USE OF SENSORY PARAMETERS AS A TOOL IN SELECTING MATOOKE HYBRIDS

Tumuhimbise Rabooni [1], Ssali Reuben Tendo [1], Namuddu Mary Gorreth[1], Lugoloobi Nsibirwa[1], wimana Brigitte [2], Batte Micheal [2] and Akech Violet [2]
[1]NARO- National Agricultural Research Laboratories, P.O.Box 7065, Kampala - Uganda
[2]IITA– International Institute of Tropical Agriculture, P.O.Box 7878, Kampala- Uganda

Introduction

Many attributes are considered by breeders in selecting promising matooke hybrids including agronomic performance, resistance to pests and diseases and end-user acceptability. However, the farmers' decision to adopt a new banana variety is influenced by both production and consumption characteristics of crop varieties (Akankwasa et al., 2013). Improved banana hybrids could thus have attributes that fail to meet the needs and preferences of end-users leading to low adoption. The crucial question that needs to be addressed before making commercialization decision is how end users, especially farmers and consumers, will react to the products of the newly developed hybrid bananas. The demand for hybrid bananas is likely to be better if , among others , varieties are selected based on producers' and consumers' preferred cooking traits (Akankwasa et al.,2013). This research is done as part of banana breeding process to enable consumers/ farmers select varieties of their choice through sensory evaluation hence helping the banana breeding program in the selection of best hybrid materials and enhancing the variety adoption rate.

Objective

To identify the best matooke hybrid varieties at various stages of field assessment using farmers' preferred sensory traits to aid the breeding programme in variety selection process.

Method

Samples are harvested mature green. Preference test method (hedonic scale six) is used to determine the likes and dislikes of consumers. Panellists are provided with questionnaires to score parameters including taste, colour, flavor, texture and general acceptability to evaluate the sensory fruit quality of the samples. Mbwazirume is used as a control.

Evaluation process flow

Results

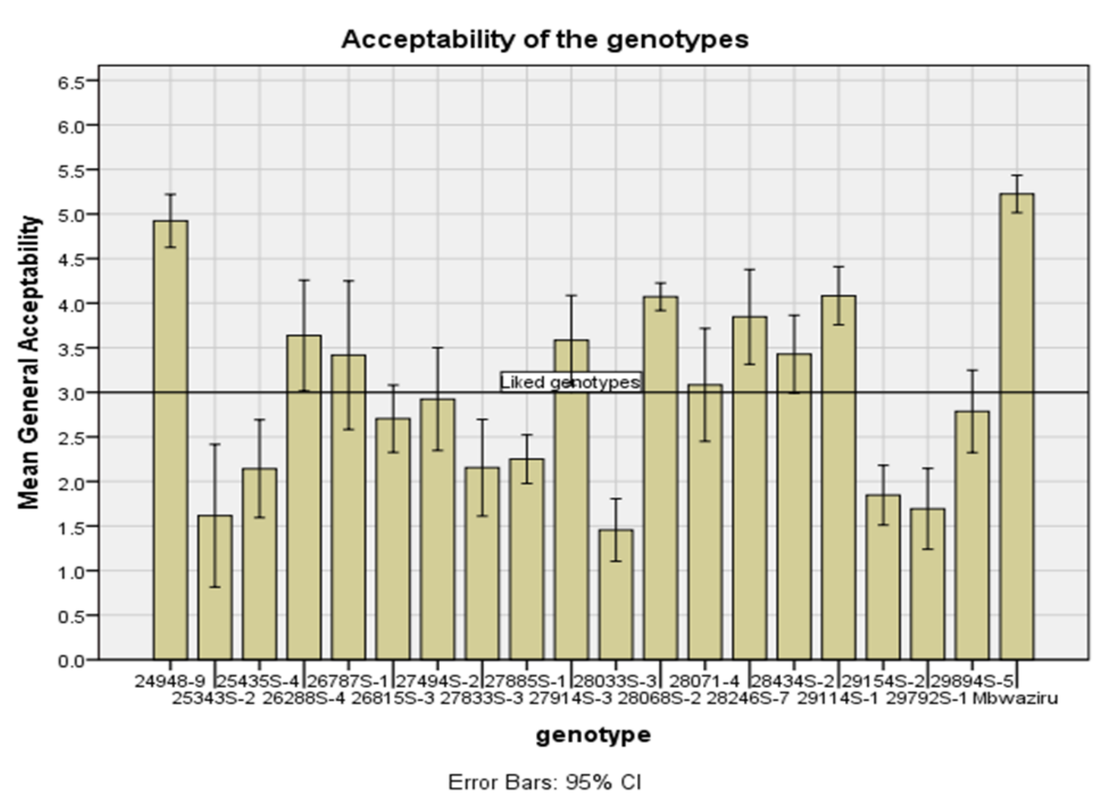
Generally the most acceptable banana genotype ranked by panelists was 24948-9 (mean score 4.92±0.137) close to the local banana variety Mbwazirume (mean score 5.22±0.104). Genotypes 29114S-1 (4.08±0.149) and 28068S-2 (4.07±0.071) ranked second and third respectively. The least acceptable banana genotype for the panelists was 28033S-3 with a mean score of 1.45±0.157.

Table 1: Mean scores of genotypes evaluated from preliminary yield trial on sensory parameters.

Genotypes	Sensory parameters				
	Taste	Flavour	Texture	Colour	General Acceptability
24948-9	5.08±0.137	4.85±0.274	4.85±0.317	4.69±0.286	4.92±0.137
25343S-2	1.85±0.373	1.77±0.378	1.85±0.355	1.85±0.451	1.62±0.368
25435S-4	2.14±0.231	2.14±0.294	2.71±0.266	1.57±0.291	2.14±0.254
26288S-4	3.73±0.195	3.00±0.302	3.36±0.310	3.27±0.237	3.64±0.279
26787S-1	3.17±0.490	3.08±0.529	3.75±0.279	3.75±0.392	3.42±0.379
26815S-3	2.81±0.220	2.52±0.202	3.11±0.216	2.37±0.221	2.70±0.183
27494S-2	3.23±0.201	3.08±0.265	2.69±0.286	2.69±0.308	2.92±0.265
27833S-3	1.69±0.237	1.69±0.286	1.46±0.243	2.62±0.385	2.15±0.249
27885S-1	2.28±0.147	2.47±0.152	2.42±0.166	1.83±0.102	2.25±0.134
27914S-3	3.50±0.230	3.33±0.256	3.83±0.207	3.75±0.250	3.58±0.229
28033S-3	1.73±0.237	1.64±0.152	1.55±0.157	1.55±0.157	1.45±0.157
28068S-2	4.14±0.177	3.93±0.195	4.00±0.210	3.64±0.133	4.07±0.071
28071-4	3.00±0.275	3.00±0.275	2.92±0.288	3.08±0.313	3.08±0.288
28246S-7	3.65±0.304	3.65±0.288	4.04±0.257	3.73±0.291	3.85±0.258
28434S-2	3.36±0.269	3.21±0.261	4.07±0.221	2.64±0.289	3.43±0.202
29114S-1	4.33±0.284	4.25±0.279	4.08±0.229	2.67±0.142	4.08±0.149
29154S-2	1.85±0.191	1.92±0.211	1.69±0.237	2.00±0.300	1.85±0.154
29792S-1	1.54±0.215	2.38±0.290	2.69±0.382	1.38±0.180	1.69±0.208
29894S-5	2.64±0.225	2.86±0.254	2.43±0.202	2.86±0.231	2.79±0.214
Mbwazirume	5.35±0.099	4.97±0.137	5.26±0.138	5.26±0.127	5.22±0.104

One out of nineteen genotypes evaluated was liked very much (i.e. 5.26% acceptability). This implies that the breeding programme should test large populations of hybrids so as to obtain highly acceptable matooke varieties.

Graph 1: Showing overall acceptability of the genotypes by the panellists.



Although sensory parameters are key for adoption, banana hybrids that offer other production advantages to the farmers but with fairly consumer acceptability may be considered by the breeding programme. For such varieties methods of preparation that make them more acceptable should be explored. From the discussions with the panelists they noted that practices like washing the banana pulp removes sap hence improving on the texture and food colour, cooking for longer hours improves on most of the sensory parameters and also cutting the banana pulp into halves improves on texture. Alternative use of those genotypes such as post harvest processing may also be considered.

Areas of improvement

- Despite the fact that sensory evaluation can gauge the actual consumer response to a particular variety, there is a need to complement the assessment with analytical (instrumental) methods to clearly define the parameters most especially taste.
- Banana breeders should also consider nutritional profiling for the promissing genotypes.

References

Akankwasa, K., Ortmann, G. F.Ortmann, E.Wale & Tushemereirwe, W. K. (2013). Adoption of new cooking banana hybrids in Uganda based on farmers perception.

Breeding Cooking Bananas: Do Men and Women's Needs and Preferences Matter?

Ssali Reuben Tendo, Nasirumbi Losira Sanya, Namuddu Mary Gorreth and Mayanja Sarah

Introduction

Cooking banana is an important food staple that supports over 17 million Ugandans; most of them rural. Banana productivity has declined due to many factors including pests and diseases infestation. Breeding new varieties is the most feasible strategy to such constraints. Farmers adopt new varieties if they provide additional benefits to them: more productivity, yield stability, better taste and quality or increased market value (Weltzien and Christinck 2005). This study sought to unravel the most important traits for various men and women actors along the banana value chain.

Purpose

To understand how the different needs and preferences of men and women shape acceptability of cooking banana varieties by value chain actors in central Uganda.

Materias and Methods

Study Area: Mukono & Wakiso Districts, Uganda.

Research design: Mixed methods both quantitative and qualitative methods (Creswell and Clark,2011).

Men and women participate in sensory evaluation of 'Matooke varieties

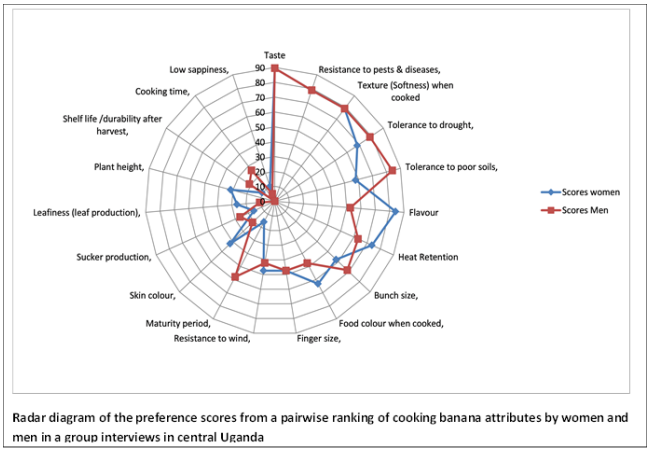
Gender disaggregated focused group discussion

Key informant interview with a restaurant operator

HH interview with main adult decision maker

Key findings

Taste of the food of a cooking banana variety is the most important trait for both men and women for accepting a banana variety in central Uganda. Adult consumers of cooking banana cannot compromise on the 'taste of matooke'.



Radar diagram of the preference scores from a pairwise ranking of cooking banana attributes by women and men in a group interviews in central Uganda

Men prize production and market related traits like tolerance to drought, tolerance to poor soils, the bunch size, maturity period and the shelf life of harvested bunches more than women. While women appreciated food quality traits like the flavor and the color of the food when cooked.

Recommendations

Taste of the food of 'Matooke' hybrids should be the driver for adopting 'matooke' hybrids. Therefore there is need to define the compound which determines the taste of cooking banana varieties.

Reference

Creswell, J. W., & Clark, V. L. P. (2011). Designing and Conducting Mixed Methods Research: Thousand Oaks, CA: Sage Publications, Inc.

Weltzien E and Christinck A.(2005). Identifying farmers' preferences and needs for varieties and specific traits, Eva Weltzien,Volker Hoffmann (eds.) Margraf Publishers GmbH, PP 83-104

Acknowledgement

We would like to thank GREAT project for the seed grant for this research

Effect of Glucose Solution on Stigma Receptivity and Subsequent Seed Set in EAHBs

Introduction

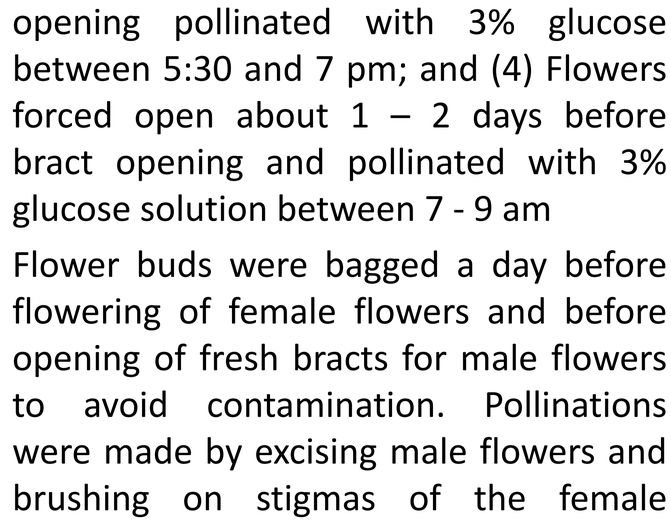
East African Highland Bananas (EAHBs) known as Matooke play a very critical socio-economic role for smallholder farmers of the Great Lakes Region of East Africa. Pests and diseases affect the crop reducing both yield and plantation life thus, hence the importance of resistance breeding. Most of the popular cultivars are sterile and hardly produce seed. This drastically slows conventional Matooke breeding. Several factors contribute to low seed set, especially stigma receptivity and the degeneration of ovules within 24 hours after anthesis. The objective of the study was therefore to explore flower manipulations that could enhance seed set and Matooke breeding efficiency.

Materials and Methods

An EAHB cultivar Enzirabahima was used as a seed fertile female parent and Calcutta 4 as the pollinator. A 3% glucose solution was applied to the stigmas using a hand sprayer before pollination. Treatments included:

(1) The customary pollination technique as control; (2) Normal flower opening pollinated with 3% glucose solution between 7 - 9 am; (3) Normal flower opening pollinated with 3% glucose between 5:30 and 7 pm; and (4) Flowers forced open about 1 – 2 days before bract opening and pollinated with 3% glucose solution between 7 - 9 am

Flower buds were bagged a day before flowering of female flowers and before opening of fresh bracts for male flowers to avoid contamination. Pollinations were made by excising male flowers and brushing on stigmas of the female flowers. Bunches were then labeled, left to fully mature in the open and seed was hand extracted from fruit pulp after ripening.



