



# Pan-African Grain Legume & World Cowpea Conference



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# Genetic Diversity Assessment of Ugandan Cowpea Scab Fungus (*Sphaceloma* sp.) Isolates Using ISSR Markers

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# Outline

- Introduction
  - Methodology
  - Results and Discussion
  - Conclusion
  - Acknowledgements
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# Introduction

- ➔ 3<sup>rd</sup> most important legume food crop in Uganda
- ➔ Northern and Eastern regions account for most of the production in the country
- ➔ Mean yield reported to be less than 400 Kg/ha
- ➔ Major fungal disease (up to 100% yield loss Mbong *et al.* (2012))
- ➔ Resurgence of scab disease

# Introduction Cont'd

- ▶ Pathogen is seed-borne
- ▶ Affects all above ground parts
- ▶ No information on diversity of pathogen
- ▶ All five recently released varieties susceptible
- ▶ Correct identification and characterization important for crop genetic improvement



# Scab infections on leaves, pod and stem



Plate A: Infected leaves

Plate B: Infected leaves and pod

Plate C: Infected stem

# Introduction Cont'd

## ➤ Objective

- Determine the genetic variability of the Scab fungus (*Sphaceloma* sp) to help inform breeding for resistance to the disease in Uganda

# Methodology

- Field survey and collection of samples from major growing areas
- Isolation and culture of pathogen
- DNA extraction from 28 isolates (Mahuku, 2004)
- PCR using ISSR markers

Table 1. List of isolates and their districts

No.	Isolate	District	No	Isolate	District
1	AmAsF1S2P	Amuria	15	ApAbF1S1L	Apac
2	AmKuF2S3L	Amuria	16	ApAdF2S4P	Apac
3	KuAtF1S1P	Kumi	17	DoAdF3S2P	Dokolo
4	KuBuF1S3P	Kumi	18	DoAgF1S4L	Dokolo
5	SeAtF1S2L	Serere	19	KiKiF3S1L	Kitgum
6	SeKyF3S4L	Serere	20	KiMaF1S4L	Kitgum
7	SoGwF1S1P	Soroti	21	LiAgF2S3L	Lira
8	SoSoF2S4P	Soroti	22	LiNgF3S3L	Lira
9	ToKiF1S4P	Tororo	23	NeNeF2S4L	Nebbi
10	ToMoF1S4L	Tororo	24	NePaF3S2L	Nebbi
11	PaKgF3S3L	Palisa	25	ArArF2S2L	Arua
12	PaOpF2S3L	Palisa	26	ArOlF2S3L	Arua
13	PaPjF2S1L	Pader	27	YuKuF1S2L	Yumbe
14	PaPuF3S3L	Pader	28	YuOdF2S3L	Yumbe

- PCR reaction conditions

- 5 mins at 94 °C

- 20 s at 94 °C

- 40 s at 30-55 °C

- 1 min at 72 °C

- 7 min at 72 °C

35 cycles



# Methodology cont'd

Table 2. List of primers, their sequence, motifs, length and annealing temperature

Primer	Nucleotide Sequence (5'-3')	Motif	Length	AT (°C)
RAMS1	CACACAACAACAACAACA	ACA	18	45
RAMS 2	GACCACCACCACCACCA	CAC	17	55
RAMS 3	ATCCGACGACGACGACGA	CGA	18	50
RAMS 4	AGGGTGTGTGTGTGTG	GT	14	35
RAMS 5	AACACACACACA	AC	12	35
→ TRI 6	GTAGTAGTAGTAGTA	GTA	15	30
				35

