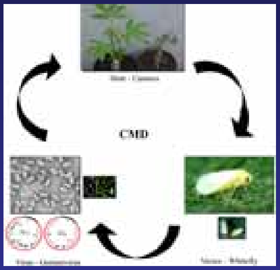
Mapping genetic loci associated with cassava mosaic disease (CMD)



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Introduction

Cassava (*Manihot esculenta* Crantz) - Euphorbiaceae

- Centre of origin - South America
- Food for 700 million people
- World production - 202 MT, Africa - 54% (FAO,2005)

India

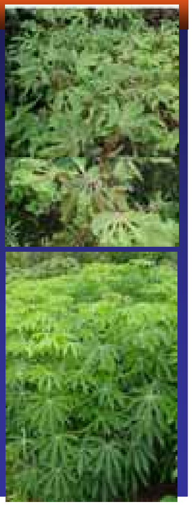
- Introduced to India by Portuguese – 17th century
- India: cultivated in Kerala, Tamil Nadu, Andhra Pradesh and Maharastra
  - Productivity – 28 t/ha : ranks 1st
  - Production – 7 MT : ranks 7th
  - 5th important starchy food crop

Problem in cassava cultivation : industrial area

- Cultivated for starch and sago purpose
- According to WTO agreement, industries should produce more quantity of starch and sago, otherwise other countries will export to India very cheap rate
- All cultivated varieties are susceptible to CMD
- Production is low, so industries in India have to improve there production
- Industries are much interested in CMD resistant, high starch, high yielding varieties in there area

Cassava mosaic disease (CMD)

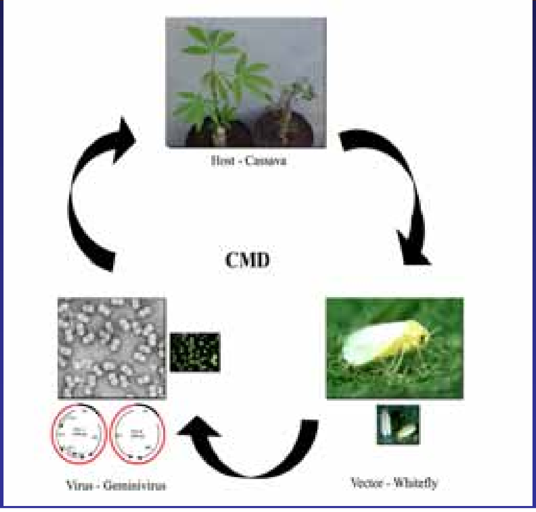
Important viral disease of cassava - ICMV



- First reported 1894 and India – 1956
- CMD caused by geminivirus and transmitted by whitefly (Vector transmitted disease)
- Yield loss ranging 20-90 per cent

Cassava mosaic disease (CMD)

Cassava and causal agents for CMD



Methods used for developing CMD resistance

- Resistance breeding - intervarietal
  - interspecific

Mapping genes using molecular markers

- For making selection at molecular level
- Method is quicker & more reliable

CMD resistant genes in cassava

Wild relative of Cassava

- *Manihot glaziovii* – ceara rubber
- Akano *et al*. (2002) – major dominant gene *CMD2* (CIAT)
- Lokko *et al*. (2004,2005) – polygenic, recessive nature (IITA)
- >500 SSR markers in cassava genome

Objective of study

- Mapping the genetic loci associated with CMD resistance using SSR markers in segregating population of CO2 x MNga-1

Breeding material

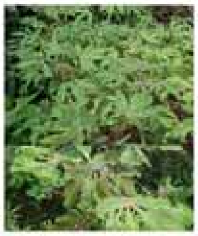
Parents Resistant - IITA Susceptible - TNAU


Leaf canopy of MNga-1


Tubers of MNga-1

X

141 F_1
Progenies


Leaf canopy of CO2


Tubers of CO2

Breeding material

Parents

Resistant : MNga-1 (TMS30001)

Susceptible : CO2

Pedigree of MNga-1

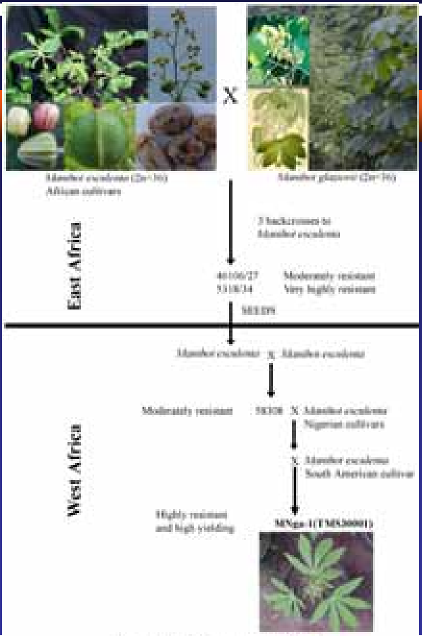


Plate 3. Pedigree of the resistant parent MNga-1

Screening for whitefly resistance

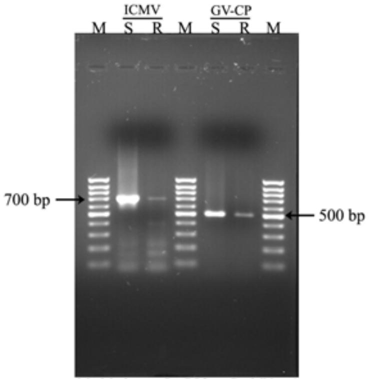
Parents

CO2 **MNga-1**

Whiteflies feeding both susceptible and resistant parents

Virus diagnosis in parents

Geminivirus specific primers



M : Marker S : CO2 R : MNga-1

(ICMV- Indian Cassava Mosaic Virus; GV-CP- Gemini virus Coat protein specific primer)