*(**A**) Female flowers (**B**) Male flowers*

*Procedure of early pollination: (**A**) Flower bract forced open and petals removed to expose stigmas for pollination (**B**) Glucose solution applied with hand sprayer (**C**) Brushing male flowers on stigmas to expel pollen (**D**) Flower bract returned in position (**E**) Inflorescence re-bagged and labeled for next pollination and (**F**) Pollinated bunch left to mature in the open*

Results

Results indicated that a 3% glucose solution applied on stigmas before pollination with Calcutta 4 increased seed set in cultivar Enzirabahima by 65%. According to Ssebuliba, *et al.* (2006), stigmas of top hands of EAHBs are less receptive compared to middle and lower hands. But results from this study showed some degree of seed set in top hands pollinated with glucose solution. On the other hand, making early pollinations and evening pollinations soon after flower opening did not increase seed set in EAHBs.

Seed set for the different pollination techniques on EAHB - Enzirabahima

Pollination Technique	Bunches Pollinated	% Bunches with seed	Seed/10,000 ovules
(1) Customary	43	33	0.62
(2) Morning + 3% Glucose solution	42	33	1.02
(3) Evening + 3% Glucose solution	42	21	0.22
(4) Early + 3% Glucose solution	27	15	0.15

Conclusion

A 3% glucose solution when applied at the right time improved Matooke stigma receptivity and subsequent seed set. However, early pollinations did not increase seed set implying that 3% glucose solution was not sufficient on its own to enable pollen germination.

References

Ssebuliba, R. *et al.*, 2006. Biological Factors Affecting Seed Production in East African Highland Bananas. *Journal of Crop Improvement,* 16(1/2) (#31/32), pp. 67-79.

Authors

A. Waniale

A.K. Tugume

R. Tumuhimbise

R. Swennen

Acknowledgement

BILL & MELINDA GATES foundation

Contact:
allanwaniale@gmail.com

Suitability of Existing *Musa* Morphological Descriptors to Characterize 'Matooke' Bananas

Batte M.[1,2], A. Mukiibi[3] , R. Swennen[4,5,6] , B. Uwimana[1] , L. Pocasangre[3] H.P. Hovmalm[2] , M.Geleta[2], R. Ortiz[2]

1 International Institute of Tropical Agriculture, Kampala, Uganda.
2 Swedish University of Agricultural Sciences, Alnarp, Sweden.
3 EARTH University, San José, Costa Rica.
4 International Institute of Tropical Agriculture, Arusha, Tanzania.
5 Laboratory of Tropical Crop Improvement, Katholieke Universiteite Leuven, Belgium.
6 Bioversity International, Heverlee, Belgium.

Introduction

Morphological traits are commonly used to characterize plant genetic resources. Germplasm characterization should be based on distinctly identifiable, stable and heritable traits that are easy to see and expressed consistently. The Taxonomy Advisory Group agreed on a list of a minimum set of 32 descriptors for characterization and documentation of bananas. However, little is known about the stability of the selected descriptors in *Musa*. In this study, the characterization of a sample of East African Highland bananas (Matooke) belonging to two clone sets was carried out. The objective was to identify stable descriptors that could be used to distinguish cultivars in germplasm collections, to select breeding materials, and to describe new cultivars developed by the breeding program.


Nfuuka bunch


purple-brown bract external face


Orange-red bract internal face


Nakasabira bunch


Purple-brown bract external face


Orange-red bract internal face


Tereza Bunch


Light green with purple stripes of bract external face


Yellow or green with orange-red towards the apex of bract internal face

Fig.1. Example of morphological descriptors for Nfuuka, Nakasabira and Tereza. The two shown descriptors distinguish Tereza from the rest of the tested cultivars

Materials and Methods

11 'Matooke' cultivars from Nakabululu and Nfuuka clone sets (Table 1) were characterized in Namulonge, Uganda. A minimum of 5 plants and a maximum of 20 plants per cultivar were described with 31 out of 32 minimum descriptors as defined by the *Musa* Taxonomic Advisory Group (2010). 28 of the descriptors were qualitative and 3 were quantitative. Data were analyzed using R-software version 3.2.0 (R Core Team 2015). Qualitative data were converted to binary scale using the mode. The mode scores were given a score of 0 while the non-mode scores were given a score of 1. The data were analyzed by binomial test at 95% confidence level. We tested the null hypothesis "the probability of getting a mode score is equal to or less than the probability of getting a non-mode score ($P \leq 0.5$)". A descriptor was considered stable if the null hypothesis was rejected. One-way ANOVA was done for the quantitative data: fruit length, number of hands per bunch, and number of fruits on the mid hand of the bunch.

Results and Discussion

Ten qualitative descriptors were stable within plants of the same cultivar, while the remaining 22 were not stable (Table 1). However, these descriptors had similar scores across the 11 tested cultivars and are therefore not suitable to distinguish between matooke clones or cultivars. These ten descriptors may be useful for distinguishing the East African highland bananas as a group from other groups of bananas but are not suitable for distinguishing between cultivars of the East African highland bananas. Only cultivar 'Tereza' had two qualitative descriptors which were unique from all the others cultivars: light green with purple stripes of the bract external face and yellow or green with orange-red towards the apex of bract internal face (Fig.1).

Results from one-way ANOVA of the quantitative descriptors (fruit length, number of hands and number of fruits on the mid hand) indicated significant variation within and between cultivars. Thus, such descriptors are not stable and thus not suitable to be used in describing the EAHB cultivars as they are highly affected by the environment.

Table 1: Probability for binomial test of 10 stable and 5 (out of 22) unstable descriptors

Descriptor	Nakabululu clone set				Nfuuka clone set						
	Kazirakwe	Nakasabira	Nakayonga	Nakyetengu	Entukura	Enyeru	Enzirabahima	Kabucuragye	Namwezi	Nfuuka	Tereza
Sap colour	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Edge of petiole margin	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Colour of cigar leaf dorsal surface	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Bract behavior before falling	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Lobe colour of compound tepal	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Bract imbrication	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Compound tepal basic colour	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Anther colour	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Dominant colour of male flower	<0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.010
Fruit shape	<0.001	<0.001	0.003	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Bunch position	0.240	0.131	0.612	0.812	0.274	0.401	0.193	0.054	0.002	0.855	0.772
Rachis position	0.592	0.005	0.612	0.812	0.886	0.598	0.806	0.054	0.002	0.855	0.401
Remains of flower relicts at fruit apex	0.951	0.748	0.806	0.187	0.886	0.038	0.072	0.376	0.323	0.363	0.038
Fruit apex	0.407	0.057	0.193	0.500	0.032	0.038	0.072	0.010	0.5	0.003	<0.001
Fruit pedicel length	0.118	0.057	0.072	0.500	<0.001	0.010	0.003	<0.001	<0.001	0.363	0.105

P ≤ 0.05: stable descriptor, P> 0.05: unstable descriptor

Conclusion

The available set of minimum morphological descriptors should be revised to include those which can efficiently distinguish the East African Highland bananas. Likewise, a minimum set of high-throughput dense DNA markers should be defined for an improved assessment of diversity in *Musa* germplasm which will complement the morphological characterization.

References

1. R Core Team (2015) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria http://www.R-project.org/
2. Taxonomy Advisory Group (2010) Minimum descriptors for banana. Bioversity International, Montpellier, France https://Sites.google.com/a/cgxchange.org/musanet/documentation/technical-guidelines

Chromosome doubling in diploid bananas for efficient breeding

Oyesigye, N.[1,2] , Rwakaharuza A. [1,2], Naturinda R.[1,2] , Tumuhimbiise, R.[1,2]
[1]National Agricultural Research Laboratories, Uganda
[2]IITA Banana Breeding

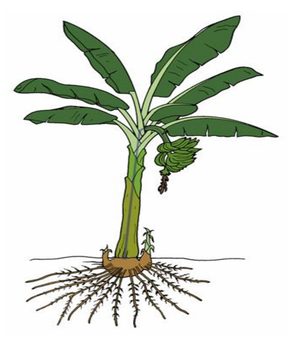
The Breeder's Challenge

Mshale (2x)hardly produce seeds after pollination to get 4x and therefore diploid chromosome doubling to form 4x could improve breeding efficiency.

Why doubling diploids?

- To reduce the breeding period by creating an alternative pathway to systematize the recovery of triploids from crosses between diploids and doubled diploids.
- The method is short to get tetraploids compared to cross breeding of 2x X 2x to get 4x.

Process of producing a tetraploid banana from a diploid



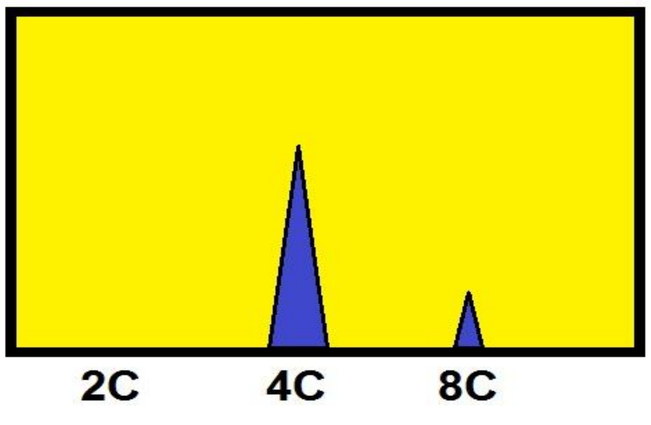
Diploid plant

***In-vitro* culture**

Shoot tip meristemic block

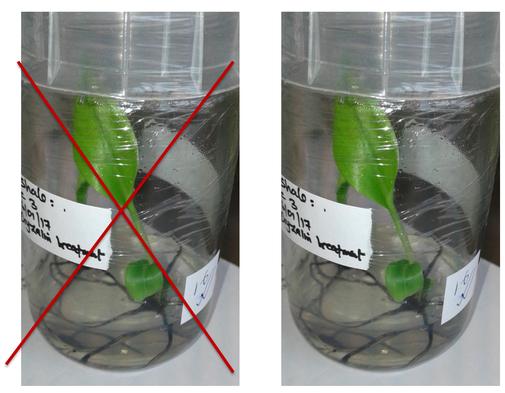
Treatmment with Oryzalin

Micro-propagation of Treated cultures



DNA ploidy screening

Elimination of other ploidy levels and mixoploids



Hardening doubled plantlets for use in breeding

Contact

Oyesigye Naboth, NARL, Kawanda
nabothoyesigye@gmail.com

Molecular markers for the detection of *Fusarium oxysporum* f. sp. *cubense* in East and Central Africa

P. Ndayihanzamaso[1], P. Karangwa[1,2], D. Mostert[1], G. Mahuku[3] and A. Viljoen[1]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

[2]Research Department, Rwanda Agriculture Board, P.O. Box 5016, Kigali, Rwanda

[3]International Institute of Tropical Agriculture (IITA), Mikocheni B, P.O. Box 34441, Dar es Salaam, Tanzania.

Corresponding author: privat@sun.ac.za

INTRODUCTION

Banana Fusarium wilt is a major banana production constraint globally and a significant threat to the livelihoods of millions of people in East and Central Africa (ECA). Management of the disease depends on a proper understanding of the diversity and population dynamics of the causal agent, *Fusarium oxysporum* f. sp. *cubense* (Foc). In this study, the genetic diversity of Foc was investigated in five countries of ECA including Rwanda, Burundi, the Democratic Republic of Congo, Tanzania and Uganda. In addition, a multiplex diagnostic tool was developed to identify Foc Lineage VI which includes closely related vegetative compatibility groups (VCG) that are present in the region.

Figure 1: Maximum parsimony phylogenetic tree of the TEF-1α gene of *Fusarium oxysporum* f. sp. *cubense* (Foc) isolates from East and Central Africa and known VCGs and lineages from other parts of the world. Isolates in Lineage VI are highlighted in blue, while those in Lineage VIII and putative new are marked VCGs in red. Non-pathogenic *F. oxysporum* isolates are marked in purple. Tree topology is statistically supported, with bootstrap values indicated at nodes, with a confidence interval (CI) of 0.900 and a retention index (RI) of 0.964. The tree is rooted with *Fusarium circinatum* isolate FCC 4880.

MATERIALS AND METHODS

The diversity of Foc in ECA was determined by VCG analysis (Leslie and Summerell, 2006), PCR-RFLP of the ribosomal DNA's Intergenic Spacer (IGS) region (Fourie *et al*., 2009), as well as phylogenetic analysis of the Translation Elongation Factor (TEF) 1α gene (Fourie *et al*., 2009). The two primer pairs were designed based on single nucleotide polymorphism (SNP) identified in the following regions: The first primer pair, (5'-CGACAATGAGCTTATCTGCCATT-3') and (5'-CATCGAGGTTGTGAGAATGGA-3'), was designed to amplify a 300-bp fragment in the TEF-1α region. The second primer pair, (5'-AGGGACTGGATTTCTACCCT-3') and (5'-GTGTCACTTGGTCCTCGTAT-3') was designed to amplify a 1002-bp in the region coding DNA-directed RNA polymerase III subunit (RPC2). The two primer pairs were tested for specificity on 84 isolates including Foc isolates representing 24 VCGs, other *formae speciales* and non-pathogenic *F. oxysporum* isolates and then combined in multiplex PCR.