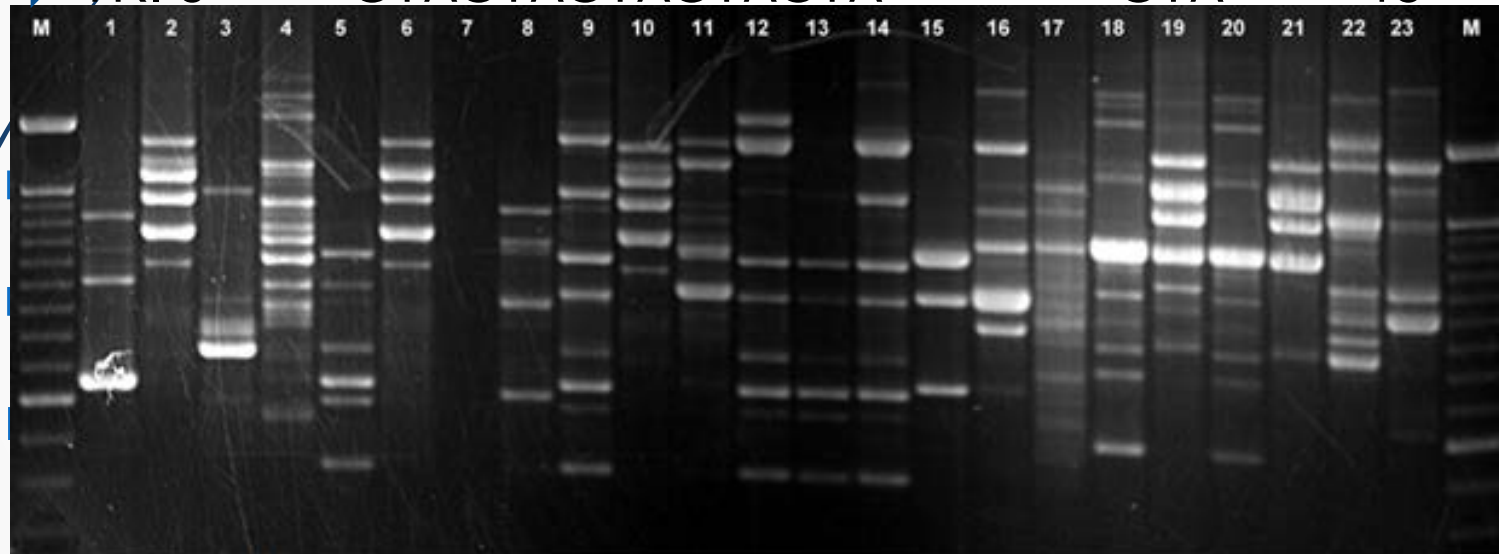


Plate D: DNA fragments of first 23 samples following PCR reaction with Tri 6 primer.  
M is DNA ladder

