THE UNIVERSITY OF QUEENSLAND
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BPAT Assessment of IITA/NARO banana, plantain programs

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Prepared by the BPAT team

Yilma Kebede, André Drenth, Mark Cooper, Christopher Lambrides

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Executive summary

In 2017, the IITA/NARO banana breeding programs operating in West and East Africa were assessed by BPAT. The assessments found that breeding teams had excellent capacity to carry out mechanistic aspects in each program with use of well-established techniques for crossing, embryo rescue, tissue culture and field testing. However, a range of issues that were potentially limiting the rate of genetic gain in the programs were identified, including a lack of formalized product profiles, the use of a complex breeding strategy, small population sizes that constrained the breeding programs particularly for quality traits, little regard to quantitative breeding approaches, long breeding cycle, poor phenotypic screens for pests and diseases, and, an inadequate supply of planting material to farmers of new released varieties.

In 2020 these programs were re-evaluated by virtual assessments using the same BPAT team of assessors (André Drenth, Yilma Kebede, Mark Cooper and Christopher Lambrides) with the exception of one new member, Vanda Morgan, whose role was to evaluate organizational effectiveness. The aim of the 2020 BPAT evaluation was to measure improvements in the breeding programs in the three years since the previous 2017 BPAT. The 2020 assessment showed the breeding teams had made several improvements to their programs. The Banana breeding Tracking Tool (BTractT), a system of electronic data capture that uses barcodes to track each step of the breeding program using hand-held devices were deployed across all programs. The East African IITA programs were able to secure additional donor funding with the award of Accelerated Breeding Better Bananas (ABBB), a three-year grant that commenced in 2019 and aims to improve production/productivity of Mchare and Matooke types in Tanzania and Uganda by focusing on pest/disease resistance and yield. All breeding programs have done a good job of rationalizing the parents used in the crossing programs. The Mchare program has introduced a backcrossing program to try and retrieve the quality attributes of the landraces quicker than was previously possible. To widen the genetic base a set of 72 genetically diverse clones were distributed to all IITA banana breeding programs. These parents are being evaluated and information shared across programs. In the triploid programs, extra use is being made (or contemplated) of diploid progeny that are observed after the primary cross and consideration is being given to 2X x 2X and 4X x 4X crosses to generate greater variation and improve the parental pool. Considerable interaction with multiple teams from the Excellence-in-Breeding platform (EiB) were evident. A new collaboration with NSIP (Nature Source Improved Plants), offers the program promising new methods for identifying parental material and enables the creation of complementary heterotic groups between 2X and 4X gene pools. The Matooke program attempted to measure genetic gain and over a 20-year period estimated increases in bunch weight (kg) and yield (t/ha/yr) of 1.4 and 1.3 %, respectively per year. While the plantain and Mchare testing programs are still in their infancy, shorter testing programs, providing the potential for a shorter breeder cycle time, have been planned by the NARO Matooke program. However, to date the breeding programs are not operating as cyclic breeding programs, where selected individuals from one cycle are used as new elite parents to initiate the next cycle in the breeding program.

Several areas of improvement were identified, and these will need to be addressed to ensure long term progress in these programs. To overcome the narrow genetic base on both the female and the male side more and better characterized germplasm for use as parents is needed. There is a need to convert the existing breeding strategy from one that relies on crossing materials of different ploidy level to one that relies predominantly on diploid germplasm. It is recommended that the recently imported diploids are characterized as a priority and their breeding values for key traits in the different programs established through testcrosses. It is also important that more material be sourced from Asia for use as female as well as male parents for the Mchare, Matooke and plantain breeding

programs. This will be a time-consuming process, but the commitment now may bypass some current limitations of the current program and may provide considerable dividends in the future. Successful breeding requires successful execution of high-performance crosses based on identification of parents with high breeding value and identification of high-performance crosses. This process is largely lacking in the banana programs currently assessed. Pedigree analyses are needed to align traits with parental lines for both male and female lines. Based on pedigree analysis more targeted crosses can be made with parents containing traits of the highest priority and with high breeding values. There is a significant opportunity to undertake an historical analysis of the banana program data sets taking into consideration available parental information. Genomic information in combination with pedigree information will improve analyses and estimation of breeding values. Two strategies for improving banana breeding are proposed for further consideration: (1) pedigree-based recurrent selection using diploid germplasm, and (2) Hybrid breeding enabled through creation of 2X and 4X heterotic pools.

The basis for developing good product profiles was evident from presentations made by the breeders but the current profiles need some refinement and linkage forward to smallholder banana growers and back to the breeding and testing effort. Pulling together information on adaptation zones, target traits, market end use etc. will provide a detailed picture that will guide the crop improvement activities and inform other team members in the various disciplines where to provide the necessary support. Breeders should examine whether they have too many product profiles/pipelines and traits for the resources at their disposal. At this point there is no alignment between on-farm performance with the trait targets that comprise the breeding product profiles. Selection for quality attributes remains a major gap in the programs. Establishing the correlation between physico-chemical components and sensory traits would aid in phenotyping of bananas for quality and NARO are making a genuine attempt to address these associations.

To achieve genetic gain for disease and pest resistance the ability to effectively screen for the presence of these resistances in hybrid progeny is essential, either in the field or in screen or glasshouses. Entomology, nematology and pathology expertise is fundamental to the banana breeding program as it is the reason why the banana breeding programs exist in the first place. The programs require access to a full-time senior pathologist with a good insight and experience of breeding and who could develop effective screening assays in collaboration with the breeders to enable selection for pest and disease resistance.