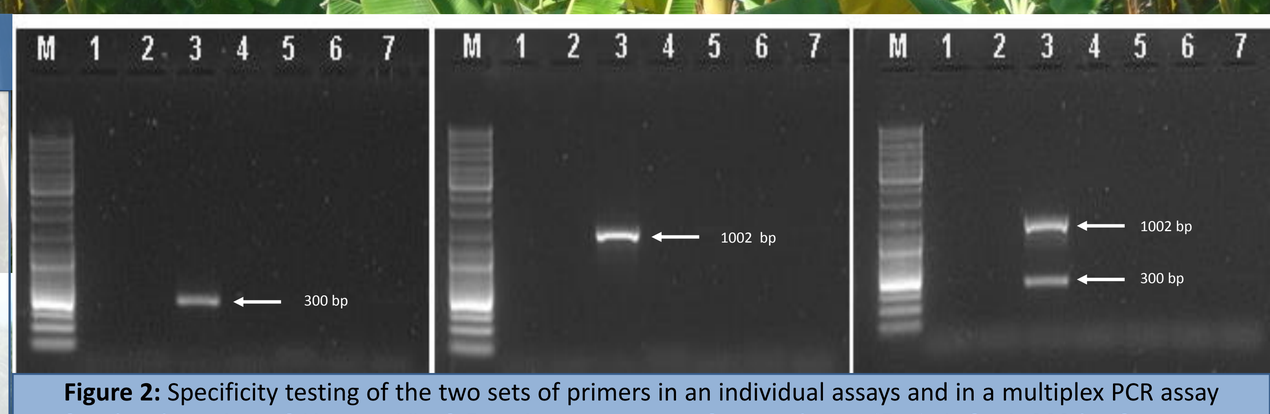


Figure 2: Specificity testing of the two sets of primers in an individual assays and in a multiplex PCR assay for the detection of Lineage VI of *Fusarium oxysporum* f. sp. *cubense* (Foc). Left: A 300-bp fragment amplified by the FocLin6b PCR assay, Middle: A 1002-bp fragment amplified by the FocLin VI PCR assay, Right: Both a 300-bp and a 1002-bp fragments of Foc amplified in a multiplex PCR assay. Lanes 1-7: isolate CAV 980, 618, 2260, 789, 871, 968 and 317; representing Foc Lineage IV, III, VI, V, VIII and *F. oxysporum* f. sp. *melonis,* respectively.

RESULTS

Genetic diversity study: Six VCGs of Foc, namely VCG 0124, 0125, 0128, 01212, 01220 and 01222 were found in ECA. VCGs 0124, 0125, 0128 and 01222 were found in relatively equal frequencies in all five countries, while the VCG 01220 was the least represented in the region. All the isolates tested are members of Foc Lineage VI according to their PCR-RFLP profiles. The phylogenetic analysis showed that all isolates analysed clustered within Foc Lineage VI (Figure 1).

PCR markers: The two primer pairs were able to amplify Foc isolates within Foc Lineage VI. Neither VCG other than Foc Lineage VI members, nor *F. oxysporum* isolates representing other *formae speciales* and non pathogenic species, were amplified (Figure 2).

DISCUSSION

- Foc in ECA proved to be more diverse than previously reported. All isolates analysed clustered Foc Lineage VI.
- Foc Lineage VI consisted of VCGs that were phylogenetically related, which allowed for the development of molecular markers to easily detect these VCGs.
- The multiplex PCR can be used to rapidly detect all isolates of Foc Lineage VI, which will significantly shorten the identification time of Foc isolates by the existing methods of VCG testing.
- The Foc Lineage VI markers will be useful for the implementation of preventive measures, such as the enforcement of quarantine regulations and the screening of banana accessions for resistance to Foc.

REFERENCES

Leslie, J. F., and B. A. Summerell. 2006. The Fusarium Laboratory Manual. Blackwell Publishing-Wiley, Hoboken, NJ., USA. 388 pp.

Fourie, G., Steenkamp, E. T., Ploetz, R. C., Gordon, T. and Viljoen, A. 2011. Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense* within the *Fusarium oxysporum* complex. *Infection, Genetics and Evolution,* 11**,** 533-542.

ACKNOWLEDGMENTS

UNIVERSITEIT STELLENBOSCH UNIVERSITY

IITA Research to Nourish Africa

ISABU Institut des Sciences Agronomiques du Burundi

A RAPID SCREENING METHOD FOR THE RESPONSE TO BANANA WEEVILS (*Cosmopolites sordidus*)

Kemigisa J, Tendo.R. S and Kubiriba J

National Agricultural Research Laboratories –Kawanda P.O. Box 7065, Kampala

Introduction

The banana weevil (*Cosmopolites sordidus Germar*) is the most damaging and yield reducing pest for the bananas globally. In Uganda, very high pest damages are observed in the EAHBs of all stages. Damage is mainly caused by the larvae when they feed and create tunnels in the corm and pseudo stem.

Weevil damage to the corm and pseudo-stem of the East African High land Bananas (EAHBs)

Corm damage

- Disrupts water and nutrient uptake by the plant.
- Interferes with root initiation and development.
- Weakens the plant and reduces its bunch weight (yield loss of 14-60%).
- Causes toppling and falling of the plant in severe cases (yield loss of 100%).

Breeding for resistance to weevils is the best control strategy. Conventional banana breeding is a slow, spacious and laborious process in selecting promising genotypes with the field screening method. A short screening bioassay with preserved excised corm components holds more promise to quickening the selection process.

Objective of the study

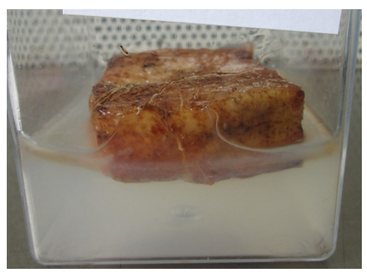
To develop a rapid and precise screening method for response to banana weevil using a set of reference genotypes.

Methods

Determining suitable medium for keeping corms fresh

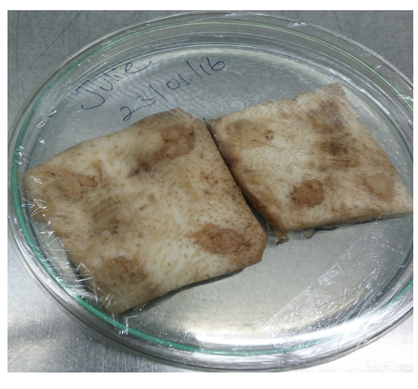
Corm from a physiologically mature banana was paired and surface sterilized

Corm discs (3x3x1) were cut out and weights (W0) determined



Corm piece (3x3x1) on water– agar medium supplemented with Giberellic Acid (GA_3)after 30 days. Corm weights (W1) were determined

Short bioassay using excised corm and larvae



Eggs are hatched on moistened tissue for 5-7 days. 1 day instar larvae are inoculated on corms placed on water-agar medium supplemented with 8mg/L of GA_3. Head capsule width (HCW), Body Length (BL) and Body weight (BW) are determined at 15 days

Field screening experiment for Validating the short bioassay

Kayinja, Calcutta 4, KM 5 , Mbwazirume, Atwalira and Kisansa genotypes at 10 months.

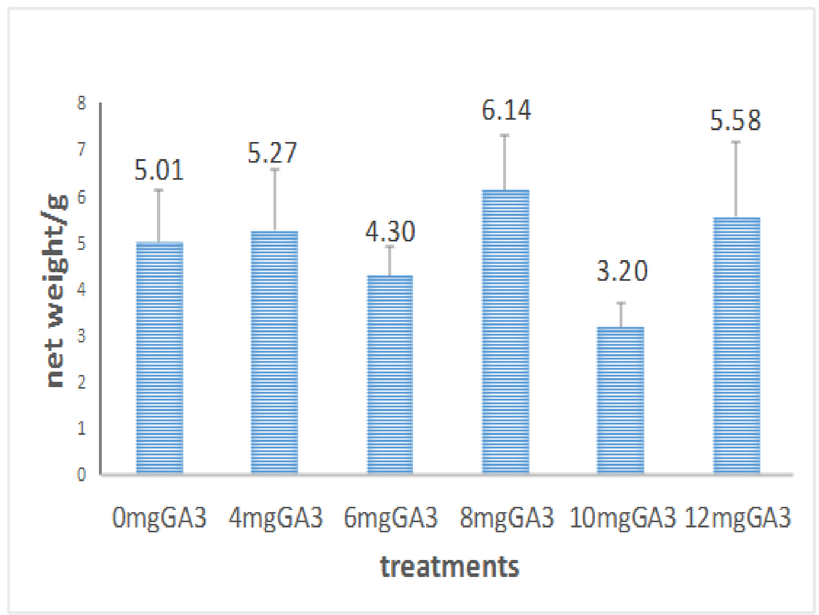
Field experiment

Planted 6 genotypes in a Randomized complete block design

Introduced 10 adult weevil s per mat at 10 months in a ratio of 1:1 males to females.

Peripheral and cross-sectional corm damage due to banana weevils will be determined at plant harvest. Weevil damage data will be correlated with the results from the short screening bioassay.

Results



Corm weights(W1-W0) on water-agar medium supplemented with different concentrations of GA_3 at 30 days

0 mg/L	4 mg/L	8 mg/L

Cross-sectional corm appearance after 30 days maintained on water-agar medium with different concentrations of GA_3

Applications of the short screening method

- Screening genome wide association studies.
- Screening promising hybrids from Early Evaluation Trials (EET) and Preliminary Yield trials (PYT).
- Screening parental materials (Mshales, Diploids, tetraploids etc).
- Any other studies that require mass screening of the genotypes for response to banana weevils.

Conclusion

- Corm discs were kept fresh on water-agar medium at 8mg/L of GA_3.
- Water agar medium is being used for preserving corms in the short screening bioassay.
- Standard protocol for the short screening bioassay is underway
- Short bioassay can predict banana response to weevils in 25days

Acknowledgment

We would like to thank members of Work package 2 of the project "**Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa"** for guiding this work

The adaptation range of black Sigatoka causal pathogen shifting towards higher altitudes in Uganda

Janet Kimunye[1,2], George Mahuku[1], Altus Viljoen[2], Allan Brown[1] and Evans Were[1]

[1]International Institute of Tropical Agriculture (IITA), [2]Deparment of Plant Pathology, Stellenbosch University, South Africa

Introduction

The hemibiotrophic fungus *Pseudocercospora fijiensis* is the causal agent for black Sigatoka a foliar disease of banana that result in fruit yield losses of between 35-85%. Defoliation reduces photosynthetic tissue hampering fruit filling and induce premature fruit ripening. In Uganda, *P. fijiensis* was restricted to below 1500 meters above sea level (m.a.s.l.). There is a likelihood that this could have changed in response to climate change. The aim of this study was to determine the current distribution of *P. fijiensis* in selected high and low altitude banana growing areas of Uganda.

Materials and methods

- Surveys were conducted in Luweero (low altitude between 1077-1243 m.a.s.l.) and Mbarara (high altitude between 1411-1877 m.a.s.l.) districts in 2016. In this study, we considered altitudes of <1200 m.a.s.l. as low,1201-1500 m.a.s.l. as mid and >1501 m.a.s.l. as high. During sampling farms 5-10 km apart were purposively selected and GPS coordinates and altitude recorded.
- For selected farms,10-15 plants were randomly selected, evaluated for black sigatoka severity using the method Gauhl et al.(1997). Disease severity index was then computed using the formula $(\mathbf{DSI}) = \{\sum \mathbf{nb} \div (\mathbf{N}-\mathbf{1})\mathbf{T}\} \times \mathbf{100}$.
- Leaf samples were collected and presence of *P. fijiensis* confirmed using PCR with species-specific primers (Arzanlou et al.,2007).

Results and discussion

- *P. fijiensis* is widespread in Uganda with 70% of samples testing positive by PCR.
- Interestingly, *P. fijiensis* was detected on samples collected from altitudes above 1500 m.a.s.l. up to 1877 m.a.s.l suggesting an expansion in habitat suitability.
- Average DSI was (34.9%) and was significantly higher in flowered (46.4%), than in pre-flowered (27.9%) and maiden (19.7%) plants.
- A weak negative correlation (*r*=-0.27) between altitude and black Sigatoka severity index was observed indicating that altitude does not significantly influence *P. fijiensis* severity in Uganda.
- .

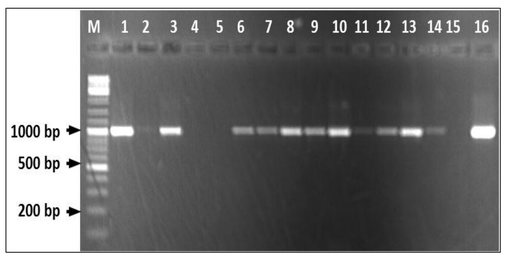


Fig.1. Gel picture of *P. fijiensis* detection on leaf samples with *P. fijiensis*-specific PCR primers.

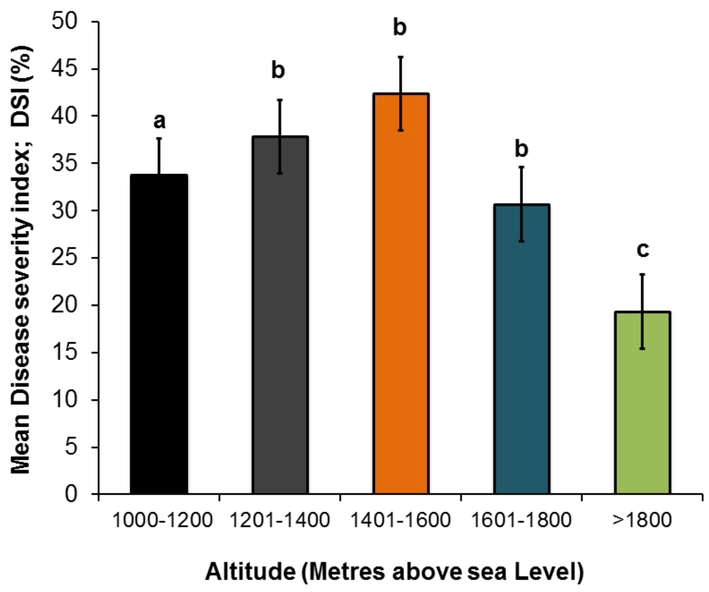


Fig. 2. Severity of black Sigatoka leaf spots along an altitude gradient in surveyed farmer fields in Uganda

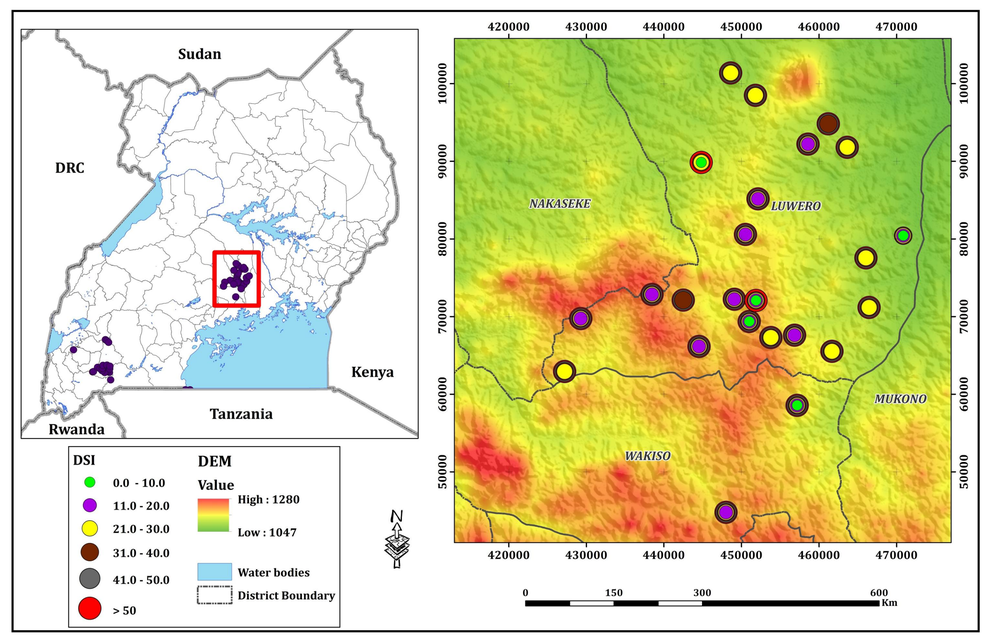


Fig. 4. Map of Luweero showing the distribution of study sites and severity of black Sigatoka per site.