# Results and Discussions

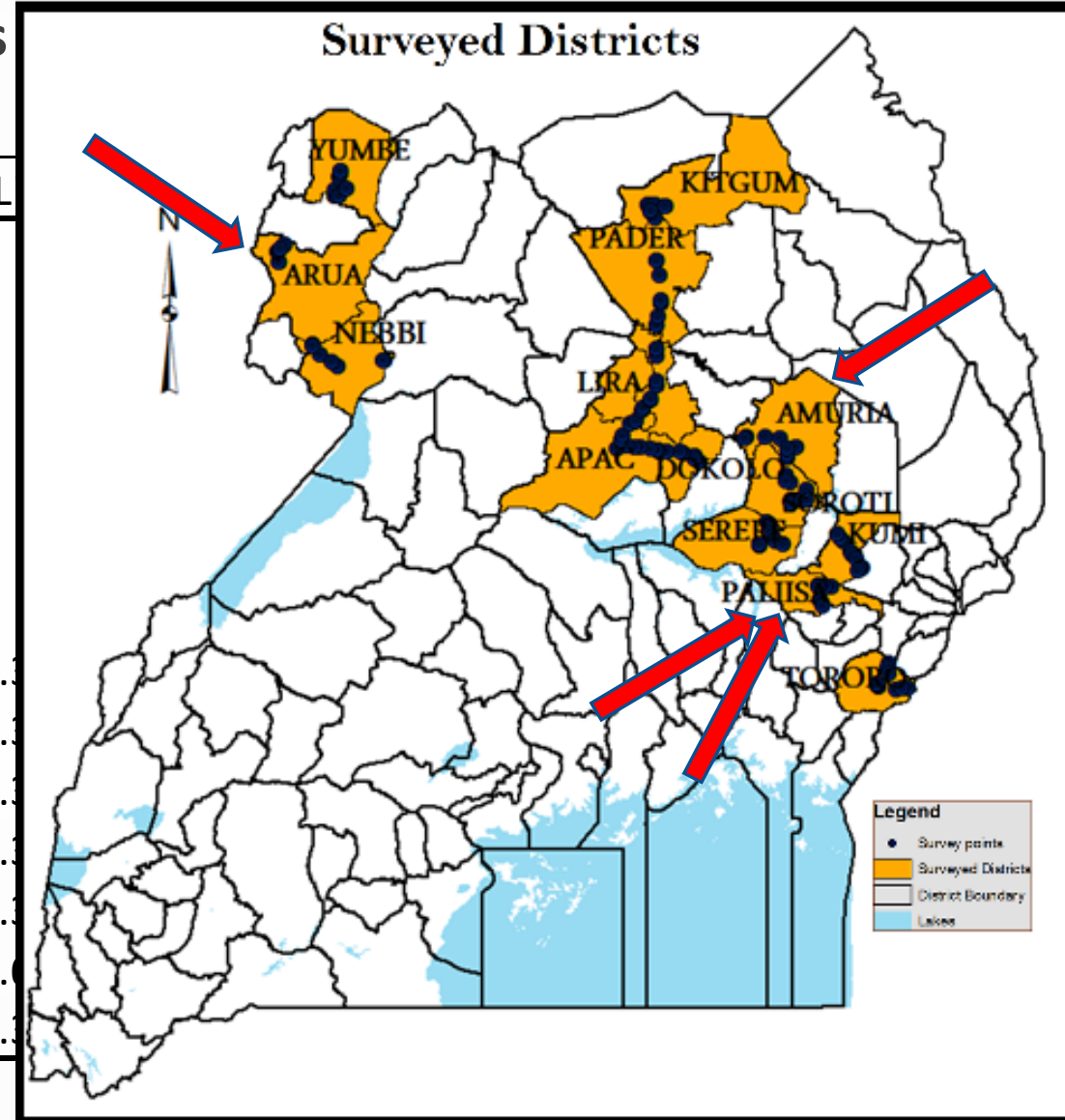
**Table 3. Three-Region nested analysis of molecular variance (AMOVA) based on 241 polymorphic loci. *P*-value estimates are based on 999 permutations.**

AMOVA analysis	df	Mean Square	Variation	% of total variation	Phi	<i>P</i> -value
Among regions	2	36.937	0	0	PhiRT -0.012	0.001
Among populations	11	40.953	1.762	4	PhiPR 0.045	0.001
Within populations	14	37.429	37.429	96	PhiPT 0.034	0.001
Total	27		39.191	100		

# Results and Discussions cont'd

Table 4: Population pairwise PhiPT values permutations.

Population	Amuria	Apac	Arua	Dokolo	Kitgum	Kumi	Lira	Nebbi	Palisa	Pader	Serere	Soroti	Tororo	Yumbe
Amuria	-													
Apac	0.362	-												
Arua	0.679	0.353	-											
Dokolo	0.316	0.673	0.67	-										
Kitgum	0.332	0.345	0.343	0.328	-									
Kumi	0.681	0.33	0.648	0.327	0.334	-								
Lira	0.341	0.336	0.334	0.339	0.332	0.335	-							
Nebbi	0.345	0.33	0.676	0.667	0.316	0.658	0.335	-						
Palisa	0.686	0.32	0.309	0.678	0.334	0.336	0.336	0.336	-					
Pader	0.339	0.323	0.327	0.329	0.343	0.328	0.336	0.336	0.336	-				
Serere	0.659	0.326	0.326	0.33	0.325	0.682	0.336	0.336	0.336	0.336	-			
Soroti	0.334	0.662	0.332	0.336	0.343	0.334	0.336	0.336	0.336	0.336	0.336	-		
Tororo	0.321	0.325	0.68	0.321	0.329	0.331	0.331	0.331	0.331	0.331	0.331	0.331	-	
Yumbe	0.67	0.32	0.329	0.32	0.332	0.658	0.332	0.332	0.332	0.332	0.332	0.332	0.332	-



