Standard Operating Procedure (SOP) for Phenotyping and Data Management of Early Evaluation Trials in Matooke (AAA) Breeding

Authors & Contributors

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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 the stage : Crossing and Screening; during the Matooke product development process as shown in figure 1.The purpose of EET is to quickly select for promising hybrids to advance for further evaluations in preliminary yield trials (PYT).

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. Stage and gates during the Matooke product development process. This SOP falls under "Crossing & screening"

1. Purpose

The purpose of this SOP is to describe a series of activities, processes, data workflow and data storage protocols carried out during the phenotyping of hybrids in EET. EET deals with field evaluation and phenotypic selection of promising hybrids to be advanced to the preliminary yield trial (PYT) for further detailed evaluation during hybrid development in banana breeding. EETs are mostly non replicated trials in which two plants per genotype are evaluated during two production cycles. The workflow is built around

www.musabase.org and the Field Book data collection App (referred to as Field Book App, here after).

2. 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.

3. Definition of terms

- **Hybrids:** Plants generated after crossing two different banana genotypes.

4. 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 banana breeding pipeline lead. The SOP manual will be revised once a year for possible updates or as soon as need to revise it arises. The list of individuals responsible for each section of the SOP in the breeding activities are listed below.

Program lead:

Breeding pipeline lead: Responsible for managing and overseeing all trials and data production conducted by the banana breeding programmes of IITA in East Africa through the stages and gates leading from product development to product introduction.

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 performing the hand pollination, harvesting, seed extraction, and field data collection or 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 the tissue culture laboratory. They also ensure that tissue culture activities are recorded with the ODK App and uploaded on 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 uploading 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

6.1 Required tools and software

- Musabase

This is the global banana breeding database and a data management tool in support of breeding (Morales *et al*., 2022). 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/.

- Android device
- Field Book App

This is an Android-based mobile application for plant phenotyping developed at Kansas State University that makes field data entry fast and flexible (Rife & Poland, 2014).The App is open source to be downloaded free from google play store: https://play.google.com/store/apps/details?id=com.fieldbook.tracker.

- Computer with internet access

6.2 Field data collection using Field Book App

After installing the Field Book App, it will create a folder called 'fieldBook' on the Android device which contains sub-folders required for the running of the App. To setup data collection on the App you require a field layout file in '.csv' format and a trait list file in '.txt' format which can be imported from your local computer storage into the App. MusaBase will automatically generate these files for any trial created or uploaded to the platform and it is now standard practice to download both files automatically from MusaBase.

6.2.1 Creating a field lay out file from MusaBase

If a trial exists on MusaBase, there are two alternative methods to create the field layout file and download it from the database to your computer following the below steps.

6.2.1.1 Using the Field Book Tools page:

- Browse to “Manage” menu and click on “Field Book App” as seen in figure 2 below.
- On the Field Book App page, at the Field layout section, click on ‘New’.
- In the next dialogue box, choose the trial from which you want to create a layout file. Choose management factor which is the treatment to be applied to the trial, choose ‘none’ if no treatment is to be applied. Choose plot as the data level since we will be taking data on each plot. In this case plot refers to a single mat or plant.
- Check if all columns required have been included and add more columns you might need if not included. See figure 3.
- Click ‘Submit’ to create the file.
- A pop-up window will appear showing that the field book layout file was saved.
- Click on the file name to immediately download the file onto your computer. The file is also available from the “Field Book Tools” page, if you need to re-download it.

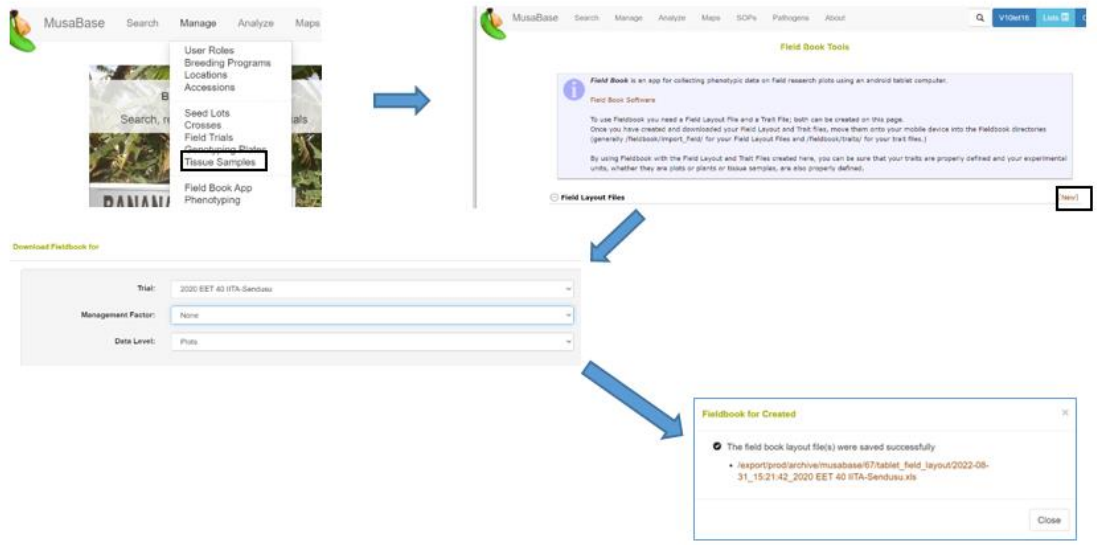


Figure 2. Workflow on creating field layout files using Field Book App on MusaBase

