# Standard Operating Procedures (SOPs) for Banana Breeding and Data Management

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**Abstract**

Banana is an important food and cash crop with a worldwide production of about 114 million metric tons. Banana is among the ten most important crops in the world and a staple food and cash crop in the Great Lakes region of East Africa, where it contributes to the livelihood of millions of people who are mainly small-scale farmers. Despite its importance, the edible yield is still low at just 9% of its yield potential. Bananas' low yield is attributed to both abiotic and biotic constraints. Breeding for host plant resistance and tolerance is the most ideal method for managing pathogens and pests and abiotic factors, respectively. Significant progress has been made in banana breeding to address these constraints through cross-hybridization and selection of recombinants at the diploid level to generate new improved hybrids by various breeding programmes like the International Institute of Tropical Agriculture (IITA), where sources of host plant resistance to various pathogens and pests are available in wild diploid species. However, the bananas grown for consumption are primarily seedless triploids and vegetatively propagated, which limits gene flow and recombination, thus hampering genetic improvement.

Seed set is generally extremely limited and seed viability and germination rates are low, which complicates breeding efforts and intensifies the resources and time required to develop superior cultivars. Crossbreeding of bananas involves a series of activities and data workflows starting with the selection of parents, the crossing of the parents (hand pollination/hybridization), tissue culture activities for embryo rescue and clonal multiplication of hybrids, and evaluation of the progeny in Early Evaluation Trials (EET) for advanced yield trials in which improved hybrids are selected for cultivar release. These processes are costly in terms of resources and time required and thus should be organised efficiently.

This manual aims to document standard operating procedures (SOPs) for banana breeding as efforts towards modernization of the breeding process pipeline. Four SOPs have been written and these are: SOP 01: Crossing to obtain hybrids for matooke breeding; SOP 02: Ploidy analysis in banana; SOP 03: Establishment of Early Evaluation Trials; and SOP 04: Phenotyping and data management of Early Evaluation Trials (EET) in matooke (AAA) breeding. These SOPs will be beneficial to the banana breeding programme by their availability as 'how-to' manuals, ensuring consistency and accuracy to reduce costly mistakes.


**Key words**

Banana, crossbreeding, hybrid, *Musa*, standard operating procedures

## 1. General introduction

### 1.1 Background

Banana (*Musa* spp.), including plantains, are the world's most popular fruit crops grown mostly in the tropics and subtropics regions and serves as a staple food in sub-Saharan Africa and the Pacific. The crop is grown worldwide in more than 130 countries by both small- and large-scale producers (Evans and Ballen, 2018). In Africa, banana is a staple food for more than 70 million people and critical for their survival, contributing to the livelihoods and income security of mostly small-scale farmers (Nowakunda & Tushemereirwe 2004; Adeniji *et al.* 2010). East African Highland Bananas (EAHB), which include the cooking types ('matooke' – AAA and 'mchare' – AA) and brewing types ('mbidde'), and plantains (AAB genome) make up approximately 70% of all bananas grown on the continent (Swennen & Wilson 1983; SU 2016), and feeding over 30 million people in East Africa. Production of cooking bananas in Uganda and Tanzania is dominated by matooke (AAA subgroup) and mchare (AA subgroup) bananas that need cooking before consumption. They represent a unique set of *Musa* germplasm mainly found in the East and Central Africa (ECA) region (Tushemereirwe *et al.* 2015). Banana produces fruit throughout the year making it important as a food security crop in sub-Saharan Africa, and particularly in its Great Lakes Region.

Bananas and plantains belong to the genus *Musa*, order Zingiberales and family *Musaceae*. The wild species of bananas are diploids ($2n = 2x$) having 14, 18, 20 and 22 chromosomes (Deepthi 2016). The edible bananas with 22, 33 ($2n = 3x$) or 44 ($2n = 4x$) chromosomes ensued from hybridization between two wild diploid species, *M. balbisiana* Colla provides the B genome and *M. acuminata* Colla that donates the A genome. These two species belong to section *Eumusa* (Simmonds & Shepherd 1955; Čížková *et al.* 2015; Deepthi 2016). Most diversity is found in *M. acuminata*, which has several subspecies including *burmannica*, *siamea, malaccensis, truncata, microcarpa, zebrina*, *errans* and *banksii* (Perrier *et al.* 2011). This hybridization led to a broad mixture of genomic combinations and ploidy levels that include diploid (AA, AB), triploid (AAA, AAB, ABB) and tetraploid (AAAB, AABB, ABBB) cultivars (Nayar 2010; Manzo-Sánchez *et al.* 2015). It is from these combinations that we have East African highland cooking (matooke) and beer bananas (both AAA), dessert bananas (AAA and AAB), plantains (AAB), and cooking bananas (AAA and AA). The East Africa region is a secondary center of banana diversity that includes both the triploid East African highland bananas (*Musa*, AAA) and the diploid mchare bananas (AA) (Kitavi *et al.* 2016; Batte *et al.* 2019).

**1.2 Banana production and importance**

The origin of cultivated banana is in southeastern Asia and the Pacific regions (Castillo & Fuller 2012) but their cultivation in sub-Saharan Africa is reported to have begun around 3,000 years ago (De Langhe *et al.* 1995; Mbida Mindzie *et al.* 2001). Bananas continue to be the world's leading fruit crop and an important export commodity (Aurore *et al.* 2009; Kilimo 2012; Ploetz & Evans 2015). Between 2008 and 2017, global banana production increased by 15.4%, from 98.8 million metric tons in 2008 to 114 million metric tons in 2017 (Evans *et al.* 2020). Asia is the leading banana-producing continent, accounting for 54.2% of the total production in 2017, followed by the American continent (26.3%), Africa (17.6%), The Pacific (1.5%), and Europe (0.4%) (FAO 2019). The Great Lakes Region of East Africa (including Burundi, Democratic Republic of Congo, Kenya, Rwanda, Uganda and Tanzania) is the leading producer of bananas (which comprise highland bananas, ABB and other cooking types) in Africa accounting for 88% of the total production. Uganda is the largest producing country in Africa with 3.1 million metric tons of harvest followed by Tanzania with 2.7 million metric tons (Lescot 2020).

The utility of bananas depends on the cultivars and area where they are grown, e.g. EAHB dominates production in the region and provides three to 22% of total daily calorie consumption estimated at 147 kcal per person. EAHBs are grown on about 30 to 40% of utilized agricultural land (Kalyebara *et al.* 2007; Tinzaara *et al.* 2018).

**1.3 Banana production challenges**

Despite its importance, matooke edible yield is just at 9% of its full potential; i.e., 60 to 80 metric tons ha$^{-1}$ (Tushemereirwe *et al.* 2015). In Uganda, the highest yield recorded is 20 metric tons ha$^{-1}$ year$^{-1}$ yet matooke has a potential yield above 70 metric tons ha$^{-1}$ year$^{-1}$ (van Asten *et al.* 2004) and in Tanzania, the yield of mchare is 7 metric tons ha$^{-1}$ year$^{-1}$ (FAOSTAT 2017). Its low yield is attributed to both abiotic and biotic constraints. The biotic constraints are pathogens and pests. Banana weevils (*Cosmopolites sordidus*) and parasitic nematodes (*Radopholus similis* and *Helicotylenchus multicinctus*) are the most important pests affecting banana production. Nutrients and water uptake are hindered due to the damage in the plant roots and corms when infested by nematodes and weevils. Furthermore, the plant is weakened by root necrosis caused by nematode infestation and the poor anchorage of the pseudostem to the corm leads to the toppling of the plant, which leads to harvest loss. The banana weevil causes up to 60% yield loss (Gold et al., 2004), while nematodes reduce productivity by 30–50% according to on-station trials (Speijer *et al.* 1999, 2000).

Banana suffers from several devastating diseases and existing cultivars are continuously challenged by new pathogens and newly virulent strains. Among the most devastating is the banana bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum* that causes up to 100% loss (Biruma *et al.* 2007). The leaf spot disease black Sigatoka or black leaf streak disease caused by the fungus *Pseudocercospora fijiensis* has been a very serious constraint in recent years with an infection commonly leading to 50% crop loss (Ferreira *et al.* 2004). Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* is widely regarded as one of the most destructive banana diseases in recorded history (Moore *et al.* 1995). Banana bunchy top virus (BBTV), which is transmitted by the aphid *Pentalonia nigronervosa*, is reported in Burundi, the Democratic Republic of Congo, Rwanda and recently in Uganda and Tanzania (Boloy *et al.* 2014; Mukwa *et al.* 2014; Ocimati *et al.* 2021; Shimwela *et al.* 2022). It is more significant on plantains than EAHB (Kumar *et al.* 2011), thus causing a significant yield decline in this crop.

The abiotic factors include declining soil fertility in the major small-scale banana growing areas affected by low fertilizer use, as well as factors associated with climate change such as drought and heat stress (van Asten *et al.* 2011). Matooke such as the cultivar 'Nakitengwa' are sensitive to seasonal drought stress, resulting in a significantly reduced yield (Uwimana *et al.* 2020).

The above-mentioned biotic constraints form the major specific objectives for banana breeding programs with the general objective of improving yield and quality to meet the market demands of consumers. In East Africa, the aim is to improve the triploid EAHB matooke and mbidde, and the diploid mchare in Tanzania. The sources of genes for the banana improvement program are available in the wild species. Where natural sources of resistance are identified within the germplasm pool, crossbreeding is used (Persley & George 1996; Ortiz & Swennen 2014) to introduce the genes of interest into the cultivar to be improved. Despite the work done to date, relatively few of the bananas cultivars in the world are from breeding programs.

## 1.4 Challenges in Banana Breeding

Crossbreeding remains technically complex and challenging in triploid *Musa* improvement because of several limitations that are often inherent characteristics of polyploid vegetatively-propagated crops that hinder rapid banana breeding (Vuylsteke 2000). The difficulty of banana breeding resides in low female and male fertility, triploidy (the homologous chromosomes cannot pair up during synapsis of meiosis as they are uneven, as a result, meiosis is affected,

and viable gametes are not produced), poor seed set, slow propagation, low seed germination rates, and time (two years from seed to seed), which makes it difficult to produce large offspring for evaluation (Vuylsteke 2000; Pillay & Tripathi 2008; Ortiz 2013; Batte *et al.* 2019). Matooke had a seed set rate ranging from an average of three to 26 seeds per bunch in early screening assessments of female fertility (Ssebuliba *et al.* 2005), while the highest average pollination success was 1.5 seeds per pollinated bunch as per the records kept by IITA matooke banana breeding in Sendusu, Namulonge, Uganda (Batte *et al.* 2019). Primary tetraploid hybrids ensuing from crosses of triploid matooke and wild or improved diploids had an average of up to 2,279 seeds per pollinated bunch (Batte *et al.* 2019). Other banana improvement programs faced similar challenges, e.g. in the Honduran Foundation for Agricultural Research (FHIA) efforts to produce improved hybrids using a cultivar from the Cavendish group (a triploid dessert banana) as a female, only 40 viable tetraploid embryos from the pollination of over 20,000 bunches were obtained. This has limited the number of primary tetraploid hybrids that can be obtained and selected from hybridizations involving triploid bananas (Aguilar 2013).

Besides male and female infertility, the triploid edible cultivars lack genetic variation and this greatly hinders the breeding process because it greatly depends on genetic variability in the source populations for recombination (Lorenzen *et al.* 2008; Pillay & Tripathi 2008). In addition, banana plants are large, so evaluation plots are equally large requiring 6 to 9m$^2$ per plant, and plants require up to three years to progress through two fruiting cycles (mother plant and first ratoon).

## 1.5 Banana and plantain breeding in Africa

Crossbreeding is the main strategy used in banana and plantain breeding to develop improved hybrids, arising from the realization that viable embryos could be obtained from crosses of certain triploid cultivars and diploid cultivars or selected diploid breeding clones (Swennen & Vuylsteke 1993; Tenkouano & Swennen 2004). The early advances in *Musa* crossbreeding demonstrated the potential of hybridization and selection to develop new plantain and banana cultivars (Ortiz & Vuylsteke 1996; Vuylsteke *et al.* 2010).

Generally, the process starts with the identification of a donor male (pollen source) carrying the gene(s) of interest and occurring naturally in the gene pool. The most commonly used diploid has been the wild *M. acuminata* accession 'Calcutta 4', which has been a major source of host plant resistance to black leaf streak, yellow Sigatoka, fusarium wilt, banana weevil, and burrowing nematode (Ortiz 2015) and despite its undesirable bunch characteristics

and inedible non-parthenocarpic fruit results in superior hybrids (Vuylsteke *et al.* 1993b; Vuylsteke *et al.* 1993c; Tenkouano *et al.* 2019; Batte *et al.* 2020).

The cultivated plantain and triploid banana cultivars were included in crossing blocks with Calcutta 4 for the production of improved diploid, triploid and tetraploid hybrids. This strategy led to the release of several fertile improved banana diploids with varying degrees of host plant resistance to pathogens (Tenkouano *et al.* 2003) and to the registration of 14 improved Tropical *Musa* Plantains (TMPx) tetraploid hybrids showing resistance to black leaf streak, by FHIA since the 1960s and IITA in the 1990s, respectively (Vuylsteke *et al.* 1993c; Tenkouano *et al.* 2003). These improved diploid and tetraploid breeding clones have continued to play a vital role in subsequent banana hybrid development by intercrossing to obtain improved secondary triploid hybrids (**Error! Reference source not found.**).
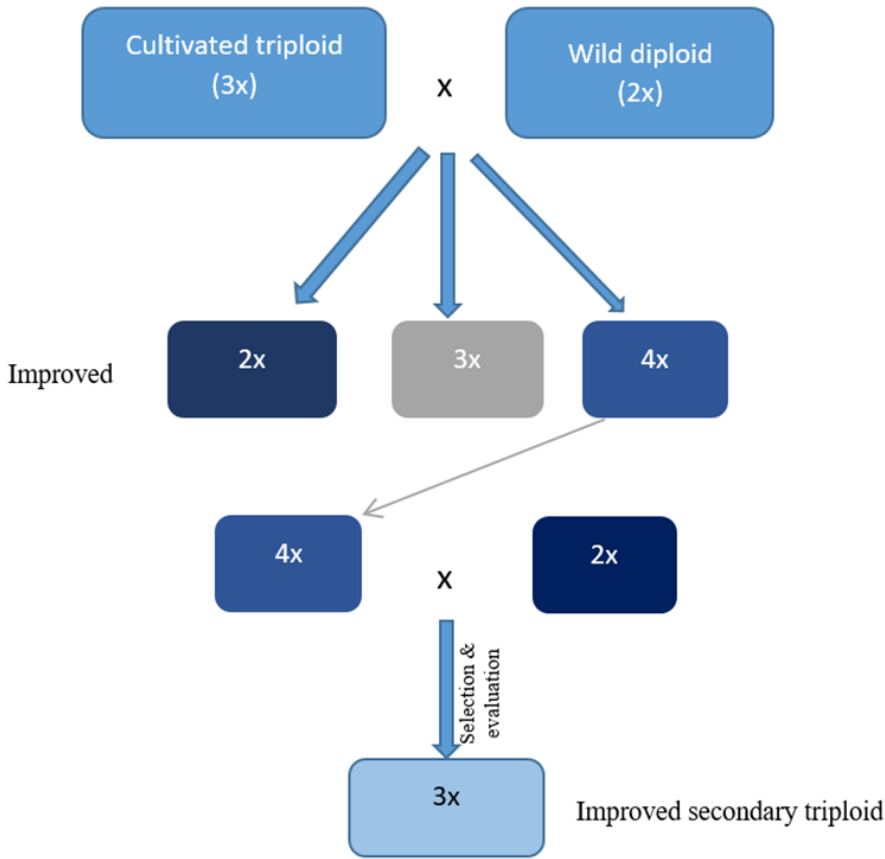


Figure 1.1. The general crossbreeding scheme for banana and plantain improvement of the International Institute of Tropical Agriculture (IITA) in sub-Saharan Africa.