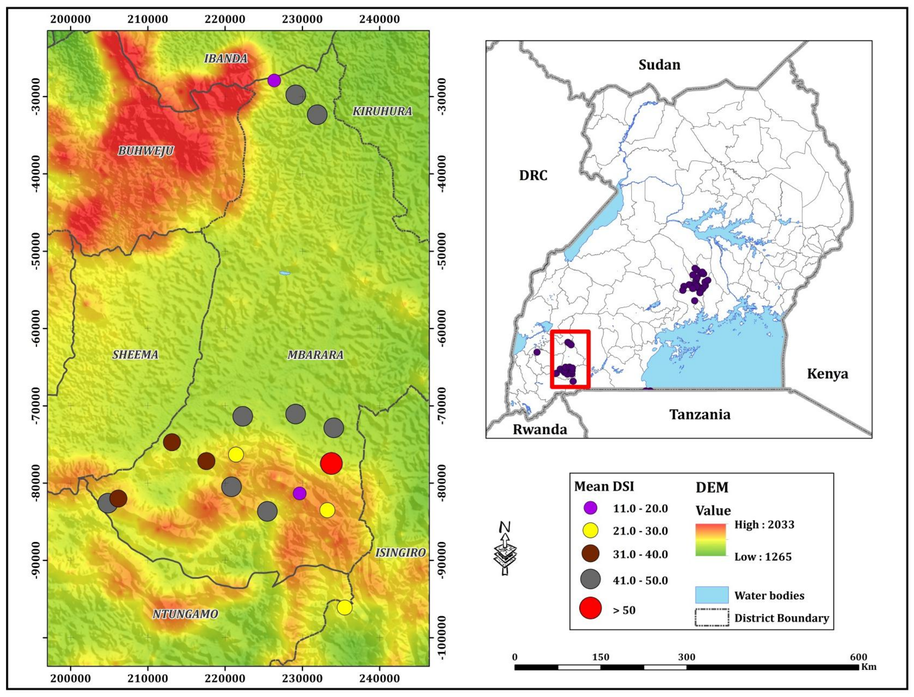


Fig. 3. Map of Mbarara showing the distribution of study sites and severity of black Sigatoka per site.

Conclusions and recommendations

- Our results indicate that black Sigatoka habitat has expanded into high altitudes.
- There is need to determine impact of black Sigatoka at high altitudes.
- Identification of other Sigatoka leaf spot pathogens will help determine their distribution and possible risks in the region .
- It is important to include more pathogen characteristics and environment related factors in future studies so as to determine the cause of the shift in distribution.

Selected references

Arzanlou, M., Abeln, E.C.A., Kema, G.H.J., Waalwijk, C., Carlier, J., de Vries, I., Guzman, M. and Crous, P.W. (2007) Molecular diagnostics for the Sigatoka disease complex of banana. *Phytopathology*, 97, 1112–1118.

Johanson A, Tushemereirwe W.K and Kamarure E.B. 2000. Distribution of Sigatoka leaf spots in Uganda as determined by Species-speciefic polymerase chain reaction PCR. Proc. Int. Symp. On banana and Plantain for Africa, Act Hort, 540, ISHS.

Acknowledgement

Bill and Melinda gates foundation for funding the work and IITA for the Fellowship

Contact: Janet Kimunye
Email:j.kimunye@cgiar.org

GENETIC DIVERSITY OF BANANA (*Musa* spp.) AND ITS RELATION TO PLANT PARASITIC NEMATODES IN TANZANIA.

Doreen M Mgonja[1,3], Gladness E Temu[1], Sylvester L Lyantagaye[1], Joseph C Ndunguru[3] Nessie D Luambano[2]

[1]University of Dar es Salaam, College of Natural and Applied Sciences, Molecular biology and Biotechnology Department, P.O. Box 35179 Dar es Salaam, Tanzania. [2]Sugarcane Research Institute, P.O. Box 30031, Kibaha, Tanzania.

[3]Mikocheni Agricultural Research Institute, Sam Nujoma Road, Box 6226, Dar es Salaam, Tanzania,

Introduction

Bananas (*Musa* spp.) are one of the most economically important crops in Tanzania. Banana production in Tanzania is largely constrained by pest and diseases mainly plant parasitic nematodes, particularly the burrowing and root lesion nematodes (FAOSTAT, 2013; Coyne , 2009). Control of nematodes through resistant varieties has been difficult due to limited information on genetic and genomic resources that may be important in supporting the development of farmers preferred varieties. In addition, names and accurate identities o f banana are not clearly known hence, limiting sustainable farming. Characterization of crops based on DNA markers is the measures for correct and quick identification of similar or closely-related cultivars. This study was conducted to assess genetic diversity of 159-banana varieties [using simple sequence repeat (SSR) markers] and their association to plant parasitic nematodes.

Materials and methods

Plant materials

A total of 159 banana varieties used in this study were collected from major banana growing areas of Tanzania (Figure 2)

Genomic DNA extraction

Genomic DNA isolation was done from the collected banana leaves using modified CTAB method.

SSR-PCR amplification

A total of 20-polymorphic banana SSR primers were used to amplify DNA isolated from banana leaf samples. The band profiles were scored and recorded. The data were analysed on NTSYS software pc 2.0 and the Poymorphic information content (PIC) values were calculated and recorded

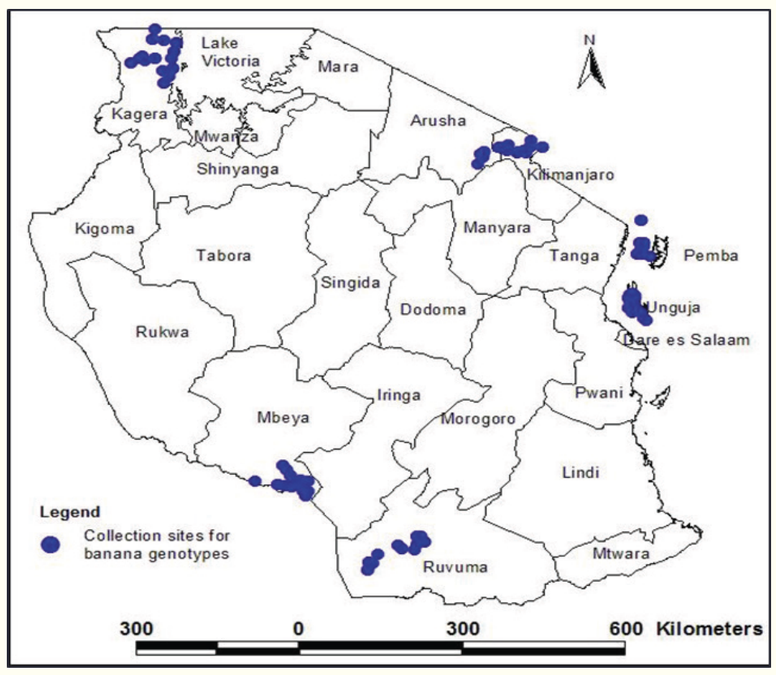


Figure 2.Map of Tanzania showing banana growing regions

Results and discussion

Genetic diversity based on PIC values

Twenty SSR primer pairs were polymorphic and generated a total of 63 distinct reproducible bands. The number of polymorphic bands detected with each primer pair ranged from 2 to 4 with an average of 3.15 per primer pair. The polymorphic information content values of each primer pair ranged from 0.50 to 0.75 with an average of 0.60.

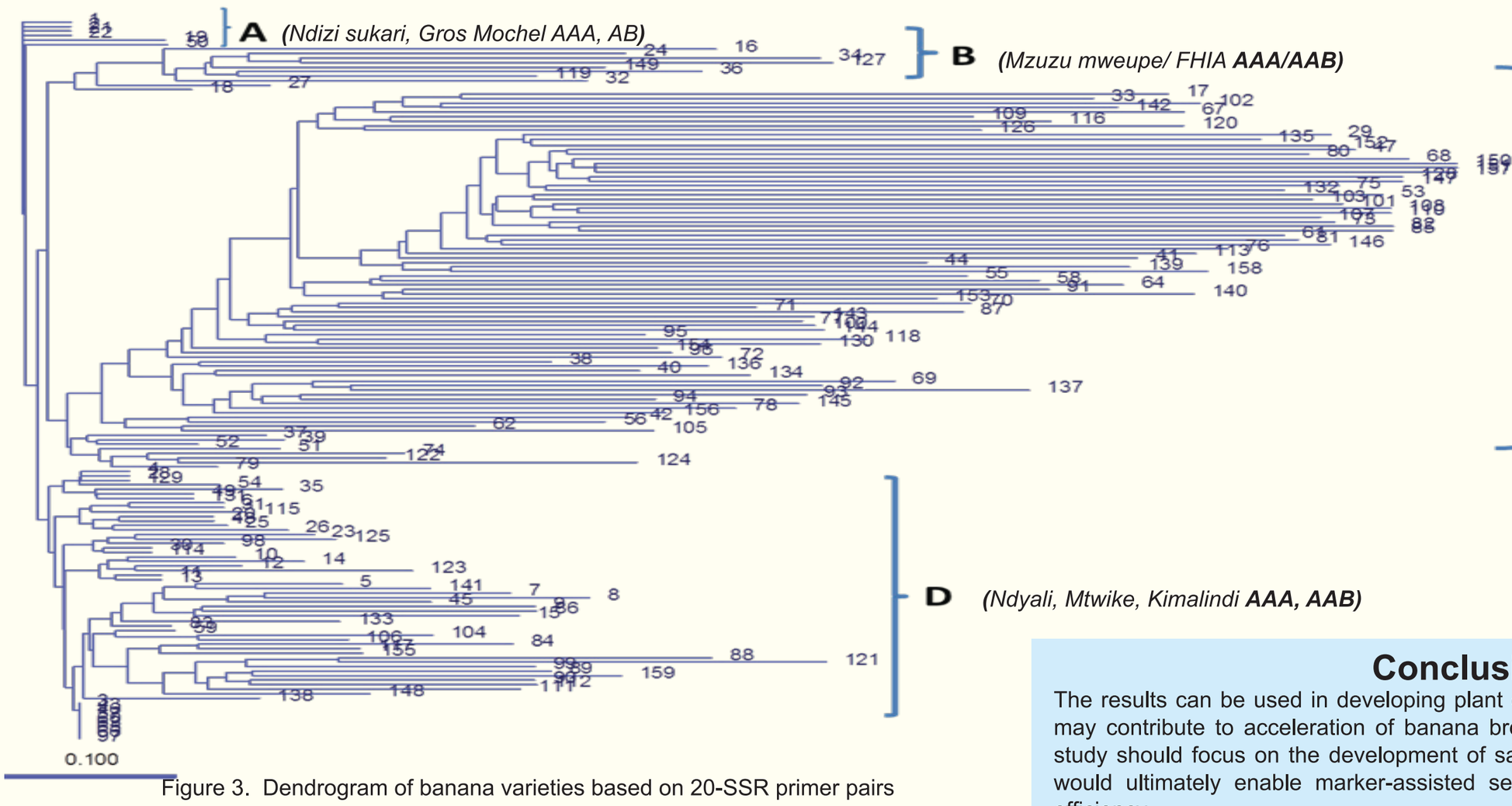


Figure 3. Dendrogram of banana varieties based on 20-SSR primer pairs

The UPGMA cluster analysis separated the 159-banana cultivars into four major groups (Figure 3) . **Cluster A** is composed of fewest genotypes (1, 19, 21, 22 and 50) - All from Kagera except genotype no. 1 from Pemba. **Cluster B** contains 10 genotypes: 3-Kagera 3-Mbeya; 2-Ruvuma; 1- Zanzibar and 1-Pemba. **Cluster C:** The largest- with 87 genotypes. **Cluster D:** 57 genotypes. The results revealed a high genetic diversity among the 159-bananacultivars.

Figure 1. Banana field showing toppling due to nematodes damage and roots damaged by nematodes

Table 2. Banana genotypes related to nematode species associated with different genotypes from different regions and altitudes of Tanzania.