Establishing a standardized process for fingerprinting individuals could be used to enhance the study of genetic diversity and genetic variation for traits at all stages of the breeding program. The challenges in banana breeding concerning low fertility, low seed set, and poor germination of the few obtained seeds remain an obstacle to progress. Any research and insights into these, making even minor improvements are worthwhile. Studies of floral biology and fertility need to focus on large segregating populations of seeded diploids. This way large numbers of progeny can be analyzed making it quicker to determine which factor(s) influence seed set, seed production and germination and the study of genetics of parthenocarpy and sterility will be simplified. The highest priority is to achieve a level of crossing success that produces large numbers of viable seeds to enable a cyclical breeding process. However, most of the breeding effort is currently going into repeating the processes that result in low crossing success with insufficient progeny numbers. While the current intensive efforts to enable crossing success can be applauded this is not compatible with enabling a cyclical breeding strategy.

Acronyms and Abbreviations

ABBB	Accelerated Breeding Better Bananas
BBB	Breeding Better Bananas
BTractT	Banana breeding Tracking Tool
EET	Early Evaluation Trial
EiB	Excellence-in-Breeding
FHIA	Fundación Hondureña de Investigación Agrícola
GS	Genomic Selection
ITC	International Musa Germplasm Transit Centre
KASP	Kompetitive Allele Specific PCR
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MEDA	Mennonite Economic Development Associates
NARO	National Agricultural Research Organization of Uganda
NSIP	Nature Source Improved Plants
PYT	Preliminary Yield Trial
RAPID	RTB Accelerator for rapid Propagation Innovations and Distribution of seeds
QC/QA	Quality Control/Quality Assurance
QTL	Quantitative Trait Locus/Loci
RTB	Roots Tuber, banana
TARI	Tanzanian Agricultural Research Institute

Introduction

Bananas (*Musa* spp) were domesticated more than 7,000 years ago from their origins in SE Asia and the Pacific region. Sterile clones of banana made their way to Africa a few thousand years ago and East and Central Africa are considered secondary centers of diversity. In Africa today, banana/plantain provide a major source of calories (about 30%) to West and East Africans and small holder farmers rely heavily on existing landraces for production. The East African Highland banana, also known as 'Matooke', consist of a number of closely related sterile triploids (AAA) used in Uganda and neighboring countries for cooking, roasting, beer production and desserts. In Tanzania, the 'Mchare' are sterile diploids (AA) and used mainly as a cooking banana in the Arusha-Kilimanjaro region. In West Africa, Plantain (AAB) is used in multiple ways as either a cooking banana or processed into a flour to make various food products. Although the genomic make up of plantain is reported as AAB, recent molecular evidence has suggested that due to past recombination events the B genome contains a large amount of A genome. The diploid species *Musa acuminata* and *M. balbisiana* are the donors of the A and B genomes, respectively.

A large yield gap exists in farmers' fields in Africa; for example, in Uganda average Matooke yields are about 10t/ha/year about a one sixth of potential production. Farmers grow mostly landraces and they are typically susceptible to pests and diseases, e.g., nematodes, weevils, black Sigatoka, Fusarium wilt (Race 1 for Mchare), and Xanthomonas wilt. Production is also affected by combinations of abiotic factors like declining soil fertility, low nutrient inputs and drought. These deficiencies combined with the introduction of black Sigatoka to Africa led to the commencement of banana breeding programs in Africa at IITA Onne, Nigeria (plantain) in 1987, NARO Kawanda, Uganda (Matooke) in 1992 and at IITA Arusha, Tanzania in 2016 (Mchare). The plantain program operated at Onne between 1987 and 2005 but ceased due to a lack of funding until a grant provided by USAID in 2013 allowed for its recommencement in Ibadan, Nigeria.

There are major challenges to banana and plantain breeding in Africa. These relate to low fertility and seed set of breeding materials, poor germination of seed and the need to select for a complex trait like parthenocarpy (fruit without seed) and most importantly a consumer quality profile that at this point is yet to be properly defined. Further, breeding triploid bananas is complicated by the breeding strategy that combines a two-step crossing procedure; the primary cross consisting of a 3X landrace x 2X clone used as a source of disease resistance giving rise to 4X progeny that are used in a secondary cross with an improved diploid (2X) to produce the final sterile triploid (3X). As a result, the genetic content of the secondary triploid only contains 25% (theoretically) of the triploid landrace used in the primary cross and consequently the specific quality attributes of the landrace are difficult to retrieve. Breeding banana of diploid and triploid types is also complicated by the existence of at least 3 sets of germplasm that are separated by structural chromosome differences that make crossing difficult across these gene pools. The difficulties in breeding sterile banana varieties are reflected in global production figures where estimates suggest that less than 5% of production is planted to improved cultivars.

In May, June and Nov of 2017, the IITA/NARO banana breeding programs operating in West and East Africa were assessed by various teams of BPAT assessors (André Drenth, Yilma Kebede, Mark Cooper, Errol Corsan and Christopher Lambrides). Four assessments were carried out: Mchare (IITA Arusha, Tanzania), Plantain (IITA Ibadan, Nigeria), Matooke (IITA Sendusu Uganda), Matooke (NARO Kawanda, Uganda). These assessments identified a range of strengths and weaknesses in the programs. The assessors found that breeding teams had excellent capacity to carry out mechanistic aspects in each program with use of well-established techniques for crossing, embryo rescue, tissue culture and field

testing. The research stations were, by and large, well equipped or had the potential to be so and were run by early to mid-career staff that were enthusiastic and showed passion for their responsibilities.

