Sub-theme

Pest Surveillance and Diagnostics in Phytosanitary Systems

Title:

SURVEILLANCE ON COCONUT LETHAL YELLOWING DISEASE IN KWALE COUNTY, KENYA.

Presented by:

JOHN MULI - KEPHIS

Introduction

Surveillance: “an official process which collects and records data on pest and disease occurrence or their absence by survey, monitoring or other procedures” (IPPC, 2021)

The coconut palm (*Cocus nucifera*) is a perennial crop grown over 90 countries globally, by over 11M farmers covering 12M Ha (Gurr *et al*, 2016).

Referred to as “tree of life” (Adkins *et al*, 2020) due to its economic importance.

In kenya, grown in Coastal counties, with few traces in Meru, Tharaka Nithi and Busia (AFA 2017)

Coconut industry potential is about 25B annually, but only 53% is utilized (AFA 2020) – sleeping giant.

Supports over 150,000 households directly and thousands indirectly with over 100 products. (AFA 2021)

Introduction cont'

Coconut lethal yellowing disease (CLYD)- Previously Lethal disease, Cape St Paul wilt, etc

Caused by Phytoplasma - vector borne and transmitted by Hemipteran insects (Solomon *et al* 2019)

Classified as *Candidatus Phytoplasma palmae* species in the bacterium domain.

Regarded as a phloem bacteria.

Globally, first reported in Cayman Islands in 1834. (Eziashi, 2010).

In East Africa, reported near Bagamoyo in 1905 (Hemmati *et al*, 2020). In 1965, it had killed 56% of the coconut population (Mpunami *et al*, 2021). Over 8M trees affected in Mozambique.

Kenya, reported in 1999 in Mpeketoni,Northern Kenya. (Mpunami *et al*, 1999).

Introduction cont'

CLYD Symptoms:

- Premature inflorescence and fruit drops
- Fronds chlorosis and senescence, forming skirt like feature
- Death of the crown – Topless bare trunks

Molecular analysis required for confirmation.

Incubation period varies with variety, geographical location etc . 844 days reported in Ghana (Nipah *et al*, 2007)

Fig 1a and 1b: CLYD symptoms
Source: KEPHIS

Problem Statement

CLYD has been reported to cause great havoc in Mozambique, Tanzania and West African countries where millions of trees have been destroyed. Since coconut trees contributes immensely to the livelihoods of Coastal communities, early detection of the disease is needed so that mitigation measures can be put in place to avoid further damages

Justification

Coconut provides livelihoolds to over 80% of Coastal population, with over 100 by products

Disease first reported in Kenya in 1999 in Northern Coast, no subsequent work has been reported.

Though not reported officially, symptoms similar to those related to CLYD have been observed and reported.

Kenya has an approximate 84,000 ha with over 15Million trees, and is the 3rd coconut producer after Tanzania and Ghana, hence the need to protect this heritage

Kwale county is the second leading coconut producing county after Kilifi and has an entry point to Kenya through Lunga lunga border.

Objectives

General objective

To establish the occurrence and extend of CLYD in Kwale county so as to inform the type of mitigation measures to be undertaken.

Specific Objective

1) To identify the *Candidatus Phytoplasma palmae* strain causing CLYD in Kwale county.

2) To determine the presence of CLYD in Kwale county.

Methodology

A) **Collection of coconut drillings for DNA extractions**

Coconut tree drillings were collected from trees sampled in the fields by boring the tree trunks (Fig 2a and 2b). Tools and materials used included rechargeable drill Bosch machine, drill bits, sterilized matchet, vials, silica gels

B) DNA extractions

Extracted using Cetyl Trimethylammonium bromide(CTAB) method. Quantification done by Nano drop and stored at -20 ºC awaiting PCR.

Fig 2a and 2b: Sample collection using a drilling machine

Source: KEPHIS

Methodology cont’

C) PCR process

Two primers used (Deng & Hiruki, 1991)

P1/P7 primers used for Nested 1 and R16F2n (forward) and R16R2 (reverse) for nested 2

D) Gel electrophoresis

DNA samples loaded into wells at the positive end of a gel, electric current applied to pull them through. Loading dye used to give illumination of color. 1kb ladder was used.

