Sub-theme:

Pest Diagnostics in Phytosanitary Systems

Title:

ON-FIELD DETECTION OF THE GENUS *PECTOBACTERIUM* AND *DICKEYA* CAUSING BLACK LEG IN TAITA TAVETA COUNTY, KENYA

Presented by:

Cyrus M. Micheni
Bramwel Wanjala
Dr. Ruth Amata – P.I

Introduction

- In Kenya, Potato (*Solanum Tuberosum*) is the second most important food crop after maize
- Contributes to 32% of overall dietary energy consumption
- Grown by 800,000 small-scale farmers
- Generating employment for an estimated 2.5 million people along the value chain

- Despite its importance potato yields are low at less than 20t/ha in Africa as compared to over 40t/ha in other continents e.g. N. America.
- Recently potato production has been hampered by several biotic constraints including brown rot caused by *R. solanacearum* and blackleg disease of potatoes
- Potato blackleg is caused by two bacteria in the genus *Pectobacterium spp* and *Dickeya spp*.
- In Kenya *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliense*, *P. wasabiae* and *D. Dianthicola* have been reported.

Introduction cont'

Symptoms

Tubers – soft rot lesions which are discolored, affected tissue becomes black and slimy

Stem – Black coloration and/ or vascular browning

Transmission

- Primary source of bacteria is through potato seed

Other sources – Aerosols, running water, rain

Problem Statement

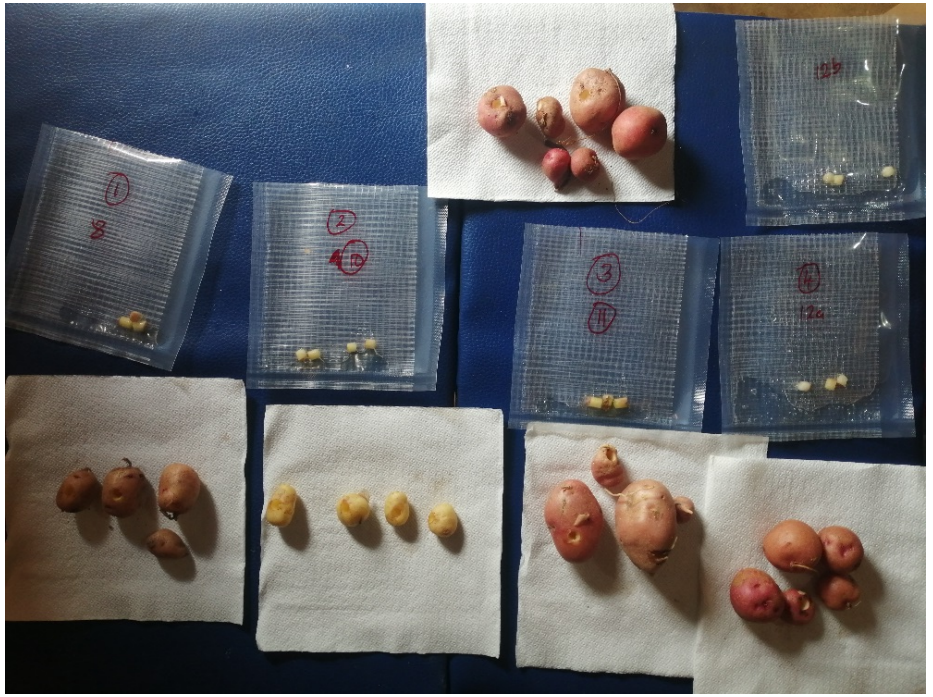
- This disease is spread mainly through movement of asymptomatically infected tubers
- It could lead to introduction of causal pathogens into disease free regions resulting in new disease outbreaks
- There's need to determine status of this disease in potato growing counties such to explore possible disease free areas for clean seed production
- Research is needed to establish the after harvest pathogen infectivity period in affected fields and tolerant varieties for proper management of the disease

Justification

- Laboratory based detection tools that have been used routinely for the detection of these pathogens are time consuming
- Further, there is a risk of samples deterioration in cases where the testing facilities are far apart from the sampling point
- In addition, the current testing regime has a high turn around time due to lengthy sample processing time
- Simple, cost effective, accurate and rapid assays are needed for detection surveys, monitoring of the phytosanitary status of pre-basic seed production and at ports of entry in quarantine or certification program

Objective

Evaluating the operability of on-site detection for the genus *Pectobacterium* and genus *Dickeya* using LAMP assay



Methodology

Survey and Detection

- Sample collection in Taita-Taveta County in January 2020
- Disease detection using PCR

LAMP validation in the Lab

- Reproducibility
- Repeatability
- Limit of detection (LOD)

