3RD PHYTOSANITARY CONFERENCE
Virtual Edition
13th-16th September 2021

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

INTERNATIONAL YEAR OF
PLANT HEALTH
2020

Sub-theme:

International Year of Plant Health

Title:

Characterization of the genetic diversity of *Cercospora zeina* as the causal pathogen of Gray Leaf Spot disease of maize in Kenya.

Presented by:

Dennis Oduor Omondi

Introduction

Maize production in Kenya

- Maize is important for food security.
- Low productivity in Kenya at 1.8t/ha^{-1} (Munialo et al., 2020)
- World average yield 6t/ha^{-1}
- Productivity is affected by threat of devastating pathogens.

GLS

Management

- **Cultural methods**
- **Chemicals**
- **Genetic resistance**
  - ✓ **Qualitative**
  - ✓ **Quantitative**

(Ngugi et al., 2000; Kinyua et al., 2010)

Introduction cont’

Gray leaf spot

Gray-rectangular shaped lesions

- Causal agent; *Cercospora zeina* or *Cercospora zeae maydis.*
- *C. zeae maydis* produces cercosporin in vitro, *C. zeina* does not, due to *CTB7* deletion.
- Both are ascomycete fungi
- Necrotrophic &polycyclic
- Mode of reproduction; asexual/ sexual
- Sexual reproduction controlled by MAT genes

Genetics of Resistance

- Risk of virulence on resistant genotypes (Muller et al., 2016).
- Differential response to GLS; necrotic lesions, chlorotic lesions and flecks (Ward et al., 1999).
- Quantitative resistance.
- Additive in nature with high heritability.

(Dunkle and Levy 2000; Kinyua et al., 2010; Shankara & Gowda 2011; Swart et al., 2017)

Problem Statement

- The pathogen has been associated with high yield losses of 60-65% on susceptible genotypes (Ward et al., 1999).
- Two species are associated with GLS, hence confusion as to the causal pathogen and it’s genetic diversity that affect management decisions.
- Based on literature, no study had exhaustively examined the genetic diversity of *Cercospora zeina* in Kenya.

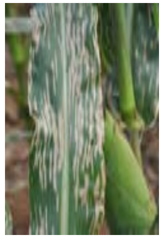
Justification

- To effectively control GLS, it is important to understand the composition & genetic diversity of *C. zeina* (Dunkle & Levy, 2000).
- Knowledge on the level of *Cercospora zeina* genetic diversity and mating type distribution will unravel whether sexual recombination is taking place in the population.

Objectives

1. To analyze the genetic diversity of *Cercospora zeina* as the causal pathogen of gray leaf spot in the four counties of Kenya.
    - H_0 *Cercospora zeina* is not the causal pathogen of GLS in Kenya and it is not diverse.
2. To determine the role sexual recombination plays in driving the genetic diversity of *C. zeina* across the four counties in Kenya.
    - H_0 There is no sexual recombination in the population of *Cercospora zeina* collected from the four counties in Kenya.

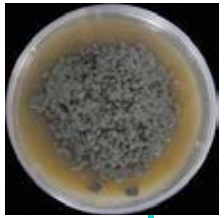
Methodology



Maize leaves with GLS symptoms collected →

Single spore isolations →

Culturing of isolates- to V8 agar →



Sub-culturing of isolates

Genomic DNA extraction with CTAB DNA extraction protocol

DNA quantification

g DNA

PCR

CTB 7 diagnostic PCR

Multiplex PCR with MAT 1-1, MAT1-2 primers

Screen isolates with neutral SSR markers

Gel electrophoresis

Gene scan analysis

(Stewart and Via, 1993)

Methodology cont’

GENETIC DIVERSITY

Multilocus genotype data

Analysed

GenAlex v6.502 (Peakall and Smouse, 2006) Excel

The total number of alleles
Private alleles
Nei’s gene diversity index (H)
Population differentiation-999 P
Genetic distance

PCoA-visualization

STRUCTURE ANALYSIS

- To determine if there are clusters in the population using multilocus genotype information