However, the assessors also identified a range of issues that were potentially limiting the rate of genetic gain in the programs. Briefly these issues included; the lack of formalized product profiles, the use of a complex breeding strategy that effectively used the same limited number of parents each year to develop recombinant progeny, small population sizes that constrain the breeding program (particularly for quality traits) where selection intensities were suboptimal for most traits, the lack of a quantitative genetics breeding approach (e.g. no determination of breeding value), long breeding cycle times (10-17 years), poor phenotypic screens for pests and diseases, and a limited knowledge of floral biology, and an inadequate supply of high quality planting material to farmers of new varieties.

In 2020 these programs were re-evaluated by virtual assessments using the same BPAT team of assessors (André Drenth, Yilma Kebede, Mark Cooper and Christopher Lambrides). The aim of these evaluations was to measure the improvements since the previous BPAT and identify areas for further improvement aimed at delivering impact in farmers' fields.

Developments since 2017

An important development across the IITA programs has been the deployment of the Banana breeding Tracking Tool (BTractT), a system of electronic data capture that uses barcodes to track each step of the breeding program using hand-held devices. A wide range of data are collected, from details of parents in the crossing program all the way through to quality assessments of advanced clones in the testing program. Every banana plant in IITA/NARO research fields receives a specific barcode where agronomic performance, disease and pest resistance, plant architecture attributes, color and quality parameters of cooked bananas can be recorded. BTracT is connected to MusaBase, a global banana breeding database which provides public access. Data uploading is done in real time allowing for managers to track breeding operations remotely and facilitate analysis and reporting. The system was developed as part of the Accelerated Breeding Better Bananas (ABBB) program and is now fully operational. In addition to the breeding programs the system also has great potential for use in the private sector engaged in seed systems operations, including tissue culture and distribution of new clones.

The East African IITA programs were able to secure additional donor funding with the award of ABBB, a three-year grant that commenced in 2019 and succeeded the Breeding Better Bananas (BBB) project. An important goal of ABBB is to improve the production and productivity of Mchare and Matooke types in Tanzania and Uganda by focusing on pest/disease resistance and yield. The target is 30% greater yield than current varieties in farmer fields grown under the same conditions. The IITA programs are working closely with TARI (Tanzanian Agricultural Research Institute) and NARO (National Agricultural Research Organization of Uganda) to achieve these goals.

The breeding programs have rationalized the number of parents used in the crossing programs. For example, both Mchare and Matooke programs have reduced the number of landrace parents after obtaining a better understanding of their flowering characteristics. The Mchare program has introduced a backcrossing program to try and retrieve the quality attributes of the landrace. It was noted that 8% of crosses with Mchare are triploids, probably because of 2n gamete formation, an attribute yet to be fully exploited in banana breeding. To widen the genetic base a set of 72 parents have been distributed to all IITA programs and these are being evaluated and information shared across programs.

In triploid programs, extra use is being made (or contemplated) of diploid progeny that are observed after the primary cross. Previously, these diploid progeny recoveries from crossing were discarded without further consideration. Consideration is now being made of 2X x 2X and 4X x 4X crosses to generate greater variation in the parental pool. The plantain program has done well to introduce a wider range of parents including 40 diploids with plantain-like fruits planted in the field to assess pollen/seed fertility and four false-horn types included in pollination blocks. A strategy to double the chromosome number of diploid plantain has been established although useful progeny have yet to be identified.

A new collaboration with Steve Tanksley, Chief Technology Officer of NSIP (Nature Source Improved Plants), offers banana breeders promising new methods for identifying parental material and creation of heterotic groups among 2X and 4X gene pools. NSIP are proposing to use genotypic data and in-house genetic algorithms and methodologies to identify parental material for inclusion in heterotic groups and speed up the banana breeding process. The process also allows for the development of optimized core collections by maximizing allelic diversity and maximizing phenotypic diversity in screening and selection of quantitative traits. IITA have provided funding to NSIP and are one of many partners that will contribute to this project. DNA of up to 1000 genotypes will be provided to NSIP by a consortium of partners to complete the first stage of this project.

The Matooke program has made a genuine attempt to measure genetic gain in the breeding program by comparing the performance of landrace (C_0), tetraploid parent (C_1) and secondary triploid hybrids (C_2). Over a 20-year period the estimates of genetic gain for bunch weight (kg) and yield (t/ha/yr) were 1.4 and 1.3 %, respectively. The Matooke molecular work has continued with some notable achievements; development of a Quality Control/Quality Assurance (QC/QA) set of KASP (Kompetitive allele specific PCR) markers using the Intertek high throughput platform, genotyping of multiple breeding populations for Genomic Selection (GS) and QTL identified for STR4/TR4 Fusarium wilt resistance following some good protocols for phenotyping of fusarium resistance screening in East Africa for Foc 1 and at University of Stellenbosch for TR4.

While the plantain and Mchare testing programs are still in their infancy, shorter testing programs, providing the potential for a shorter breeder cycle time, have been planned by the NARO Matooke program. The previous cycle time of 12 years will be reduced to 8-9 years by reducing the EET (Early Evaluation Trial) from 4 to 3 years, the PYT (Preliminary Yield Trial) from 4 to 2.5 years and the on-farm trial scheme from 3 to 1.5 years. However, to date the breeding program is not operating as a cyclic breeding program, where selected individuals from one cycle are used as new parents to initiate the next cycle of the breeding program. The topic of recycling selected individuals as new parents to enable ongoing genetic gain was discussed during the first BPAT review and was a considered in more detail during the follow-up BPAT. The NSIP project has the potential to provide breeding value information on individuals created within a breeding cycle to support selection for their recycling as parents for a subsequent cycle.

Considerable interaction with multiple teams from the Excellence-in-Breeding platform (EiB) were clearly evident since the 2017 BPAT. There have been breeding optimization simulations conducted and advice given on genotyping, phenotyping and development of product profiles. To date the breeding optimization simulations provided by the EiB have provided preliminary information to facilitate discussion of the assumptions used in the simulations and their applicability to the specifics of the banana breeding programs. To progress towards an operational cyclical banana breeding strategy there are important opportunities to align subsequent use of the EiB methods for modelling the banana breeding programs and the breeding approaches that will be applied to the banana data by NSIP.

