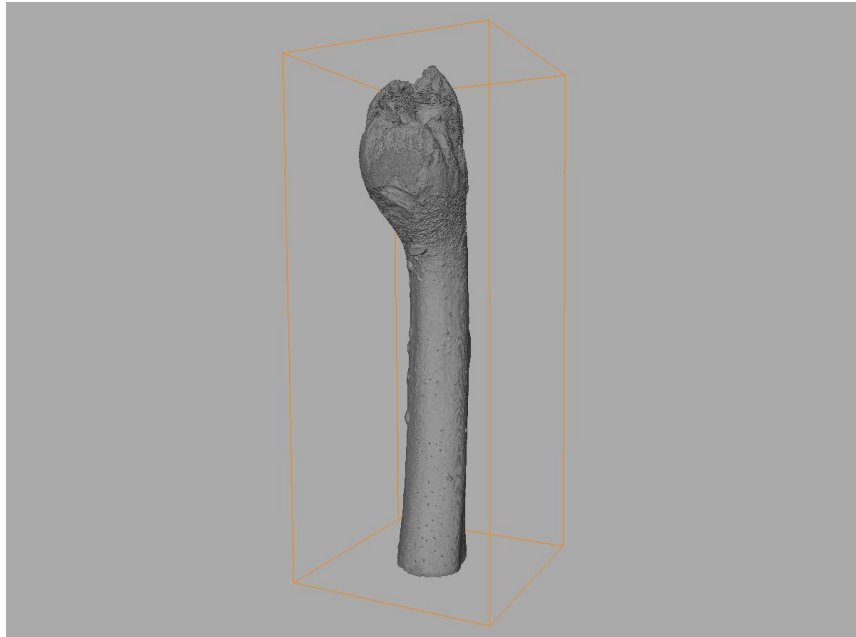


Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa

A project supported by the Bill & Melinda Gates Foundation

PROGRESS NARRATIVE/ TECHNICAL REPORT by KU Leuven

Reporting Period: October 1, 2016 - March 30, 2017



Micro-tomography of style and stigma

1 Executive Summary

This report provides information on the development of micro-tomography imaging of style and stigma.

KU Leuven is a subcontracted partner of IITA (Principal Investigator) and participates in work package 1 viz. Banana Breeding.

The role of KU Leuven is to develop a methodology to study bract opening and flower/stigma development under field conditions to allow optimization of pollination to get higher seed set. This method was first developed on greenhouse plants at KU Leuven and transferred to the field

We investigated now via micro-tomography the styles of flowers at anthesis and at day -2 of greenhouse grown Cavendish. 11,575 pictures were made amounting to approximately 650,000 KB observations. At anthesis the stigma is clearly trilobular but an intercellular canal in the style could not be seen. Pending more observations, we hypothesize that pollen tubes have not much room to grow in the style.

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2 Primary Outcomes, Intermediate Outcomes, Outputs and Milestones

Table 1 below provides information on primary outcomes, intermediate outcomes, outputs and milestones/targets.

Table 1: Framework and Results Tracker for all Work Packages wherein KUL participates

	Primary Outcomes		Intermediate Outcomes		Outputs	Targets/ Milestones				
						YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
1	Primary Outcome 1 Matoke and Mchare breeding pipeline performance increased by a 15-20% higher production of seeds facilitating a wider selection for the delivery of pest and disease resistant hybrids	1.1	Intermediate outcome 1 Female fertile cultivar profile widened in EAH bananas and Mchare : from 14 to 37 cultivars	1.1.1	Output 1 Floral development stages at and after anthesis characterized in 2 Matoke varieties (IITA-NARO-KU Leuven)	Inventory of seed set in Matoke varieties and selection of 2 Matoke varieties that are already field planted Floral development stages in 2 Matoke varieties identified and characterized Pictorial catalogue on flower developmental initiated	Characterisation of floral development stages of 2 Matoke varieties (weekly basis) Pictorial catalogue on flower developmental finalized Microscopy: stigma development and pollen tube growth correlated with flower developmental stages	Floral development stages of 2 Matoke varieties characterised at the moment of pollination (daily basis) Microscopy: stigma development and pollen tube growth correlated with flower developmental stages (weekly basis)	Floral development stages of 2 Matoke varieties characterised at pollination (daily basis)	-
				1.1.3	Output 3 Floral development stages at and after anthesis characterized in 2 Mchare varieties (IITA-NARO-KU Leuven)	Inventory of seed set in Mchare varieties and selection of 2 varieties for in vitro multiplication and field planting Floral development	Characterisation of floral development stages of 2 Mchare varieties (weekly basis) Pictorial catalogue on flower	Characterisation of floral development stages of 2 Mchare varieties (weekly basis) Pictorial catalogue on flower developmental	Characterisation of floral development stages of 2 Mchare varieties (at pollination) Completion of microscopic study on stigma development and	Characterisation of floral development stages of 2 Mchare varieties (at pollination) Comparison of Matoke and Mchare flower/stigma/