<u>Parameters in STRUCTURE v2.3.4</u>
K values ranging from 1 to 20
Iterations = 20
Burn in length 100,000
Simulations; 1,000,000 MCMC

DISTRUCT for cluster visualization

CLUMPP 1.1.2 generate bar graphs

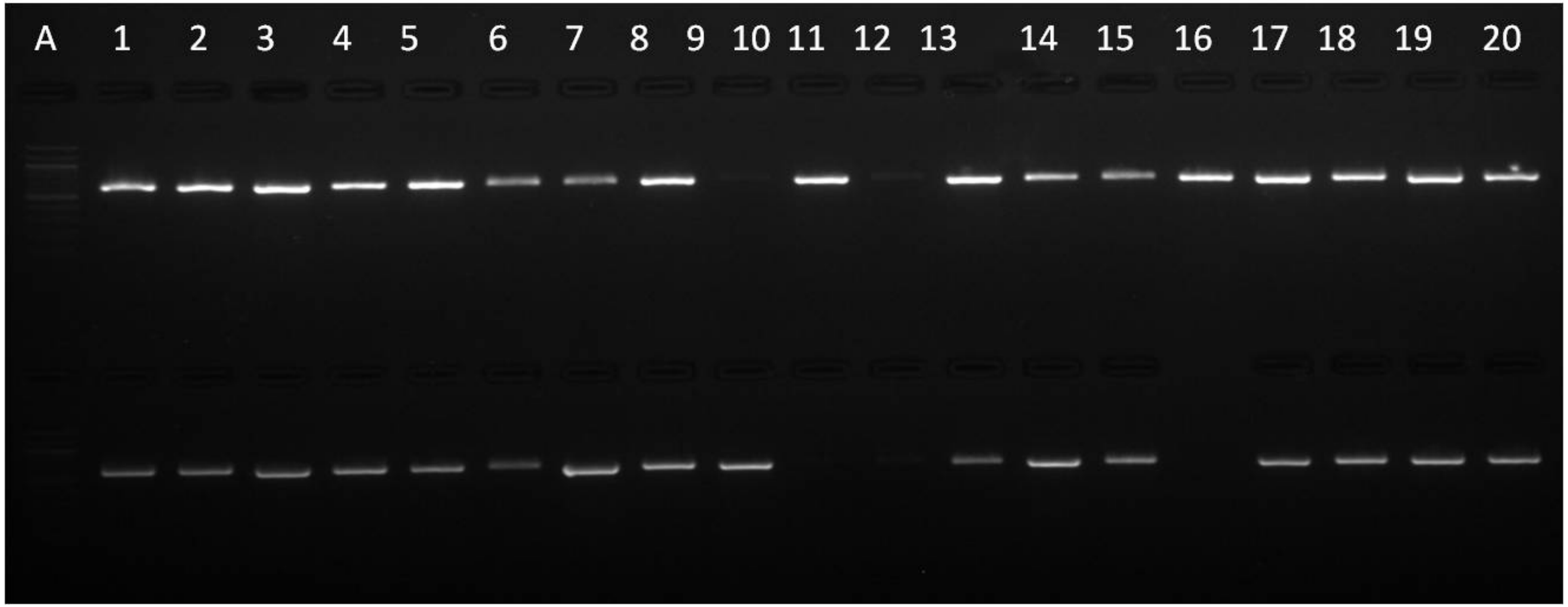
STRUCTURE HARVESTER generate ΔK

MULTILOCUS v1.3b to determine population differentiation

(Pritchard et al., 2010; Evanno et al., 2005; Peakall & Smouse, 2006)

Results

Cercospora zeina is the causal pathogen of GLS



618 bp

All the isolates produced PCR products of 618 bp same as positive control hence all are *C. zeina*.

Figure 1. Appearance of isolates on a gel

Lane A: 100 bp DNA ladder
Lane 1: CMW 25467 (Positive control)
Lane 2-20*: Cercospora zeina* isolates

(Swart et al., 2017)

Results cont’

Cercospora zeina is highly diverse in Kenya

Figure 1. Indicators of genetic diversity for *C. zeina*.