On-site LAMP validation

- Precision
- Accuracy
- Range Validation/ Verification

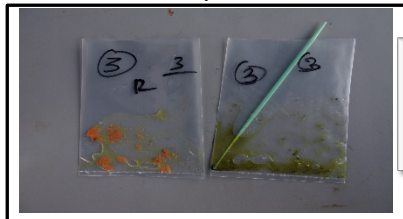
Methodology

- Both *Dickeya spp* and *Pectobactrium spp* were identified from the survey.
- *D. dianthicola* and *D. solani* ; *P. wasabiae* and *P. carotovorum* subsp. *brasilience*
- LAMP assay was designed using genus primers to be able to pick all the species present

LAMP primers	**Sequence (5' - 3')**
	Genus *Dickeya*
Oligonucleotide Primer	**Sequence**
Mglc-F3	TCGCTATCGGCGGTAACC
Mglc-B3	ACCACCGGCAAAAGACAC
MglC-FIP	GCCGGACAGCATATACACCCAAGGAAGCCGCCAAAGTGTC
MglC- BIP	CCTTCGGCGGTATGCTGGAAGCGATGGCGTCAAGTTCGTA
MglC-Loop	CGGTAGTGCCACTAACAACCTGG
MglC- Loop probe	56-FAM/ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGATTTTCGGTAGTGCCACTAACAACCTG
Quencher probe	TCGGCATCCGCATCCGCATTCGCATCCGGGTCCTCAGCGT/3BHQ_1/
	Genus *Pectobacterium*
P4HA-F3	CATGAAACCCCGTTCCAGT
P4HA-B3	AAGGCGTCAGAGGTCAGC
P4HA -FIP	CGCCGTTACGTTCACGGTAGTTTATGGCGTAACAGCAGCATC
P4HA-BIP	TTCCTTTAGCCTCCGGCAAAGTTCGTTACACATTCCCAGCC
P4HA -Loop	GGAACTCATGGGCAAGCG
P4HA- Loop probe	/56-FAM/ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGAGGAACTCATGGGCAAGCG
Quencher probe	TCGGCATCCGCATCCGCATTCGCATCCGGGTCCTCAGCGT/3BHQ_1/

Methodology cont'

Cut three stem pieces/ three tuber pieces



Grind in 1ml PEG buffer (sap/buffer)

Dilute sap 1:10 and add to Isothermal master mix

Run LAMP - Real time Results

3-5 Mins Sample preparation

4 - 30 mins time to results

Results

SYMPTOMATOLOGY

Black coloration on stem

Stem lodging

Lack of tuber production

Leaf rolling and chlorosis

Results cont'

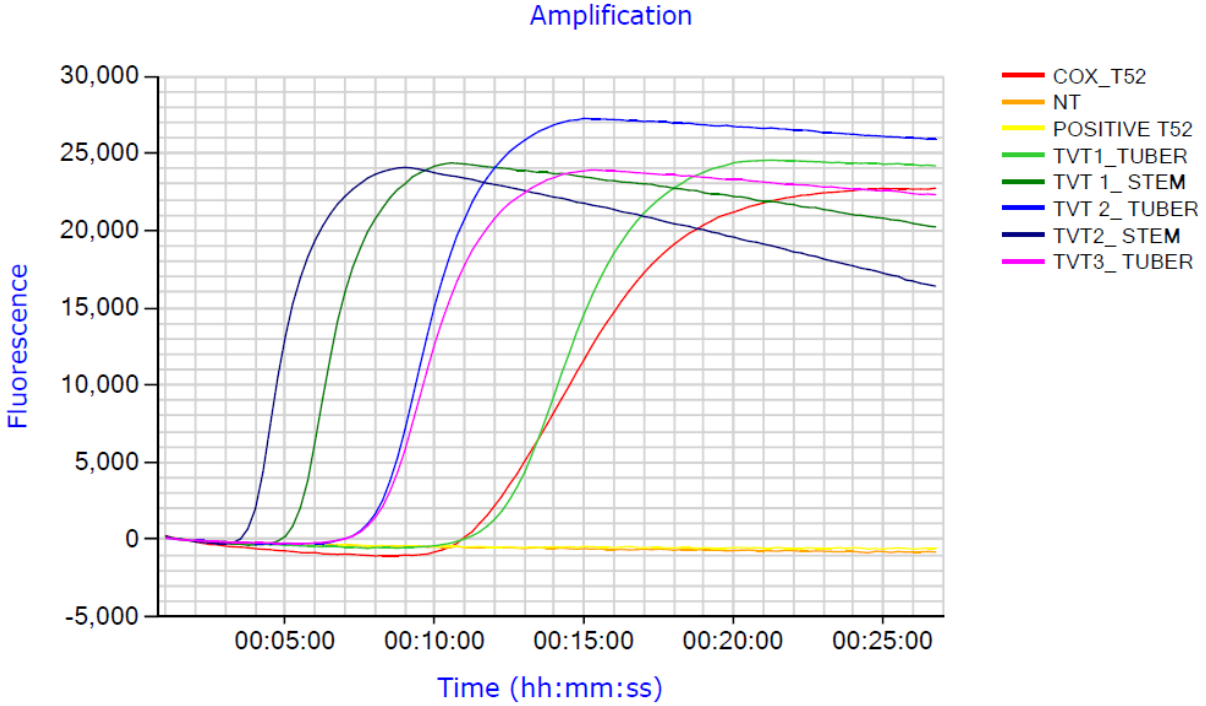
- A total of 8 farms were visited
- 20 tuber and 14 stem samples were tested for both pathogens