The developed hybrids undergo a series of phenotypic evaluations at different levels. They are first established in an Early Evaluation Trial (EET), in which they are assessed for at least two crop cycles for bunch characteristics and host plant resistance to black leaf streak. Selected hybrids from this trial are then planted in a replicated Preliminary Yield Trial (PYT) where the yield potential, and agronomic and quality traits are evaluated for two crop cycles. Selections from PYT are thereafter propagated and evaluated in Advanced Yield Trials (AYT), which often is multi-site and off-station (thus is sometimes named Multi-Environment Trial or MET for short). Under AYT or MET, hybrids are evaluated for their stability across different environmental conditions (Madalla *et al.* 2022b). Hybrids that perform relatively well across all environments are highly preferred since their yield is stable.

Figure *1.2* summarizes the salient features of the crossbreeding process followed by IITA and the National Agricultural Research Organization (NARO) of Uganda.
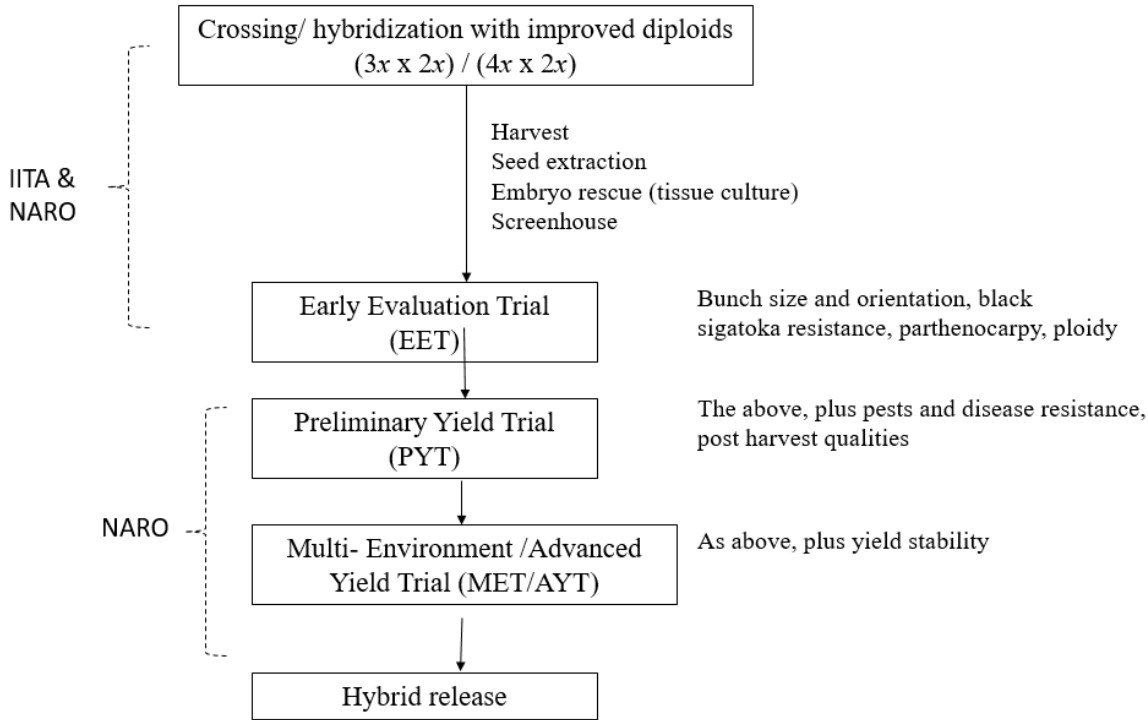


Figure 1.2. Typical flow diagram of the banana evaluation and selection process used by the International Institute of Tropical Agriculture (IITA) and the National Agricultural Research Organization (NARO) of Uganda.

### 1.5.1 Plantain breeding in West Africa

Plantain (AAB) is the most prevalent cultivar grown in the humid lowlands of West and Central Africa. This region is considered a secondary centre of plantain diversification (Swennen 1990) because of the remarkable amount of variability of plantain it harbours (Adheka *et al.* 2016a; Adheka *et al.* 2016b; Adheka *et al.* 2018). Research on bananas/plantains started in 1976 in Nigeria by IITA (Ray 2002). The actual breeding work on *Musa* genetic improvement is reported to have commenced in 1986 by crossing plantains with wild bananas. IITA accepted the responsibility of breeding plantain and cooking bananas for Africa with resistance to black leaf streak in 1987 and a Plantain and Banana Improvement Program (PBIP) was established in 1991 (Vuylsteke *et al.* 1993a).

The first registration of 14 improved tropical *Musa* plantain hybrids with resistance to black leaf streak in 1993 (Vuylsteke *et al.* 1993c) is a milestone in the history of *Musa* breeding. The plantain breeding programme continues to develop several secondary triploid hybrids that combine high yield with resistance to black leaf streak, and distribute them to national breeding programs elsewhere, particularly in West and Central Africa (Tenkouano *et al.* 2019).

The Centre de coopération internationale en recherché agronomique pour le développement (CIRAD) was established in 1983 in France and set up a station in Cameroon in the late 1980s, focusing on plantains and other dessert bananas. CIRAD developed four synthetic dessert banana hybrids (FB918, FB919, FB920 and FB924) with partial resistance to two nematode species *R. similis* and *P. coffeae* (Quénéhervé 2009). Similarly, the Centre Africain de recherches sur bananiers et plantains (CARBAP) in Cameroon, which focuses more on plantain improvement, was inaugurated after an agreement among the Ministers of Research and Development for West and Central Africa in 2001 (Ortiz 2013). A new hybrid 'CARBAP K47' was developed and registered by CIRAD in partnership with CARBAP. The variety is resilient in marginal growing conditions, has very high yield potential, is resistant to black leaf streak, and has very high tolerance to banana weevil and nematode (Depigny *et al.* 2016).

### 1.5.2 Banana breeding in East Africa

IITA banana breeding in East Africa started in 1994 in Uganda by the late Dirk Vuylsteke, with the initiative to improve the matooke landraces (Vuylsteke 2001). IITA initiated the collaboration with the NARO in the 1990s using some of the banana hybrids it had developed at its High Rainfall Station at Onne in Nigeria (Vuylsteke *et al.* 1993a), where IITA has already developed the first matooke hybrids (pers. comm. R. Swennen, IITA). The breeding efforts

continued in collaboration with NARO focusing on developing hybrids with a combination of high yield, and host plant resistance to pathogens and pests as the major breeding objectives. This cooperative program has led to the development of 27 improved hybrids called NARITA over the past 20 years of joint breeding efforts.

These hybrids with host plant resistance to the black leaf streak and high yield were developed after two crossing cycles, i.e., the triploid female fertile matooke cultivars with the wild diploid Calcutta 4 to obtain tetraploids, which thereafter were included in crossing blocks with improved diploid bananas to obtain secondary triploids or NARITAs (Tushemereirwe *et al.* 2015). This was enabled by the initial work and findings of Ssebuliba *et al.* (2005). They screened the matooke germplasm available in the field gene bank in Kawanda for female fertility and found 37 out of the 78 tested matooke cultivars set seed with a maximum of 25 seeds per pollinated bunch. Of the 27 hybrids, 25 were evaluated for three crop cycles at the research station in Namulonge (central Uganda), which led to the release of NARITA 7 (syn. Kabana 6H, M9, Kiwangaazi) as a new cultivar in 2010 by NARO and four NARITA hybrids as TARIBAN cultivars in Tanzania (Madalla *et al.* 2022a). NARITA 7 is already being grown by 15% of banana farms in Uganda (Tushemereirwe *et al.* 2015).

IITA started banana breeding in Arusha (Tanzania) in 2011. Today the breeding of diploid mchare bananas continues (IITA 2019). The focus of this breeding program is on improving mchare diploid bananas for yield and host plant resistance to pathogens since banana production in Tanzania is significantly threatened by weevils, parasitic nematodes, black leaf streak, fusarium wilt and banana xanthomonas wilt (Swennen *et al.* 2013; Jackson *et al.* 2015; Ploetz & Evans 2015 ; Shimwela *et al.* 2016; Viljoen *et al.* 2017). Interestingly, the mchare cooking bananas are the closest living diploid relative to the important dessert bananas such as Cavendish and Gros Michel (Perrier *et al.* 2018).

In 2018, consumer acceptability tests of NARITA hybrids were conducted in three sites (Maruku, Mitalula and Moshi) in Tanzania to provide results that could help inform the selection of the best NARITAs to take on-farm and subsequent varietal release (Marimo *et al.* 2019). Tanzania officially released four NARITA hybrids, now called TARIBAN 1, TARIBAN 2, TARIBAN 3 and TARIBAN 4 of the important staple cooking banana, "matooke" (Madalla *et al.* 2022a) which are the first-ever matooke banana hybrids released in Tanzania. These new cultivars are resistant to black leaf streak and their potential yield ranges from 18 to 35 metric

tons ha$^{-1}$ year$^{-1}$ versus the current farmers' average productivity of 7 metric tons ha$^{-1}$ year$^{-1}$ (IITA 2021; Madalla *et al.* 2022b).

The NARITA hybrids are now being multiplied and tested in Kenya by researchers at Kenyatta University to test their agronomic performance and their potential for adoption by farmers, consumers, and traders in this East African country. This is seen as a step towards collaborative research to improve banana production in more East African countries.

## 1.6 Standard Operating Procedures and their application in crop improvement

Standard operating procedures (SOPs) are formal procedures that describe how an individual or organization performs a task and documents the performance of that task. SOPs provide a step-by-step set of how-to instructions for those performing a specific task e.g. on what to do, how to do it, when to do it, and how to record it. Each SOP should be clear and specific enough to be understandable, but flexible enough to be useful (Gough & Hamrell 2009). SOPs in banana breeding are efforts towards modernization of the breeding process by ensuring accuracy, consistency and quality of all breeding steps throughout the process, thus increasing the repeatability of results by decreasing the number of errors and ensuring efficient communication as detailed and validated step-by-step. SOPs leave no space for debate. Additionally, they can be used as training tools for technical tasks allowing for continuity in programs when leadership and other personnel change.

This paper outlines SOPs for banana breeding and related data management at the stages of hybridization or making crosses (SOP 01), determination of the ploidy level of the hybrids from the crosses (SOP 02), the establishment of these crosses in the field as Early Evaluation Trials (EET) (SOP 03), and the phenotyping and data management of the EET (SOP 04) as done by IITA. They are written systematically and logically but independent of each other, to reflect the logical order in which these processes are carried out during banana breeding.

## 2. Bibliographic approach

This report included a literature review on the introduction, importance, taxonomy, production and breeding challenges of bananas as well as, banana breeding programs in Africa. Its focus was placed on the banana breeding scheme of IITA, particularly in East Africa. This was intended to lead to the specific steps and methods throughout the banana breeding cycle for which the standard operating procedures (SOPs) ensued. Literature was sought and used for some specific procedures such as the flow cytometry to determine ploidy levels in bananas. The open search for journal articles and other relevant literature from the Web of Science, Scopus, ScienceDirect, Google Scholar, Musalit, ProMusa and the CGIAR databases was based on the terms "banana production in Africa", "banana breeding, process and challenges", "banana hybridization", "conventional banana breeding", and "standard operating procedures in plant breeding". These terms included words from the titles and the keywords. A total of 275 scientific articles resulted from the open search. There was very limited literature on the application of SOPs in plant breeding with only one notable reference on sweet potatoes (CIP 2020).

### 3. Standard Operating Procedures (SOPs) for Banana Breeding and Related Data Management

#### 3.1 Purpose

The purpose of this document is to describe the SOPs for banana breeding and related data management. These SOPs include crossing, ploidy analysis, evaluation trial establishment, phenotyping and selection in both Early Evaluation and Preliminary Yield Trials. Four SOPs have been developed for different purposes as outlined below. The tasks in these SOPs are under the stage: Crossing and Screening; during the matooke product development process as shown in

Figure *3.1*. Stage, as depicted in the figure refers to the major steps in the breeding pipeline processes from product conception until the product is launched in the market for final users or consumers. The stages are linked to steps in the banana evaluation and selection process depicted in Figure *Figure 1.2*.  as used in the matooke product development process, which represents the decisions and actions made to transition from one stage to the next, e.g. the gate between 'crossing and screening' stage to 'early testing' is 'to select clones for PYT'.

#### 3.1.1 SOP 01: Crossing to obtain hybrids for matooke breeding

This SOP describes the tasks carried out before, during and after pollination or crossing is done when developing hybrids for the crossbreeding of matooke.

#### 3.1.2 SOP 02: Ploidy analysis in banana

In this SOP details are given regarding the tasks, data recording and interpretation during the ploidy analysis of bananas using the DNA flow cytometry method based on the operation protocol of the Sysmex ploidy analyzer machine. Ploidy analysis is carried out at all stages of banana improvement, either on the products of hybridization for pre-breeding and breeding ( *Figure 3.1*) or on any newly acquired germplasm from genebanks or other banana breeding programs.

#### 3.1.3 SOP 03: Establishment of early evaluation trial

The purpose of this SOP is to describe a series of tasks undertaken 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. Some of these procedures also apply to plants that for various purposes go through the tissue culture process.

| 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 |

Stage          Gate

Figure 3.1. Stage and gates during the matooke product development process. This standard operating procedure (SOP) falls under "Crossing & screening"

### 3.1.4 SOP 04: Phenotyping and data management of early evaluation trials (EET) in matooke (AAA) breeding

This SOP describes tasks, data workflow and data storage protocols used for phenotyping breeding clones along with checks in early evaluation trials (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. The workflow is built around [www.musabase.org](http://www.musabase.org) and the field book data collection app (hereafter referred to as the Field Book App).

### 3.2 Scope

The SOPs apply to all breeding steps implemented under the IITA banana breeding program in Sendusu, Namulonge, Uganda in collaboration with NARO. It aims to reach the broader banana breeding community in Uganda and for use as a blueprint in other banana breeding programs elsewhere.

### 3.3 Roles and responsibilities

All staff involved in implementing breeding tasks in the IITA and NARO banana breeding programs 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 a need for revision arises. The list of individuals responsible for each section of the SOP in the breeding activities is given below.

*Line or product manager*. Responsible for managing and overseeing all trials and data production conducted by the banana breeding programmes, through the stages and gates leading from product development to product introduction.

*Data manager*. Responsible for establishing of trials and all aspects of the trial data recording thereafter. This includes trial design (incl. field plot techniques), planting, its management and data recording, initial statistical analysis and checks on the implementation of defined protocols on sites ensuring that metadata for all trials is accurately measured, have no outliers, are completed and uploaded in Musabase. S/he provides end-user support and training on the usage of Musabase and digital tools.