Areas for Improvement

Breeding Strategy

Currently, tetraploids that result from the primary cross (3X x 2X) for breeding Matooke and Plantain, are crossed with an improved diploid to produce a secondary triploid. In this process the combination of favourable alleles for quality, which existed in the landrace used as female is lost to a large degree. The highest priority is to achieve a level of crossing success that produces large numbers of viable seeds to enable a cyclical breeding process. However, the majority of the breeding effort is currently going into repeating the processes that result in low crossing success and insufficient progeny numbers. While the current intensive efforts to enable crossing success can be applauded this is not compatible with enabling a cyclical breeding strategy.

The current breeding strategy, which has been used for a long time, has not produced varieties which have given rise to major changes in farmers' fields anywhere in the world. The vast majority of banana varieties grown in Africa, and globally, are simple selections from the wild, and have not undergone any form of domestication. At present the breeding programs are not operating to improve clones over multiple cycles but rather are largely operating to continually resample first cycle breeding crosses from a narrow genetic base of landraces.

An additional challenge in banana breeding is the lack of detailed information concerning the genetics and breeding population estimated components of genetic variation of the traits needed for the product profile, which prevents reliable estimates of their heritability. Not only the lack of genetic studies and in general very low numbers of progenies, but also the different levels of ploidy and difficulty of making crosses have exacerbated this problem. The heritability of traits and estimation of breeding value in relevant breeding populations should start at the diploid level, which will aid the breeders at making not more, but better crosses targeted at the product profiles, defined as combinations with the highest probability of combining favourable alleles. Especially in the Matooke breeding there seems to be an emphasis on selecting parents based on fertility and not the breeding value of key traits needed to meet the product profile. Thus, parents with high crossing success have the potential to dominate the initial stages of the breeding program regardless of their breeding value. Priority should be given to quantifying the level of pedigree diversity entering the crossing nursery and the level of diversity created from the successful crosses.

To overcome the lack of progress in banana breeding it is important to consider alternative breeding strategies that enable cycling of parents with superior breeding value for product profile target traits. Beyond pedigree-based recurrent selection strategies an alternative breeding strategy to deliver long-term genetic gain would be to move towards hybrid breeding at the diploid level. For this to work different pools of parental germplasm (heterotic groups) need to be developed at the diploid and the tetraploid level which show trait performance complementarity when crossed. This way additivity can be exploited both within and across heterotic groups based on breeding value. Heterotic groups established at the tetraploid level can be used for crossing with the diploid pool to produce triploid cultivars.

We strongly support the approach put forward in collaboration with the NSIP and the associated collaborations with other banana breeding programs e.g., India and EMBRAPA in Brazil. While we see little organizational or technical difficulties in obtaining genotypes from DNA samples from a large set of germplasm currently distributed across Africa, Asia, and Latin America, obtaining relevant and reliable phenotypic data to a high standard will be a huge challenge. The other challenge concerns

movement of this germplasm. Since pollen cannot be transported, crosses planned based on data gathered in this approach can only be made when the material is brought together in one place and grown to produces flowers at around the same time to enable pollination. The successful crossability of desirable combinations and recovery of sufficient numbers of progeny will require careful considerations to prioritize germplasm movement. The presence of plant viruses and bacteria in some localities means that material need to pass quarantine before being moved to crossing blocks, taking additional time. To avoid delays training sets based on specific crosses may initially need to be only based on material which is at hand in one location to get the program underway with a longer-term horizon for material sourced from other continents.

Recommendation: Two strategies for improving banana breeding are proposed for further consideration and both will depend on introgression of a large number of diploid clones preferably from the centers of origin of the species; (1) pedigree-based recurrent selection utilizing breeding value, and (2) hybrid breeding enabled through a combination of development of heterotic pools and utilization of pedigree information and breeding values. In these pools, traits can be combined at the diploid level to create improved diploids and breeding values established. These diploids can then be crossed with material from another heterotic pool, either diploid or tetraploid exploiting heterotic effects. The diploid strategy would not preclude the possibility of breeding sterile diploid cultivars. Working backwards from the product profiles, traits that are more complex may need to be pried apart to work out which combinations of alleles are required and how heterotic pools need to be established to achieve favourable allele combinations after crosses between individuals of the different pools. The information generated from the NSIP collaboration can be used to evaluate the merits of the alternative breeding strategies. Both methods can be compared to the current strategy that is largely based on resampling from the same first cycle of potential parents. The availability of genetic fingerprinting capability from the NSIP collaboration can be used to assist and implement the breeding strategy that provides the best breeding outcomes.

Should IITA/NARO agree to the change in breeding strategy, we recommend that change champions be appointed to oversee the transition to the new breeding approaches as they represent a significant departure from the traditional methods of breeding African banana/plantain. The change champions will need to have a detailed knowledge of banana breeding and training in quantitative genetics to oversee the estimation of breeding values in the programs using sophisticated statistical analyses that take into consideration pedigree and genotypic information. Institutionally, significant resources will need to be committed to each of the programs as there will be additional costs of importing diploid materials and establishing new field nurseries and crossing programs. However, there should be considerable savings made as the old breeding system is unraveled mainly because the need to cross all year around should be lessened, assuming that new fertile diploid material will be easier to cross during a defined part of the growing season.

Product Profiles

In 2017 BPAT recommended that IITA/NARO engage in a comprehensive program of developing product profiles in each breeding program pulling together information on geographical zones, target traits, market end use etc. to provide a detailed picture that will guide the crop improvement activities and inform all team members in the various disciplines to align the needed support.