	N	MLG	Pa	%P	CF	I	He
Chogoria	8	8	2	82	0	0.67	0.46
Kiambu	34	27	12	91	0.21	0.68	0.36
Meru	76	61	14	100	0.20	0.83	0.48
Nakuru	11	11	4	91	0	0.87	0.48
Total	129	107	32	91	0.17	0.76	0.46

N-sum total of genotypes per county, MLG-Multilocus genotypes, Pa-Private alleles, %P-Percent polymorphism, CF-Clonal Fraction, I-Shannon-Wiener index, He-Gene diversity.

Nei’s unbiased gene diversity was comparatively high across all regions

(Nei, 1978)

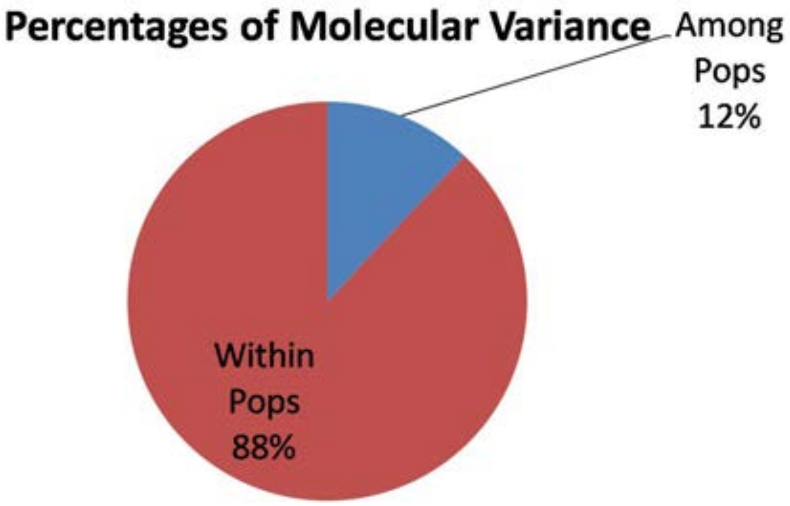
Results cont’

Cercospora zeina populations exhibit partial population structure

Table 2. Analysis of the molecular variance data for the SSR data

Source	df	SS	MS	Estimated Variance	% of total variance	PhiPT(p-value)	Nm
Among Populations	3	29	10	0.34	12	0.12 (0.001)	3.85
Within Populations	103	256	2	2.48	88		

df-degrees of freedom, SS-Sum of squares, MS-Mean squares, PHiPT-A measure of the population differentiation, Nm-Estimate of gene flow



H_o : No population differentiation
H_o : PhiPT (ϕ) = 0
Decision; Reject the null hypothesis

AMOVA revealed that sources of variation from within and among the counties contributed to the genetic variance. Gene flow was also relatively high.

Results cont’