*Field technicians*. Perform field tasks such as performing 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 the quality of data within defined periods.

*Laboratory technician*. Tissue culture laboratory technicians perform laboratory tasks such as embryo *in vitro* germination, 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 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 recorded data and ensure that field technicians follow the protocol of each trial. Ensure that recorded data are daily backed up, implement efficient and safe procedures for data handling and sharing with the relevant research team. Work with the database curators to organize and maintain data in the correct formats for uploading and storage on Musabase. Keep good care and maintenance of data collection gadgets and apps, and identify, justify, and report any need for an upgrade, update, or replacement of the data collection gadgets. Assist with reports and data extraction when needed.

## 3.4 Botanical characteristics of the banana plant
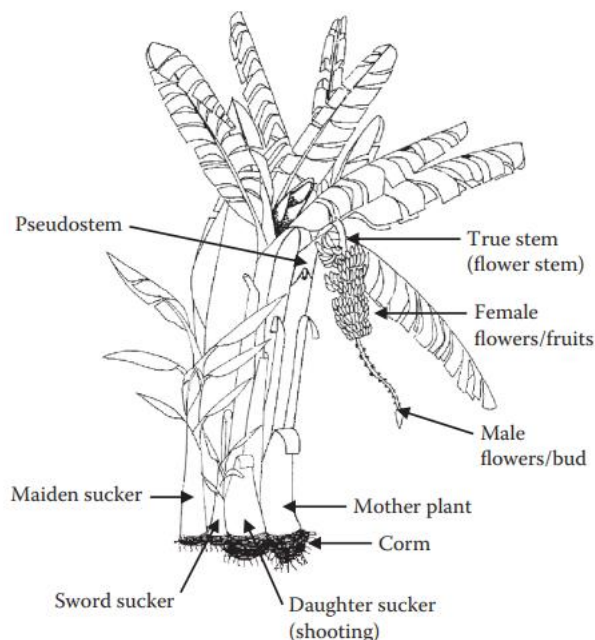


Figure 3.2. The morphology of the *Musa* plant: a mat or stool (after Karamura et al., 2011). The mat is the clump formed by the corm, the fruit-bearing stem(s) and the suckers.

## 4.  SOP 1:  Crossing to obtain hybrids for matooke breeding

## 4.1 Required tools and equipment

4.1.1 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/.

4.1.2 Banana Tracking Tool (BTracT)

The banana tracking system for crosses, seeds and plantlets is accessed at http://btract.sgn.cornell.edu/btract/. This tool is enabled through the Open Data Kit (ODK), which is an open-source suite of tools that allows data collection using Android mobile devices and data submission to an online server, even without an internet connection or mobile carrier service at the time of data collection.

4.1.3 Mobile Android device

4.1.4 Plant ID field labels

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

4.1.5 Zebra mobile printer

Zebra ZQ520 $^{©.}$

4.1.6 Zebra desktop printer

Zebra ZT411$^{©}$ industrial printer.

4.1.7 Zebra designer software

Zebra designer professional version 3.5.

4.1.8 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.

4.1.9 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.

4.1.10 Barcode scanner

Zebra DS2208-SR handheld 2D omnidirectional barcode scanner

4.1.11 Computer with internet access

## 4.2 Parent identification and establishment of crossing blocks

4.2.1 Parent identification and creation of crossing plan

The list of parents to be used as males and females are provided by the breeder together with the crossing plan referred to as 'wish list' hereafter. The breeding programmes perform triploid ($3x$) × improved diploid ($2x$) crossing to develop tetraploid hybrids ($4x$) with desirable attributes of both $3x$ and $2x$ parents. However, these $4x$ hybrids are very fertile and contain seeds. So secondary $3x$ sterile offspring with parthenocarpic fruit with additional favourable alleles are developed by crossing the tetraploids with improved diploids. These crosses are also given an ascending order of priority, with '1' being the highest priority. This means the crossing plan with priority = 1 should be performed first if a female plant is ready for crossing and pollen is available. The breeder determines priority based on the attributes of the product profile and past performance of the parents. The choice of parents and priority order can be changed if the breeder suggests the need to. An example of this is seen in Table 4.1. Follow this link to organize a crossing plan.

https://solgenomics.github.io/sgn/03_managing_breeding_data/03_06.html#cross-wishlist

Table 4.1. An example of a crossing plan.

| Female | Ploidy | 10969S-1 | 5265-1 | 5610S-1 | 8075-7 | 9128-3 | cv. Rose | *malaccensis*_250 | SH3217 |
|---|---|---|---|---|---|---|---|---|---|
| Entukura | $3x$ | | × | | × | × | × | × | × |
| Enzirabahima | $3x$ | | × | | × | × | × | × | × |
| Kabucuragye | $3x$ | | × | | × | × | × | × | × |
| Kazirakwe | $3x$ | | × | | × | × | × | × | × |
| 25974S-17 | $4x$ | × | | × | × | | × | × | |
| 29275S-5 | $4x$ | × | | × | × | | × | | |
| 29364S-2 | $4x$ | × | | × | × | | | × | |

(Header spanning "Male (all 2$x$)" over the male columns.)

Key

| Priority 1 |
| Priority 2 |
| Priority 3 |
| Priority 4 |
| Priority 5 |

### 4.2.2 Adding a trial to Musabase

Detailed step-by-step instructions on how to organize or add a trial in Musabase are available at: https://solgenomics.github.io/sgn/03_managing_breeding_data/03_07.html#adding-trials

- No experimental design is followed for pollination blocks.
- Organize defined lists of accessions to be used as male and female parents.
- Add the lists to the database.
- Use the lists to add the accessions to the database if they have not been added yet.
- Open an Excel file in '.csv' format of the field layout.
- Add the trial to Musabase entering all relevant metadata in "Trial Information" like trial name using naming convention, location, and plot and field dimensions.

### 4.2.3 Field preparation

- Choose land that is relatively flat for efficient cultivation and management.
- Clear the land of overgrown bushes by hand slashing.
- Do a first plough of the land to break the hard surface and open it up with a tractor.
- Do a second plough 3–7 days after the first plough using a tractor mounted with a harrow disc that cuts deep to loosen up and mix the soil thoroughly.
- Measure and field mark to a spacing of 3 m (between plants) × 3 m (between rows) for triploid and tetraploid parents, giving a plant density of 1,111 plants ha$^{-1}$.
- Measure and field mark to a spacing of 2 m (between plants) × 3 m (between rows) for diploid parents, giving a plant density of 1,667 plants/ha.
- Dig or drill planting holes measuring 60 cm wide and 60 cm deep.
- Separate the topsoil from the bottom soil while digging the planting holes to be used at planting.

### 4.2.4 Preparation of planting material

- If using tissue culture plants, choose healthy and strong-looking plants with a girth of 5 cm and above for planting. Use girth and not height because the plants should not break during transportation and planting exercises.
- If using suckers, pick the sword suckers, pare corm by removing several thin layers, and discoloured tissue to reduce nematode infestation.
- Dip the pared corms in boiling water (100° C) for 30 seconds to kill any other pathogens left on the surface.

- Alternatively dip the pared suckers in a pesticide solution containing 50 ml of Dursban (active ingredient 75 % chlorpyrifos) in 20 L of water.

4.2.5 Planting crossing blocks

- Planting is done at the start of the rainy season (March−April for season one and August-September for season two in Uganda).
- Mix earlier separated topsoil with 20 kg of well-dried farmyard manure (cow dung is commonly used).
- Place the tissue culture plantlet or pared and treated corm in the middle of the planting hole and add the soil-manure mix until the plantlet is firm or the corm is well covered with at least 15 cm of soil.
- Place mulch around the plant (ring mulching) to conserve soil moisture and control weeds. Be careful that the mulch does not touch the plant.

4.2.6 Creating and printing field labels

A detailed step-by-step process of how to organize and print barcodes is available at http://solgenomics.github.io/sgn/03_managing_breeding_data/03_12.html. The general steps are:

- Open the manage tool on Musabase.
- Click on label designer.
- Select the trial for which you want to write labels.
- Organize 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 barcode label papers mentioned in section 4.1.4 and shown in Figure 4.1.
- Download the PDF file of all barcodes of the trial.
- Print the barcodes using a laser jet office desktop printer. The saved design 'Sendusu labels' mentioned above is customized to print one barcode label per plot.
- Stick the barcodes on 0.5 m long PVC pieces, flattened at the area of barcode placement.
- Label the plants by placing the labelled PVC pieces in front of the plant



17-ZebrinaG.F_R24C13_plot358

Figure 4.1. Example of a Sendusu label design for bar codes.

4.2.7 Field maintenance

- Mulch the fields with dry grass to a thickness of about 8-15 cm to control weeds and conserve moisture. Placed the mulch 1.5–2 ft (45–60 cm) from the mats to allow space for forking to loosen the soil and for effective weevil control.

- Control weeds by manual removal of weeds using hand hoes as frequently as possible or by herbicides (50 ml of 2, 4-D amine salt 720g $L^{-1}$ and 150 ml of Agrosate – 480 g $L^{-1}$ glyphosate, mixed in 15 L of water) using a Knapsack sprayer. If using herbicide, wear protective gear such as a waterproof suit, gumboots, gloves and nose masks to protect yourself against the harmful effects of the herbicide.

- De-trash the plants by cutting off the broken, dead and dry leaves to keep the fields clean and reduce *P. fijiensis* inoculum.

- Routinely scout for banana xanthomonas wilt infected plants and destroy them by cutting, collecting them in one place and burning them. Disinfect all tools used with bleach to prevent spread.

- De-suck the female pollination blocks to maintain three plants, mother, daughter and granddaughter plants (MDG) per mat regularly.

- Cover male buds of the pollen source plants as soon as they come out, with a cotton mesh bag to prevent pollen contamination as seen in Figure 4.2.

- Remove the male buds from female parents after the last cluster has formed to control banana xanthomas wilt.

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



a                                          b

Figure 4.2. Male bud to be used as pollen source (a) and male bud covered with a bag to prevent contamination by foreign pollen (b).

**4.3 Using BTracT for data recording**

4.3.1 Setting up ODK data collection form

- This is done as soon as the "wish list" and pollination blocks field layouts have been uploaded and synchronized in MusaBase.

- Export the earlier organized crossing plan from Musabase to the ONA server.

- This is done on Musabase via the link https://musabase.org/breeders/odk.

- Choose an ODK form appropriate for the breeding programme, for example, 'BTracTSendusu' for the breeding programme in Namulonge as seen in Figure 4.3.

- Import the form and this will make the crossing plan available on the device to be used.
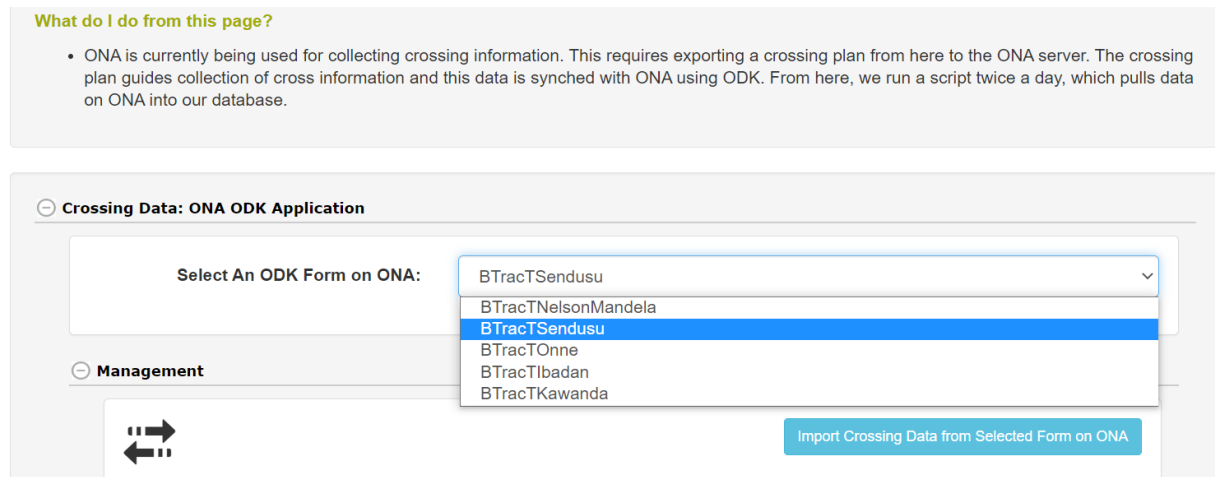


Figure 4.3. Example of ODK forms available on Musabase.

4.3.2 Setting up BTracT and data recording

- This is done for field and nursery activities and requires an internet connection.

- Download and install ODK collect, ODK Sensors Framework and ODK print drivers from the play store on the Android tablet. BTracT operates using these three softwares.

- Detailed step-by-step process of device and account set-up can be accessed at http://btract.sgn.cornell.edu/btract/_w_357fae98de45f75473effcfb22e6f879646238197 da00b2f/docs/usingbtract.html.

- Open the main menu and click 'Get Blank Form' to load the data recording form on the device as highlighted in Figure 4.4.

- Select the form to be used for the specific site e.g. BTracTSendusu for the Sendusu site.

- Go back to the main menu and select 'Fill Blank Form'.

- Select the form displayed, for this case 'BTracTSendusu' to start recording information.
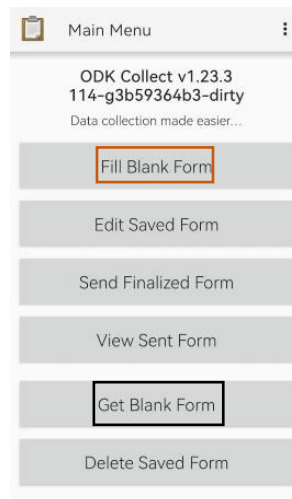
Figure 4.4. Screenshot of the main menu of the ODK collect App on a device.

## 4.4 Crossing

- Scout for flowering female plants and record them. This is done daily in the evening of the day before pollination or the day of pollination.
- Collect pollen as early as possible (recommended 6:00 – 6:30 am) from male parents, since pollen viability is known to decline with time (Tenkouano *et al.* 2011).
- Place the pollen from each genotype in separate polyethene bags. Pollen from different plants of the same genotype can be placed in the same bag.
- Scan the field label of the pollen source plant (male plant) and reprint the barcode label on the mobile printer. See the process in Figure 4.5.
- Place the reprinted barcode on the bag containing the pollen.



Figure 4.5. Process of barcode labelling of pollen collected for pollination.

- Find the female flowering plants from the previous scouting records.
- Open field activity 'first pollination' on ODK collect app.
- Scan the female plant barcode to be pollinated.
- Select the male genotype according to the pollen available (earlier collected) and order of priority stipulated in the cross plan.

- Scan the male plant barcode of the genotype chosen above (barcode on polyethene bag).

- Pollinate the female plant by rubbing the pollen collected gently over the female flowers with open bracts as early as possible in the morning between 7:00 and 10.30 am because pollen viability reduces with time (Swennen & Vuylsteke 1993).

- Spray the pollinated flower buds with a glucose solution made by dissolving 30g of glucose in 1L of water.

- Proceed to print an automatically generated cross ID barcode and label the cross made.

- Cover the pollinated flower bud to avoid contamination from foreign pollen (Figure 4.6).