	Primary Outcomes		Intermediate Outcomes		Outputs	Targets/ Milestones				
						YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
						stages in Mchare in the field collection identified/characterised	developmental stages initiated	finalized Microscopy started on stigma development and pollen tube growth with simultaneous flower characterisation	pollen tube growth with simultaneous flower characterisation	developmental stages

3 Results to Date

Table 2 below provides the progress made by KU Leuven

Table 2: Progress

	Primary Outcomes		Intermediate Outcomes		Outputs	Targets/ Milestones	Progress
ri	Primary Outcome 1 Matoke and Mchare breeding pipeline performance increased by a 15-20% higher production of seeds facilitating a wider selection for the delivery of pest and disease resistant hybrids	1.1	Intermediate outcome 1 Female fertile cultivar profile widened in EAH bananas and Mchare : from 14 to 37 cultivars	1.1.1	Output 1 Floral development stages at and after anthesis characterized in 2 Matoke varieties (IITA-NARO-KU Leuven)	Microscopy: stigma development and pollen tube growth correlated with flower developmental stages (weekly basis)	Variance: 50% Microtomographical method developed on Cavendish But need to observe flowers at different developmental stages
				1.1.3	Output 3 Floral development stages at and after anthesis characterized in 2 Mchare varieties (IITA-NARO-KU Leuven)	Microscopy: stigma development and pollen tube growth correlated with flower developmental stages (weekly basis)	Variance: 50% Microtomographical method developed on Cavendish But need to observe flowers at different developmental stages

In previous reports, we provided info on flowering in banana. We demonstrated that more than one bract opens and that mostly the curling of the bract happens at night. We also showed that when the bract opens, stigmas can be either immature or are in the process of degeneration. We showed that the opening of the bracts follows the same patterns in the field as in the greenhouse where the camera-based recording was developed. Therefore we can tentatively conclude that pollination should be done on more than 1 hand (as sometimes 2 hands open) and that they should be pollinated during 2 consecutive days. Pollination should be done as early as possible in the morning, as bracts open normally at night.

Now we have focused on developing another technology to study the morphology of the style via micro-tomography. Indeed pollen germinate on the stigma and the pollen tube needs to grow fast through the style into the ovary where it grows towards an ovule. And we want to know whether there is intercellular space, if any, to allow pollen tubes to grow.

It is generally known that very few seeds develop per bunch and if so they are mostly found at the apical side of the fruits. There might be several reasons like for example the long length of the style. Based on previous observations in plantain at IITA in the 1980s, we now explore whether we can confirm the existence of a large intercellular canal along the entire length of the style and observe its development over time.

We therefore investigated via micro-tomography the styles of flowers at anthesis (Figure 1 and 3) and at day -2 of greenhouse grown Cavendish. We ignored possible variation between the flowers of the top and bottom row of the hand (Figure 2) as the focus was on the development of the technology, and time effect of flowering.



Figure 1 Freshly harvested bunch of Cavendish with the first bract opening(day 0)



Figure 2 Fresh flowers of hand 1 at day 0. Green (right) ovaries come from flowers in the top row of the hand 1, while yellow (left) ovaries come from the lower row of hand 1



Figure 3 Close up of flowers at anthesis (day 0)

11,575 pictures were made amounting to approximately 650,000 KB observations. These allowed us to reassemble the shape of the style and stigma of flowers at anthesis and at -2 days (Figure 4).

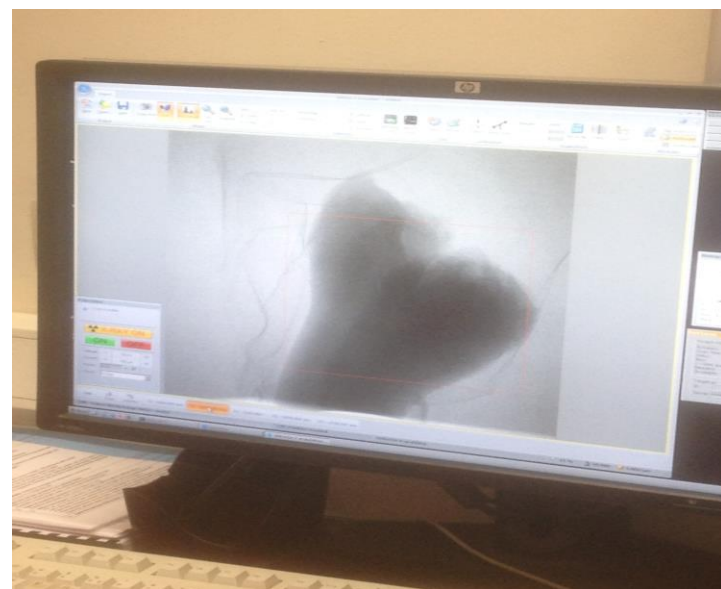


Figure 4 Computer imaging after reassembling pictures from micro-tomography

At anthesis the stigma is clearly trilobular (Figure 5-6) but an intercellular canal in the style could not be seen (Figure 5-6). This canal in the style could also not be seen at day -2.