		Genus *Pectobacterium* +ve	Genus *Dickeya* +ve
Plant Part	**Tuber**	9	14
	Stem	6	8

- Earliest time to positivity for the genus Dickeya was 4.30 and 6.30 minutes for stem and tuber respectively
- Earliest time to positivity for genus Pectobacterium was 6.00 and 8.00 minutes for stems and tubers respectively.

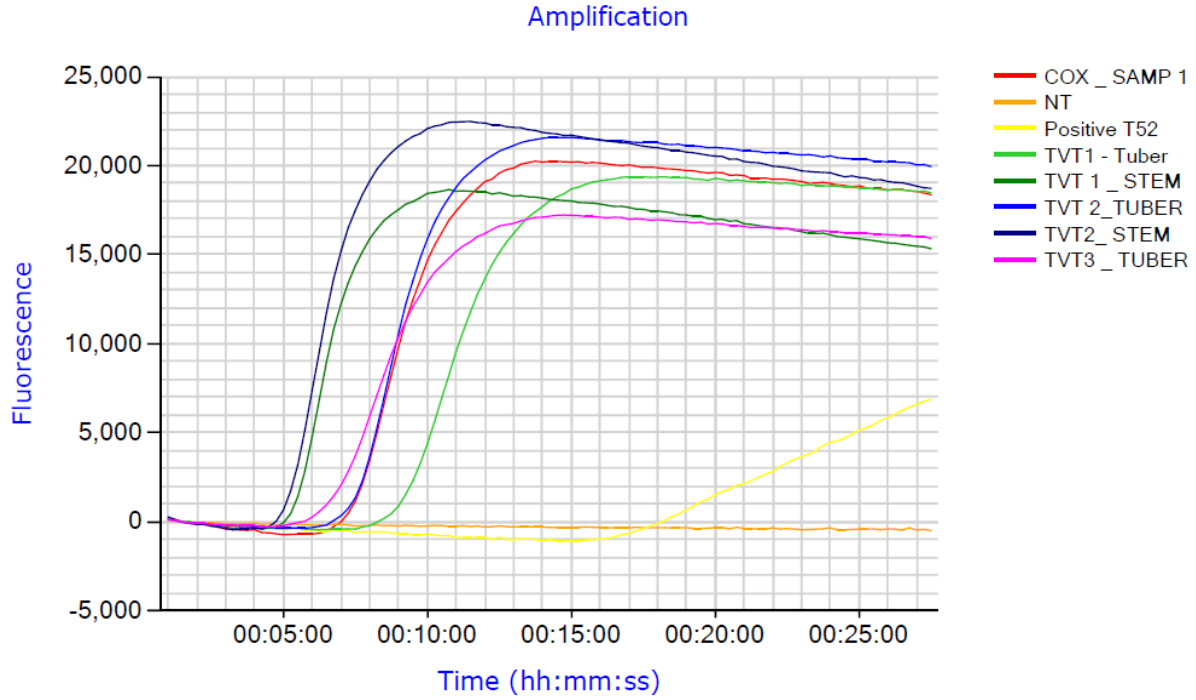
Results cont’

Genus *Dickeya*



Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	COX_T52	12:45
2	NT	
3	POSITIVE T52	
4	TVT1_TUBER	13:30
5	TVT 1_ STEM	06:00
6	TVT 2_ TUBER	09:15
7	TVT2_ STEM	04:30
8	TVT3_ TUBER	09:15

Genus *Pectobacterium*



Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	COX _ SAMP 1	08:15
2	NT	
3	Positive T52	
4	TVT1 - Tuber	10:15
5	TVT 1 _ STEM	06:00
6	TVT 2_TUBER	08:15
7	TVT2_ STEM	06:00
8	TVT3 _ TUBER	08:00

Conclusion

- LAMP assay offers a quick and sensitive on-site diagnostic tool for the detection of Blackleg
- LAMP is highly specific and has an advantage over conventional PCR in that it utilises crude DNA for amplification
- LAMP is highly versatile tool in that it can be used on cultures, extracted DNA and crude extracts from the stem and tuber

Recommendations

- This robust and cost effective tool could be used by Phytosanitary agencies to;

i. Conduct Routine surveillance for the detection of both genus *Dickeya* and *Pectobacterium*

ii. Inspect seed stock production farms to prevent unwanted introduction of Blackleg to farmer fields

iii. Rapid testing at ports of entry

iv. Field testing on farmer fields

Acknowledgements

Theme: *"Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*

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