- Continue to pollinate the plant as bracts open until all flowers are pollinated in a process referred to as 'repeat pollination'. To record data for repeat pollination do as follows;
  - Under field activities on ODK collect app, open 'repeat pollination'.
  - Scan the cross ID previously generated in the first pollination.
  - Male genotype to use will then be displayed, as you must use the same pollen as during the first pollination.
  - Scan the male genotype displayed as a pollen source.
  - Select and record the date of repeat pollination (should be the day of repeat pollination).
  - Save and exit to save records.
  - Cover the bud every time with the mesh bunch cover bag until all pollinations on the plant are made.



a b c

Figure 4.6. Female flowers ready for pollination (a), hand pollination (b) and, pollinated bunch covered (c).

**4.5 Harvesting**

- Harvest the bunch when one of the fingers starts to ripen.
- Harvest by cutting the pseudostem and gently lowering the bunch towards the ground to avoid damaging the fingers.
- Cut the bunch off the main plant at about 10 cm of the rachis.
- Use BTracT to record the date of harvest by scanning the harvested bunch (cross ID) barcode.
- Finalize the harvest by acknowledging that the bunch has been taken to the ripening chamber, using BTracT.

**4.6 Ripening**

- Place the harvested bunch in a basin or bucket.
- Reprint the cross-ID barcode label of the bunch and place it on the basin or bucket containing the bunch to avoid mix-ups.
- Place the bunch in a dark room with no ventilation referred to as a ripening chamber until bunches are fully yellow ripe as seen in Figure 4.7.
- Check bunches daily to just about full ripening and not rotting.

**4.7 Seed extraction**

- Collect ripe bunches from the ripening chamber.
- Pluck off the fingers from the bunch.
- Peel one finger at a time while squeezing through the soft pulp to feel for seeds until the bunch is completed.
- Place all seeds extracted in a soft mesh bag/cheese cloth.
- Wash the seeds with clean running water to remove all the pulp.
- Air dry the seeds.
- Go to BTracT activity of 'seed extraction', scan the barcode of the extracted bunch and record all necessary information such as date of extraction, the total number of seeds extracted and where seeds will be taken.
- If many seeds have been extracted, the utmost 50 seeds should be sent for embryo rescue and the rest taken to the nursery for early germination unless otherwise stated for specific needs.

- For seeds to be sent for the embryo *in vitro* germination, re-print the cross ID and label the envelopes containing the seeds as seen in Figure 4.7.
- Keep seeds awaiting embryo rescue in dry storage shelves at room temperature for a maximum of one week only.
- See below for a demonstration of workflow (Figure 4.7)



Figure 4.7. Banana ripening and seed extraction.

## 4.8 Tissue culture laboratory activities

Other standard operating procedures for the processes of embryo rescue, germination, subculture, and rooting, are followed in the laboratory.

- Record data using BTracT for the laboratory tasks following the questionnaire-like prompts as seen in Figure 4.8.
- This web form is available at https://ona.io/home/
- The detailed systematic process for how to record each activity is described in detail at http://btract.sgn.cornell.edu/btract/_w_357fae98de45f75473effcfb22e6f879646238197da00b2f/docs/usingbtract.html

Figure 4.8. Process of recording data for laboratory activities on ONA.

### 4.9 Data synchronizing to server

- This process requires an internet connection to synchronize activities that were recorded without an internet connection.
- It ensures recorded data is exported to the server for cloud storage and access.
- Go to the main menu.
- Click on 'Send Finalized Form' (Figure 4.9 a).
- Select all forms that need to be sent and click 'Send selected' (Figure 4.9 b).

- Successful data upload is confirmed with a pop-up message as seen in (Figure 4.9 c).
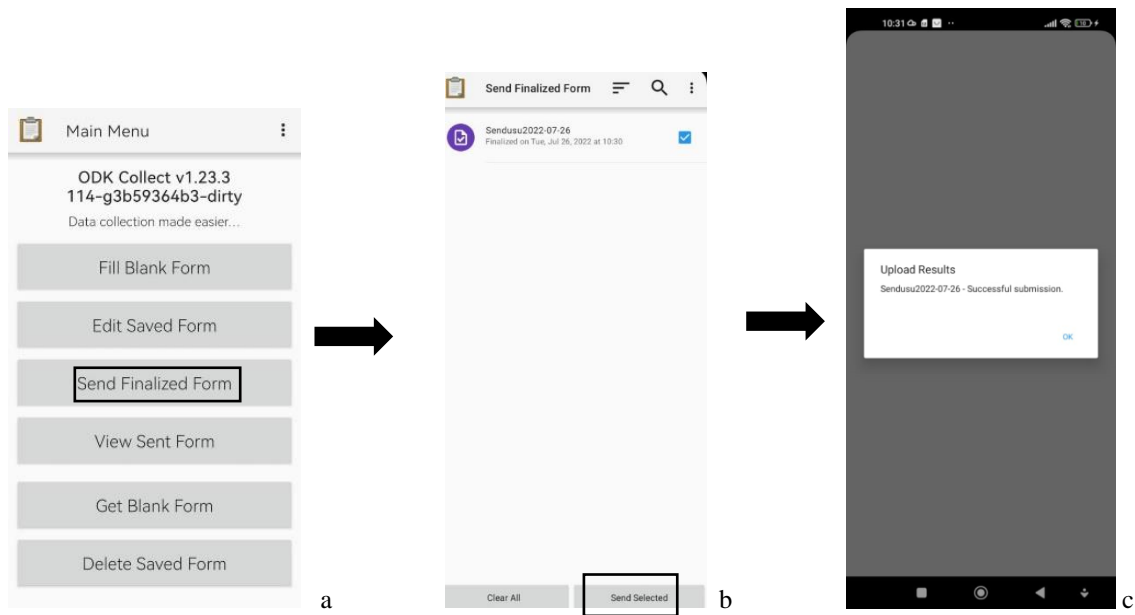


Figure 4.9. Data synchronizing process on ODK main menu highlighting how to send a form (Fig 10 a), select a form for submission (Fig 10 b) and successfully upload a message (Fig 10 c).

## 5.   SOP 02: Ploidy analysis in banana

Banana is a natural multi-ploidy crop. Wild bananas are seeded diploids ($2x$), while banana cultivars are seedless, mostly triploid ($3x$) and sometimes diploid. Because of the sterility of triploid bananas, improvement entails the generation of tetraploid intermediate hybrids ($4x$), which are fertile. Hence, the determination of ploidy level is important for the informative use and management of banana germplasm for different breeding objectives and targets. It guides the breeder on the choice of parents to include in a breeding scheme based on ploidy among other considerations. Upon hybridization, bananas produce hybrids of mixed ploidy levels such as diploids, triploids and tetraploids. Therefore, it is very important to determine their ploidy level along with their performance in field trials so that hybrids are compared within their ploidy levels.

Ploidy can be determined by several methods with the most consistent ones being chromosome counting and DNA flow cytometry. Chromosome counting is accurate but very laborious. DNA flow cytometry has been demonstrated to be rapid and reliable for ploidy screening in *Musa* (Dolezel 1997). A critical aspect for obtaining accurate, reliable, and high-resolution estimates of nuclear DNA content is the release of nuclei from the cytoplasm in sufficient amounts, while maintaining their integrity throughout the analysis, protecting their DNA from degradation by endonucleases, and enabling stoichiometric DNA staining (Loureiro *et al.* 2021).

### 5.1 Requirements

5.1.1 Equipment and apparatus

- Ploidy Analyser Machine (the CyFlow® ploidy analyser, Software- CyView™ 1.6, REF-CY-S-3039).

  A ploidy analyser is a fully equipped desktop flow cytometer (FCM) and features a modular optical concept that allows the use of different lasers as light sources and the detection of up to six optical channels (parameters). It operates with an internal PC (personal computer), and data acquisition, instrument control, and data analysis are controlled and performed by the CyView™ software (CyView™ 1.6)

- Waste bottle
- Sheath fluid bottle
- Sterile falcon tubes
- Cuvettes

- Filter paper (Whatman paper)

- Stirring machine

- CellTrics® sieves

- Glass Petri dishes

- Forceps

- Sharp razor blades

- Scissors

- Marker pen

- Fridge (+4 °C)

- Deionized water

- Paper Towels

- Android device installed with ODK Collect App and a QR bar code reader App.

## 5.1.2 Buffers and solutions

- Otto I buffer: Citric acid monohydrate (0.1 M). This is used to break the nucleic membrane to release the nucleic contents from the plant cell nucleus.

- Tween 20: This is a surfactant that removes surface tension and therefore facilitates dissolving of hydrophobic particles, which helps in the spreading of reagents reducing the tendency of nuclei and debris to aggregate.

- Otto II buffer: 0.4 M Anhydrous Sodium hydrogen phosphate ($Na_2HPO_4$) added to the isolated nuclei to enable staining.

- DAPI (4', 6-diamidino-2-phenylndole): This is a fluorescent dye used to stain nucleic acids.

- $Na_2S_2O_5$ (sodium metabisulphite). This is an anti-oxidant.

- 70 % Ethanol.

## 5.1.3 Software and Apps

- CyView™ software (CyView$^{TM}$ 1.6)

This is the software installed in the ploidy analyser that controls data acquisition, instrument control, and data analysis. It is purchased together with the machine.

- ODK Collect App

This is the App that includes the banana tracking tool (BTracT) used to organize the barcodes for the samples from the tissue culture laboratory and to record the ploidy

analysis results. This can be downloaded from google play services using the link

https://play.google.com/store/apps/details?id=org.odk.collect.android&hl=en&gl=US&pli=1

- QR barcode reader App

  This is needed for reading of the QR barcodes to identify the samples for analysis and recording.

## 5.2 Buffer preparation

5.2.1 Otto I Buffer (500 ml)

- Weigh 10.5 g of citric acid monohydrate (0.1 M) and dissolve it in 400 ml of deionized water.
- Add 2.5 ml of Tween 20 to the solution.
- Stir the solution using a magnetic stirrer to dissolve solids.
- Filter the solution using Whatman filter paper to remove small particles.
- Adjust the volume of the solution to 500 ml with deionized water.
- Store the solution at 4°C.

5.2.2 Otto II Buffer (500 ml)

- Weigh 28.4 g of anhydrous $Na_2HPO_4$ (0.4M) and dissolve in 400 ml of deionized water.
- Heat the mixture while stirring until $Na_2HPO_4$ dissolves.
- On cooling, filter the solution using Whatman filter paper to remove small particles.
- Adjust the volume to 500 ml with deionized water.
- Store the solution at room temperature in the dark.

**Note:**

- At the time of running the samples, add 4 µl of DAPI (4', 6-diamidino-2-phenylindole) for every 10 ml of Otto II buffer (20 µl of DAPI for every 50 ml of Otto II buffer).
- Add 1 ml of $Na_2S_2O_5$ (Sodium Metabisulphite) to 50 ml of Otto II buffer.

**5.3 Ploidy machine set up**
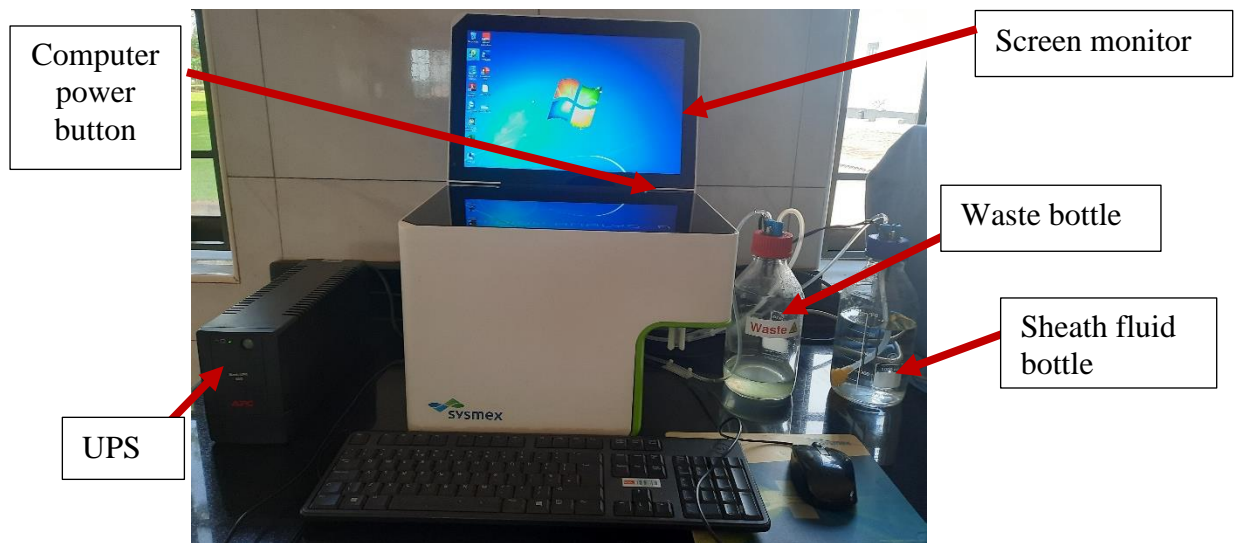


Figure 5.1. The CyFlow® ploidy analyser machine.

5.3.1 Starting the machine and analyzer software

- Switch on the uninterrupted power supply (UPS).

- Plug the ploidy analyzer into the UPS (UPS protects the machine from the unstable power supply).

- Switch on the ploidy analyzer with the power button found at the lower back panel of the machine.

- Lift open the screen monitor of the machine.

- Start the computer of the machine by pressing the computer power button shown in Figure 5.1.

- Click open the 'Cube 1.6' icon on the desktop to start running the CyView ™ software as seen in Figure 5.2.



Figure 5.2. Ploidy machine display with cube 1.6 software icon highlighted.

- Login into the software using the details; Login: **USER**, Password: **Cube1** as shown in Figure 5.3. Both username and password are case-sensitive.
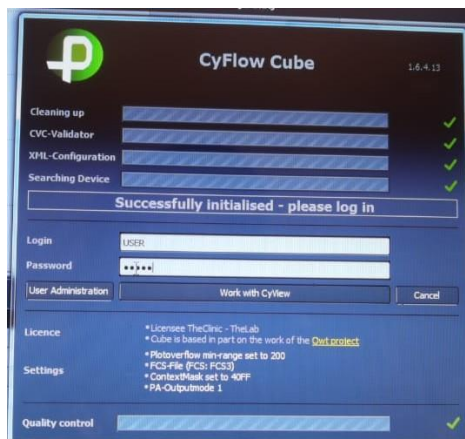


Figure 5.3. Display of the CyFlow® Cube 1.6 software login details page.



Figure 5.4. Icons for loading and saving data files (a) and for saving and retrieving the configuration files (b).

5.3.2 Priming the machine

Priming is the initial process carried out to not only get the machine ready but also to clean the entire flow cytometry system of the machine like the tubes, getting rid of blocked air and stabilising the system. Priming is done when starting the instrument for the first time (daily initialization) after the sheath fluid bottle is filled or as a troubleshooting procedure e.g. when there is no signal or bad signal and blockage.

- Load the configuration script Cfg-file for priming (this script is present) from the CFG folder as shown in Figure 5.4 b.
- Select the initialization icon highlighted in Figure 5.5 and click 'play'.



Figure 5.5. Icons for Prime (highlighted for use during priming), work, and clean mode.