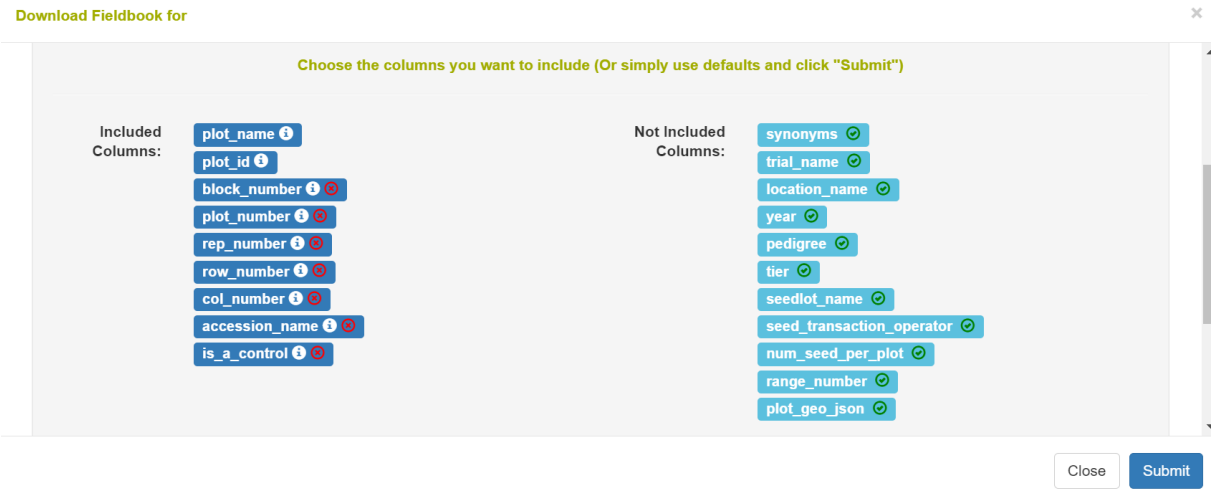
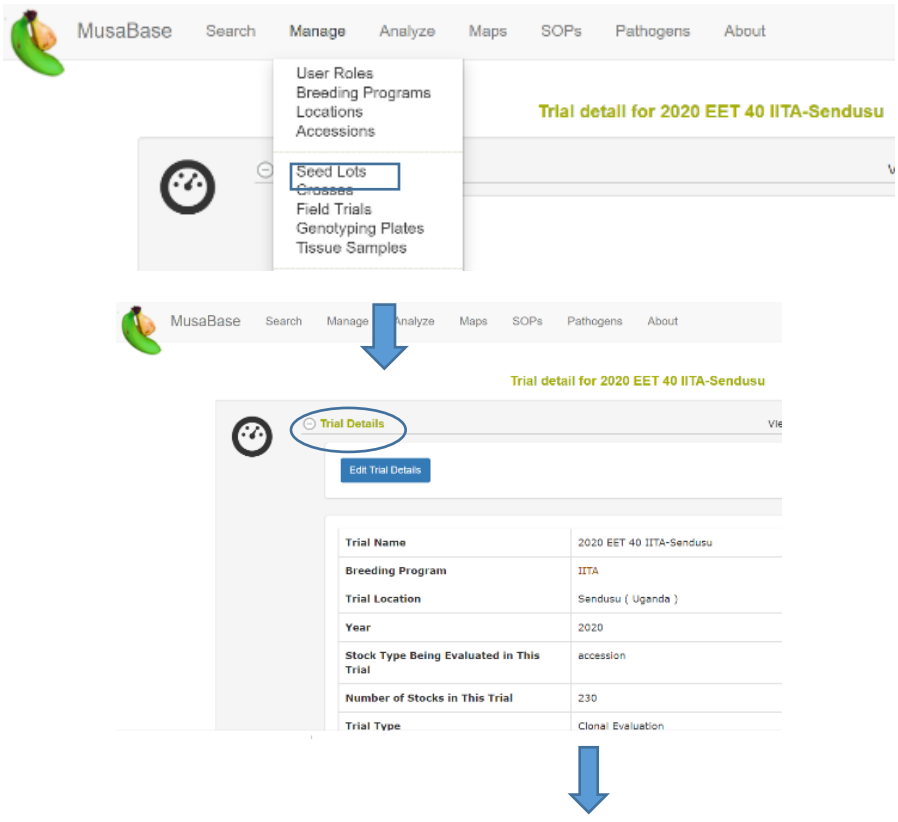