Cercospora zeina populations exhibit partial population structure

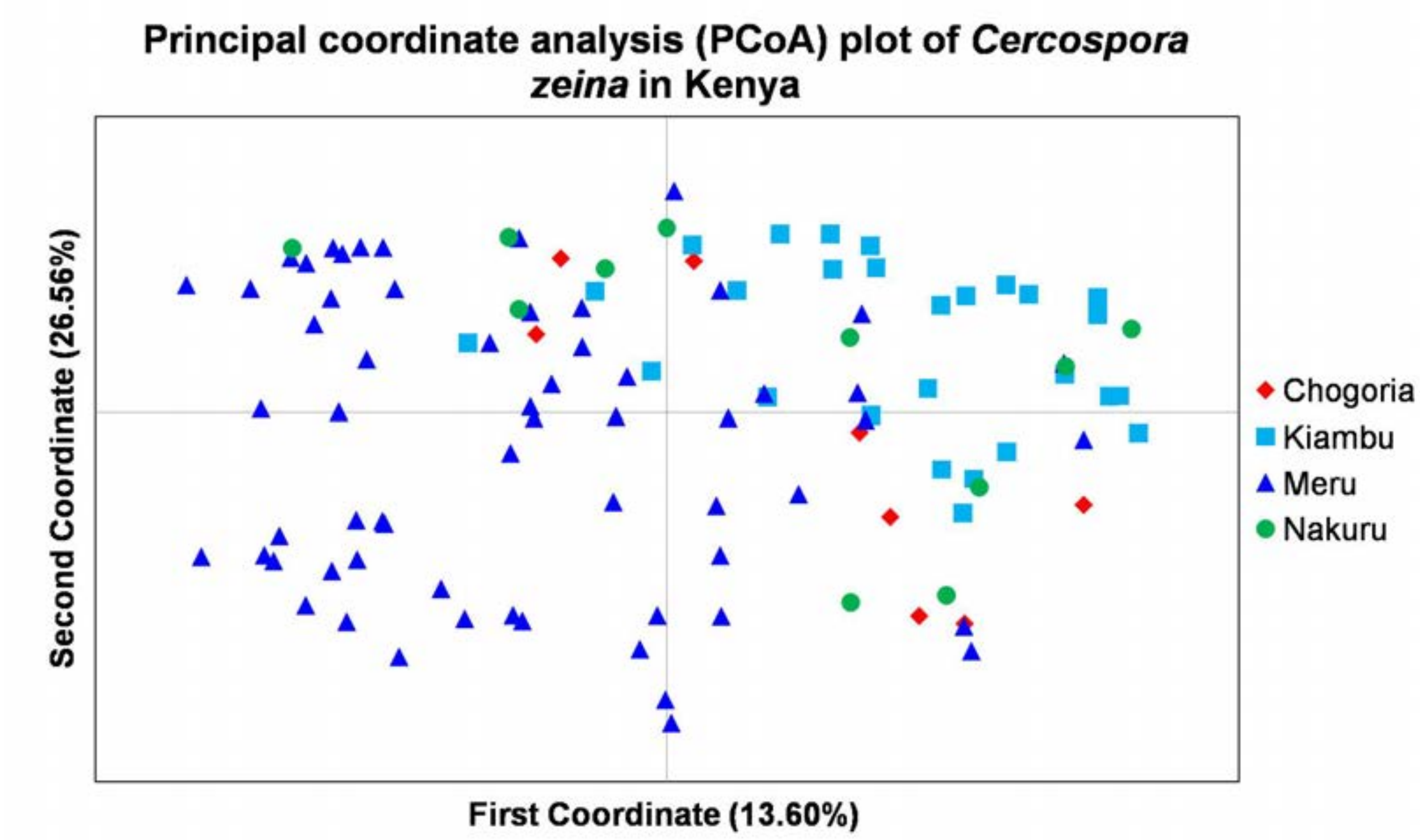
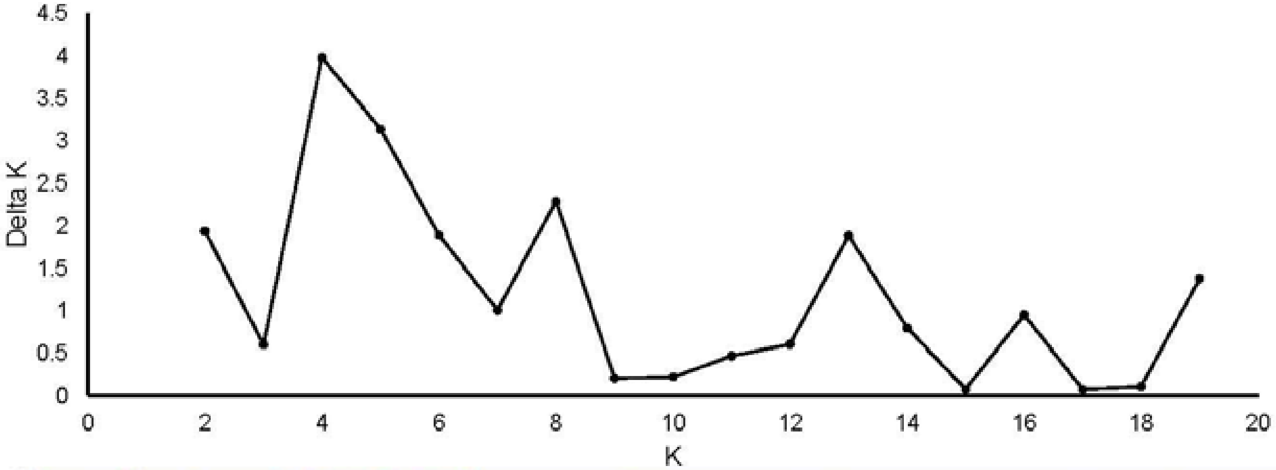