- This will start the priming process and a guide dialogue series will follow.
- Connect a sample tube with the decontamination solution (violet solution), press "continue" and wait until the system has finished running.



Figure 5.6. Step 1 of the priming process.

- Disconnect the sample tube, press "Continue" and wait until the system has finished running.



Figure 5.7. Step 2 of the priming process.

- Reconnect the sample tube with the decontamination solution, and press "Continue".

Figure 5.8. Step 3 of the priming process.

- Connect the sample tube containing sterile distilled water or sheath fluid and press "Continue".

- When the machine stops running, it is ready for use. A message to confirm the end of the priming process will be displayed as in Figure 5.9.



Figure 5.9. Step 4 and the final stage of the priming process.

5.3.3 Calibration of the machine

Calibration is done using fish DNA with known ploidy (diploid), UV calibration beads to ensure that the UV lamps are working, and banana cultivars of known ploidy called standards.

- Switch from Prime to the "WORK" mode by clicking on the work icon as shown in FigureFigure 5.10.



Figure 5.10 Work mode activated.

- Load the calibration files from the CFG folder by clicking on the icon highlighted in Figure 5.11.
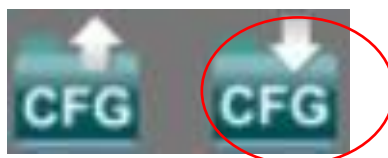


Figure 5.11. Icon for loading configuration files.

- Select the DNA calibration script.

- Put 1 ml of fish DNA in a cuvette and load it in the sample chamber.

- Run the sample by clicking 'play' in work mode.

- Adjust the gains to set the control peak at channel 200.

- Then select the UV calibration beads script from the CFG folder.
- Mix one drop of the UV calibration beads solution into 50 ml of sterile deionized water and shake gently, to mix well.
- Add l ml of the solution into a cuvette and run it.
- Adjust the gains to set the control peak at channel 200 in region one as seen in Figure 5.12.



Figure 5.12. A peak curve at channel 200 in region one indicates a well-calibrated machine.

- Once both fish DNA and UV beads have peaked at frequency channel 200, the machine has been calibrated.
- Further calibration can be done using accessions with known ploidy like Calcutta 4 as a diploid, any of the East African highland bananas as triploid and 1201K-1 as tetraploid.

## 5.4 Sample collection, preparation and analysis

5.4.1 Sample collection from tissue culture plantlets

- Select plantlets in the tissue culture laboratory before the rooting stage.
- Pick 2–3 leaves from each plantlet using a disinfected surgical blade.
- Wrap the leaves in aluminum foil and stick the respective sample ID barcode on the sample as seen in Figure 5.13.
- Take the samples to the molecular laboratory.
- The samples can be kept in the $+ 4^{\circ}$C refrigerator for a maximum of three days if they are not going to be worked on immediately.

Figure 5.13. Example of a sample labelled with a barcode.

- Sterilize the blade by wiping it with a paper towel soaked in ethanol before taking the next sample.

5.4.2 Sample collection from the field

This is done for plants that are already established in the field (not in the tissue culture laboratory) but whose ploidy level needs to be determined as needed.

- Identify a healthy clean green cigar leaf (the cigar leaf is the youngest leaf on the plant, still rolled like a cigar) on the banana plant.
- Scan the barcode on the plant to record the sample to be collected using the Coordinate App.
- Cut about 6 cm of the leaf from the leaf apex (tip of the leaf) using sterile scissors.
- Wrap the leaf sample in aluminium foil and stick the sample ID barcode within it.
- Sterilize the scissors by wiping the cutting edges with a paper towel soaked in ethanol before sampling the next plant.

5.4.3 Sample preparation in the laboratory

- Hold the fresh leaf sample with a pair of forceps in a Petri dish containing 1 ml of cold Otto I buffer and chop the midrib into very fine pieces, using a sharp razor blade taking care not to crush the sample (Figure 5.14). This is to release nuclei into the isolation buffer.
- Mix the homogenate in the Petri dish and then filter it to remove leaf debris, using a cellTrics® sieve into a cuvette.
- Incubate the solution for 1-5 minutes to allow the cell suspension to thoroughly pass through the cellTrics® sieve.
- Since the samples are stable in Otto I buffer, several samples can be prepared while still fresh, cell suspension in cuvette tubes covered and stored at +4°C in the fridge.
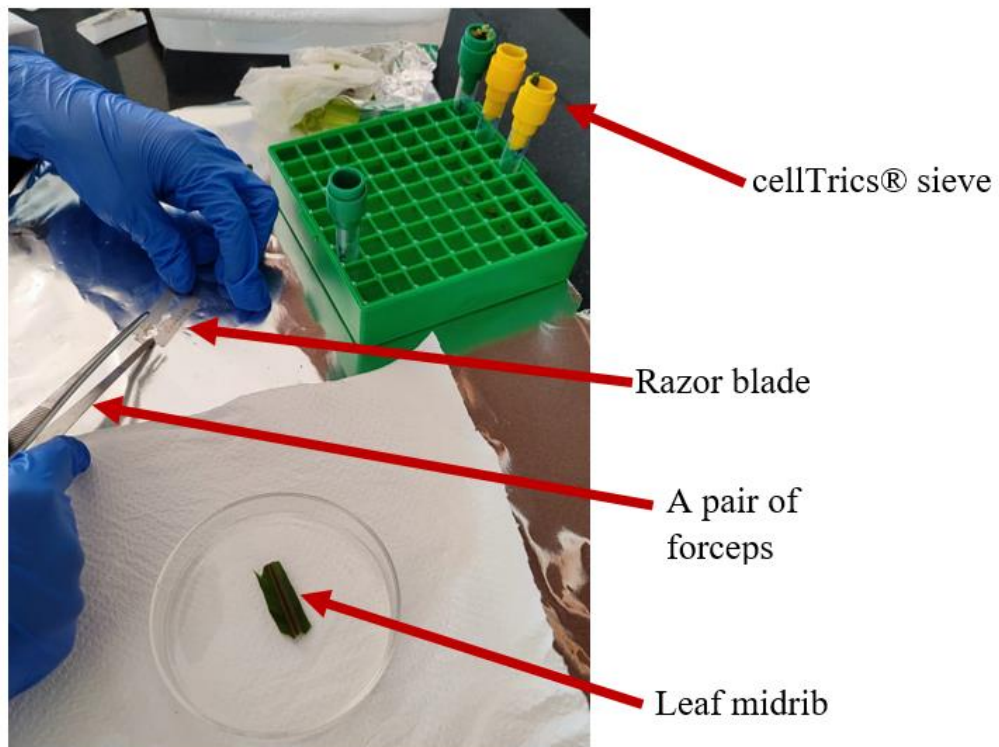
Figure 5.14. Sample preparation for ploidy analysis.

5.4.4 Sample analysis

- Choose the work mode as in Figure 11 in section 6.4.3 to measure and acquire data from the samples measured.

- Add 1 ml of Otto II buffer (containing DAPI) into the cuvette tube containing the sample

- Attach the cuvette tube to the ploidy analyzer machine and run the sample.

- Observe the peak formed. Samples of different ploidy will show corresponding peaks as shown in Figure 5.15.



a
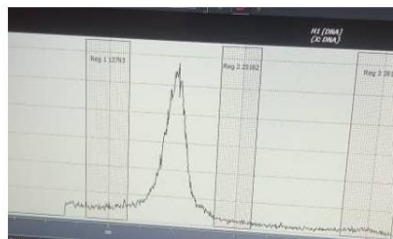


b
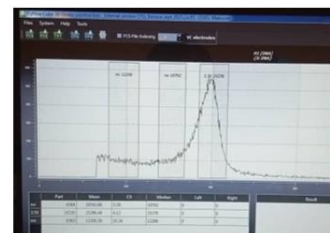


c

Figure 5.15. Peaks showing ploidy level of diploid (a), triploid (b) and tetraploid (c) banana.

- Record the ploidy level results, as they appear using the ODK collect app as illustrated in steps 1 to 5 in Figure 5.16.

- Save the sample data results (peaks) by clicking the FCS ⬇ icon shown in Figure 5.4A above, for saving the .fcs files on the computer.
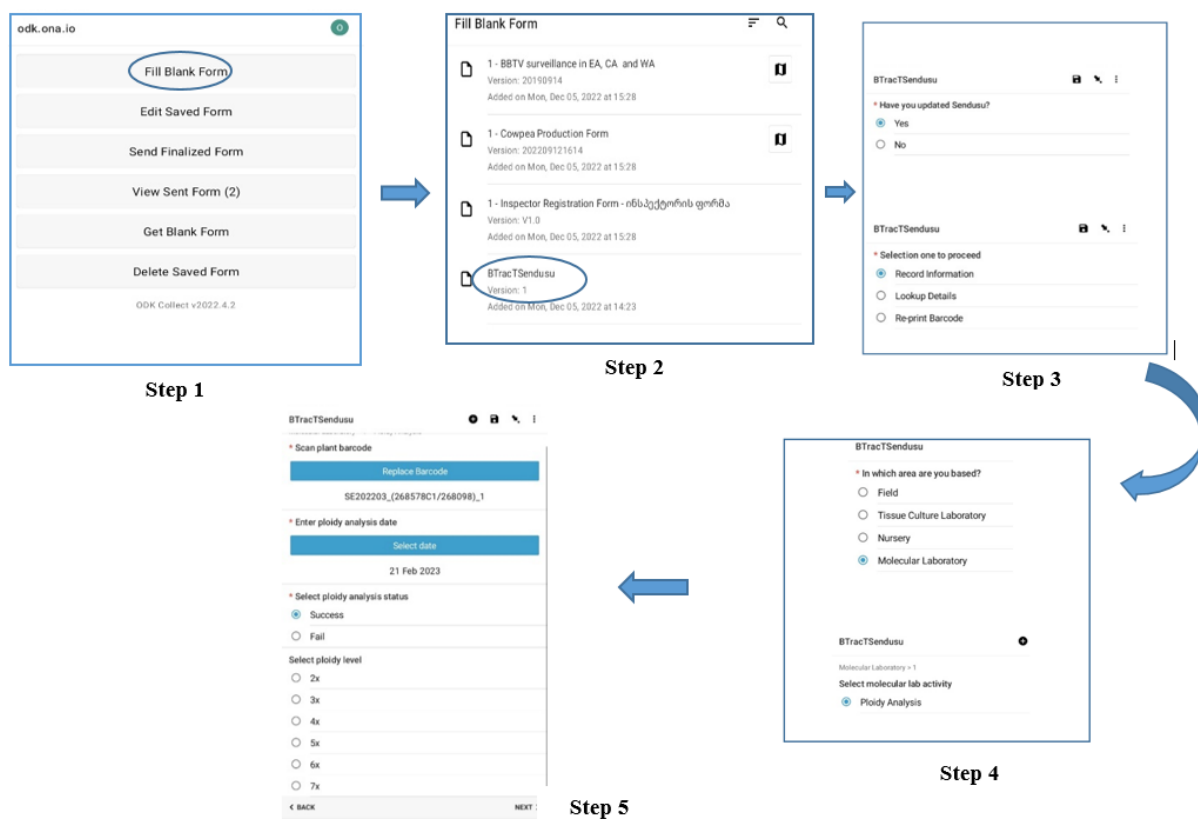


Figure 5.16. The process (steps 1-5) of data recording of ploidy results using the BTracT system.

## 5.5 Clean mode (Shutdown process)

This mode is activated to clean the sample port and tubes, and shut down the process. It follows the steps below:

- Select the "CLEAN" mode by clicking on the "CLEAN" icon highlighted in Figure 5.17.
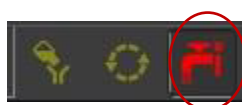


Figure 5.17. Activated clean mode icon.

- Press start (play icon), and the system will start the priming process automatically and guide you through the program
- Connect a sample tube with the cleaning solution (green solution) shown in Figure 5.18 and press continue.



Figure 5.18. Decontamination solution (violet) and cleaning solution (green).
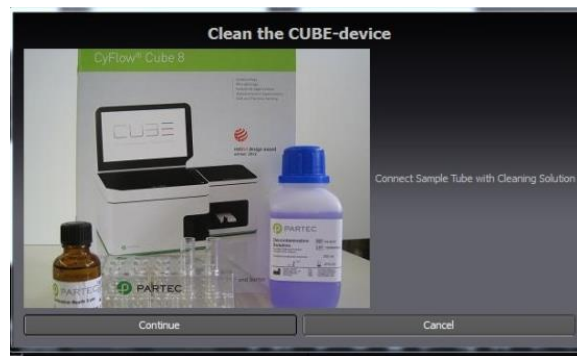


Figure 5.19. Step 1 of the cleaning process.

- Connect a sample tube with decontamination solution (violet solution) and press "Continue".



Figure 5.20. Step 2 of the cleaning process.

- Connect a sample tube with water (sterile distilled water or sheath fluid) and press "Continue".



Figure 5.21. Step 3 of the cleaning process.

- After completing the cleaning process, CyView™ will be shut down automatically. Laser sources will be switched off.
- Switch off the PC, close windows (start-Exit-switch computer off).
- To power off completely, use the main power switch at the back panel.
- Close the machine and cover it with dustproof protection.

**5.6 Critical control points to note and guide when troubleshooting**

5.6.1 Sample preparation

- In the chopping procedure, it is important that the razor blade is sharp, and the material is chopped into very fine pieces and not just crushed.

5.6.2 Levels of waste and water bottles

- Always check the levels of the sheath fluid and waste bottles before switching on the machine. The sheath bottle should be filled with at least 700 ml of sheath fluid and closed with a screw top. Please note that a higher level of sheath fluid could lead to unstable sample flow. The level of waste in the waste bottle and sheath fluid in the sheath fluid bottle should not be the same at any given time to avoid a buildup of pressure in either bottle.
- When filling up the sheath fluid bottle, ensure that no air bubbles are trapped in the yellow filter unit inside the bottle. Get rid of any air bubbles that appear.

- It is recommended to replace the sheath fluid at least once a week or before any daily use.

- The waste bottle must be emptied after and before each user session and the screw top tightly closed.

5.6.3 Repeat - priming

The Prime mode should be performed in the following cases;

- Starting the machine for the first time (daily initialization).

- After the sheath fluid bottle was refilled.

- As troubleshooting procedure (when no/bad signals were observed, blocking, etc.).

- When a daily checkup is needed, please select the "Calibration Beads" configuration file for the correct settings for the calibration beads (very crucial settings for the correct verification of the performance of the machine).

5.6.4 Intermediate cleaning process (cleaning between samples)

- After running a sample, load the sample chamber with a cuvette containing deionized sterile water.

- Click the "clean" mode icon to clean the system between samples.

**5.7 Maintenance of the Ploidy Analyser machine**

- The machine should be placed on a flat surface, free from shaking forces.

- Clean the machine casing regularly and carefully with a soft dry cloth. Water must not enter the machine or peripheral devices or come into contact with electric conditions and switches.

- Use a special screen cleaner and a soft cloth for cleaning the screen. Do not use any organic solvents, nitro thinner, benzol, alcohol, highly concentrated bleach etc**.,** to clean the ploidy analyzer or its screen.

- Do not use tools to clean the flow cuvette. In case it is blocked, enquire Partec for rapid exchange.

- Regularly empty the waste bottle and clean it with a warm detergent solution and a brush.