Highest mean nematode count/100 mls							Lowest mean nematode count/100 mls				
Altitude zone	Nematode spp.	Genotype	Region	Altitude level (masl)	Mean nematode count/100 mls	Cluster	Genotype	Region	Altitude level (masl)	Mean nematode count/100 mls	Cluster
1	*R. similis*	MzuMwWZ	Zanzibar	42	617	D	MtwNZ	Zanzibar	17	17	C
	P. goodeyi	GurZRMb	Mbeya	477	5317	D	MtwMP	Pemba	34.7472	33	C
	P. coffeae	KijCZ	Zanzibar	46	700	C	PukWZ	Zanzibar	7	17	C
2	*R. similis*	TokKMb	Mbeya	581	223	C	HarRMb	Mbeya	565	17	B
	P. goodeyi	TokKMb	Mbeya	581	650	C	HarRMb	Mbeya	565	17	B
	P. coffeae	MshNR	Ruvuma	1080	717	C	KiskNR	Ruvuma	955	13	C
3	*R. similis*	TokRMb	Mbeya	1250	3783	C	JamAA	Arusha	1255	17	D
	P. goodeyi	MwamMR	Mbeya	1358	3183	C	BokSR	Ruvuma	1014	17	C
	P. coffeae	BukSRR	Ruvuma	1077	533	D	BokSR	Ruvuma	1014	17	C
4	*R. similis*	NshaKK	Kagera	1603	1567	C	KanKK	Kagera	1603	17	C
	P. goodeyi	MkojMKi	Kilimanjaro	1527	1500	C	MzuMR	Ruvuma	1557	10	D
	P. coffeae	KatMR	Ruvuma	1557	66	C	MkojMKi	Kilimanjaro	1527	0	C

The results shows that the highest mean count of nematodes were from genotype GurZRMb - altitude zone 1, followed by TokRMb– Altitude zone 3 (Table 2.) which are commonly cultivated genotypes. However, some of the introduced genotypes such as FHIA 23 were moderately affected by *R. similis* and *P. goodeyi*. This supports the possibility of the PPN to adapt and spread into different environments regardless of their original environments. The results shows that *P. goodeyi* and *R. similis* are highly distributed in all zones, although zone 2 had the lowest count o these nematode populations. In addition, *P. goodeyi* were highly abundant in altitude zone 1, the low land and humid climate while the previous report showed that *P. goodeyi* are confined in high cool altitudes. This indicates that these nematodes are spreading and able to adapt to new environmental conditions.

Conclusion

The results can be used in developing plant disease management strategies and may contribute to acceleration of banana breeding programs worldwide. Further study should focus on the development of saturated genetic linkage maps which would ultimately enable marker-assisted selection and improve crop selection efficiency

Acknowledgements

This work is sponsored by Sugarcane Research Institute (SRI) Kibaha through PEARL banana nematode project coordinated by Dr. Nessie Luambano

Reference

1. Food and Agricultural Organisation of the United Nations (FAOSTAT) 2013.
2. Coyne D. 2009 Pre-Empting Plant-Parasitic Nematode Losses on Banana in Africa: Which Species Do We Target? *Proc. IS on Banana Crop Protection Sustainable Productivity & Improved Livelihoods*.

DISTRIBUTION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH BANANA CROPS IN TANZANIA

Nessie Luambano[1], Charlie Opperman[2], Doreen Mgonja[3] and Joseph Ndunguru[3]

[1]Sugarcane Research Institute-Kibaha, TANZANIA, [2]North Carolina University, USA, [3]Mikocheni Agricultural Research Institute, TANZANIA,

SUMMARY

Plant parasitic nematodes significantly affect banana production in Tanzania. The general objective of this project is to generating information on occurrence, abundance, diversity and distribution of nematodes in banana-growing regions useful in development of management strategies for nematodes.

INTRODUCTION

- Banana is one of the major food crops in Tanzania and about 30% of the population depend on it
- However, the yield is low due to many factors including plant parasitic nematodes (PPN)
- PPN invade and destroy roots of banana and in severe cases resulting in falling of plants
- **Specific Objective:** To establish the geographical distribution of the key PPN of banana in major banana-growing areas of Tanzania.

Fig. 1:Nematode and effects on banana plants and roots

MATERIALS AND METHODS

- Survey was conducted in 4 agro-ecological zones comprising 10 major banana growing regions of Tanzania .
- Soil, roots and geographical data were collected.
- Nematode incidence was assessed before identification and counting of nematodes.
- The information was used to generate nematodes distribution Maps.

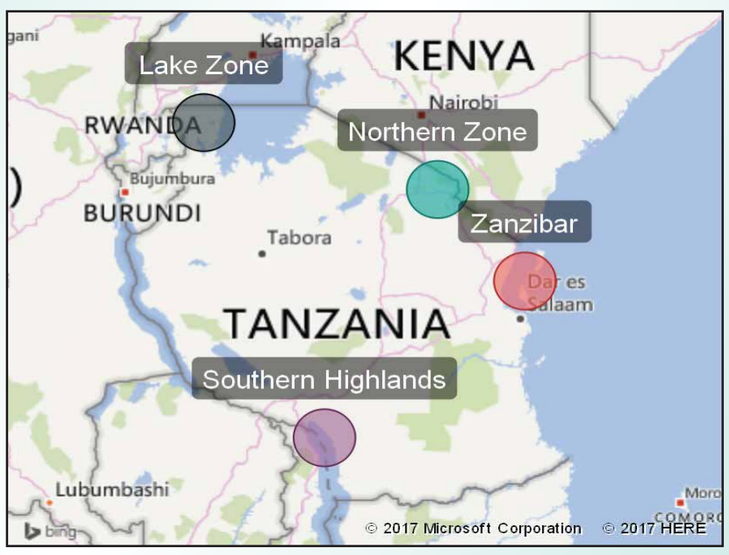


Fig. 2: Agro-ecological zones sampled. BLACK: Lake Zone; PUPLE: Southern Highlands; GREEN: Northen Zone; RED: Zanzibar