E) Sanger sequencing

Preparation for Sanger sequencing done using QiaGen gel extraction procedure for the positive DNA fragments. The gene sequences obtained were compared with existing phytoplasmas using Basic local Alignment search Tool (BLAST) from the National Centre for Biotechnology Information (NCBI)

Results

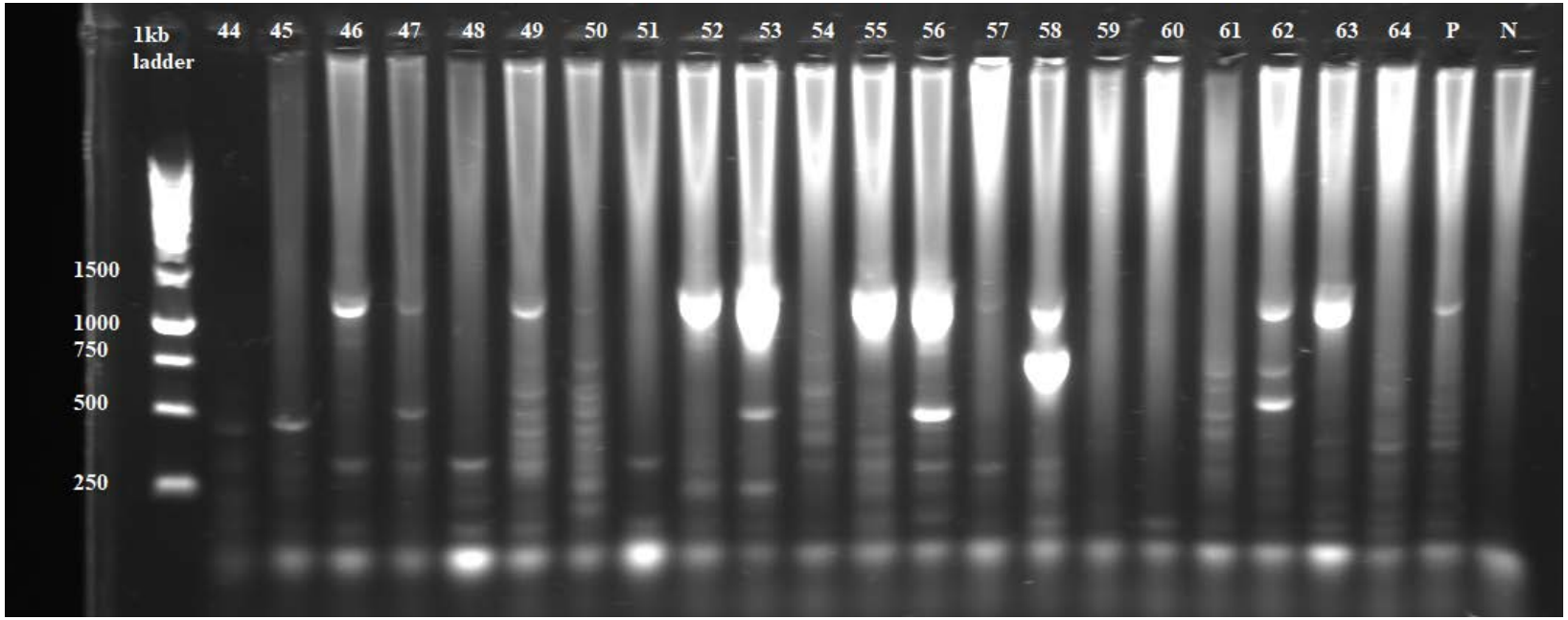

Fig 3: Amplification of phytoplasma DNA using universal primer pairs (P1/P7) for samples collected in Msambweni, Matuga and Vanga sub counties. Sample No 46, 47, 49, 52, 53, 55, 56, 58, 62 and 63 tested positive for PCR.

Results cont’

Table 1: Table showing the wards which tested positive for CLYD

Sub county	Ward	Village	Sanger (16S: F2/R2) results (Forward /Reverse)	Gene bank Accession No
Lunga lunga	Vanga	Kidembe	CLY iso Tanz08	GU952107.1
Lunga lunga	Pongwe	Mwazaro	CLY iso Tanz08	GU952107.1
Lunga lunga	Vanga	Migombani	CLY iso Tanz08	GU952107.1
Lunga lunga	Vanga	Migombani	CLY iso Tanz08	GU952107.1
Lunga lunga	Vanga	Juakali	CLY iso Tanz08	GU952107.1
Msambweni	Kinondo	Ganja la simba	CLY iso Tanz08	GU952107.1

Results cont'

