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

Sub-theme:

Pest diagnostics in phytosanitary systems

Rapid molecular identification of thrips transmitting maize infecting viruses in Murang'a County of Kenya

By

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Introduction

Thrips as vectors

- 5800 thrips species have been described worldwide
- Size ranges 1.5 - 3 mm in length
- Thrips transmit tospoviruses and machlomoviruses
- Five genera are viral vectors: Thrips, Frankliniella,
- Scirtothrips,   Microcephalothrips and Ceratothripoides

Introduction cont’

Thrips transmit *Maize chlorotic mottle virus* (MCMV) than can co-infect with a cereal Potyvirus (SCMV, MDMV, WSMV) to cause maize lethal necrosis (MLN)

Sugarcane mosaic virus **By aphids**	**Maize chlorotic mottle virus** **By thrips**	**Maize lethal necrosis** **(Severe chlorosis)**
		

Problem Statement

- *Frankliniella williamsi* and *F. occidentalis* (known vectors of MCMV) occur in all maize-growing regions of Kenya and Uganda (Mahuku et al 2015)
- Virus transmission competence vary with thrips species (Ananthakrishnan and Annadurai, 2007) which necessitates exploration of their genetic diversity.
- Studies of Western flower thrips, *F. occidentalis* on French beans in Kenya have shown genetic differentiation and host preference (Hondelmann et. al., 2017)
- The genetic diversity of thrips infesting maize as well as their competence in MCMV transmission has not been conclusive in Kenya

Justification

- ❑Identification of thrips can be difficult because they are tiny with little morphological differentiation (Brunner et al., 2002; Murai and Toda 2001)
- ❑Molecular identification complements morphological analysis to help overcome morphological variation challenges within life stages (Brunner et al., 2004)
- ❑Mitochondrial gene cytochrome c oxidase I (mtCOI*)* marker differentiates interspecies variation (Savolainen et al., 2005)
- ❑Accurate vector identification is critical for both vector and virus disease management. It also guides establishment of bio-assay protocols for virus transmissions (Armstrong and Ball, 2005)

Objectives

To determine the genetic diversity of thrips species infesting maize/corn in different agro-ecological zones affected with MCMV in Kenya.

Specific objectives

- ☐ To determine the range of thrips species infesting maize in Kenya using mtCOI barcode.
- ☐ To describe the genetic diversity of the thrips species infesting maize in Kenya using barcodes.
- ☐ To determine the relationship between the genetic diversity of thrips species and MCMV incidence in different agro-ecological zones of Kenya.

Methodology

Sample collection

- Thrip samples collected from MLN affected maize fields at low, mid and high altitude agro-ecological zones
- Adult and late instar larvae sampled on maize in each of the six randomly selected Sub Counties of Murang’a
- Sample collection points considered at least 2 km apart so as to minimize chances of collecting clones from the same parthenogenetic female thrip
- Thrips mechanically aspirated onto collecting bottles before placing them in 95% ethanol inside 2 ml eppendorf tubes and shipped into the laboratory for genotyping

Methodology cont’

mtCOI barcoding

DNA extraction and PCR

- DNA extraction by chelex method
- mtCOI amplification using target primers
- Gel electrophoresis