RESULTS

Nematodes Distribution and abundancy by zone in Tanzania

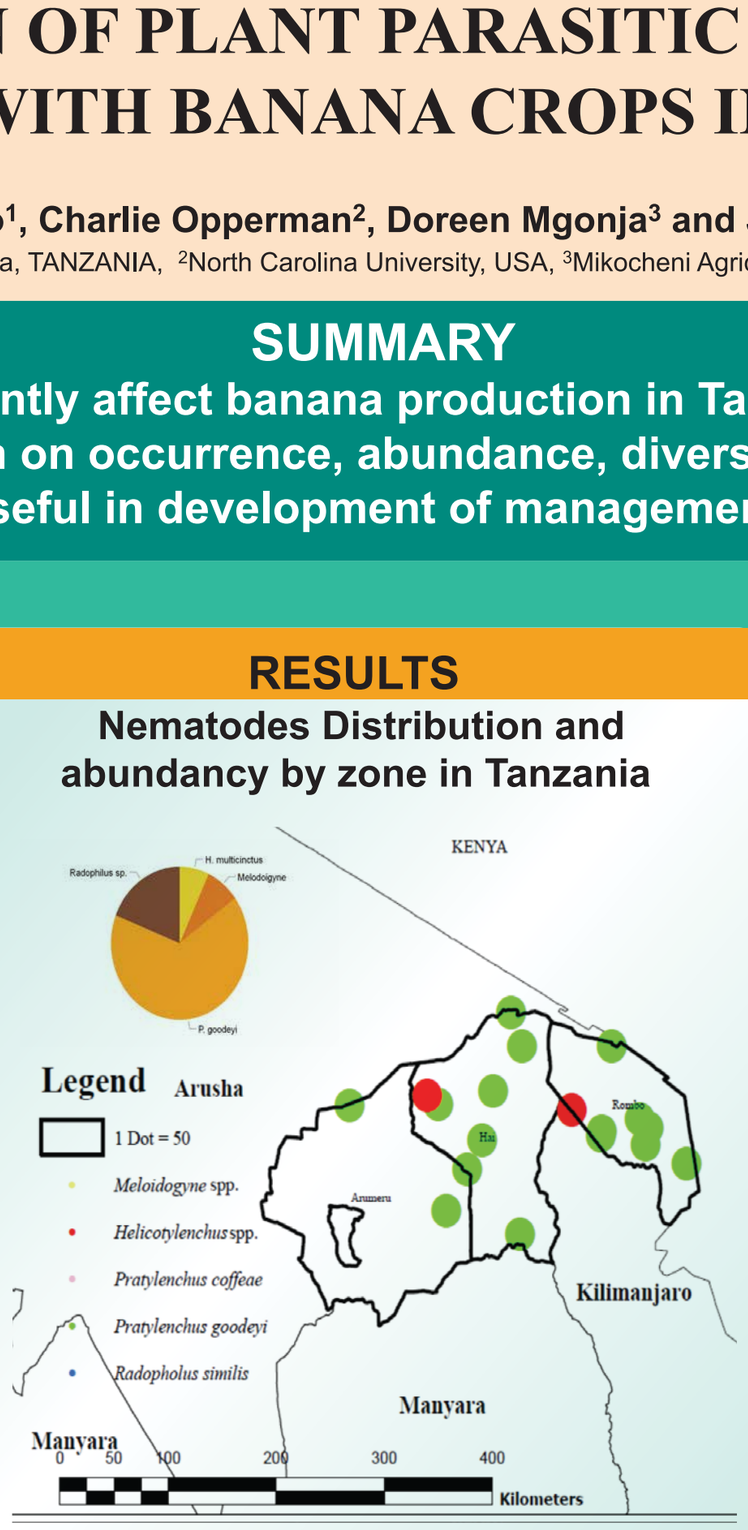


Fig. 3: Nematode distribution-Northern Zone

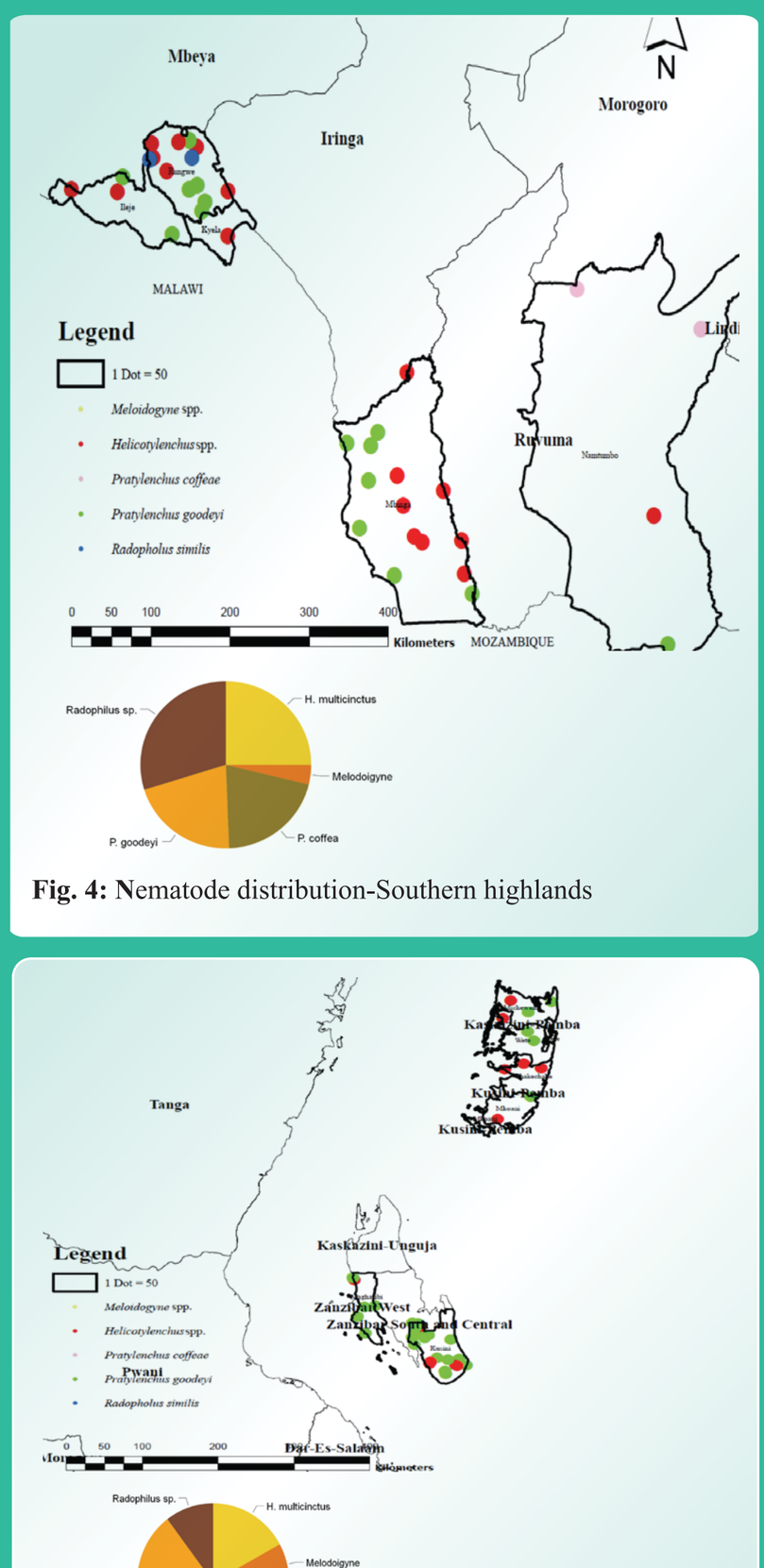


Fig. 4: Nematode distribution-Southern highlands

Fig.5: Nematode distribution-Zanzibar

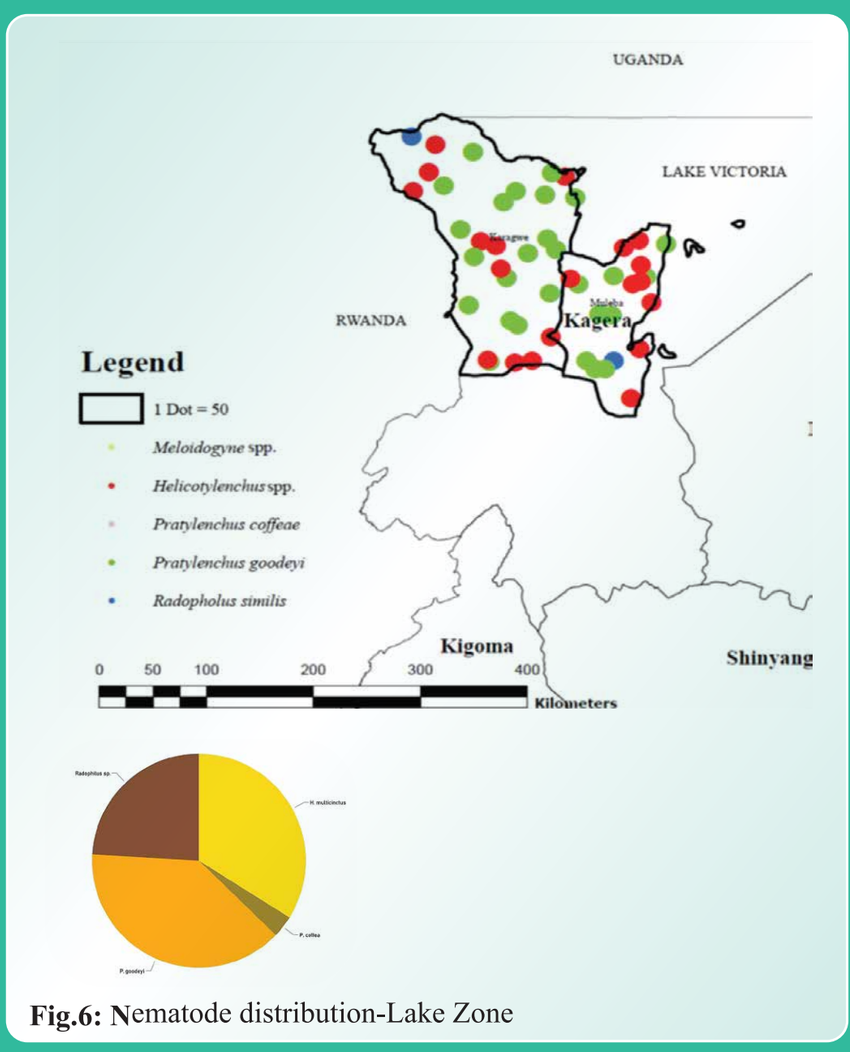


Fig.6: Nematode distribution-Lake Zone

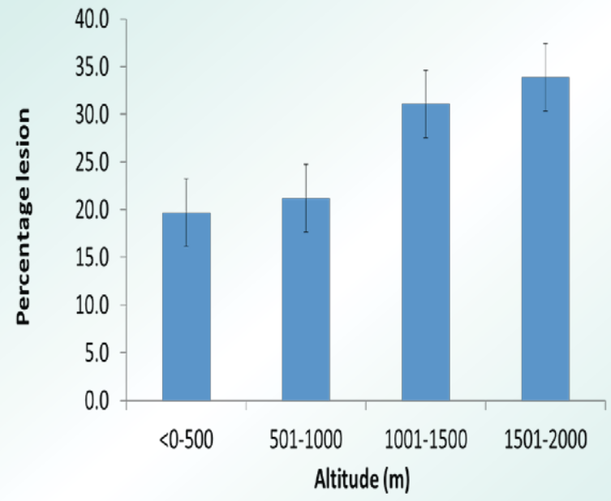


Fig.7: Percentage root lesions recorded at various altitude ranges

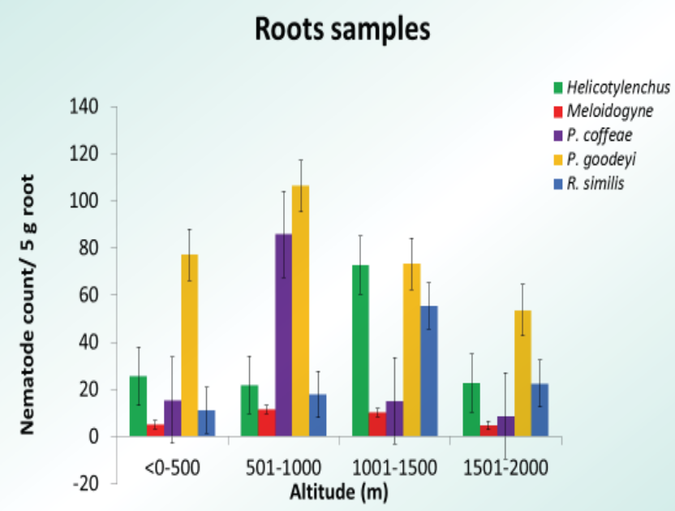


Fig. 8: Nematode abundancy in different altitude ranges

Acknowledgement

- To Bill and Melinda Gates Foundation for financial support
- Agshare Today Project
- Government of Tanzania

Trait Variation in a Banana Training Population for Genomic Selection

Nyine M[1,3], Uwimana B[1], Swennen R[2], Batte M[1], Brown A[2], Christelová P[3], Hřibová E[3], Lorenzen J[1*], Doležel J[3]

[1] International Institute of Tropical Agriculture (IITA), Kampala, Uganda

[2] International Institute of Tropical Agriculture (IITA), Arusha, Tanzania

[3] Institute of Experimental Botany & Palacký University, Czech Republic

* Currently with the Bill and Melinda Gates Foundation

Introduction

Conventional crossbreeding is the main approach used in banana improvement. However, the method requires up to two decades of crossing and field evaluation to develop a new hybrid. This is because selection is carried out at different levels (Fig 1). At every level, plants are evaluated after three crop cycles, each taking about a year. Yield traits can only be scored at harvest while organoleptic traits are recorded after harvesting, making the selection process slow, expensive and labour intensive. Molecular tools with the potential to improve banana breeding efficiency are being investigated. These include genomic selection (GS), which will benefit breeding through increased genetic gain per unit time (Meuwissen et al. 2001; Nakaya and Isobe 2009). Understanding trait variation and the correlation among economically important traits is an essential first step in the development of GS models. In this study we tested the hypothesis that trait variations in bananas are not affected by cross combination, cycle, field management and their interaction with genotype.

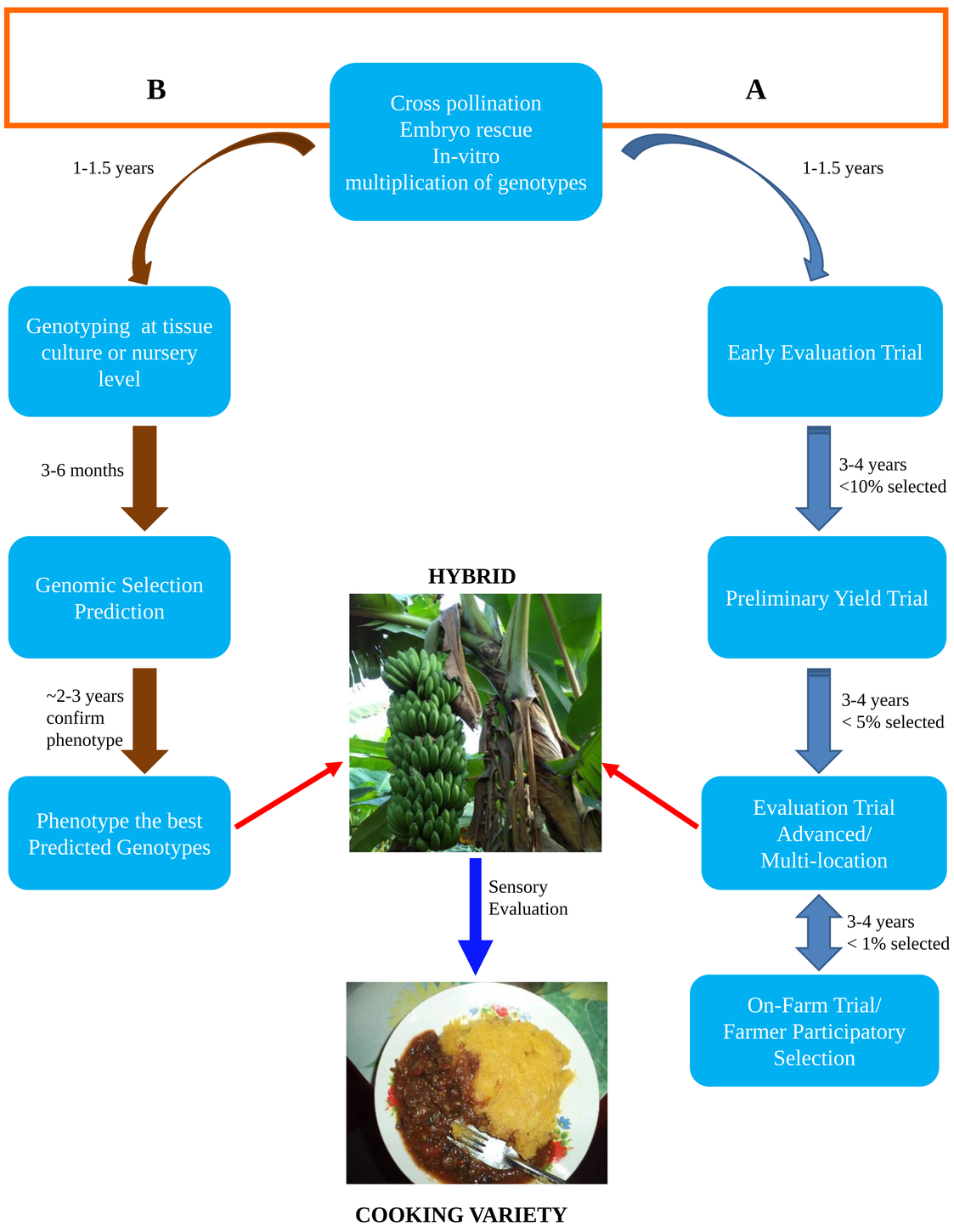


Fig 1: Approaches to hybrid selection in banana breeding program. (A) the classical phenotypic selection of banana hybrids and (B) integrated genomic selection and phenotypic selection approach being investigated.

Materials and Methods

The training population consists of 307 genotypes that include parents and the resulting hybrids from the East African Highland banana breeding program of the International Institute of Tropical Agriculture (IITA) and National Agricultural Research Organization (NARO). The population was phenotyped under low (no mulch and NPK fertilizer) and high (mulch + NPK) field input management at Namulonge research station. Data collected on two crop cycles were analysed using R statistical software. The correlations and significance of correlations were determined using R package Hmisc. Analysis of variance was performed to understand the effect of genotype and the interaction between genotype and cycle, and genotype and field management on trait variation.

Results and Discussion

A high level of correlation among vegetative and yield related traits was observed (Table 1). This could mean that the predictive ability of traits that are difficult to phenotype will be similar to less difficult traits they are highly correlated with. Therefore, genomic selection models could be developed for traits that are easily measured. Table 2 summarizes the genotypic effects and the interaction between genotype and cycle and genotype and field management on the traits. Black Sigatoka-related traits were not affected by crop cycle. These could be measured in the first cycle thus reducing on phenotyping burden. Growth traits such as plant height and girth were the least affected by field input management. Conversely, yield-related traits such as bunch weight, number of hands and number of fingers were significantly affected by both crop cycle and field input management. The variation in traits observed suggest that different genomic selection models should be tested. For traits affected by cycle and field management, models that account for non-additive genetic effect are likely to have better predictive ability on them. Integration of genomic selection in crossbreeding allows simultaneous prediction and selection of best hybrids. This is likely to reduce the selection cycle and increase genetic gain per unit time.

Table 1: Pearson's correlation coefficients of traits under high input field management

	Pant height	Plant girth	Index of non-spotted leaf	Bunch weight	Number of hands	Number of fruits	Fruit length	Fruit circumference	Fruit diameter
Plant girth	0.77*								
Index of non-spotted leaf	0.21	0.27							
Bunch weight	0.37*	0.62*	−0.13						
Number of hands	0.22	0.42*	0.10	0.52*					
Number of fruits	0.37*	0.58*	0.19	0.57*	0.84*				
Fruit length	0.20	0.44*	−0.15	0.83*	0.28*	0.27*			
Fruit circumference	0.33*	0.45*	−0.15	0.81*	0.15	0.15	0.85*		
Fruit diameter	0.39*	0.48*	−0.16	0.79*	0.16	0.18	0.80*	0.97*	
Pulp diameter	0.39*	0.45*	−0.16	0.74*	0.11	0.13	0.76*	0.94*	0.99*

* Significant correlation with P-value < 0.05

Table 2: Effect of genotype and genotype interaction with cycle and field management on the traits

Trait	Indep. variable	Sum Sq	Df	F value	*P* value
Plant height	Genotype	2222889	306	3.77	<0.0001
	Genotype x Field	432297	284	0.79	0.995
	Genotype x Cycle	332846	299	1.05	0.266
Plant girth	Genotype	73176	306	4.30	<0.0001
	Genotype x Field	12061	284	0.76	0.998
	Genotype x Cycle	13057	299	1.51	<0.0001
Index of non-spotted leaf	Genotype	116602	306	2.44	<0.0001
	Genotype x Field	58584	284	1.32	0.0005
	Genotype x Cycle	51026	299	0.95	0.695
Bunch weight*	Genotype	1214	303	12.55	<0.0001
	Genotype x Field	127	269	1.48	<0.0001
	Genotype x Cycle	109	276	1.49	<0.0001
Number of hands	Genotype	3334	303	8.67	<0.0001
	Genotype x Field	570	269	1.67	<0.0001
	Genotype x Cycle	429	276	1.26	0.005
Number of fruits	Genotype	1380509	303	5.46	<0.0001
	Genotype x Field	333081	269	1.49	<0.0001
	Genotype x Cycle	262981	276	1.23	0.009

* Original data was square-root transformed

Conclusion

Genomic selection as a form of marker assisted selection is a non-stand alone approach but if integrated into conventional crossbreeding it has the potential to accelerate the breeding process. The effectiveness of genomic selection in banana will greatly depend on the prediction accuracy of the genomic selection models. Understanding the correlation between traits, the genetic effect and its interaction with the environment (field management and crop cycle) will pave the way towards choosing the right model for each trait.

References

1. Meuwissen THE, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 2001;157: 1819-1829.

2. Nakaya A, Isobe SN. Will genomic selection be a practical method for plant breeding? Ann Bot. 2012;110: 1303-1316.

Genetic dissection of *FOC* resistance using *Musa acuminata* ssp. *Malaccensis*

Andrew Chen[1], Jessica Dalton Morgan[1], Sam Fraser-Smith[1], Manual Zander[1], Pradeep Ruperao[3], Jacqueline Batley[3], Sharon Hamill[2], David Edwards[3], Elizabeth Aitken[1].

[1] School of Agriculture and Food Science, University of Queensland, St Lucia, Brisbane, QLD, Australia.
[2] Department of Agriculture, Fisheries & Forestry (DAFF), Maroochy Research Station, Nambour, QLD, Australia.
[3] School of Plant Biology, University of Western Australia, Perth, WA, Australia.