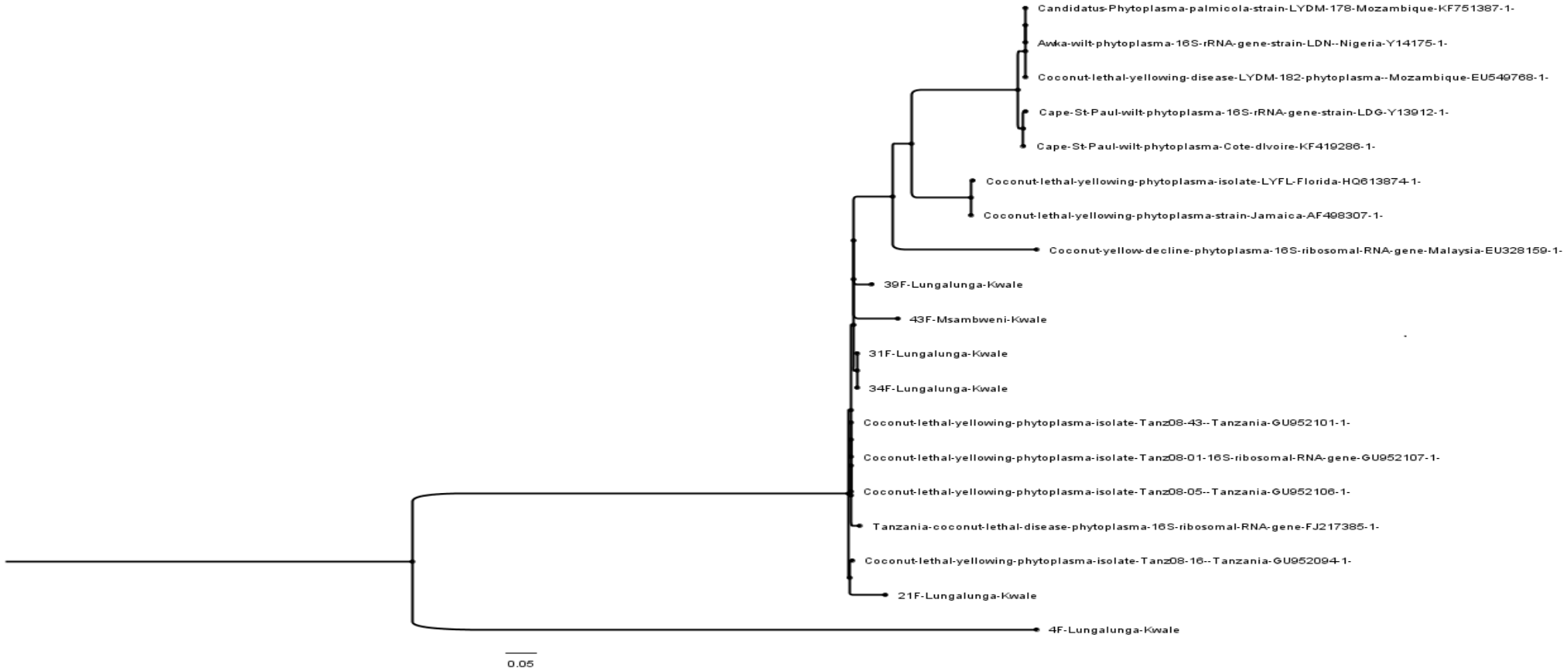

Fig 4: A Phylogenetic tree constructed using the maximum likelihood method showing the phylogenetic relationship among the *Candidatus Phytoplasma palmae* detected in the coconut plants (39F, 31F, 21F, 34F, 43F and 4F) and representatives from existing different 16sr groups from Tanzania, Mozambique, West Africa and Florida. The existing GenBank accession numbers are indicated next to the names of the existing 16sr groups. Sample is noted to be quite divergent from the rest, which may be due to poor sequencing quality or presence of multiple isolates.

Conclusion

CLYD was first reported in Mpeketoni (Lamu county), and from this study, CLYD presence was evident in Kwale.

The Phytoplasma strain was identified as CLY iso Tanz08, which is closely related to the strains identified in Tanzania.

The presence of the same phytoplasma strain in Kenya and Tanzania may be associated either by material exchange between the two countries or some common vectors.

Though Mozambique borders Tanzania to the South, it's interesting to note that Mozambique has strain is related to those found in West Africa.

Recommendations

There is need to undertake more studies to determine the following:

- Varieties most affected by the disease. (East Africa Tall was reported as the most prone)
- Vectors transmitting the disease, characterization and genetic diversity.
- Alternative hosts and the incubation period. Some hosts such as limes have been reported to harbor the phytoplasma without showing any symptoms.
- There is also need to undertake social economic surveys to ascertain the estimated coconut trees which have been destroyed by the disease in Kenya since 1999.
- Optimal and current management of CLYD is through strict quarantine, prompt detection and destruction of symptomatic palms, planting varieties less susceptible, proper weeds control to get rid of the alternate hosts and possible control of the alternate hosts.

Acknowledgements

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

www.africa-cope.org

For more information, please contact:

www.africa-cope.org
www.kephis.org
Facebook.com/3rd phytosanitary Conference 2020
Twitter: @3rdphytoconf

Theme: *Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*
www.africa-cope.org