- Clean the sheath bottle (reservoir) with distilled water and a clean brush and flush with clean distilled water several times. A sheath bottle is critical for the optimum operation of the machine.

- The machine should be primed once a week when not used regularly as a form of routine maintenance. Specifically: clean the flow system by using distilled water.

- Always keep the electrodes of the sample chamber dipped in distilled sterile water or sheath fluid at the sample port.

- Certified technicians should also service the machine accordingly as a form of routine maintenance.

## 6. SOP 03: Establishment of early evaluation trial (EET)

The hybrids developed from the different cross combinations undergo a series of trials starting with the EET, in which only two (IITA) and one (NARO) replication(s) per genotype are planted and evaluated for traits such as black leaf streak resistance, bunch size, fruit parthenocarpy, and dwarfness for over two production cycles. This activity is under stage: crossing and screening; during the matooke product development process as shown in Figure 3.1. The purpose of EET is to quickly select promising hybrids to advance for further evaluations in preliminary yield trials (PYT).

### 6.1 Required tools and materials

6.1.1 MusaBase

This is the global banana breeding database and a data management tool in support of breeding. Use this system to generate design trials, upload existing trials and design and print barcode labels. It is accessed at https://musabase.org/.

6.1.2 Banana Tracking Tool (BTracT)

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

6.1.3 Android device

6.1.4 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).

6.1.5 Zebra desktop printer

Zebra ZT411 industrial printer.

6.1.6 Zebra designer software

Zebra designer Professional version 3.5.

6.1.7 Cross ID 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.

6.1.8 Computer with Internet access.

6.1.9  Potting soil

Sterile forest soil or loam soil, dry manure and sawdust mixed in a ratio of 4:2:1 respectively.

6.1.10 Transparent 100 ml volume plastic ice cream cups with holes in the bottom, used as weaning cups.

6.1.11 Black polyethene potting bags (21.8 cm × 26.4 cm) with holes in the bottom.

6.1.12 Rigid white plastic plant labels, 12 cm length by 1.4 cm 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* 6.1 a.
- Fill the weaning cups with potting soil.
- Re-print the barcode labels shown on the baby jars (number of reprinted barcode labels = number of plantlets to be transferred, usually four).
- Place the barcode labels on the rigid white plastic tags and stick them into the cup that will contain the plantlets as seen in Figures b and 6.1 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 watering them.
- Scan plantlet barcode ID to record the 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 transfer them into a humidity chamber standing under a shade as in
- *Figure* 6.1 d.
- Continue mist spraying the plantlets and chamber to maintain a high humidity to prevent plantlets from drying out.
- Leave the plantlets in the humidity chamber for 3–4 weeks until fully weaned. Fully weaned plants should have at least 3–4 fully open green standing leaves.

Figure 6.1. Barcode-labelled baby jars with plantlets (a), plants in weaning cups (b and c) and plantlets in a humidity chamber (d).

### 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 should be fitted with an automatic irrigation and misting system.

- Fill to 3/4 capacity of the black polyethene potting bags or 5 L plastic buckets, with potting soil.
- In the morning transfer one plantlet with its bar code label/tag to 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 the status of the potted plant into BTracT.
- Transfer the potted plants into the nursery, placing them in an orderly manner systematically.
- Water them after transfer until water passes through the bottom holes regardless of the irrigation schedule of the nursery.

### 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 a week before planting in the field.

- Remove the plants from the screen house and place them under shade.
- Scan plantlet barcode ID to record the date of hardening, number of hardened plantlets, status (contaminated or not contaminated) and photo of the contaminated 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 flow

cytometry. A complete standard operating protocol for the ploidy analysis process is described above.

## 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 disc that cuts deep to loosen up and mix the soil thoroughly.

- Measure and field mark to a spacing of 3 m (between plants) × 3 m (between rows) for triploid and tetraploid hybrids, giving a plant density of 1,111 plants/ha.

- Measure and field mark to a spacing of 2 m (between plants) × 3 m (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 topsoil from the bottom soil while digging the planting holes, to be used at planting.

### 6.4.2 Trial design

In 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 breeding clones to be evaluated, the large space requirements for banana plants and the limited number of replicates per breeding clone. 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 organize 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 the user status of 'submitter' can organize 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 Figure 6.2.



Figure 6.2. How to design a 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 6.3 and Figure 6.4). 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.
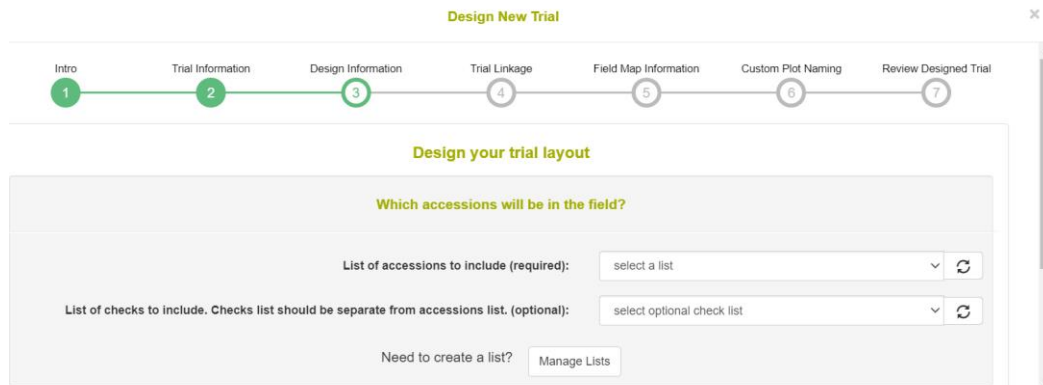


Figure 6.3. Workflow on how to design a new trial in MusaBase.
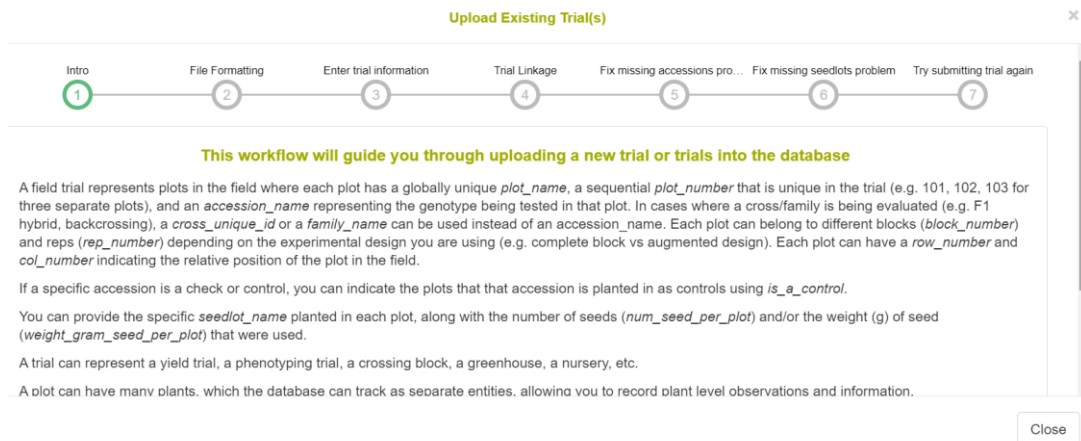
Figure 6.4. Workflow on how to upload an existing trial in MusaBase.

- When uploading an already existing trial (layout in a spreadsheet), be sure to use the required format by MusaBase (Figure 6.5).
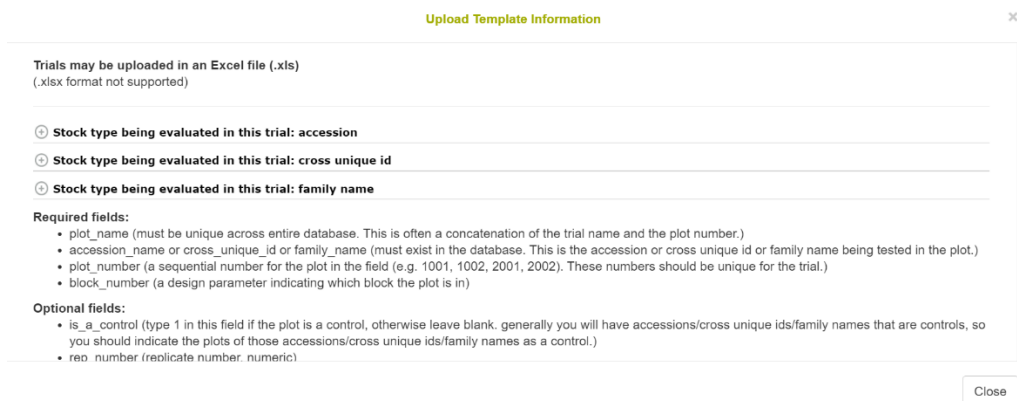


Figure 6.5. Template and required headers for formatting existing trial layout spreadsheet.

- On completion of all required steps with the required information, upload the trial. If all necessary information and format are correct, you will get a message as seen in Figure 6.6.
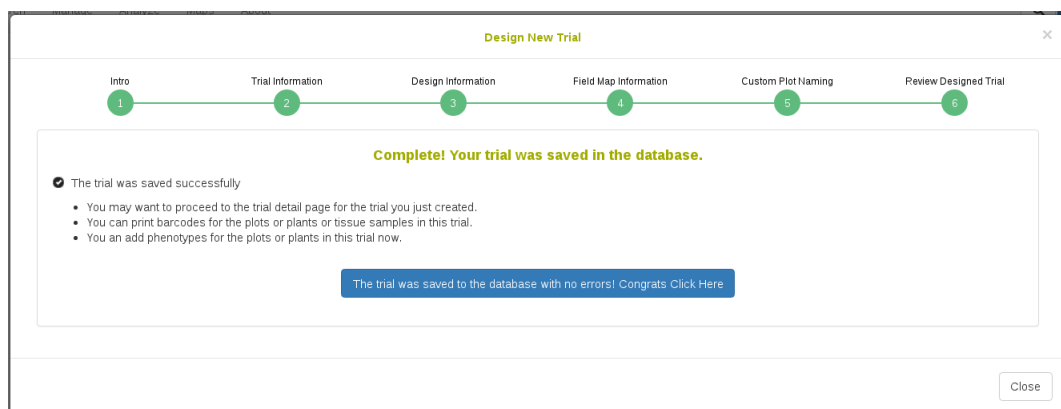


Figure 6.6. Confirmation of successful trial upload on MusaBase.

6.4.4   Organizing and printing field labels

Detailed step-by-step process of how to organize and print barcode 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 print labels for.
- Establish a new design or load a saved design. The saved design for field labels used at Sendusu is named ''Sendusu labels'' in the database.
- Download the PDF file of all barcodes of the trial.
- Print the barcodes using a laser jet office desktop printer.
- Stick the barcodes on 0.5 m long PVC pieces, flattened at the area of barcode placement for easier QR code reading as seen in Figure 6.7.



Figure 6.7.  Barcode label on a PVC piece.

**6.5  Planting Early Evaluation Trials (EET)**

- Choose healthy and strong-looking plants with a girth of 5 cm and above for planting. Use girth and not height because plants 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 the 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.

**6.6  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 720 g/L and 150 ml of Agrosate – 480 g/L glyphosate, mixed in 15 L of water).

- De-trash the fields by cutting off the broken, dead and dry leaves to keep the fields clean and reduce *P. fijiensis* inoculum.

- Routinely scout for banana xanthomonas wilt 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 the spreading of the disease from one plant to another.

- Remove the male buds to control banana xanthomonas wilt.

- De-sucker after taking cycle one data for several 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 next to the right plants and replace any damaged barcodes.

# 7    SOP 04: Phenotyping and data management of early evaluation trials (EET) in matooke breeding.

The hybrids developed from the different cross combinations undergo a series of trials starting with the EET, in which only two replications per genotype are planted and evaluated for traits such as black leaf streak 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 2. The purpose of EET is to quickly select promising hybrids to advance for further evaluations in PYT.

## 7.1 Required tools and software

7.1.1 Musabase

Banana breeding database for curation, data collection and storage, https://musabase.org/.

7.1.2 Android device

7.1.3 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 and can be downloaded for free from the google play store: Field book.

7.1.4 Computer with internet access

## 7.2 Field data collection using Field Book App

After installing the Field Book App, it will open a folder called 'fieldBook' on the Android device, which contains sub-folders required for running the App. To set up 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 established or uploaded to the platform and it is now standard practice to download both files automatically from MusaBase.

7.2.1 Creating a field layout file from MusaBase

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

7.2.1.1 Using the Field Book Tools page:

- Browse to the "Manage" menu and click on "Field Book App" as seen in Figure 7.1.

- 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 organize a layout file. Choose the management factor, which is the treatment to be applied to the trial, and 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, a plot refers to a single mat or plant.

- Check if all columns required have been included and add more columns if needed. See Figure 7.2.

- Click 'Submit' to open 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.
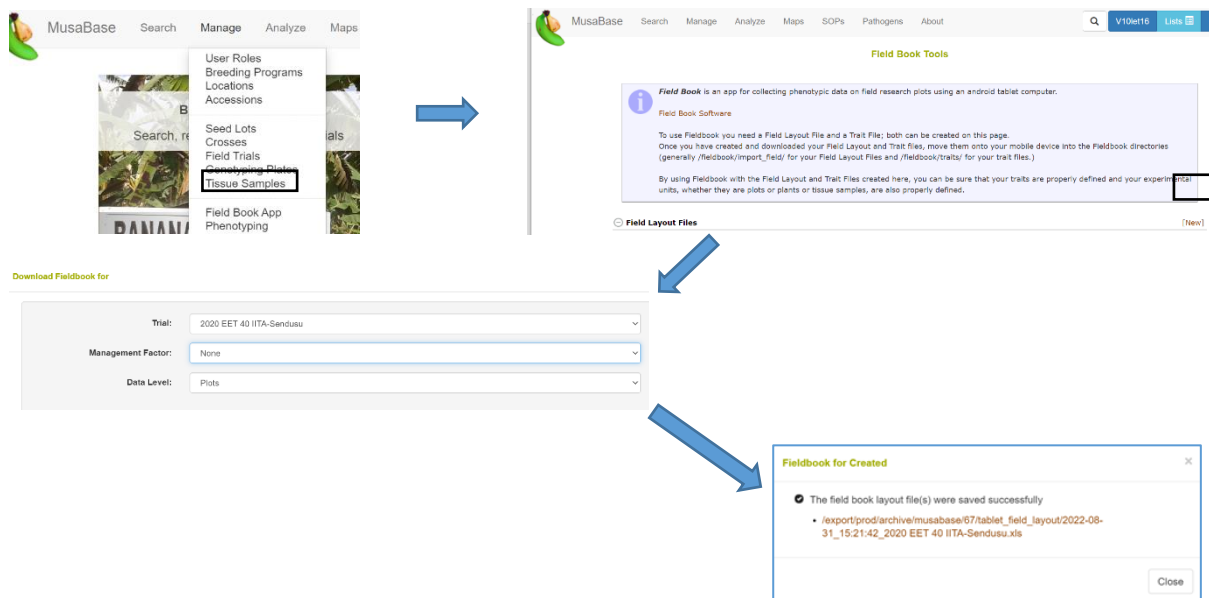


Figure 7.1. Workflow to create field layout files using Field Book App on MusaBase.
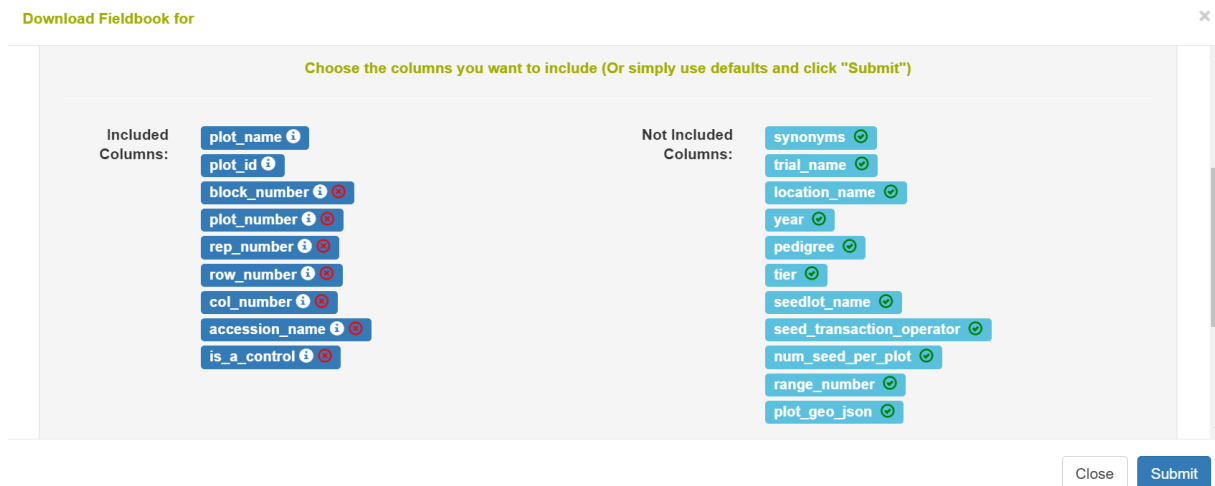
Figure 7.2. List of required (left) and optional (right) columns for the Field Book template.