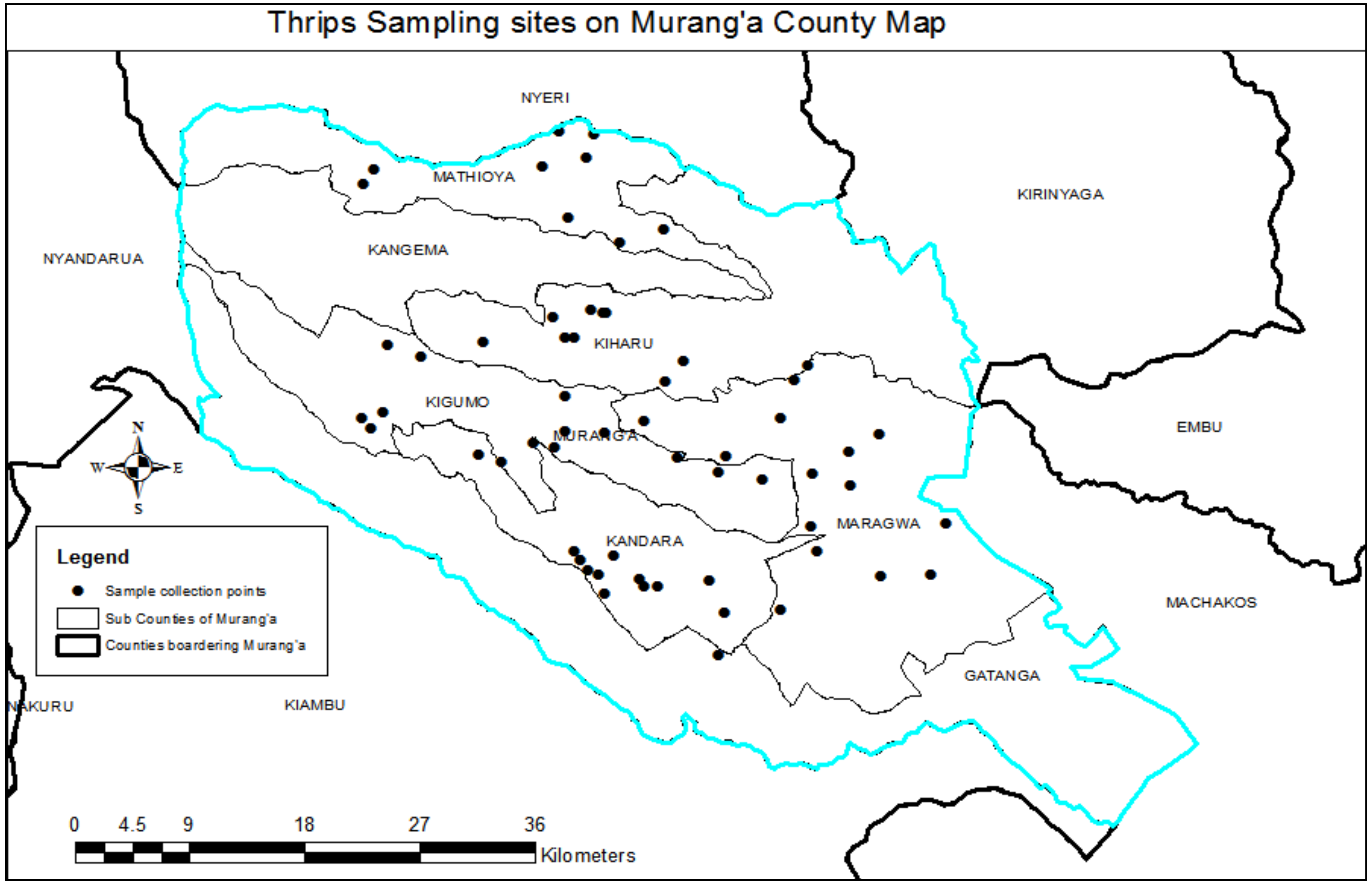
PCR product purification and

- Amplified DNA purification using purification kit
- Quantification of purified DNA using spectrophotometer

DNA sequencing and analysis

- Sequencing of DNA
- Analysis of sequences and vector identification by matching sequences with online databases in gene-bank