Figure 3. List of required (left) and optional (right) columns for the Field Book template

6.2.1.2 Using the trial details page

- Go to the "Trial Detail" page of the trial that you want to create Field Book data collection file for, via the 'Manage' tool as seen in figure 4 below.
- On the "Trial Detail" page, scroll down to the bottom of the page to find "Android Field Book Layout" in the "Files" section, then click on the "Create Field Book" link.
- This will open a new window showing the name of the trial that you selected, as well as data level (plots or plants) as in section 3.7.2.2.1 above. Click to submit.
- A pop-up window will appear showing that the field book layout file was saved successfully with a link to download the file, as seen in figure 4 below.
  Click on the file name to download the file onto your computer.



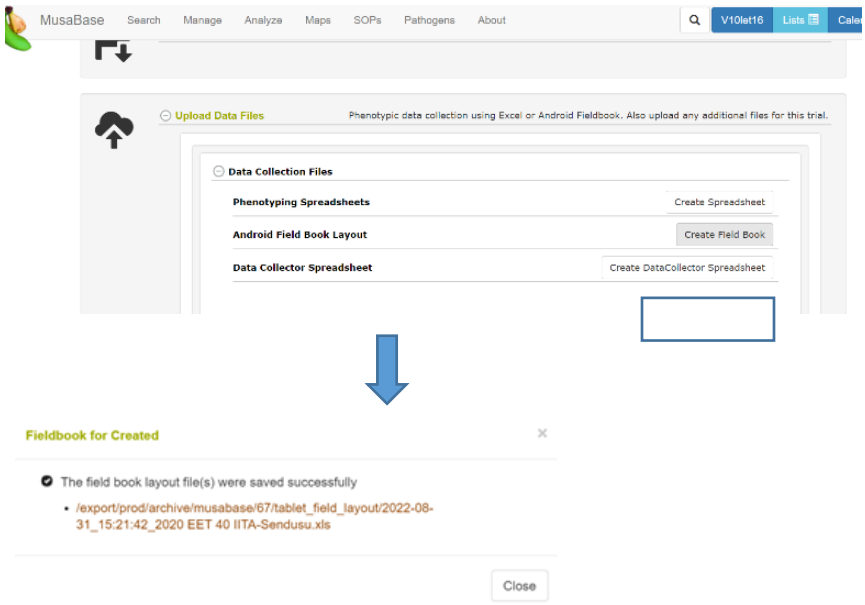


Figure 4. Workflow of how to create field layout files using Trial details page on MusaBase

6.2.2 Creating a trait file from the list of traits on MusaBase

A minimum set of breeder's list traits for EET has been developed by the IITA and the National Banana Programme breeding teams and is attached in Annex 1. This list is also available from MusaBase (figure 5).

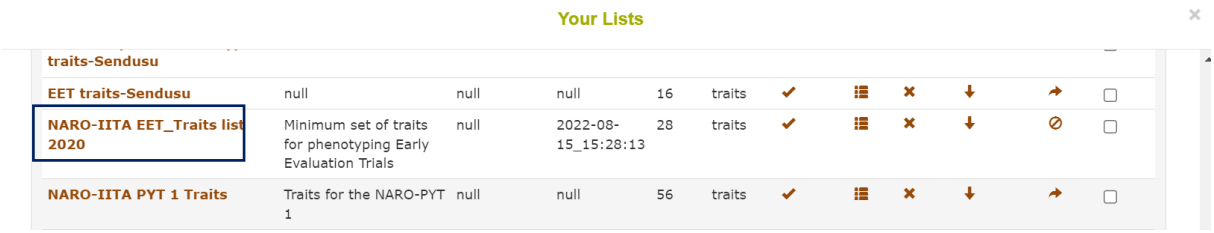


Figure 5. Screenshot highlighting a minimum set of breeder's list traits for EET from MusaBase

To create a trait file follow the steps below

- Browse to "Manage" menu and click on "Field Book App".

- Scroll down to Trait files section and click on 'New' (figure 6).