7.2.1.2 Using the trial details page

- Go to the "Trial Detail" page of the trial that you want to organize a Field Book data collection file via the 'Manage' tool as seen in Figure 4.

- 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 the data level (plots or plants) as in section 7.2.1.1 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 the final step of Figure 7.3. Click on the file name to download the file onto your computer.
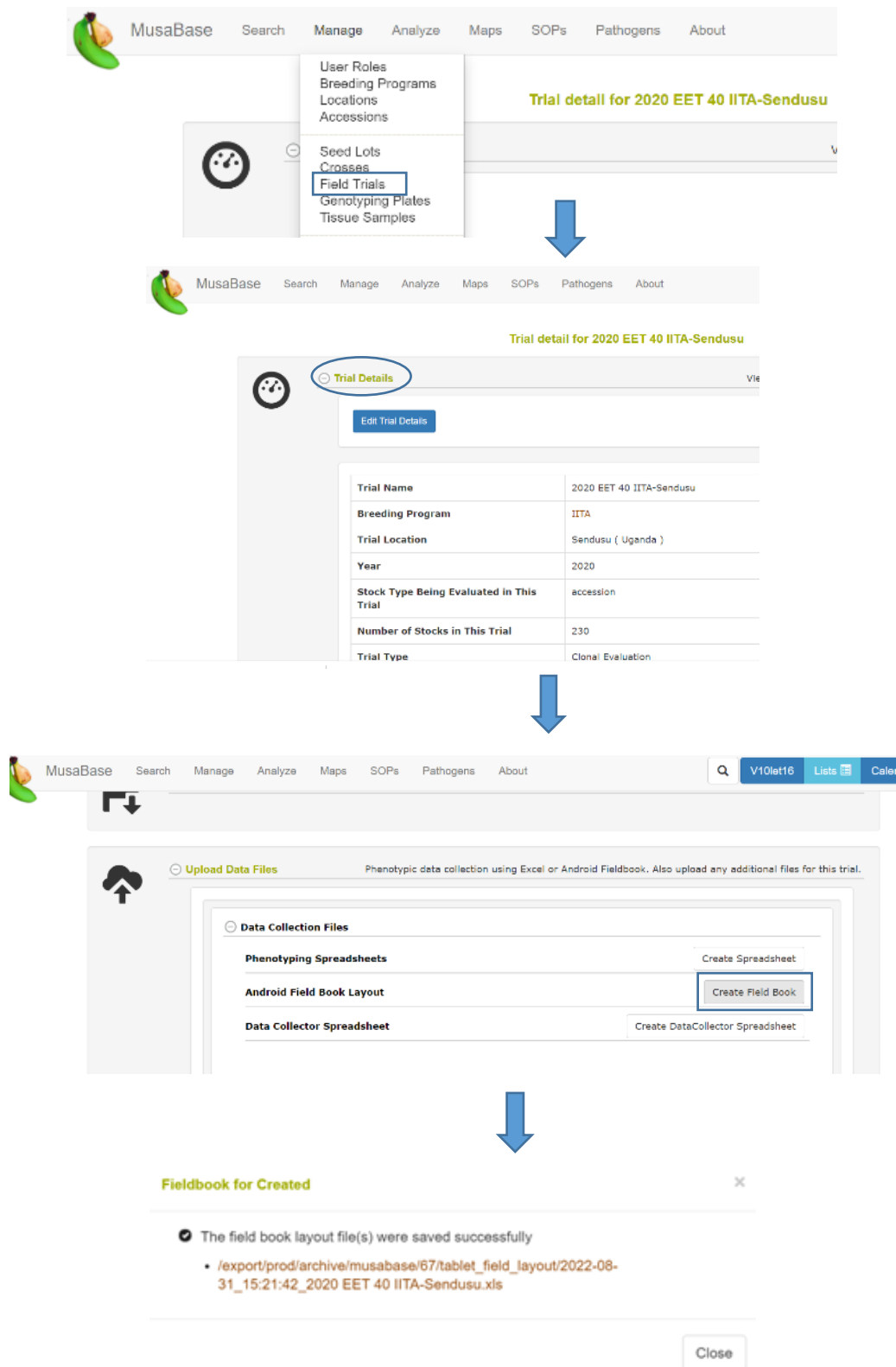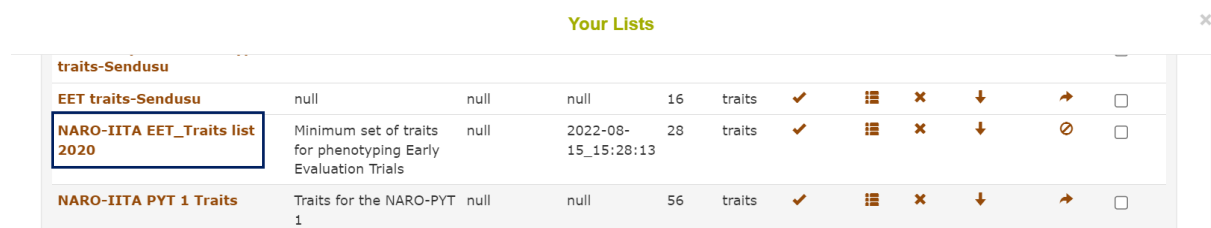
Figure 7.3. Workflow of how to organize field layout files using the Trial details page on MusaBase.

### 7.2.2 Organizing 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 NARO's National Banana Program breeding teams and is attached in Annex 1. This list is also available from MusaBase (Figure 7.4).



Figure 7.4. Screenshot highlighting a minimum set of breeder's list traits for EET from MusaBase.

To organize a trait file follow the steps below

- Browse to the "Manage" menu and click on "Field Book App".
- Scroll down to the Trait files section and click on 'New' (
- Figure 7.5).
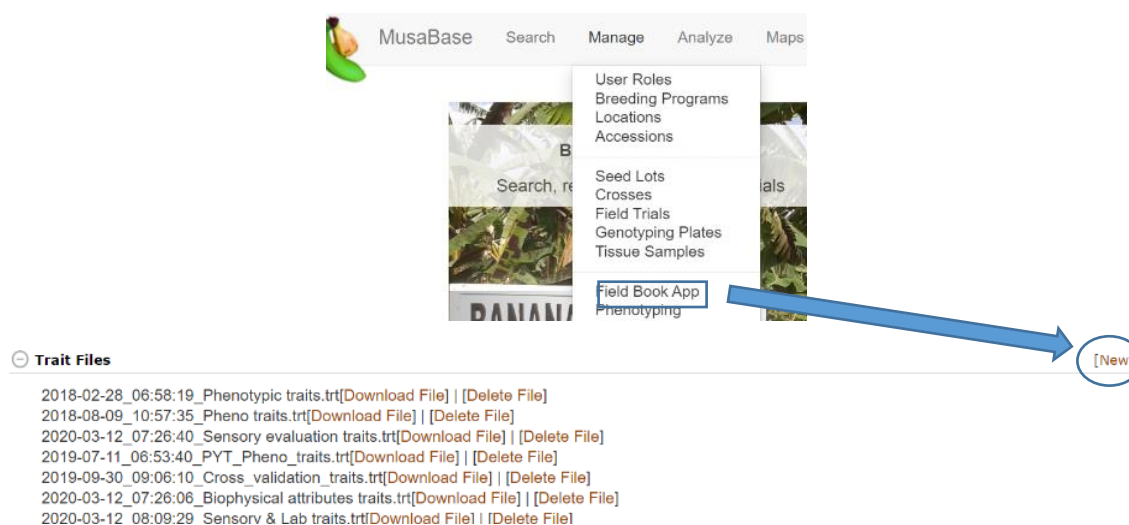


Figure 7.5. Steps 1 and 2 of how to organize a file using a trial list on MusaBase.

- From the drop-down list, select the list of traits you organized or the one available, in our case the "NARO-IITA_Traits list 2020" as in Figure 7.6.
- 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.
- Click on 'create file'.
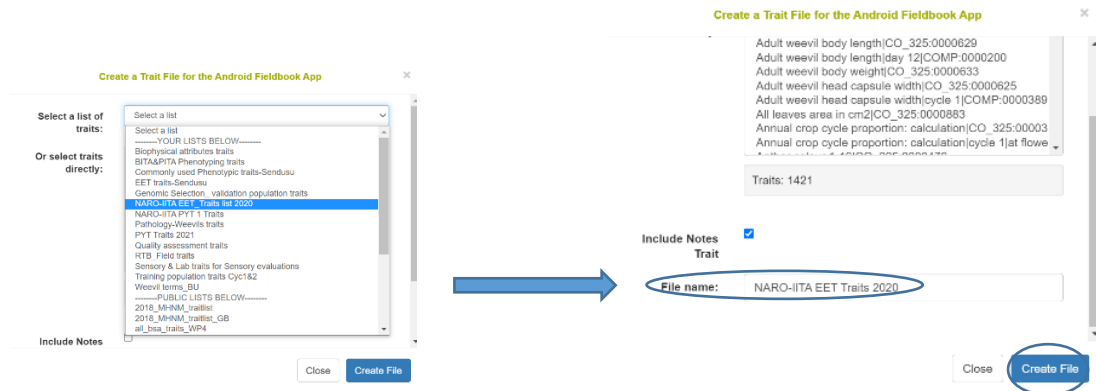
Figure 7.6. Steps 3, 4 and 5 of how to organize a file using a trial list on MusaBase.

- A pop-up window will appear showing that the trait file was organized with a link to download the file (Figure 7.7).
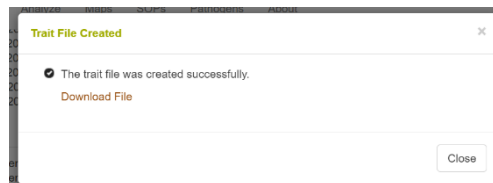


Figure 7.7. Pop-up message showing successful creation of trait file on MusaBase.

- Click on the '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 the '.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 7.8).
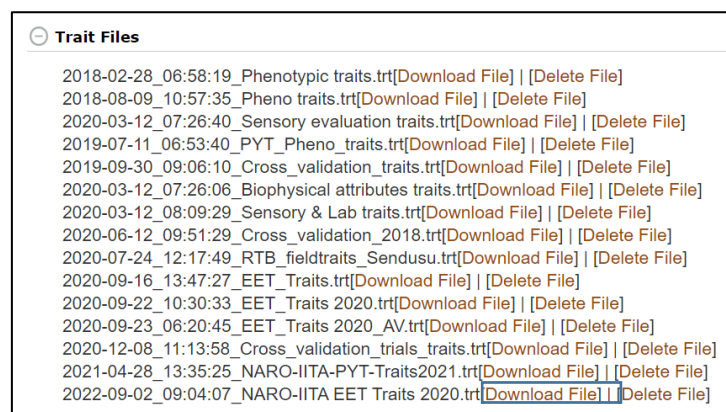


Figure 7.8. Existing lists of traits files on MusaBase, downloadable for use in the Field Book App.

### 7.2.3 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 7.9).
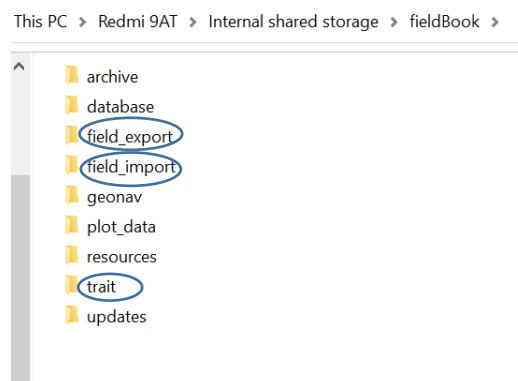


Figure 7.9. Sub-folders for operating the Field Book App.

- 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.

### 7.2.4 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.

7.2.4.1 Importing field layout file

- Open the Field Book App on the Android device to display the main menu.

- Click on the 'Fields' option and click on the '+' in the upper right corner as highlighted in Figure 7.10 b, 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 7.10 c).

- 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 a unique identifier, 'row_number' as the primary identifier and 'col_number' as a secondary identifier (Figure 7.10 e). 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 7.10 f.
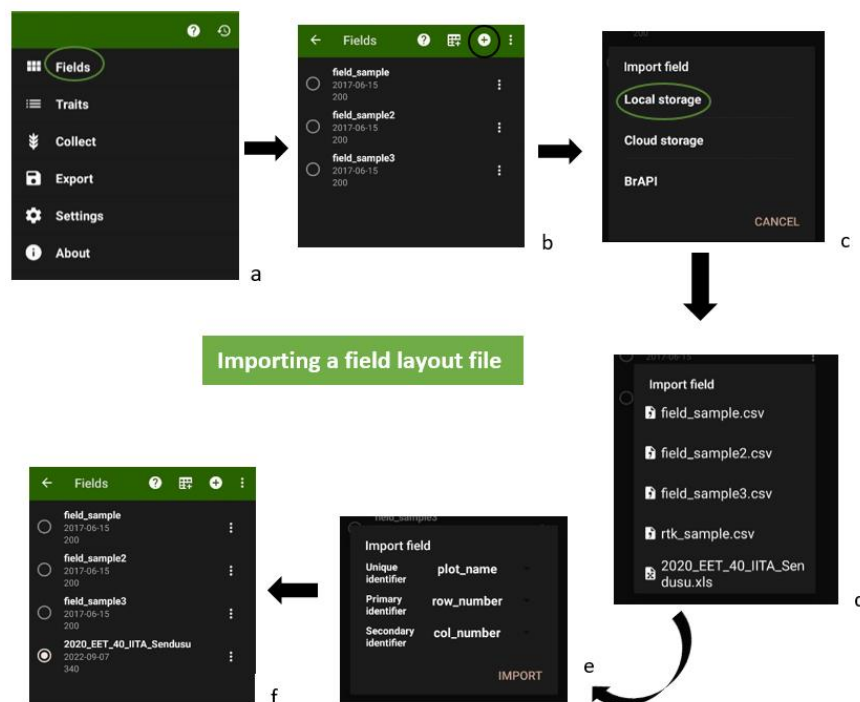


Figure 7.10. How to import a field layout file in the Field Book App.