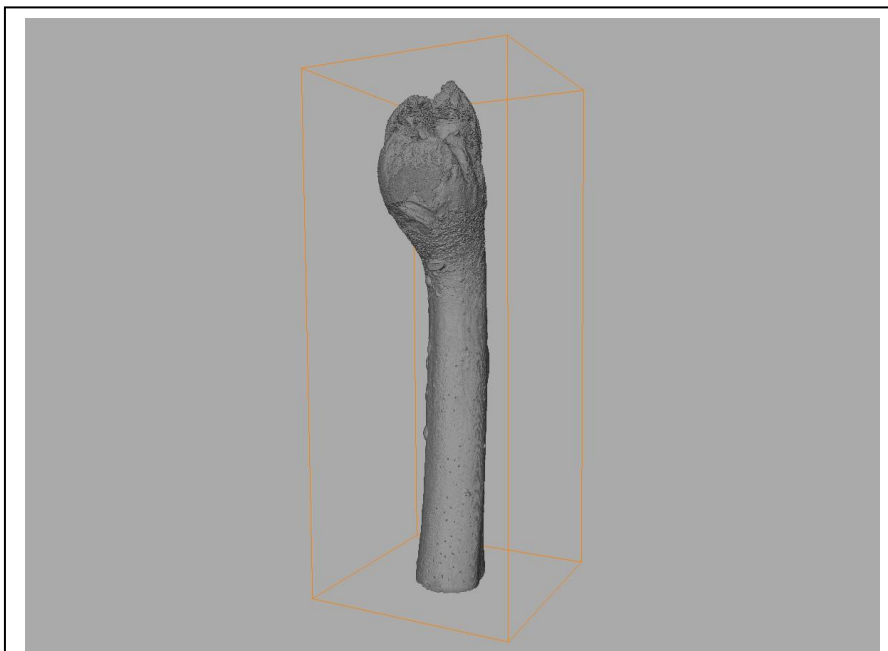


Figure 5 Longitudinal image of the style and stigma at day 0; note the trilocular structure of the stigma

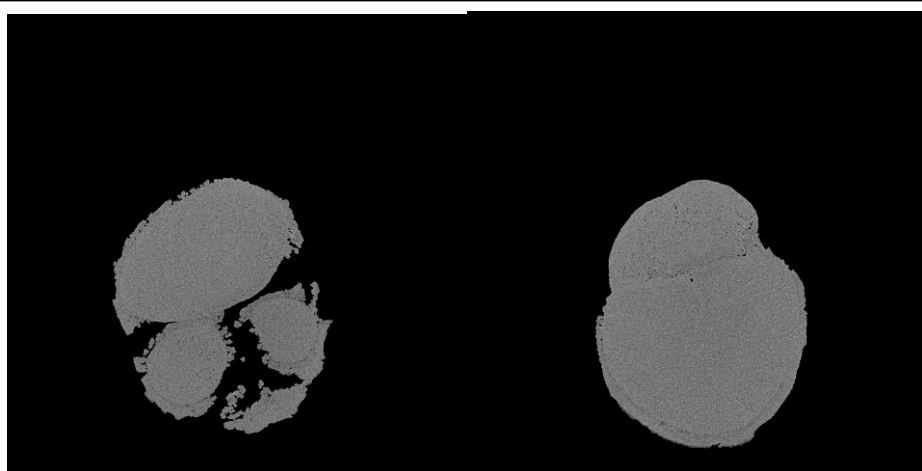


Figure 6 Cross section at day 0 at the level of the stigma (left) and at the level of the style (right)

Therefore pending further observations on other plants of Cavendish at anthesis, and at day -1, -2, +1, +2, we need for the moment to hypothesize that pollen tubes have not much room to grow in the style.

4 Challenges Encountered

As the technology was developed at KULeuven with very expensive equipment, we are bound to use banana varieties that grown in the greenhouse and are rather short. Therefore the observations were made on Cavendish and not on Matoke nor Mchare.

5 Lessons Learned

We should not have harvested the entire bunch, but hand per hand, to make observations over different days.

6 Work Plan

The overall work plan, including timeline was not adjusted.

7 Other Relevant Project Information

None.