# Results and Discussions cont'd

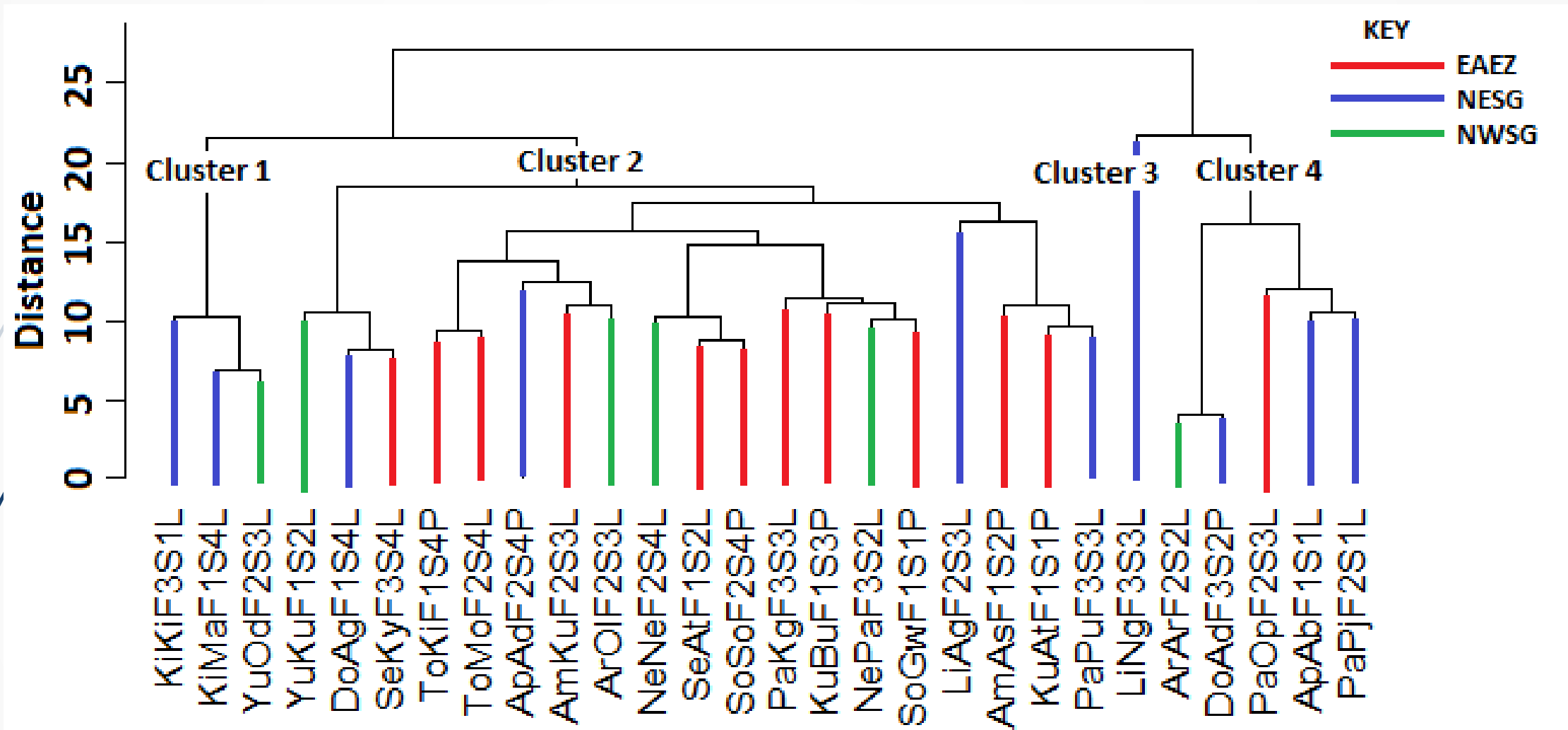


Figure 1: Dendrogram showing the clustering patterns among the 28 isolates

# Results and Discussions cont'd

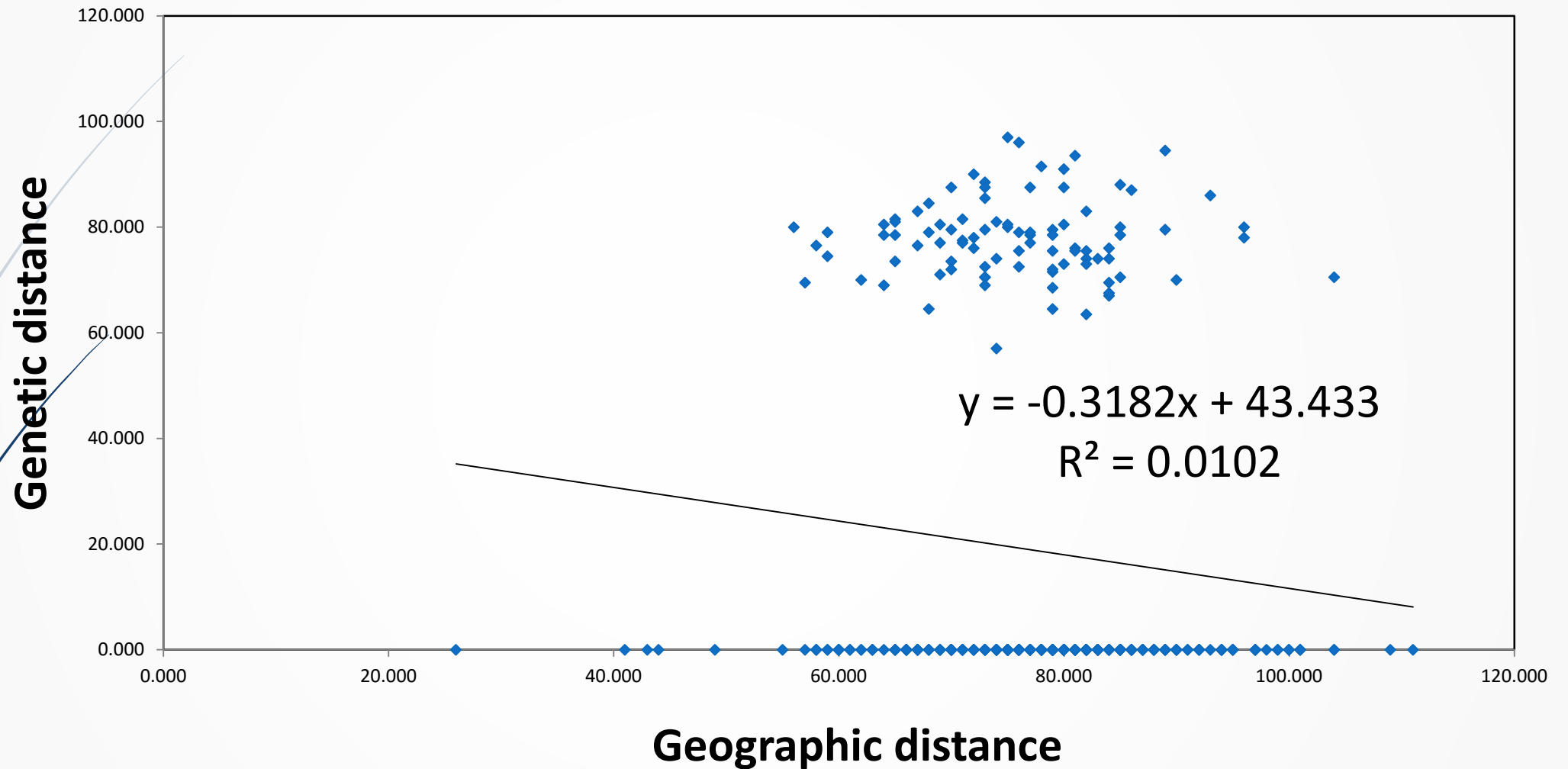


Figure 2. Mantel test of genetic distance matrix vs. geographic distance matrix for 14 populations of *Sphaceloma* sp. (Prand  $\geq$  data = 0.204)

# Conclusions

- High genetic differentiation among the *Sphaceloma* sp. populations
- Genetic diversity within populations was high while low among populations
- Geographic distance had no significant influence on the genetic variability
- One isolate (LiNgF3S3L) appeared to be the most isolated (unique) in the populations





**Thank you**

**Je vous remercie**

**شكرا جزىلا**

# Acknowledgements



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