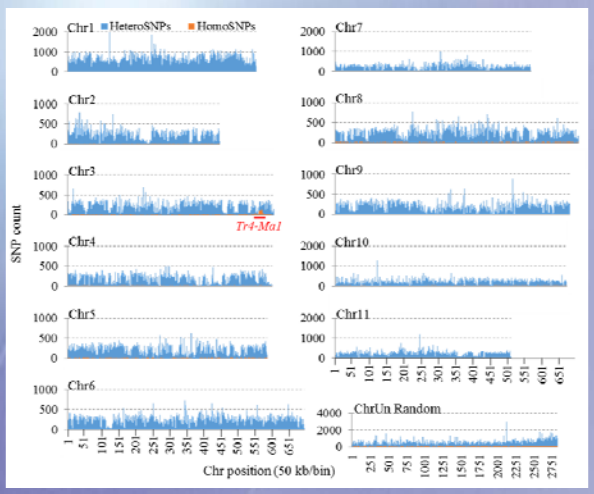
Background

Fusarium wilt is one of the major diseases threatening banana production worldwide. The causal agent is the soil borne fungus *Fusarium oxysporum* sp. *cubense* (*Foc*) which enters the roots and then colonises the vascular tissues to induce a lethal wilt. We have identified *Foc* resistance in the wild banana *Musa acuminata* subsp *malaccensis*.

Results and Progress

1. QTL identified for resistance to Race 1 and 4

Genotyping-By-Sequencing was performed on resistant and susceptible progeny to identify SNPs on a genome-wide scale. A strong level of association between SNP zygosity and resistance was identified in a region on chromosome 3.

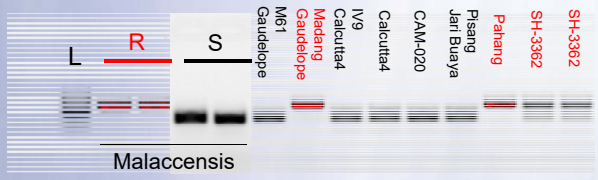


2. Identification of candidate genes

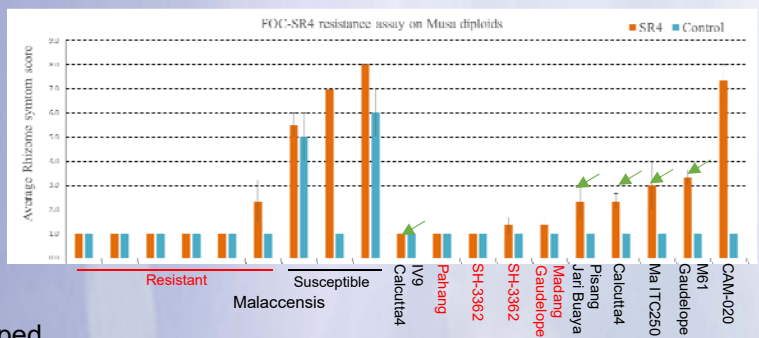
- Using SNP PCR markers, this region was fine-mapped.
- The QTL was delimited to a 157 kb nucleotide sequence containing 28 putatively defined genes.
- Only 15 genes are predicted to carry non-synonymous SNPs leading to changes in the encoded amino acid sequence.

3. Assessing the distribution of resistance gene

Molecular markers closely linked to the FOC SR4 locus can be used to detect the presence/absence of the resistance gene in other cultivars/genotypes



FOC-SR4 colonised millet and spore suspension cultures were used to assess plant resistance against FOC race 4



4. New sources of resistance identified.

The green arrows indicate genotypes that lack our resistance gene marker but possess moderate to strong level of resistance. These lines may carry sources of resistance unlinked to our locus.

Conclusions

- malaccensis is an excellent genetic tool for studying Foc resistance owing to a completely sequenced genome, high fertility and a rich gene pool.
- SNP discovery is important for mapping, positional cloning and allelic diversity studies
- A reliable resistance assay method is developed for glasshouse pot trials. Rhizome discoloration ositively correlated with the observed level of resistance.

References

- Bayer, P.E., Ruperao, P., Mason, A.S. et al. Theor Appl Genet (2015) 128: 1039. doi:10.1007/s00122-015-2488-y
- Fraser-Smith S., Czislowski E., Daly A. et al. Acta Hortic 1114 ISHS (2016). DOI 10.17660/ActaHortic.2016.1114.13

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

BILL & MELINDA GATES foundation
IITA
Research to Nourish Africa

Genetic Analysis of Resistance against *Fusarium Oxysporum F. Sp. Cubense*

Arinaitwe I.K.[1, 2, 4], C.H. Teo[4], K.S. Wai[5], Ali M.[2], F. Kayat[3], B. Uwimana[6], R. Tumuhimbise[2], J.A. Harikrishna[1, 4], R.Y. Othman[1, 4]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia; [2]National Agricultural Research Organisation (NARO), Uganda; [3]Faculty of Agro-based Industries, Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia; [4]Center for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur, Malaysia; [5]School of Biosciences, Taylor's University, Subang Jaya, Selangor, Malaysia; [6]International Institute of Tropical Agriculture, Kampala, Uganda

Introduction

Fusarium wilt, a fungal disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most disastrous diseases of banana, causing an estimated annual yield loss of 60 to 90% [1]. Attempts to control *Foc* using chemical, cultural and biological methods have not been very effective. Host plant resistance found in wild bananas (diploids) is the most appropriate and cost effective intervention to control *Foc* because it is durable and environmentally friendly [2]. NARO-Uganda and IITA have already successfully utilised wild bananas to improve susceptible triploid *Musa acuminata* 'Matooke' [3]. Conventional breeding in *Musa* is hampered by many factors, key of which is low number or complete absence of seeds in fruits, size of the plants, the crop's long life cycles, the long breeding cycle (10-12 yrs) coupled with limited knowledge of the genetics of resistance to diseases such as *Foc*. Understanding genetics of resistance to *Foc* and application of marker assisted selection (MAS) in breeding will aid in shortening the banana breeding cycle for resistance to *Foc* in *Musa*.

This study aims at elucidating the genetics of *Foc* resistance in at least 2 diploid banana populations and mapping Quantitative Trait Loci (QTL) associated with resistance, as a first step towards marker assisted selection for *Foc* in banana.

Materials and Methods

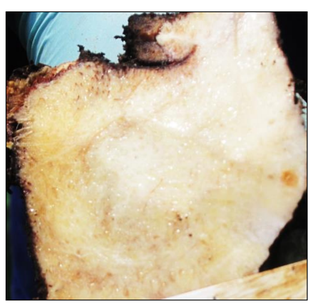
Screening diploid parents with Foc

Table 1. Screening of potential diploid parents

Material screened	Reaction to Foc race 1
Kokopo	Susceptible
Monyet	Tolerant
Mshale	Susceptible
Calcutta 4	Tolerant
TMB2X614-1	Tolerant
Pahang	Tolerant
Zebrina	Tolerant
Kasaska	Tolerant
Borneo	Tolerant
Pisang Lilin	Tolerant
Mwitu Pemba	Tolerant
Long tavoy	Resistant
OP-Malaccensis (R)	6 Resistant, 18 Tolerant
OP-Malaccensis (S)	18 Susceptible, 3 Highly susceptible

Crossing parents with relative degrees of Foc resistance to generate segregating populations

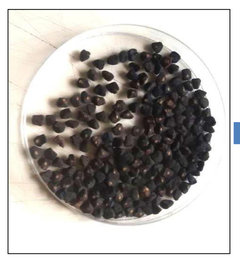
Monyet



Kokopo

Phenotyping the generated populations

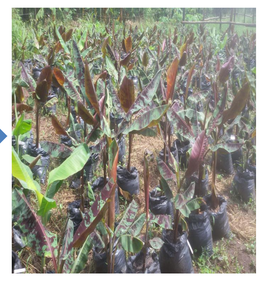
Seeds



Multiplication

Inoculation

Pot evaluation



Genotyping the segregating populations

Construction of linkage maps and QTL analysis

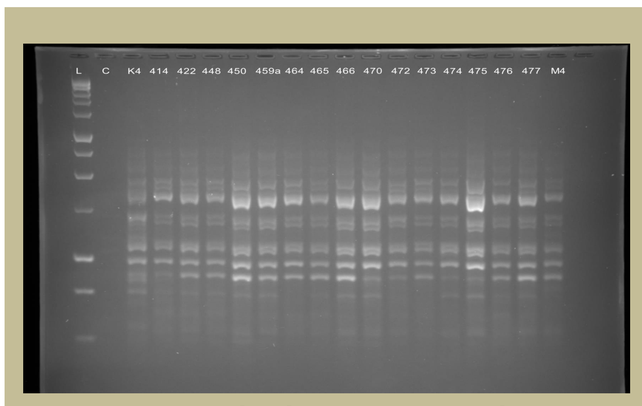
Progress so far:

Screening of the 13 parents resulted in identification of parents resistant and susceptible to *Foc* (Table 1). Parental combinations of Monyet x Kokopo, and Calcutta 4 x Mchare were chosen as potential parents of the mapping populations. Currently F1 (Monyet x Kokopo) lines are being screened in a pot experiments.

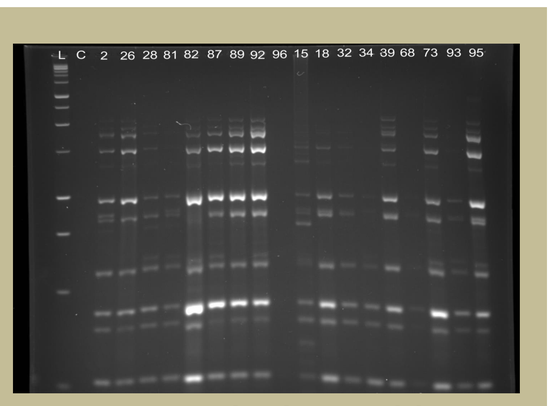
Preliminary results of genotyping the Monyet and Kokopo parents, their 13F_1's and 45 OP *Malaccensis* plants using 1 IRAP and (4 out of 20) ISSR markers revealed important polymorpism (Table 2)

Table 2. Preliminary genotyping by SSR and IRAP markers

Marker	Polymorphism	
	Kokopo X Monyet	OP-Malaccensis plants
CTC6T (ISSR)	26.7%	18.8%
CAC6T(ISSR)	27.3%	-
CTC6G (ISSR)	14.3%	18.8%
GTG6A (ISSR)	36.4%	20.0%
GLyTrev (IRAP)	43.7%	35.0%



CTC6T (Kokopo X Monyet)



GLyTrev (*Malaccensis*)

Figure 1. Example of a gel photo for IRAP and ISSR genotyping

Conclusion

The markers tested so far have potential for identifying *Foc* resistant and susceptible bananas lines of the monyet x Kokopo population. The marker results will be supplemented with SNP markers and phenotypic data for marker trait association.

References

1. Bhuvanendra, K. H., Udaya Shankar, A. C., Chandra, N. S., & Prakash, H. S. (2010). Biochemical characterization of *Fusarium oxysporum f.sp. cubense* isolates from India. *African Journal of Biotechnology*, 9(4)523-530.
2. Subbaraya, U. (2006). Potential and constraints of using wild Musa. In I. Buddenhagen, *Farmers' knowledge of wild Musa in India* (pp. 33-36.). Rome, Italy: FAO.
3. Tumuhimbise, R., Buregyeya, H., Barekye, A., Ssali, R.T., Talengera, D., Kubiriba, J., Muhangi, S., Namagembe, B., Namanya, P., Arinaitwe, G. and Tushemereirwe, W.K., 2016. Selection of cooking banana genotypes for yield and black Sigatoka resistance in different locations in Uganda. *Journal of Plant Breeding and Crop Science*, *8*(5), pp.60-7

Acknowledgements

This work is funded by the **Bill & Melinda Gates Foundation**

Contacts

Ivan Arinaitwe Kabiita
ivanarinaitwe@yahoo.co.uk

Phenotyping of a Diploid Banana Population for Resistance to *Radopholus similis*

Batte M.[1,2], J. Kisaakye[1], B. Uwimana[1], R. Swennen[4,5,6], D. Coyne[3], H.P. Hovmalm[2], M.Geleta[2], R. Ortiz[2]

1 International Institute of Tropical Agriculture, Kampala, Uganda
2 Swedish University of Agricultural Sciences, Alnarp, Sweden
3 International Institute of Tropical Agriculture, Nairobi, Kenya
4 International Institute of Tropical Agriculture, Arusha, Tanzania
5 Katholieke Universiteite, Leuven, Belgium
6 Bioversity International, Heverlee, Belgium

Introduction

The burrowing nematode (*Radopholus similis)* is one of the main banana pests, causing a yield loss of up to 50%. Breeding for host plant resistance is the most sustainable option to control this pest. Banana conventional breeding takes about two decades to produce a cultivar. Therefore, molecular tools are needed to speed up the process through marker assisted selection. This study aims at mapping quantitative traits associated with resistance to *R. similis* in a diploid banana population. We report here about genetic variation of the phenotypic data for 137 genotypes of the population.