Figure 2. The Principal coordinates analysis illustrates the occurrence of partial population differentiation across the 4 counties

Results cont’

Population structure



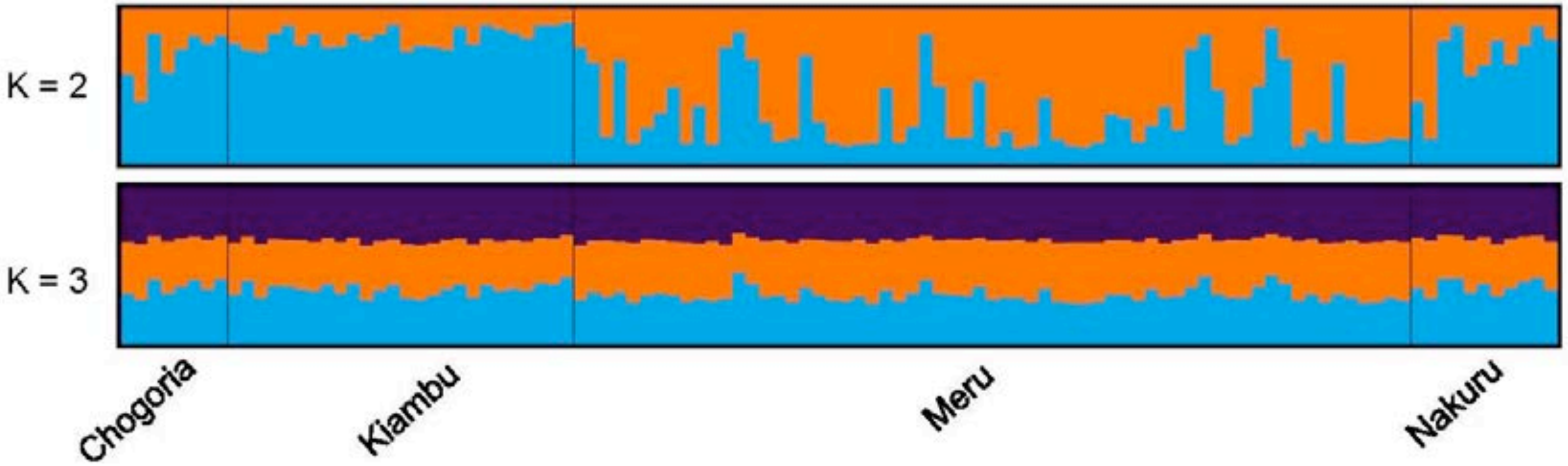


Figure 3 & Fig 4 illustrate delta K graph and STRUCTURE analysis

Delta K indicates that the most likely number of clusters in the population is four. STRUCTURE reveals the existence of partial population structure across the 4 counties.