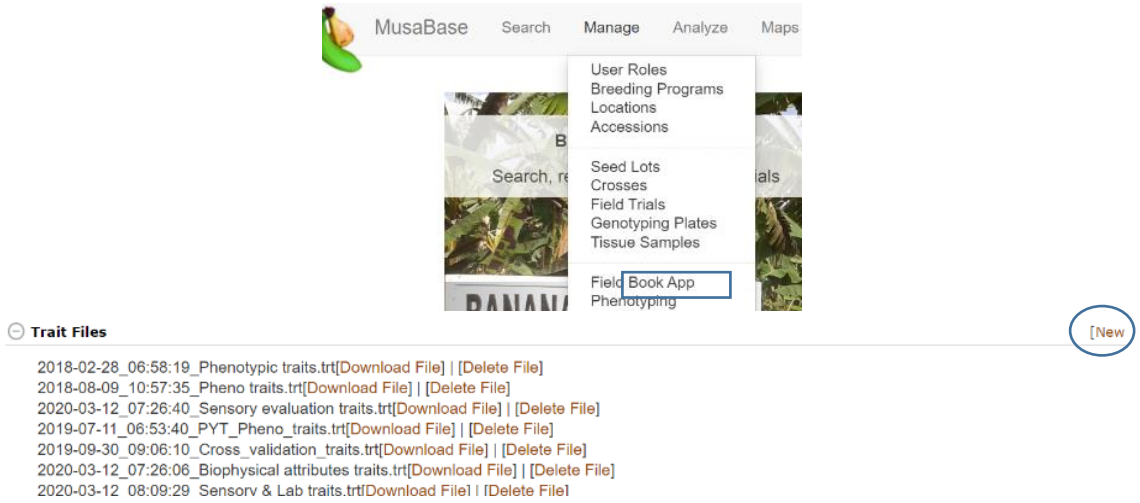


Figure 6. Steps 1 and 2 of how to create a trait file using trial list on MusaBase

- From the drop down list select the list of traits you created or the one available, in our case the "NARO-IITA_Traits list 2020" as in figure 7a below.
- Then name your trait list file and check the box 'Include Notes Trait' to be able to add and upload notes and comments on plots as seen in figure 7b.
- Click on 'create file'.

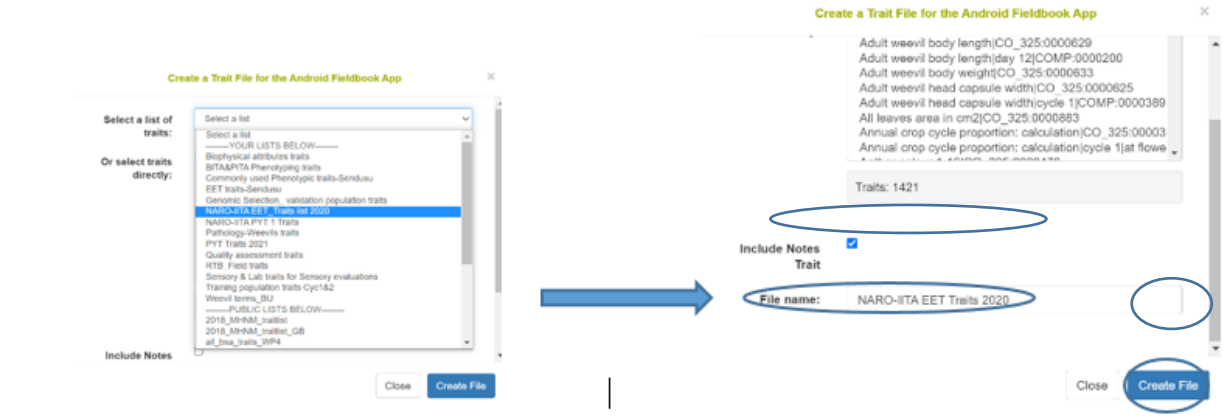


Figure 7. Steps 3, 4 and 5 of how to create a trait file using trial list on MusaBase

- A pop-up window will appear showing that the trait file was created with a link to download the file (figure 8).

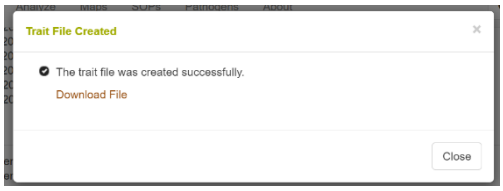


Figure 8. Pop up message showing successful creation of trait file on MusaBase

- Click on 'Download file' link as seen in figure 8 above to download the trait file on your computer. Trait files are in text format and saved with '.trt' extension on the file name.
- After the trait file is saved, you can also see your file listed in the "Field Book Tools" page under trait files and download it to your computer (figure 9).

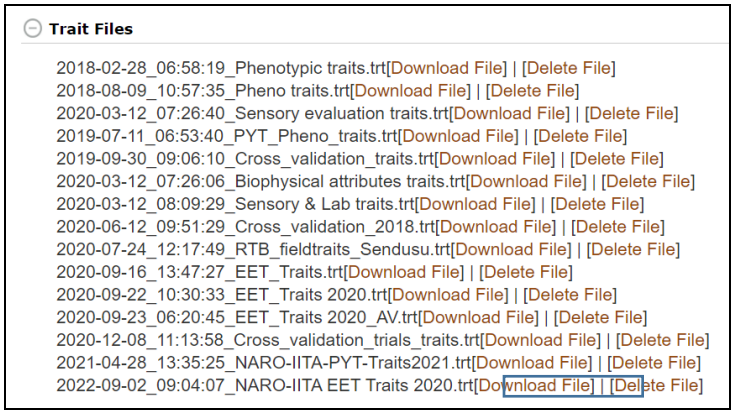


Figure 9. Existing lists of traits files on MusaBase downloadable for use in Field Book App

6.2.3 Transferring field layout and trait files from computer to Android device

- The downloaded files are found in the “Downloads” folder on your computer unless you moved them from there and saved them elsewhere on your computer.
- The field layout files always have a prefix ‘fieldbook_layout_’ added to the original name. For example trial 2020_EET_40_IITA-Sendusu will be renamed fieldbook_layout_2022-08-31_15:21:42_2020_EET_40_IITA-Sendusu.
- Connect the Android device to the computer.
- Open the internal storage of the Android device and open the ‘fieldBook’ folder. This folder contains nine sub-folders but only three (field_export, field_import and trait folders) are of importance for data collection (figure 10).