Results

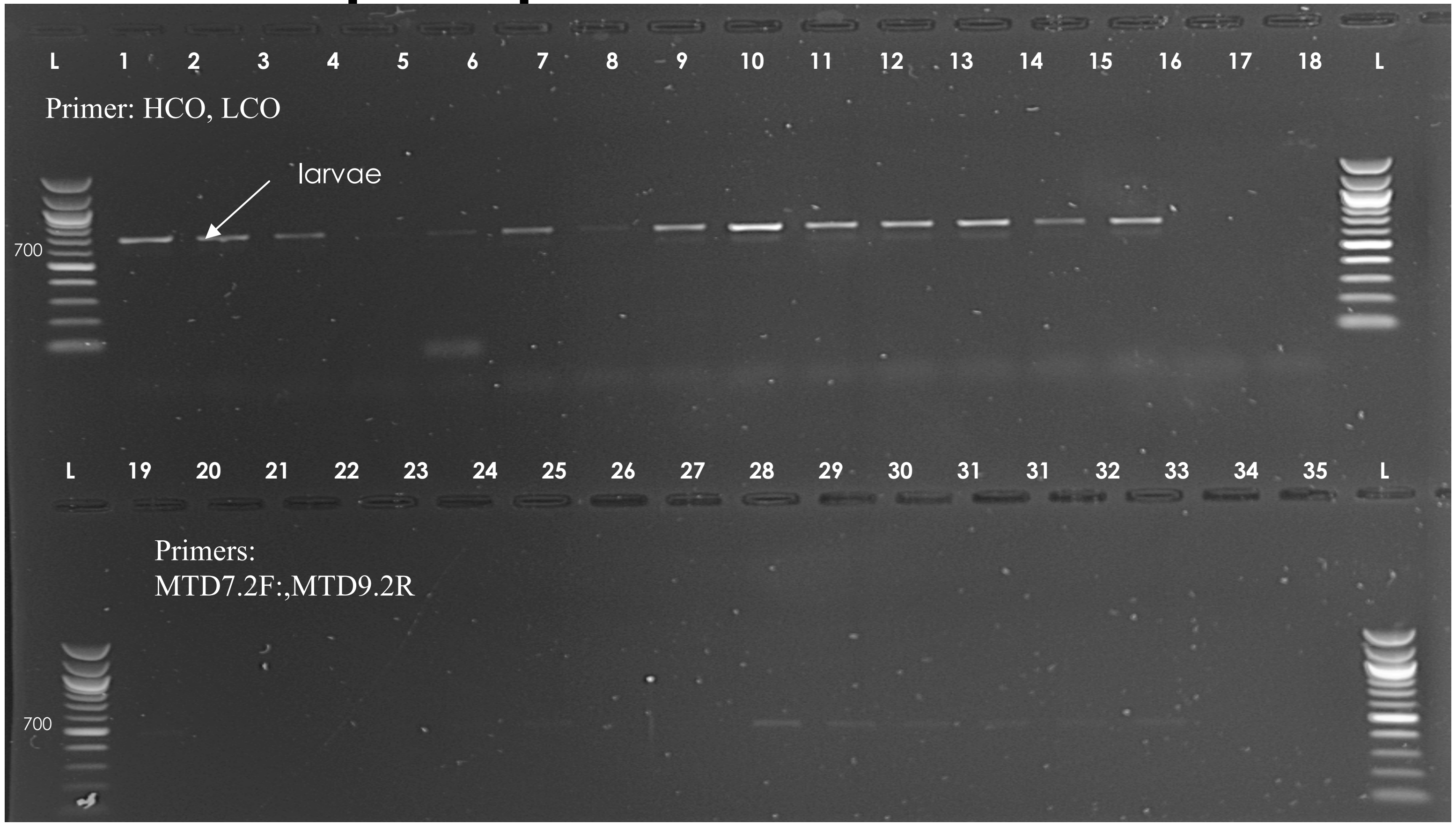

Thrips Sampling sites on Murang'a County Map

Samples

- Thrips were collected from MLN symptomatic maize plants
- 306 samples were collected from 56 sites
- 11 sites in each of the five sub counties were sampled
- 112 samples were processed for DNA extraction

Results cont’

mtCOI PCR amplified products



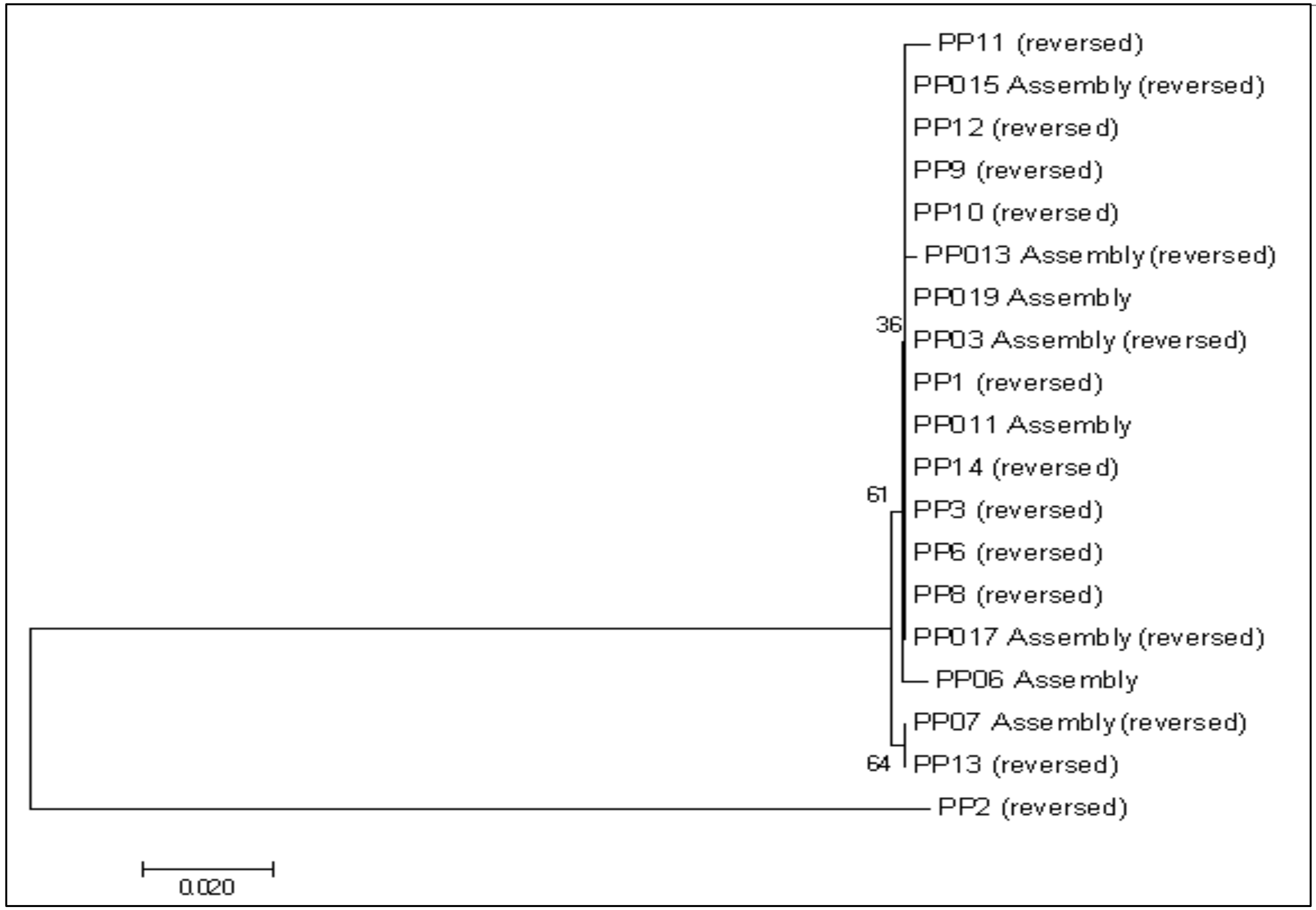
- DNA was extracted from samples of thrips larvae
- About 700bp of DNA were extracted
- Two primer sets were used
- Universal primers: HCO, LCO generated clear DNA bands than thrips specific primers: MTD7; MTD9.2R