The progress to date based on presentations made by the lead breeders revealed that the different programs have started collating information on product profiles. The banana product profiles have been put together with due consideration of key target traits. The product profiles resulted from discussions with EiB assistance. The basis for developing good product profiles was evident from

presentations made by the breeders but the current profiles need some refinement and linkage to the breeding and testing effort. Traits need to be clearly defined. For example, yield should be stated in in ton/ha/year. In addition, some thresholds need to be determined such as length of fingers, number of fingers, cycle time, pseudostem diameter etc. so that they fall within the acceptable agronomic range. The breeding teams are still at an early stage of internalizing the product profiles into the design and operation of the breeding programs. The status of development and internalization of product profiles for each program is discussed further below.

2021 Mchare assessment

Yield, disease resistance (Fusarium (race 1 and TR4), Sigatoka, weevil, nematodes, BXW, quality (Flavor, Color, Texture) shorter stature and cycle time were listed under product profile. What was presented however, was not complete. Several traits were listed but need to be more specific. Further specification of the target or threshold levels of the traits for effective product advancement would improve the clarity of the target product profile for Mchares. An advantage of defining these trait level targets is to help with trait prioritization with the breeding programs to minimize the burden that can occur when a breeding program attempts selection on large numbers of traits without information on the extent of the genetic correlations among the different traits. In addition, it is important to define the production zones for which the final products will be recommended. Market research and more details are required to make the product profiles more specific. Seeking additional support from IITA socio economics team and continued engagement with EiB is essential.

2021 Matooke assessment

The program has put together a list of mainly important agronomic traits and target levels including yield, maturity, plant stature, height, suckering behavior, bunch orientation and resistance to black Sigatoka. However, it is unclear which adaptation zones are targeted. A lot of work is yet to be done to define clear profiles with supporting information from producer surveys.

2021 Plantain assessment

Currently going through a product design based on fresh market or other uses, and ecological zones, using RTB foods user survey and working with Peter Coaldrake of EiB to define market segments using IITA market economist input. Whether different product profiles are required for different regions, should be informed by feedback from growers/consumers in those regions and an analysis of current landraces grown across those regions.

2021 NARO assessment

NARO has put together a list of traits with some target levels and prioritization. The challenge is to develop product profiles that target the needs of agro-ecologies to serve as a blueprint for the crop improvement teams. The market needs should be considered in targeting production zones and prioritized based on market size. The NARO breeding program should request support from IITA to complete and align their target product profiles with those developed by IITA.

Recommendation: Pulling together information on adaptation zones, target traits, market end use etc. will provide a detailed picture that will guide the crop improvement activities and inform other team members in the various disciplines where to provide the necessary support. A focus on product profile specification related to the main production areas and consumers will enable a more effective alignment of the breeding program by addressing the right environments and client needs.

Each profile should be based on a unique product specification taking into account major environments (area of adaptation), defensive traits (biotic, abiotic), and farmer/consumer preferences. In addition, the product profile specification should include the relative size of the

market for a specified profile as well as the current benchmark products that the breeding program is attempting to improve/replace.

Breeders should examine whether they have too many product profiles/pipelines and traits for the resources at their disposal. The formal mechanisms for implementing a product-focused breeding pipeline reflecting market size were not clearly visible in any of the programs.

Where there was information provided around product profiles, they do not appear to be market-data driven, something that will require additional effort to get such information documented. Some of the information is available but needs to be organized to direct the breeding and testing activities. The profile needs to be developed from the ground up with consumers, growers, and economists etc. to define what is actually required.

Market penetration

In 2017 BPAT recommended that the performance of new varieties ought to be monitored and followed on a regular basis. Specific data on amount of planting material produced, yield and area under production of released varieties are required. A data driven approach to monitor the performance of new varieties to gain confidence on the contribution of the breeding program as well as to better align with what is required by growers and consumers was considered essential. It is especially important that the bananas meet consumer preferred attributes (taste, aroma, color, and texture) found in local bananas.

Feedback on the performance of new released varieties, adoption and rate of expansion was not provided but has been prioritized by NARO for study.

The current BPAT assessment revealed that not much progress has been made on this front. Involvement of the public extension system in popularizing new varieties appears low. A stronger interaction between NARO, extension, and planting material producers will be essential for popularization of new varieties and ensure that farmers are aware and educated about improved varieties. Assessments of on-farm performance of new varieties must be aligned with the target traits that comprise the breeding product profiles. This would provide required feedback to the breeding teams on the internalization and operational alignment of the breeding programs with their product profiles. At present these connections have not been established.

Recommendation: Planting material provided through a network of public and private entities requires involvement and resources from the breeding program in the form of multiplication of true to type elite planting material of a high health status. To be effective, formal interventions need to be coordinated among governmental, private sector, and non-governmental implementation partners. Each intervention requires activities to be owned by different stakeholders in the planting material production chain. These stakeholders include NARO, extension service, public/private seed enterprises, farmers, and regulatory bodies. This also includes effectively identifying the target farmers and agro-ecologies that will benefit from the new variety. This will require sustained engagement with stakeholders to develop strategies for varietal dissemination and monitoring performance of variety releases.

Seed systems, variety release

The 2017 BPAT recommended that variety release and registration needs to be organized in an effective manner. It is essential to develop variety release and registration guidelines detailing steps and processes of varietal evaluation, release and registration. Best practice requires that older varieties should be retired from the system as newer and higher performing varieties are released. A big hurdle for commercial tissue culture plant production is the limited use of certification for quality and health, which is important to avoid the spread of pathogen and pests. In the absence of a well-developed regulatory system, the current system faces various challenges. It is essential to establish

standards to enforce quality control for planting material producers. Engagement with regulatory bodies tasked with enforcement of guidelines for quality control is critical. This will require updating the existing relationships, facilities, and personnel.