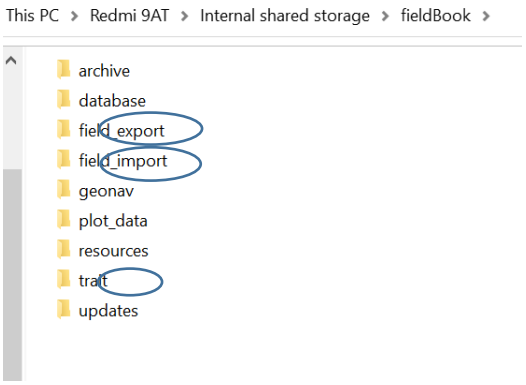


Figure 10. Sub-folders for operating the Field Book App

- Copy the field layout file from the computer into the field_import folder of the Android device.
- Copy the traits file from the computer into the trait folder of the Android device.
- You can copy and paste the files or drag and drop them into the folders specified above.

6.3 Setting up Field Book App on the Android device for data collection

After transferring the files into the 'fieldBook' folder on the Android device, the App needs to be set up for data collection and storage by importing both field layout and trait files into the App on the Android device.

6.3.1 Importing field layout file

- Open the Field Book App on the Android device to display the main menu.
- Click on 'Fields' option and click on the '+' in the upper right corner as highlighted in figure 11b below, to import the field layout file.
- Choose the option 'Local storage' since the field to be imported is already in the field book folder on the Android device (figure 11c).
- Select the field you want to import, in this example, "2020_EET_40_IITA-Sendusu".

- The next dialogue box requires you to choose the unique identifier for the trial. Always choose 'plot_name' as unique identifier, 'row_number' as primary identifier and 'col_number' as secondary identifier (figure 11e). This ensures that data recording moves by plot, row and column in the field, so no plot is skipped unless by choice.
- Click 'IMPORT'. The imported field will appear in the list of fields as seen in figure 11f.

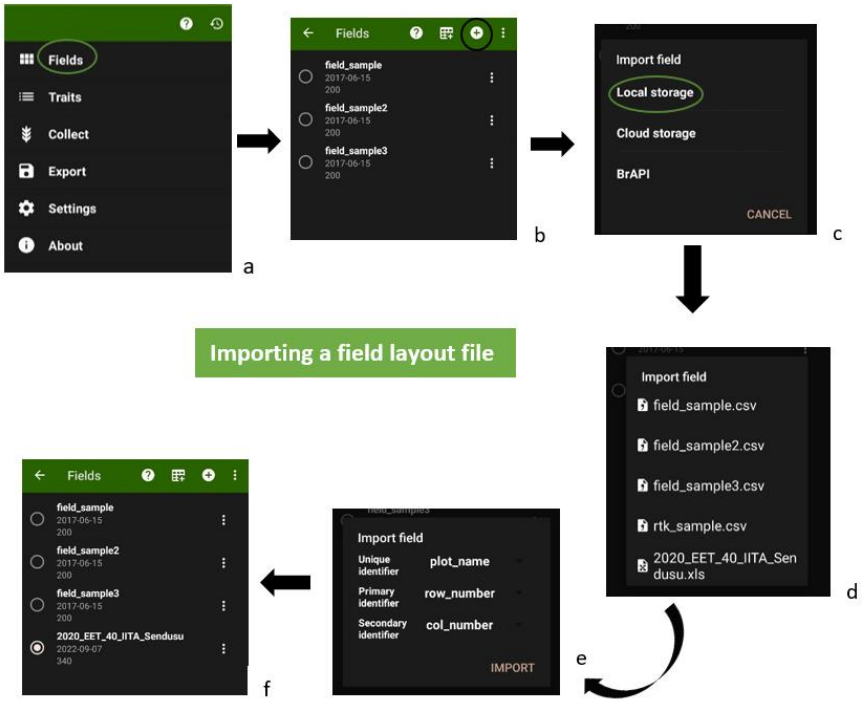


Figure 11. How to import a field layout file in Field Book App

6.3.2 Importing the trait file

- Open the Field Book App on the Android device to display the main menu as seen in figure 12.
- Click on ‘Traits’ option and click on the three dots in the upper right corner as highlighted in figure 12b below.
- Click on ‘Import/Export’ option and then ‘Import’ option (figure 12 c and d).
- Choose the option ‘Local storage’ since the trait file to be imported is already in the field book folder on the Android device.
- Select the trait file you want to import, in this example, “NARO-IITA EET Traits 2020.trt”.
- The traits in the trait file will appear in a list of traits as seen in figure 12g.

