PhD Research Progress Report

TITLE: Floral Biology and Crossability Studies for Improving Matooke and Mchare Banana (*Musa* ssp.) Breeding in East Africa

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Timeline of study: Aug 2015 – Jan 2020

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Research Objectives

1. To determine the time of flowering opening and influence of weather on flower opening so as to determine the best plausible time for controlled pollination
2. To develop more efficient methods for in vitro and *in vivo* germination of banana pollen for quick evaluation of male fertility and stigma receptivity enhancement
3. To determine the effective pollination period in East African cooking banana for effective controlled pollination
4. Ascertain the seed set patterns in East African cooking bananas and influence of weather on seed set as well as get insights on post-zygotic barriers
5. To develop appropriate *in vivo* pollination techniques that can be adopted for increase of seed set and/or overcome female sterility

Achievements

- Results from objective 2 have been accepted for publication as,
  "Simple sugars perform better than sucrose for *in vitro* and *in vivo* germination of banana pollen" in *ActaHorticulturae* journal

- Two paper have been written from results of objective 4;

1. Seed set patterns in East African Cooking Bananas are asymmetric in bunches and fruits

2. Weather influences seed set in East African cooking bananas (*Musa* spp.) before, during and after pollination

The two papers are ready for submission to *'Botanical Studies'*

- Final thesis compilation ongoing.

Background

Matooke and Mchare cultivars of banana play a very important socio-economic role in the livelihoods of smallholder farmers of the great lakes region in East Africa. However, pests and diseases significantly reduce the crop yield thus, directly affecting the livelihoods of the communities involved in banana production. Low female fertility in bananas is the biggest hurdle for breeding these bananas. Some land races especially in the Matooke category have been rendered "infertile" and their breeding potential is unknown.Breeding programs rely on residual fertility in cultivars that have been selected for breeding. Residual fertility yields about 0-25 seeds after pollination (in EAHBs) yet the potential can be over 30,000 seeds per bunch. The aim of this PhD research is therefore to increase seed set in East African cooking bananas (EACBs) and subsequently hasten theirbreeding pipelines.Overcoming sterility will in essence broaden the parental base to include the previously unutilized land races of EACBs whereas give breeders a wide progeny base for selection. Having deeper understanding of sterility will also enable banana improvement programs to use it to their advantage as it is a prerequisite in the developed hybrids for consumers.

Summary of the study

- Results from the flower opening study revealed that flower opening in bananas starts late afternoon and continues through the night up to dawn in some genotypes.
- Total time of bract opening events in female flowers was noted to be longer than male flowers, besides, male flowers start opening at a later time compared to female flowers.
- Glucose was found to germinate more banana pollen faster compared to fructose, sucrose and the combinations of the three energy sources. Glucose had 49% average pollen germination of Calcutta 4 and Tmpx8075, fructose had 34% germination while sucrose had 26% after 3 hours of incubation. Use of glucose for *in vitro* banana pollen germination studies will therefore give more accurate results.
- When liquid pollen germination media (PGM) was applied on banana stigmas, receptivity was enhanced. Therefore use ofPGM to enhance fertility is expected to increase seed set especially in crosses that have low compatibility even if pollen viability is high.
- Pollen tube evidence was found even in the midsection of fruits from proximal hands yet literature has it that seed set is biased to middle and distal hands and the distal end of the fruit. This strongly suggested ovule abortion. Ovules from pollinated fruits noticeably grow larger and started to wither after about 10 days compared to those from unpollinated fruits which wither after a week.
- Flowers of two Matooke, two Mchare and Calcutta 4 were therefore pollinated at one day intervals from a day before opening to 4 days after opening. There was evidence of ovule growth up to four days after flower opening implying that stigmas can remain receptive four days after flower opening.
- Seed set was found to be biased in midsection hands and distal hands in all bunch size categories of EACBs. Seed set was also biased towards the distal end of the fruit. Estimate of distances pollen tubes covered in different cultivars strongly suggest thetube reach ovules but they abort after fertilization.
- Larger bunches were found to be more fertile with higher pollination success compared to smaller bunches.

- Weather was found to affect seed set before, during and after pollination. The most critical stage that had the highest association of weather with seed set was 15 days before pollination.
- Using correlation matrix PCA, average temperature and maximum temperature were found to have the highest variability in seed set data. A plot of correlation coefficients against time of flower development in two Mchare and one Matooke revealed a similar pattern which can explain the pattern in other banana cultivars.
- Pollination of EAHB with PGM to enhance stigma receptivity increased seed set by 42% in Enzirabahima, 57% in Mshale and 136% in Nshonowa. Since there was evidence of ovule abortion, ovule culture was tried but it was futile in EAHBs. Hormonal treatments including salicylic acid, ABA, TIBA, and cytokinin inhibitors have all been futile in overcoming sterility.

Conclusion / next steps

There are a number of factors that affect seed set in banana. The study revealed that flower opening starts late afternoon, stigma receptivity can be a hindrance to seed set, there is strong evidence of ovule abortion and that weather affects seed set before and after pollination. A holistic approach to overcoming sterility in bananas will therefore have to be used. This will include pollinating at the right time, enhancing stigma receptivity, ensuring optimum weather conditions at the pollination station during bunch development and use of plant growth regulators. Since seed fertility varies between cultivars and seasons, it implies that finding the right pollination technique can fundamentally yield seed in any banana genotype. Future studies therefore have to put all known factors that affect seed set into consideration. Banana sterility is a perquisite in hybrids released to consumers thus it is paramount that breeders understand and use it to their advantage.