The progress to date has seen a proposal submitted to EiB to mainstream a sustainable national roots, tubers, and banana (RTB) seed system in Uganda. NARO has partnered with MEDA (Mennonite Economic Development Associates) under the RAPID (RTB Accelerator for rapid Propagation Innovations and Distribution of seeds). The banana project aims to improve seed systems in Uganda and Tanzania focused on addressing key bottlenecks in banana's early plant material generation at the tissue culture and nursery operations level: multiplication rate and distribution of plantlets. The project is focused on identifying and scaling new technologies that improve the multiplication rate and distribution efficiencies which will be combined with new and innovative business models and opportunities to improve the operations. NARO will virus index (banana streak virus, banana bunchy top virus, banana cumber mosaic virus and banana bract mosaic virus) all planting material to be given out to tissue culture labs for multiplication. NARO will develop molecular fingerprints for all releases.

Variety registration and licensing agreements are being developed by the commercialization unit of NARO. Quality assurance with the Ministry of Agriculture Animal Industry and Fisheries through direct training and facilitation of QA for private lab facilities is emphasized. Standard operating procedures (SOP) are being developed for quality assurance and control in partnership with NARO, MAAIF (Ministry of Agriculture, Animal Industry and Fisheries Uganda) and tissue culture labs.

The current assessment identified a number of activities on this front and these appear encouraging with NARO partnering with stakeholders to address the recommendations. This is commendable and ought to be sustained.

Recommendation: NARO has the mandate to release new varieties and should spearhead this and provide the necessary support.

Quality assessment

The 2017 BPAT recommended that "Matokeness" needed to be better understood and clearly defined so it can be selected for to improve and ensure marketability of future variety releases. Preference traits are hard to quantify, though NARO has made significant efforts using taste panels to get a better handle on this issue. This testing involves a lot of time, effort and material and is not suitable for high throughput. Since the different components of the desired quality characteristics are not clearly defined or quantified, quality cannot be accurately evaluated nor effectively selected for.

A major challenge in banana breeding is selection for fruit quality. The problem is that there are several traits, including texture, aroma, and flavor among others which are not clearly defined and most likely underpinned by complex genetics making selection for quality very challenging. This is a challenging problem for any breeding program but is particularly challenging for the banana breeding programs that are not recycling parents. The question is how to translate these quality components into parameters one can select for within and over cycles of breeding. In case of bananas in Africa, fruit quality is not an attribute that was selected for in the plant, as the diversity of germplasm that came to Africa was extremely limited. Instead, the human population adapted to the characteristics of this newly introduced plant and as such require new varieties resulting from the breeding program, to fit within a narrow bandwidth of quality traits. As such, the breeding program cannot improve on these traits but needs to produce products that fall within this bandwidth. This provides an opportunity to define clear quality targets as integrated components of the product profiles. This level of definition of quality within the product profiles has not yet been achieved.

Recommendation: Protocols are needed to reliably assess fruit quality and consumer preference. It is important to obtain an understanding of the breeding value of fruit quality for the various diploids

used in crosses if progress is to be made in this area. Whether or not quality can be associated with markers needs to be investigated but is unlikely to succeed and will not succeed without clear and highly reproducible phenotyping procedures. Given the current stage of the banana breeding programs it is recommended to focus on ensuring quality traits are maintained to fall within the acceptable bandwidth of quality.

The current situation suggests that farmers prefer local varieties, explained to be due to their superior quality characteristics. Work is being done at NARO and supported by an RTB foods project to define quality characteristics of Matooke. Stakeholder (farmers, traders, input suppliers, extension, consumers/processors) consultation meetings to identify and define matooke end-user preferred traits have been conducted. Out of consultations with end-users NARO has identified and defined 11 end user-preferred traits for cooked product and 6 traits of raw fruits (based on appearance, size, shape, texture, taste, impression, and aroma. Physico-chemical components related to sensory traits are being studied in partnership with food scientists and biochemists at the food biosciences lab at NARL.

In the breeding programs of NARO Uganda quality characteristics consist of color, texture, firmness, and taste and are determined through sensory evaluation by testing panels. Preliminary correlation data which seeks to establish the physical-chemical bases of essential consumer traits has been obtained. This is in line with earlier recommendations and is a step in the right direction.

Recommendation: Data that would establish the correlation between physical-chemical components and sensory traits would aid in phenotyping of bananas for quality. NARO need to allocate more resources to this task as it will underpin any high-through put phenotyping strategy that will be needed to evaluate large numbers of new progeny.

Genetic Base and Choice of parents

Mchare (AA), Matoke (AAA) and Plantain (AAB) came to Africa from Asia as three independent introductions 2-3,000 years ago through human movement between these two continents. As only a few clones were brought across for each variety, the bananas currently grown in Africa went through an extreme genetic bottleneck. Continuous vegetative propagation by African farmers and the lack of genetic recombination gave rise to a series of genetically very closely related landraces suffering from varietal stagnation.

It is of interest to note that material highly similar to Mchare, Matoke and Plantain has not yet been found in the center of origin. In Southeast Asia, smallholder farmers grow over 1,000 different varieties of bananas but we do not know how old these varieties are and if there have been many shifts in cultivation of different varieties over time. In the center of origin, new hybrid varieties likely occur on a regular basis due to the large numbers of seeded bananas growing in the wild.