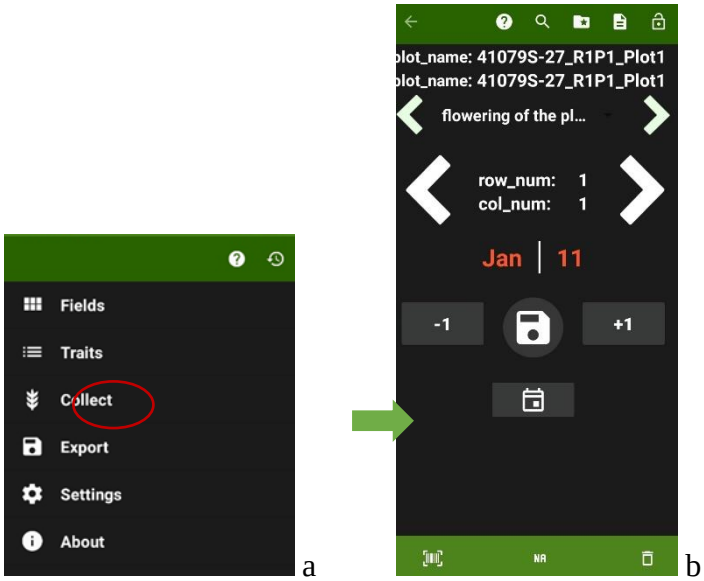
Figure 12 How to import a trait file in Field Book App

6.4 Collecting data with Field Book App

After importing the field layout and trait files, the Android device is now ready for collecting and storing data. To collect data:

- Go to main field book menu.
- Click ‘Collect’ as seen in figure 13a.

- A data entry area will appear displaying the first trait, plot, first row and first column as seen in figure 13b below.
- Scroll through plots and traits using the back and forward arrow.
- For traits in 'date' format you must click the save icon highlighted to save the date (saved date will appear highlighted in red). Use the -1 and +1 to back date or forward date.
- All other traits are automatically saved when you scroll to the next plot or next trait.



a b

Figure 13 Data collection area in Field Book

6.5 Exporting files from Field Book App

Data collected using the App is sent to the 'field_export' folder and can then be transferred to the computer from this folder. To do this

- Go to main menu display of Field Book App.
- Click on the 'Export' option.

- The data is available for export in two formats, the Database and Table format. Database format is preferred for uploading the data to MusaBase but the Table format is preferred for quick and easy read in excel. The Table format can also be uploaded to MusaBase with some formatting.
- After choosing appropriate format, click 'Ok' to export the data.
- The exported data file is found in the field_export sub folder of fieldBook folder where it can be copied and saved to other locations on the computer.

6.6 Data curation

Phenotype data files should be uploaded on your computer as back up. This should be done as regularly as possible since data collection is continuous over a long period of time and by several people. Check the data for inconsistencies, outliers and correct if necessary, before uploading. This should be done once a month at the time of uploading the data on MusaBase. Be careful not to merge data files. It is recommended that each data file is saved with the time stamp of when it was curated for purposes of tracking when necessary. If you decide to merge files with different data points, make sure to correctly align the column names for all the files.

6.7 Uploading data to MusaBase

It is standard practice to upload the data on MusaBase for cloud storage and access.

- Browse to "Manage" menu and click on "Field Book App".
- Scroll to the "Uploaded Phenotype Files" section and click on "Upload" link.
- Clicking on the "Upload" link will open a new dialogue box asking you to choose a file that you want to upload to the database website.