7.2.4.2 Importing the trait file

- Open the Field Book App on the Android device to display the main menu as seen in Figure 7.11 a.

- Click on the 'Traits' option and click on the three dots in the upper right corner as highlighted in Figure 7.11 b.

- Click on the 'Import/Export' option and then the 'Import' option (Figure 7.11 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 7.11 g.
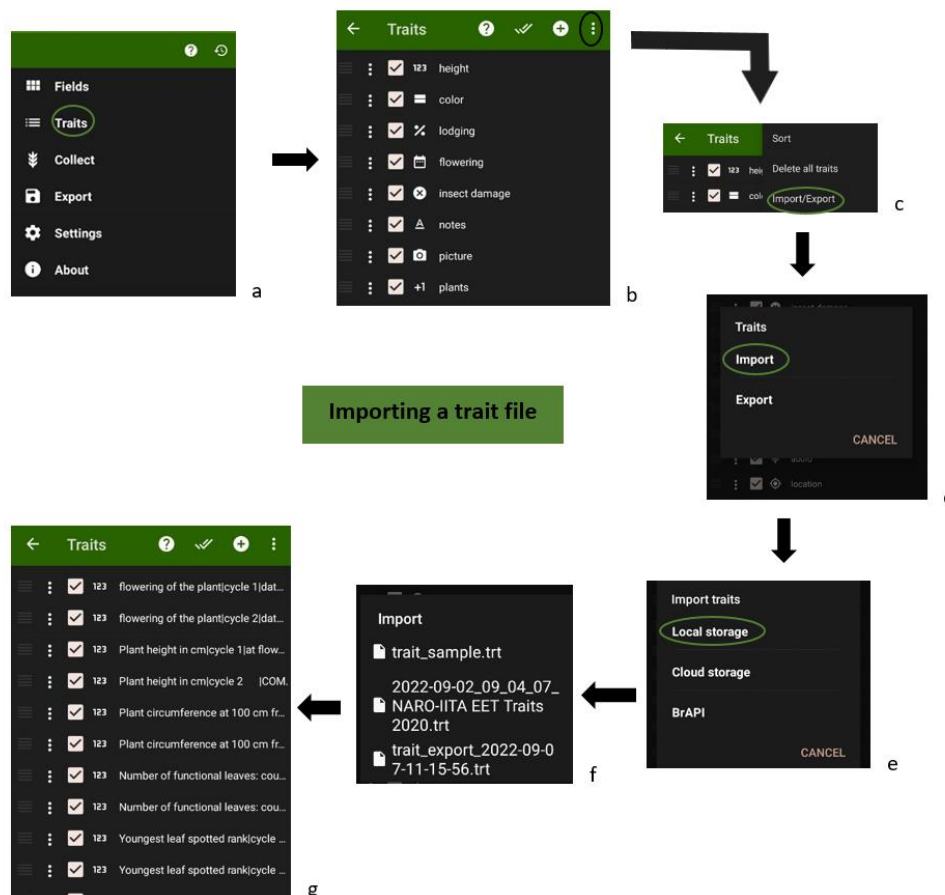


Figure 7.11 How to import a trait file in the Field Book App.


**7.2.5 Collecting data with the 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 the main field book menu.
- Click 'Collect' as seen in Figure 7.12 a.

- A data entry area will appear displaying the first trait, plot, first row and first column as seen in Figure 7.12 b.
- Scroll through plots and traits using the back and forward arrows.
- For traits in 'date' format you must click the save icon highlighted to save the date (the saved date will appear highlighted in red). Use the -1 and +1 for an earlier or a later date, respectively.
- All other traits are automatically saved when you scroll to the next plot or next trait.
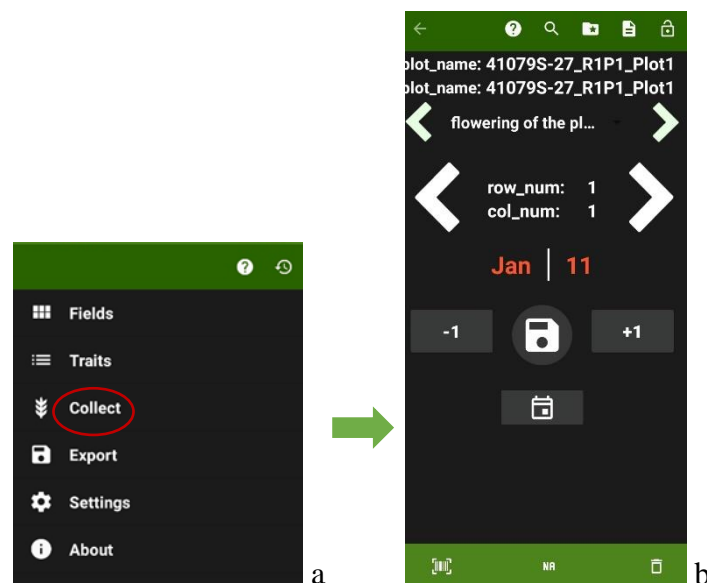


Figure 7.12 Data collection area in the Field Book.

## 7.2.6 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 the main menu display of the Field Book App.
- Click on the 'Export' option.
- The data is available for export in two formats, the Database and Table format. The Database format is preferred for uploading the data to MusaBase but the Table format is preferred for quick and easy reading in Excel. The Table format can also be uploaded to MusaBase with some formatting.
- After choosing an appropriate format, click 'Ok' to export the data.
- The exported data file is found in the field_export subfolder of the fieldBook folder where it can be copied and saved to other locations on the computer.

## 7.3 Data curation

Phenotype data files should be uploaded on your computer as a backup. 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 and outliers, and correct, if necessary, before uploading. This should be done once a month at the time of data uploading 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.

## 7.4 Uploading data to MusaBase

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

- Browse to the "Manage" menu and click on the "Field Book App".
- Scroll to the "Uploaded Phenotype Files" section and click on the "Upload" link (Figure 7.13).
- 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.
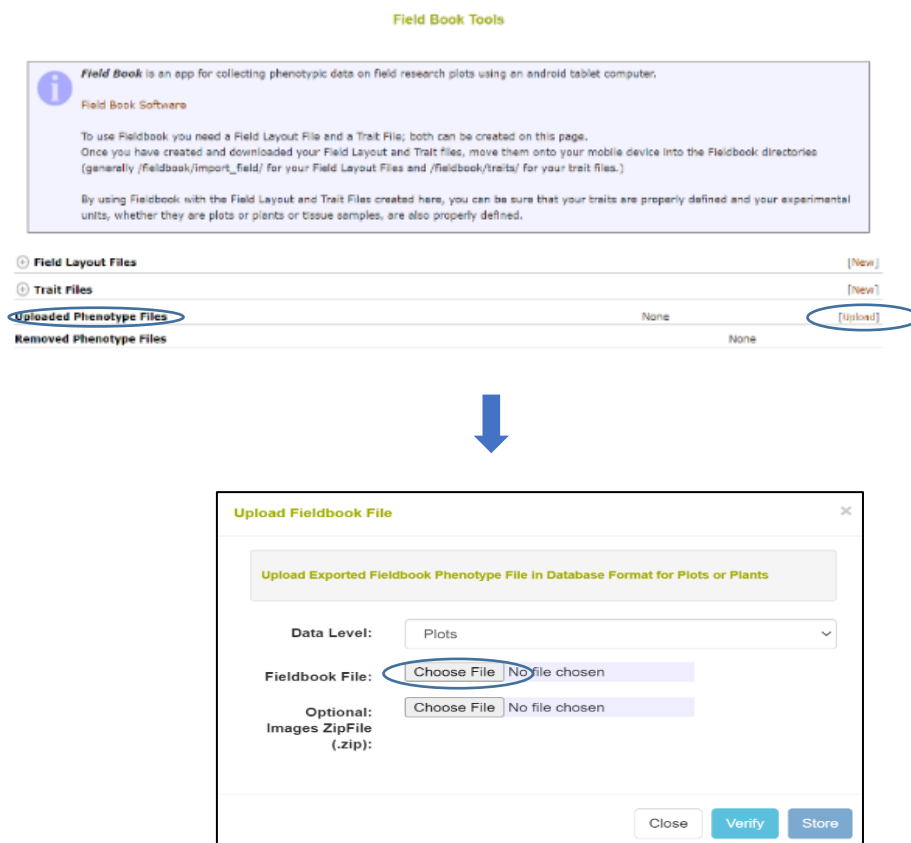


Figure 7.13. Steps 1 and 2 of uploading data to MusaBase.

- Please ensure that "plot_name" is the first column of the file to be uploaded as this ensures the uniqueness of each plot.
- To make sure that the file has the correct format for uploading, click on the "Verify" button (Figure 7.14).
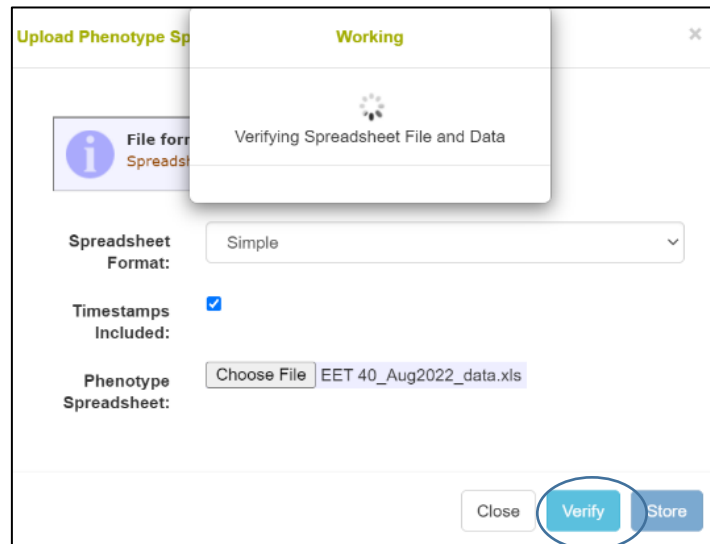


Figure 7.14. Step 3 verifying the data file to be uploaded to Musabase.

- After the file format has been verified, click on the "Store" button as seen in Figure 7.14 above to upload the data. For successful upload, a pop-up message 'successful upload' will appear as seen in Figure 7.15.
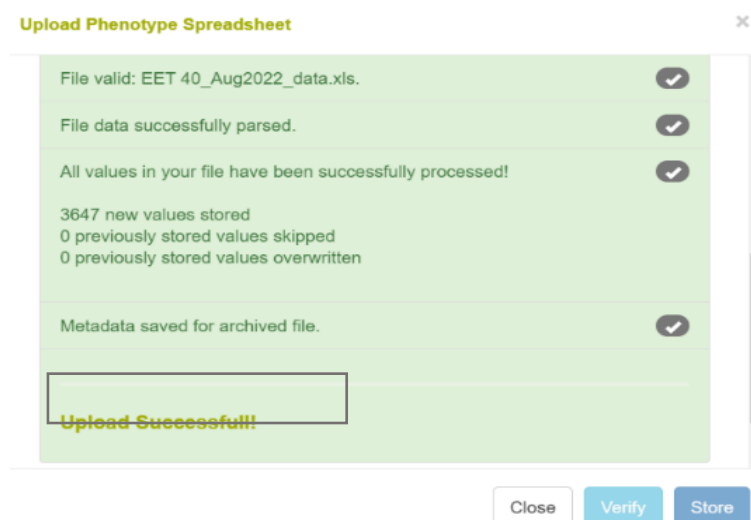


Figure 7.15. Pop-up message of successful upload of data to MusaBase.

- The uploaded data can be found on the 'Field Book Tools' and the 'Trial Details' page as seen in Figure 7.16 where it can be viewed or downloaded.



Figure 7.16. Location of the uploaded data file on Musabase.

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

## 7.5 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 signpost and by scanning one of the barcode 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 data of 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, was 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 back up the data on the local storage of the Android device.

## 7.6 Phenotyping and selection in EET

Different traits are collected at the flowering and harvest stages. 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 the '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 filling, raw pulp color and sap amount. The detailed list of descriptions, format, database codes and links to these traits on MusaBase is attached in Annex 1.

At the EET level, the focus is on black leaf streak resistance, bunch size, fruit parthenocarpy, and dwarfness. Black leaf streak resistance is evaluated under field conditions using the youngest leaf spotted (YLS) method of Vakili (1968). Standard operating procedures of phenotyping for black leaf streak host plant resistance or susceptibility in the field can be accessed at SOP04-Sigatoka.

Bunch size is used as an indicative measure of yield potential, determined from records of average bunch weight in kg (BWT, kg) with a more accurate yield potential calculation done at the PYT. 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 indicates a stouter 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 one and two), using a measuring pole while plant girth is measured as the plant circumference at 100 cm from the collar.

In the past, the 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 and orientation, black leaf streak resistance, astringency and pulp colour. This method is now combined with data records. Calculated breeding clone's means are used as a reference to the product profile with the most popular 'matooke' cultivar Mbwazirume as a control for the selection of hybrids to advance to the PYT.

## 8. Discussion and outlook

Current efforts to increase efficiency in banana breeding processes for quicker and higher genetic gains need to rely on standard operating procedures. Vast amounts of data are generated

during the breeding process from creating the crosses, and evaluations to the eventual release of improved hybrids. The breeding program depends on these data for fast decision-making. This proper documentation of these activities and data is also useful after staff changes as SOPs maintain consistency in the workflows.

This report documents only four SOPs. More breeding SOPs are being developed to document the establishment of PYT, data analysis at advanced levels of selection, sensory and quality evaluation, biophysical and biochemical analysis, selection to advanced yield trial, participatory on-farm testing using the mother-baby trial approach, variety release procedure and cultivar registration, germplasm management and quality checks to ensure germplasm is still true to type, DNA extraction and genotyping of new hybrids. Other SOPs for screening for resistance to pests and diseases such as the evaluation of diseases (black leaf streak, banana xanthomonas bacterial wilt, and fusarium wilt race 1) and pests (nematodes and weevils) have been developed by the IITA pathology team and are available online at [MusaBase](#).

## 9. Conclusion

Banana breeding is costly in terms of time and other resources such as land (6-9 $m^2$ per plant). Implementation and compliance with the proposed standard operating procedures will ensure the standardization of the breeding and data management workflows, quality control and minimize costly mistakes. Processes typically change as methods, materials, equipment, personnel, and practices change. Procedures need to be reviewed at regular intervals to ensure they continue to reflect actual practices. Hence, SOPs are referred to as 'living documents'. It is recommended that these SOPs are updated at least once a year by the product manager and are validated by the breeding team.

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