Results cont’

Sample no	Location	code	ncbi blast %id	Sequence accession	Identity match
1	Muranga	PP1	81.98%	MF993432.1	*Frankliniella occidentalis*
2	Muranga	PP2	86.62%	HM246184.1	*Chirothrips manicatus*
3	Muranga	PP3	81.98%	MF993432.1	*Frankliniella occidentalis*
4	Muranga	PP6	81.54%	MF993432.1	*Frankliniella occidentalis*
5	Muranga	PP8	81.98%	MF993432.1	*Frankliniella occidentalis*
6	Muranga	PP9	81.98%	MF993432.1	*Frankliniella occidentalis*
7	Muranga	PP10	81.98%	MF993432.1	*Frankliniella occidentalis*
8	Muranga	PP11	82.29%	MF747143.1	*Frankliniella williamsi*
9	Muranga	PP12	81.98%	MF993432.1	*Frankliniella occidentalis*
10	Muranga	PP13	81.98%	MF993432.1	*Frankliniella occidentalis*
11	Muranga	PP14	81.98%	MF993432.1	*Frankliniella occidentalis*
12	Muranga	PP03	81.98%	MF993432.1	*F. occidentalis*
13	Muranga	PP04	80.87%	KM532315.1	*Thripidae*
14	Muranga	PP06	81.12%	KM532315.1	*Thripidae*
15	Muranga	PP07	80.58%	KM532315.1	*Thripidae*
16	Muranga	PP011	81.87%	MF993432.1	*F. occidentalis*
17	Muranga	PP013	80.04%	KM532315.1	*Thripidae*
18	Muranga	PP015	81.98%	MF993432.1	*F.occidentalis*
19	Kiambu	PP017	81.83%	MF993432.1	*F. occidentalis*
20	Kiambu	PP019	79.60%	KM532315.1	*Thripidae*

- *Frankliniella* thrips on maize in Murang’a have a genetic similarity of 80.04 - 82.29%
- *Frankliniella* genus is widely distributed
- Only one sample matched with *F. williamsi*

Results cont’



- *Frankliniella* thrips on maize in Murang’a have a close genetic similarity
- All samples collected were closely related
- Only one sample distantly related with Frankliniella genus

Conclusion

- Universal primers: HCO, LCO produce clear DNA bands than thrips specific primers: MTD7; MTD9.2R
- Thrips in *Frankliniella* genus on maize in Murang’a have a genetic similarity of 80.04 - 82.29%
- *Frankliniella* genus is more prevalent and could play a major role in virus transmission
- *Frankliniella occidentalis* is widely distributed than *F. williamsi*
- 26% of the samples were identified up to genus level - Tripideae

Recommendations

- More studies on genetic diversity of Thrips in *Frankliniella* genus in Kenya
- There is need for more information on the thrips species complexes infesting maize in Kenya as well as their genetic lineages through development of a comprehensive DNA barcode library for Thysanoptera

Acknowledgements

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