Results cont’

The role of sexual recombination in the genetic structure of *Cercospora zeina*

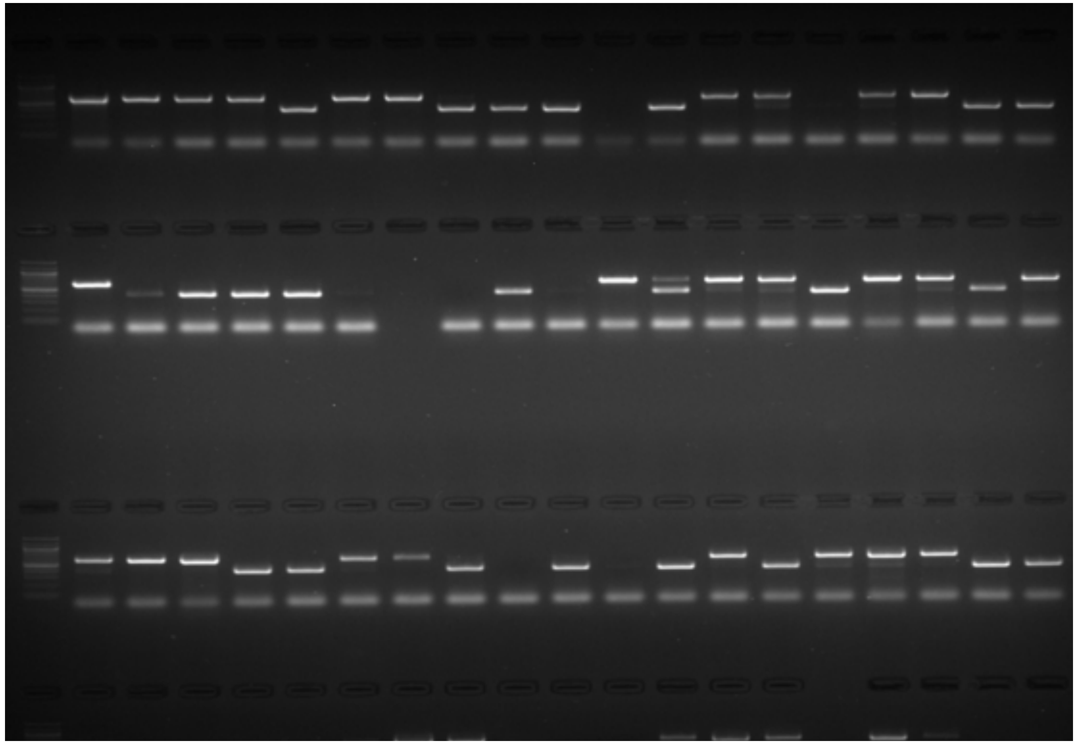


Fig 5. Gel image of *Cercospora zeina* mating types MAT1-1 yielded 630 bp and MAT1-2 yielded 400 bp PCR products.

Table 3. Annual frequency distribution of MAT1-1-1 & MAT1-2-1

Location	Number of isolates	MAT 1-1	MAT 1-2	MAT1-1/ MAT1-2	χ^2
Chogoria	8	4	4	1.00	1.00 (0.32)
Kiambu	34	16	12	1.33	0.57 (0.50)
Meru	76	36	32	1.13	0.24 (0.35)
Nakuru	11	6	4	1.50	0.4 (0.47)
Subtotal	129	62	52	1.19	0.877

H_O: Populations do not significantly deviate from the expected 1:1 ratio
Decision: Fail to reject H_O at $P<0.05$

Conclusion

- *Cercospora zeina* is the causal pathogen of GLS in Kenya.
- *C. zeina* is highly diverse in Kenya.
- Existence of partial population structure among *C. zeina* populations.
- Sexual recombination plays a role in the genetic structure of *C. zeina.*
- The high genetic diversity in the population from the 4 counties in Kenya is attributed to the occurrence of sexual recombination.

Recommendations

- ✓ *Cercospora zeae maydis* was not identified among the isolates collected in this study. Stringent sanitary and phytosanitary measures to be put in place to prevent entry of this species into the country.
- ✓ Information on the diversity of *C. zeina* is an important tool for maize breeding programs working on resistance to gray leaf spot and management decisions regarding the disease.
- ✓ For subsequent monitoring studies and researchers aiming to identify the causal pathogen of gray leaf spot, CTB7 diagnostic test is a powerful tool that would improve identification of the species.
- ✓ Disease management practices may look at preventing the occurrence of sexual reproduction this could assist in the control of gray leaf spot.

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Acknowledgements

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www.africa-cope.org

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