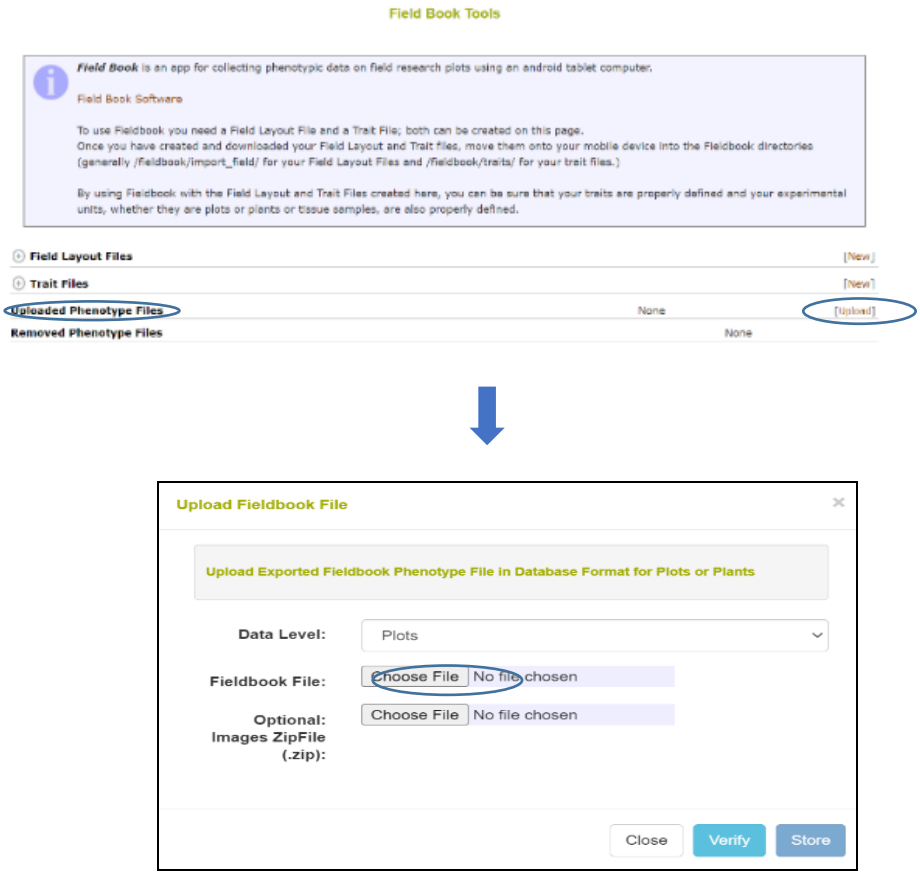


Figure 14. Step 1 and 2 of uploading data to MusaBase

- Please ensure that "plot_name" is the first column of the file to be uploaded as this ensure the uniqueness of each plot.
- To make sure that the file has the correct format for uploading, click on the "Verify" button (figure 15).

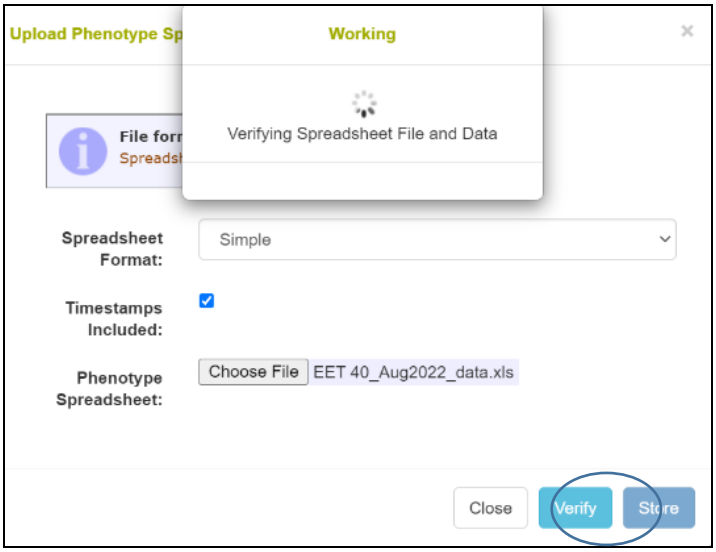


Figure 15. Step 3-verifying the data file to be uploaded to Musabase

- After the file format has been verified, click on the "Store" button as seen in figure 15 above to upload the data. For successful upload, a pop up message 'successful upload' will appear as seen in figure 16 below.

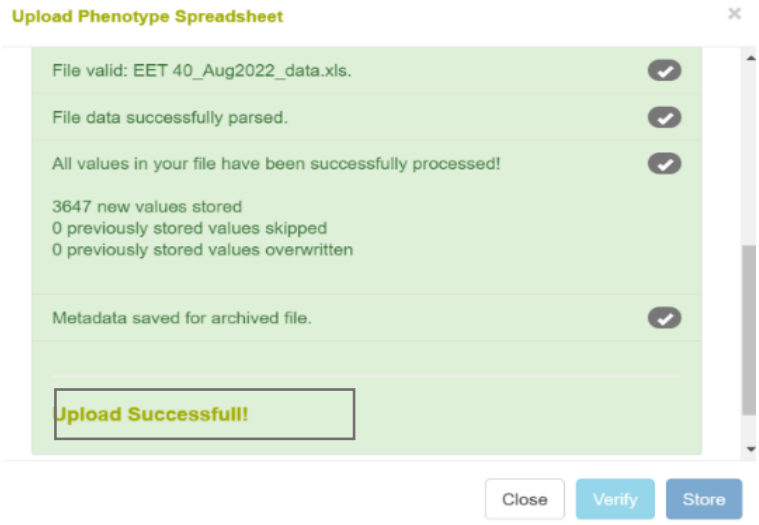


Figure 16. Pop up message of successful upload of data to MusaBase

- The uploaded data can be found on the Field Book Tools and the 'Trial Details' page as seen in figure 17 where it can viewed or downloaded.