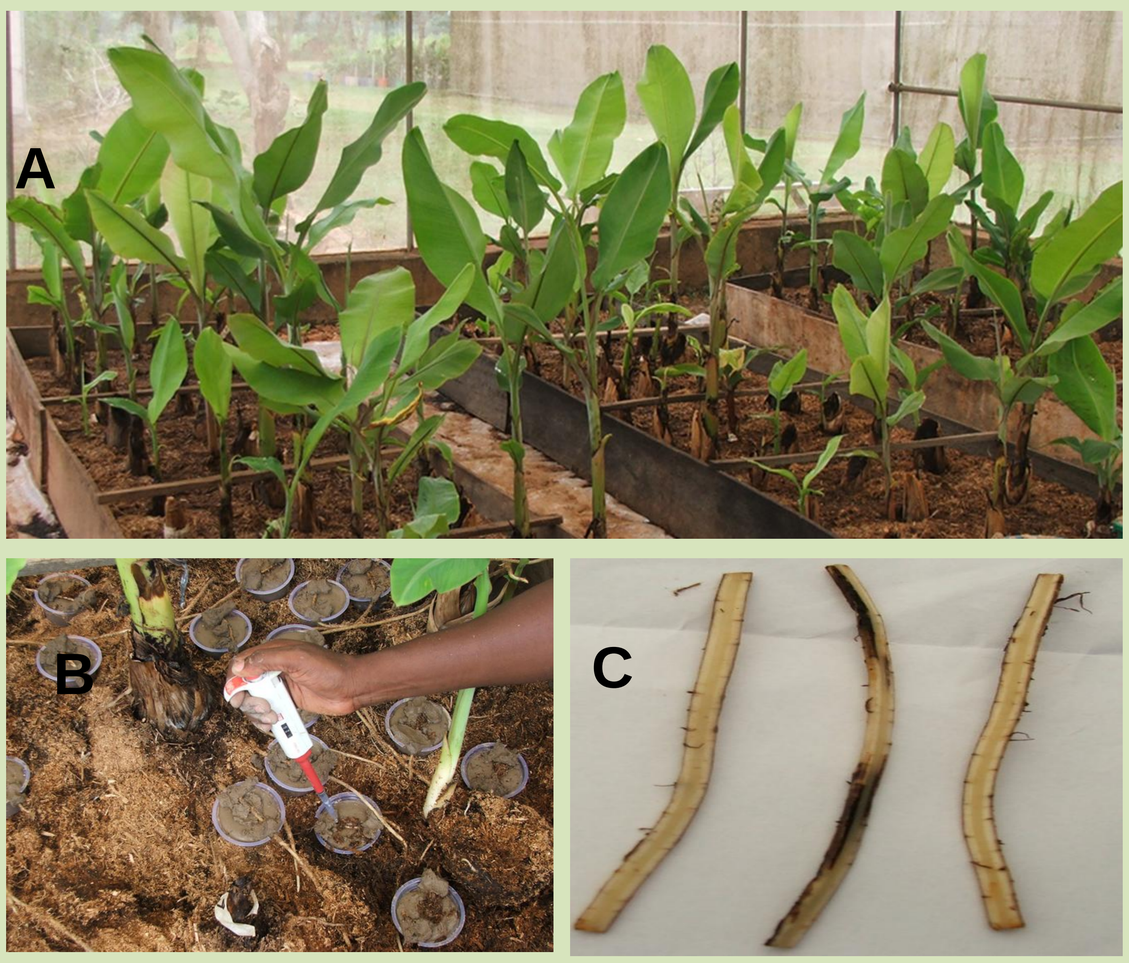


Figure 1. Experimental set up in the screen house (A), inoculation 8 weeks after planting (B), scoring for root necrosis (C)

Materials and Methods

A diploid population derived from crossing Kasaska and Borneo was phenotyped in the screenhouse in a randomized complete block design with 3 replications (Figure 1A). The experiment was set up in series of 33 plants, including the parents, and the susceptible (Valery) and resistant (Km5) controls. Four to six roots were inoculated with 50 nematodes 8 weeks after planting (Figure 1B). Each experiment was terminated 8 weeks after inoculation. Phenotypic data were recorded on percentage root necrosis and total nematode count per inoculated root. Data were analyzed using SAS software and genotypes were compared with resistant and susceptible checks using Dunnet's test.

Results and Discussion

There was a significant positive correlation (r=0.56 at P<0.001) between nematode count and percentage root necrosis. This was close to what was observed by Moens et al. (2001) and Nega and Fetena (2015), who found a significant correlation between *R. similis* counts and root necrosis, ranging from 0.62 to 0.75 in root samples of banana plantations. Total number of nematode showed a continuous variation. Some genotypes are as resistant as Km5, and others as susceptible as Valery with a whole range in-between (Table 1). Such variation is fit for QTL mapping, a step toward MAS in breeding for resistance to *R. similis* in banana. Genotyping of the population with 16 SSRs revealed pollination mistakes which resulted in admixture. Once SNP genotypic data are available, QTL mapping will be assayed using the GWAS method.

Table 1. Comparison of mean total nematode count for the genotypes with the controls using Dunnet's test

Comparison with Valery	Comparison with Km5	Host response	Number of genotypes
Significantly different	Not significantly different	Resistant	28
Significantly different	Significantly different	Partially resistant	7
Not significantly different	Not significantly different	Inconclusive	75
Not significantly different	Significantly different	Susceptible	27
Total			137

Conclusion

The screening results show that the population segregates for *R. similis* resistance and therefore suitable for use in QTL mapping.

Acknowledgements

Banana research programme at the National Agricultural Research Organization (NARO).
Bill and Melinda Gates Foundation (BMGF)

References

Moens T., M. Araya & D. De Waele. 2001. Correlations between nematodes numbers and damage to banana (*Musa* AAA) roots under commercial conditions. Nematropica 31:55-65.

Nega G., Fetena S. 2015. Root necrosis assessment of plant parasitic nematodes of banana (*Musa spp.*) at Arbaminch, Ethiopia. Journal of Biology, Agriculture and Healthcare 5: 76-80.

Building a breeding database for African banana programs: Musabase

Guillaume Bauchet[1]
Alex Ogbonna[1]
Bryan Ellerbrock[1]
Nick Morales[1]
Naama Menda[1]
Brigitte Uwimana[2]
Allan Brown[2]
Trushar Shah[2]
Margaret Karanja[2]
Robooni Tumuhimbise[3]
Inge van den Bergh[5]
Rhiannon Crichton[5]
Rony Swennen[4]
Lukas Mueller[1]

[1] *Boyce Thompson Institute for Plant Research (BTI), Ithaca, New York, U.S.A.;*

[2] *International Institute of Tropical Agriculture (IITA)*, Ibadan, Nigeria

[3] *National Agricultural Research Organisation (NARO)*

[4] *KU Leuven, Leuven, Belgium*

[5] *Bioversity International, Rome, Italy*

Introduction

The East African Highland Banana project aims to significantly accelerate genetic improvement of banana and unlock the full potential of Mchare and Matooke, two major cooking banana type central to food security and livelihoods across Eastern Africa.

Musabase[1] (figure1) was created to centralize information tracking, genotypic and phenotypic data, and provide trial analyses. Banana has several specificities such as Cycles (or Ratoon) and multiple ploidy level that require particular software modifications.

Defining a controlled vocabulary with banana breeders is an important step to standardize data collection and storage in Musabase. Musabase is hosted at SGN lab[2].

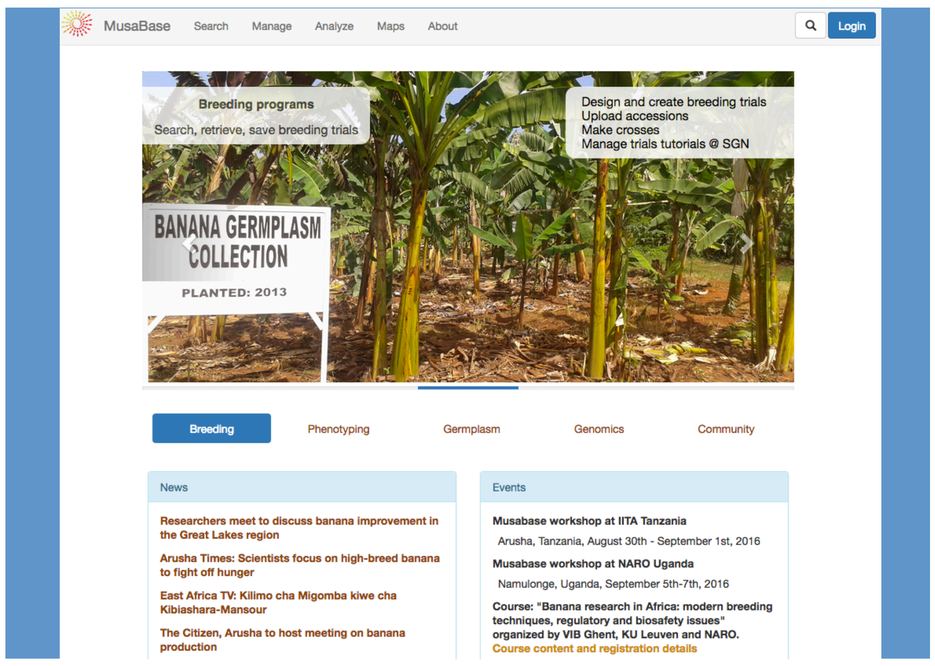


Figure1: Musabase homepage: www.musabase.org

Main objectives of Musabase:

-Develop a data management routine for banana breeding data collection.

-Provide relevant analysis for breeding decision.

-Initiate crossings and nurseries for next generation.

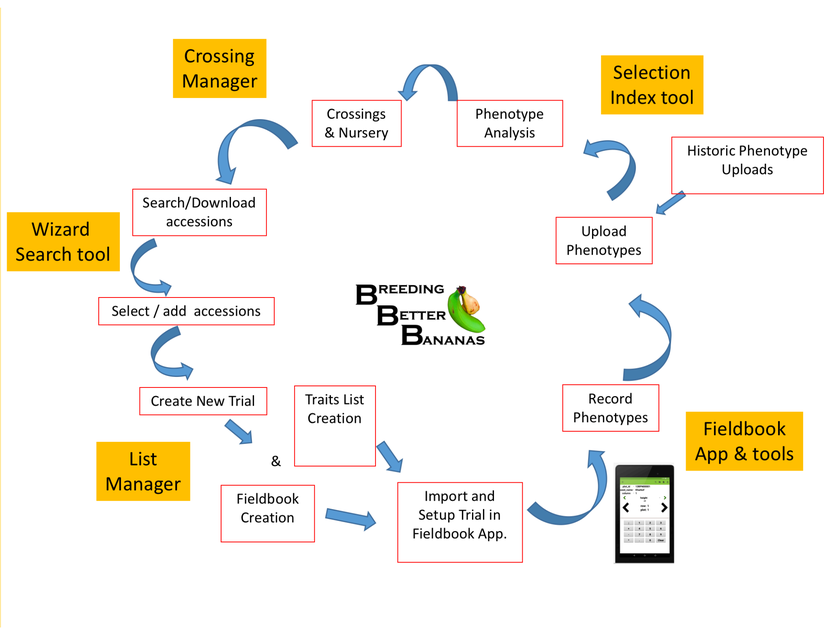


Figure2: Tools and pipeline for phenotypic data collection, trial and crossing management

Materials and Methods

Using Musabase and the Fieldbook app[3] data collection pipeline (Figure 2)

Step 1: Search and select existing germplasm using the Wizard search and/or add accessions using the List manager.

Step 2: Select the desired accession set to create a trial with a relevant design.

Step 3: Generate Field book templates and export them from Musabase to an android tablet device.

Step 4: Perform field data collection and upload Fieldbook template back to Musabase.

Step 5: Visualize trial data and accession performance through descriptive statistics of collected phenotypic traits .

Step 6: Analyze accession performance for multiple traits using the selection index and make list of accessions to advance.

Step 7: Design the next crossing blocks and trials including your findings .

--

Results and Discussion

-In 2016, two workshops where held in Tanzania and Uganda to train field technicians and breeders. The breeding data collection using tablets and Musabase test site (www.musabase-test.sgn.cornell.edu) was initiated at Arusha and Sendusu IITA stations as in NARO Uganda.

-Existing banana ontology was reviewed, updated with breeders and uploaded to Musabase test site (http://musabase-test.sgn.cornell.edu/tools/onto/), allowing phenotypic data storage, 97 breeding variable were defined.

-Banana breeding includes specificities such as multiple ploidy levels and plant cycles Ploidy information level was added to the database. Cycles can now be handled using the fieldbook.

-Musabase currently contains about 1100 germplasm entries. When available, accessions in Musabase are linked to the Musa Germplasm Information System (MGIS) https://www.crop-diversity.org/mgis and provide user more details.

-For germplasm characterization, it is now possible to upload simultaneously phenotypic data together with photo taken on a plot.

These developments in Musabase make the database able to handle banana breeding specificities.

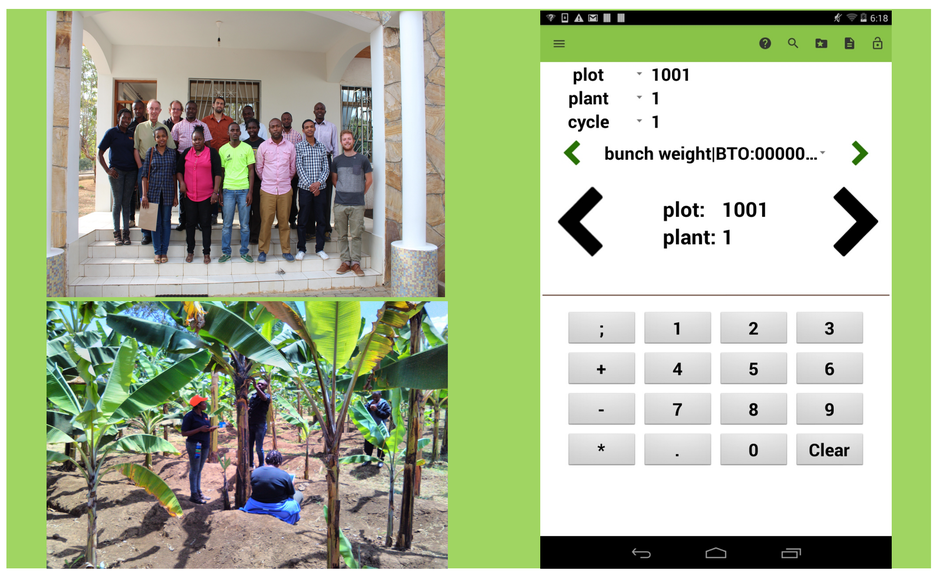


Figure3: Workshop at IITA Arusha, August 2016, phenotyping session using the Fieldbook app.

Conclusions and Recommendations

Musabase is now available to the community and functional for banana breeding data collection.

Upcoming trainings and development could cover other database components such as crossing block and nursery management, phenotypic data analysis and genomic data storage.

Recommendations can be formulated on different aspects such as:

Germplasm:

Using Musabase can be beneficial for breeding germplam curation across stations. Breeders can work collaborate through the database to exchange trial information or germplasm list

Ontology:

The development of the ontology will be continued, adding new traits on breeder's demand.

Pedigree and Trial data:

Musabase offers an opportunity to take advantage of historical trial and pedigree data through it's efficient and user friendly search wizard.

References

1. www.musabase.org and www.musabase-test.sgn.cornell.edu
2. Fernandez-Pozo, N., The Sol Genomics Network (SGN)--from genotype to phenotype to breeding, *Nucleic Acids Research*, 2014.
3. http://wheatgenetics.org/fieldbook

Contacts:

gjb99@cornell.edu
lam87@cornell.edu