record Information' as figure as depicted in figure 4.

Figure 5: Part of the webform showing general information.

- Select the Station
- Select the activity for example embryo germination as in figure 5
- Scan the barcode and select the appropriate activity options
- Submit the information entered

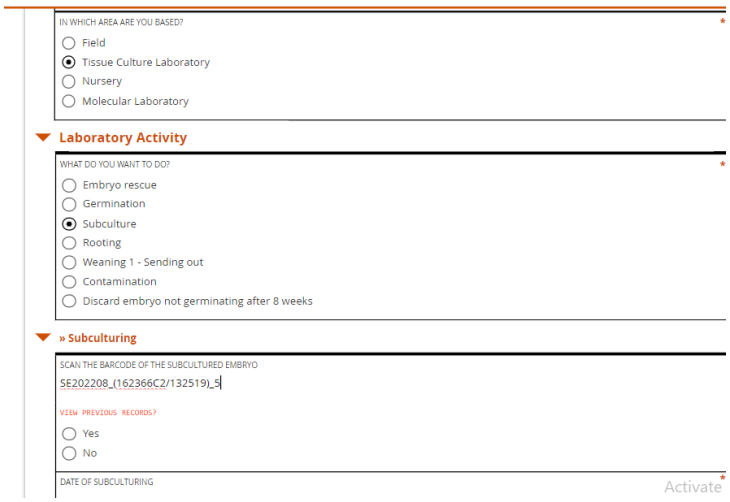


Figure 6: Shows a webform with different activities

How to print barcodes

- Select the BTrac-T icon.
- Go to barcode labels.
- Select the activity, site, the date range when the activity was done.
- Download as depicted in figure 7.

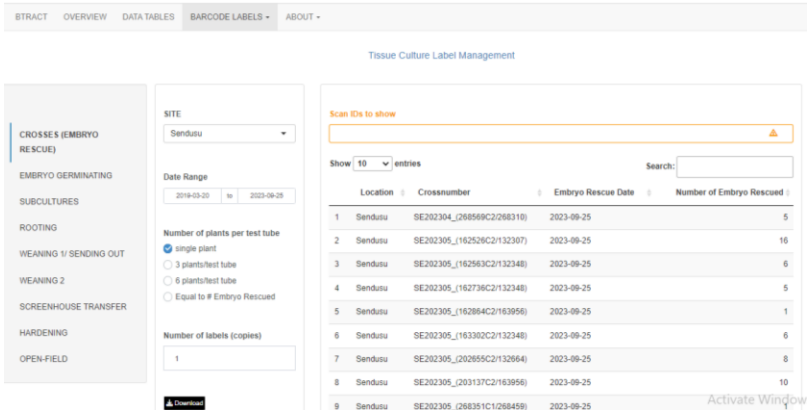


Figure 7: Shows a list of entries to be downloaded for barcode generation

7. References

Asif, M.J., Mak, C. and Othman, R.Y., (2001). In vitro zygotic embryo culture of wild Musa acuminata ssp. malaccensis and factors affecting germination and seedling growth. Plant Cell, Tissue and Organ culture, 67, pp.267-270.

Bakry, F., (2008). Zygotic embryo rescue in bananas. Fruits, 63(2), pp.111-115.

Luyiga, J. (2022). Male fertility and zygotic embryo germination in bananas (MSc dissertation, Makerere University).

Uma, S., Lakshmi, S., Saraswathi, M.S. *et al.* (2011). Embryo rescue and plant regeneration in banana (*Musa* spp.). Plant Cell, Tissue Organ Cult 105, 105-111 https://doi.org/10.1007/s11240-010-9847-9

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