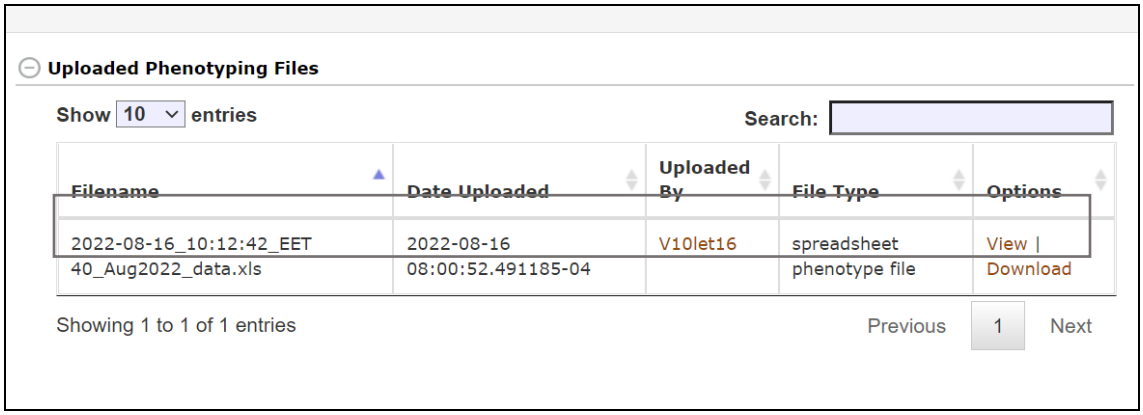


Figure 17. Location of uploaded data file on Musabase

- Data upload to MusaBase should be done at least once every month, preferably the last Friday in a month.

6.8 Phenotyping quality control points

- Load the right field on the data collection tablet since one Android device can hold more than just one field. Always cross check with the field sign post and by scanning one of the bar codes labels from a random plant in that field.
- Label cycle numbers on plants with a tag (ribbon or plastic tag) and note the flowering date on the tag, to keep track and avoid mixing up of data for cycles for plants on the same mat.
- Differentiate between the value '**zero**' and **missing values** due to missing plants. There might be empty plots due to external reasons, for example if the plant died, was damaged, fell before flowering or harvest, stolen, etc. These become missing values and should be left blank and the reason noted under the trait 'notes'.
- Always export the data after every data collection session, this saves the data on the local storage of the Android device.

6.9 Phenotyping and selection in EET

Different traits are collected at the flowering and harvest stage. Flowering is when the inflorescence containing the female and male flowers appears at the top of the plant and harvest is when the fingers are at full size maturity. These traits are collected for two cycles starting with the mother plant as cycle one and the first ratoon commonly referred to as 'daughter' plant as cycle two. Traits collected at the flowering stage are: date of flowering (later used to calculate days to flowering), plant height in cm, plant circumference at 100 cm from the collar in cm, number of functional leaves, youngest leaf spotted and number of suckers for cycle one only since de-suckering is done after flowering. Traits collected at harvest are: date of harvest (later used to compute days to maturity), number of functional leaves, youngest leaf spotted, bunch position, bunch weight, fruit parthenocarpy, fruit fill, raw pulp color and sap amount. The detailed list of description, format, database codes and links to these traits on MusaBase is attached in Annex 1.

At EET level, the focus is on black sigatoka resistance, bunch size, fruit parthenocarpy, and dwarfness. Black sigatoka resistance is evaluated under field conditions using the youngest leaf spotted (YLS) method of Vakili (1968).

Standard operating procedures of phenotyping for black sigatoka in the field can be accessed at https://musabase.org/ftp/SOPs/Sigatoka%20SOP%20-%20220822.pdf or the IITA data base at SOP04-Sigatoka.

Bunch size is used as an indicative measure of yield potential, determined from records of average bunch weight in kilogrammes (BWT, kg) with a more accurate yield potential calculation done at the preliminary yield trial (PYT) level.

Dwarfness or plant stature is determined by the ratio of plant girth (PGF) to plant height (PHF) in cm and at flowering. A higher ratio is preferred as it is signifies a more stout plant structure capable of withstanding strong winds. Plant height is measured as the

distance from the collar, or from the pseudostem base at the ground if the collar is not visible, to the intersection of the petioles of the two youngest leaves (leaf ranks 1 and 2), using a measuring pole while plant girth is measured as the plant circumference at 100 cm from the collar.

In the past, selection of promising hybrids at the EET level was by visual subjective selection by a team led by the breeder. This was based on visual bunch size, black sigatoka levels, astringency and pulp colour. This method is now combined with data records taken as described in section 3.7.6 above. Calculated means are used in reference to the product profile with the most popular landrace Mbwazirume as a control for selection of hybrids to advance to the PYT.

Reference

Rife, T. W., & Poland, J. A. (2014). Field book: an open-source application for field data collection on android. Crop Science, 54(4), 1624-1627. https://doi.org/10.2135/cropsci2013.08.0579

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