Recent genetic and genomic studies have shed some light on the origin of the progenitors that initially gave rise to the African banana landraces. Detailed genomic studies on wild Musa species may provide more insight and an in depth understanding of the genetic make-up of the African banana varieties. This information may help to identify female as well as male parents that contain alleles, which would be useful to breed improved varieties.

Mchare (AA), Matoke (AAA) and Plantain (AAB) have a different origin and genetic makeup but in the current breeding programs they are crossed with the same set of male parents. It is highly likely that the breeding value of the male parents is quite different for the three different banana types grown in Africa. The male parents are chosen mainly based on the presence of pest and disease resistances and pollen fertility but typically provide a genetic drag when it comes to productivity and fruit quality.

Genetic gain in a breeding program is achieved through careful selection of parental material, an experimental design involving specific crosses resulting in progeny, which are subjected to several rounds of selection for key traits to end up with products containing combinations of favourable alleles meeting the product profile. One of the problems in banana breeding is that the number of diploids, which have traditionally been used as male parents, is rather small. This combined with the narrow genetic base of the African landraces does raise the question if the genetic material used in the African banana breeding programs at present is suitable to achieve genetic gain. Improvements in genetic gain are possible but may require better parents with high breeding values for the priority traits of the product profiles.

To overcome the narrow genetic base on both the female and the male side more and better characterized germplasm is needed. The genus *Musa* contains a lot of diversity in the form of species and subspecies and occurs over a wide geographic range in South and Southeast Asia. We note that the program has introduced more diploids from the ITC (International Musa Germplasm Transit Centre) over the last three years and now has about 60 2n parents of which 70% are seeded. To make more targeted crosses parental selection not only requires information concerning the phenotype of the parent but more importantly information concerning what the newly introduced germplasm contributes to its progeny expressed as breeding value for the target traits of the product profiles. This is especially the case for fruit quality, which cannot be assessed for seeded diploids.

Recommendation: It is recommended that the recently imported diploids are characterized as a priority and their breeding values for the different programs established through testcrosses. It is also important that more diploid material be sourced from Asia for use as female as well as male parents for the Mchare, Matoke and plantain breeding programs. Careful attention needs to be paid to genetic and genomic studies involving banana germplasm, as ideally one needs germplasm for use as female parents, which shows a high level of homology to the African landraces.

Pedigree analysis

Successful breeding requires successful execution of high-performance crosses based on identification of parents with high breeding value and identification of high-performance crosses. Successful execution of a breeding program cycle relies on being able to successfully recover sufficient progeny from the promising crosses. Execution of a crossing plan for banana is challenging due to limitations on the crosses that can be made in any breeding nursery and the heterogeneous numbers of viable progeny recovered from the crosses that are made. Crossing challenges in banana breeding relate to low fertility, poor seed set in breeding materials and poor germination of these seeds. These problems are further exacerbated by the need to have parthenocarpy and a certain level of fruit quality characteristics of which the genetic basis and heritability is not known. Progress to achieve genetic gain is further constrained by not knowing the breeding value of the germplasm used as pollen donors.

Traits such as disease resistance, productivity and quality are scattered among numerous wild diploid banana species. To achieve genetic gain these traits need to be brought together in so-called improved diploids. Some of this pre-breeding has been done in the past in the FHIA (Fundación Hondureña de Investigación Agrícola) program in Honduras and pre-breeding is currently also being conducted by Embrapa and the African programs have access to some of this material through collaboration.

To get better insight into the breeding value of potential parents past crosses need to be interrogated to identify parental material with a high breeding value. At the same time parental lines with low breeding values, which produce inferior hybrids ought to be removed from the breeding program.

Some form of recurrent selection through recycling some of the progeny from the primary crosses may need to be considered to improve the parents. It is now well established that a Mchare clone in the centre of origin in Southeast Asia was the source of the unreduced gamete, which produced the high-quality Gros Michel and Cavendish banana varieties. Hence, the diploid Mchare, and improved Mchare germplasm may be useful for breeding of replacements for Cavendish varieties.

Recommendation: Pedigree analyses are needed to align traits with parental lines for both male and female lines. Based on pedigree analysis more targeted crosses can be made with parents containing traits of the highest priority and with high breeding values. Recycling of newly developed material from the primary and secondary pools need to be considered to reduce the genetic drag experienced when crossing with wild seeded diploids. There is a significant opportunity to undertake an historical analysis of the banana program data sets taking into consideration available parental information. Going forward parental information should be captured as a component of the electronic data capture process to assist timely analysis of data as they are generated. As fingerprinting is available this enables genomic information to be used in combination with the pedigree information to improve analyses and quantify breeding values of clones generated from the breeding programs to assess their potential as parents for future breeding cycles.

Disease screening

The overall aim of the banana and plantain breeding program in Africa is to raise yields in farmers' fields, which at present are very low, and in the range of 15-25 % of their yield potential. The reasons for this yield gap are a combination of biotic factors which have invaded and/or spread in Africa (pests; weevils and nematodes; diseases, black Sigatoka, Xanthomonas wilt, Fusarium wilt), abiotic factors (drought stress and low soil fertility) and management (lack of agronomic inputs such as fertilizer and irrigation water often combined with a lack of access to clean planting material).

Some of these problems require a genetic solution while others require a change in management of the crop. Therefore, it is important that traits are prioritized based on a detailed analysis of the importance of pests and diseases and abiotic factors and in combination with the potential opportunities for improved agronomic management to close yield gaps. Based on the product profiles, priority traits for pest and diseases include black Sigatoka, Fusarium wilt, weevil, and nematode resistance. Genes expressing these traits can be found in diploids such as the seeded Calcutta 4, Borneo, cultivar Rose and several improved diploids from the FHIA and Embrapa breeding programs.

To achieve genetic gain for disease and pest resistance the ability to effectively screen for the presence of these resistances in hybrid progeny is essential, either in the field or in screen or glasshouses. Entomology, nematology and pathology expertise is fundamental to the banana breeding program as the incursion of pathogens into Africa is the reason why the programs exist in the first place.

The breeding program has established a screen-house in Arusha to obtain suitable and uniform conditions to screen germplasm for black Sigatoka resistance. The disease pressure for black Sigatoka is too low in Arusha to be able to screen in the field. Screens for Fusarium wilt race 1 can take place in certain parts of Tanzania in the field (but not Arusha) and are required for Mchare as they are susceptible to Fusarium wilt race 1 and in Uganda. Screens for Fusarium wilt TR4 are now being conducted in secure glasshouses at the University of Stellenbosch in South Africa. Screening for TR4 is important as this has the potential to spread all over Africa as it has recently established a foothold in Mozambique. Although Matooke is resistant to race 1, and plantain has been found to be resistant to Fusarium wilt race 1 and TR4, testing of progeny is still required to ensure that no susceptibility is introduced in the hybrid progeny.

The lack of rapid and effective phenotyping for pest and disease resistance and the absence of a full-time pathologist experienced in developing and implementing screening of germplasm, is of concern. Although pathology services are provided from afar, there is a lack of training and capacity building at the local level to improve screening for diseases within the breeding program. With regards to nematodes, some screening is conducted in Uganda but there is no effective screening for nematode resistance in the Plantain breeding program while it is known to cause problems with mats growing out of the ground.

Recommendation: The program requires access to a full-time senior pathologist with a good insight and experience of breeding and who can develop effective screening assays in collaboration with the breeders to enable selection for pest and disease resistance. A streamlined resistance screening program needs to be developed using relevant pathogen strains and be able to deal with large numbers of progeny from breeding and test populations in a timely manner. Data collection also needs to be standardized among the different sites the program operates in to be able to compare data across the various breeding locations.

Utilization of genetic markers

Establishing a standardized process for fingerprinting individuals could be used to enhance the study of genetic diversity and genetic variation for traits at all stages of the breeding program. This would also enable detailed pedigree analysis within the breeding program. For the initial stage of developing fingerprinting capability, dense marker coverage of the genome is not required. The banana team need to be able to track chromosomal contributions of parents and develop a base quantification of the levels of linkage disequilibrium operating across the breeding populations and the extent to which recombination is influencing the genetic variation sampled from successful crosses. The NSIP collaboration has the potential to be able to deliver this foundational capability to the banana programs.

The same marker platform used for fingerprinting, as discussed above to facilitate pedigree breeding, could also assist targeting of the markers to be used to assist trait selection. It is important to clearly articulate for which priority traits genetic markers are required. Several biotic phenotypes can be reliably obtained in the field or in screen or glasshouses and require no markers. For diseases like fusarium wilt R1, which displays resistance through a single dominant gene, parental improvement in Mchare can potentially yield homozygous resistant parents reducing the need for further extensive phenotyping or the use of genetic markers.

For TR4 it would be good to have a marker to be able to remove susceptible lines very early in the breeding cycle. Reliable markers for TR4 are not yet available. At the same time, a TR4 phenotyping system is set up at Stellenbosch, which should be used fully and may be able to help with validating markers for TR4. Although markers for Fusarium wilt TR4 are under development through international collaborations, there is yet no evidence that they show utility for parents used in the breeding program. Hence, these markers when they become available still need to be rigorously validated in test populations involving parental lines to increase the chance, they can be effectively utilized in the breeding program. A better approach would be to develop genetic markers in the target populations as that would potentially increase their utility in the breeding program. This is an area where the fingerprinting strategy discussed above could provide access to suitable alternative markers once regions are identified and the breeding program parental sources of resistance are identified through screening.

The breeding program invests in marker assisted selection for resistance to Fusarium wilt Race 1 and TR4 but at present none of these markers are used to advance material in the breeding programs.

Some work is done in house in Uganda while collaborators conduct other work. One concern is that especially for QTL markers, the collaborators are working with different genetic material and crosses than those used in the breeding program. It is likely that these markers will not carry across even after a significant amount of validation. Although scientifically interesting they burn up a lot of time and resources, while their utility for the breeding program is questionable.

Recommendation: It is important that collaborators closely liaise with the lead breeder to ensure that the genetic material they use for their research on marker-assisted selection is of value and potentially useful for the breeding programs and that a clear pathway exists for their implementation. Establishing a robust fingerprinting strategy for the different stages of the breeding program would enable identification of effective markers in breeding populations after parental sources of resistance contributing to the breeding crosses entering a breeding program cycle have been identified. A proof of concept for marker-assisted breeding is not needed. What is needed is markers linked to priority traits that can be used in the breeding program to allow for rapid screening of the germplasm and advancement of material in the breeding pipeline.

Pollination Biology

The challenges in banana breeding concerning low fertility, low seed set, and poor germination of the few obtained seeds remain an obstacle to progress. Any research and insights into these making even minor improvements are worthwhile. Analysis of past pollinations and seed production can be analyzed to gain some insight into factors, but it may be more effective to study pollination, seed set and seed germination in seeded diploids first as a separate project. Using seeded diploids, large numbers of seeds can be obtained, and as such, differences in treatment effects can be identified much more readily. Issues such as weather during pollination, timing of pollination, ripeness of the fruit when harvested for seed and treatment of the seed postharvest may have an influence on germination and this can be easily assessed in fertile diploids that produce large numbers of seed that can be used for experimental purposes.

Recommendation: Study floral biology and fertility in seeded diploids. This way large numbers of seeds can be analyzed, and it will be much quicker to determine which factor(s) influence seed set, seed production and germination.

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