Survey of resistant and susceptible parents for the presence of virus using ICMV and geminivirus coat protein specific primers

Nature of virus resistance in the parents

- Both the parents are susceptible to the vector
- Presence of virus detected in resistant and susceptible parents
- In MNga-1 virus spread & symptom expression blocked
- There by effect of virus in growth and physiological activity of the plant is nullified in MNga-1

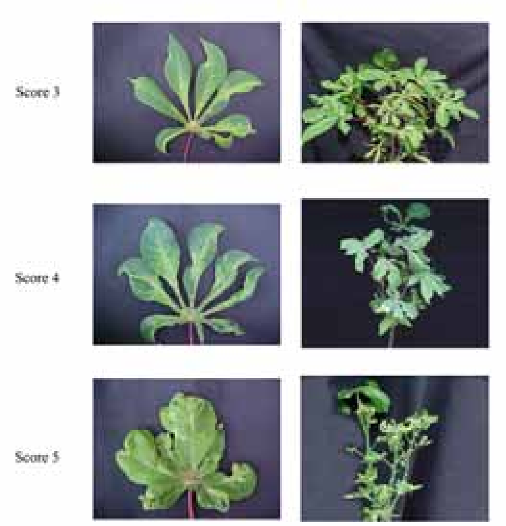
Phenotypic scoring using symptom expression

Hahn *et al.* (1980)

- 6th month phenotypic scoring
- 7th month pruning
- 8th month phenotypic scoring

Phenotypic scoring using symptom expression

Hahn *et al.* (1980)

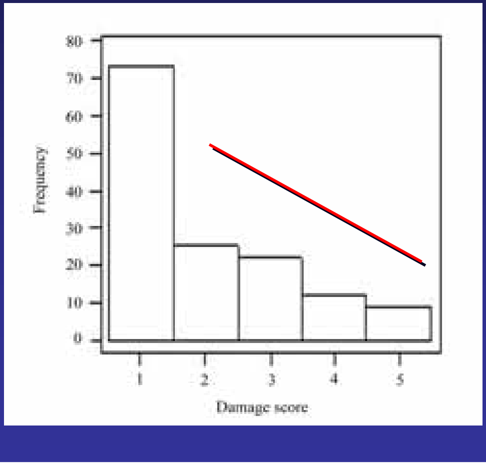


Phenotypic scoring

Severity of symptom expression

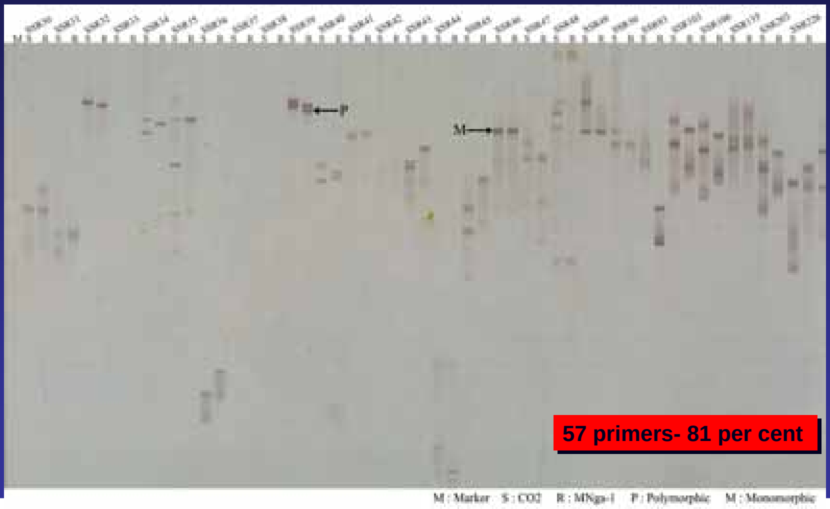
73

69



Parental screening

70 SSR primers – PCR amplification (Mba *et al*., 2001)



Genotyping with SSR primers

S- Susceptible parent R – Resistant parent

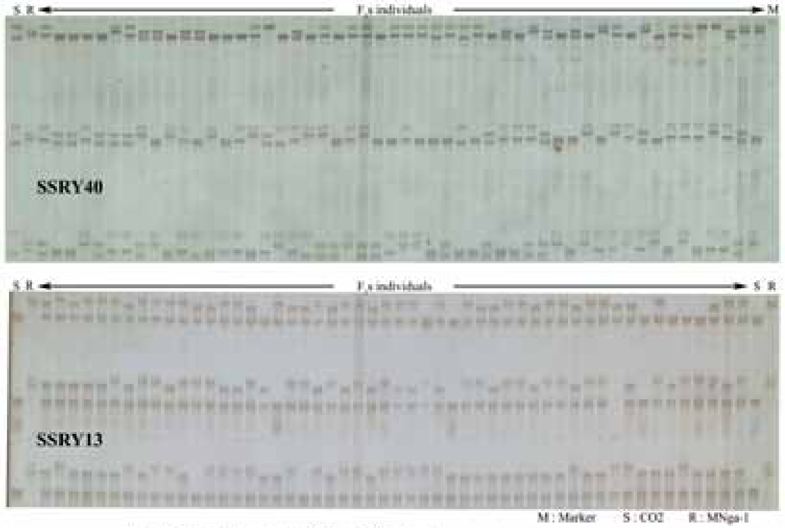
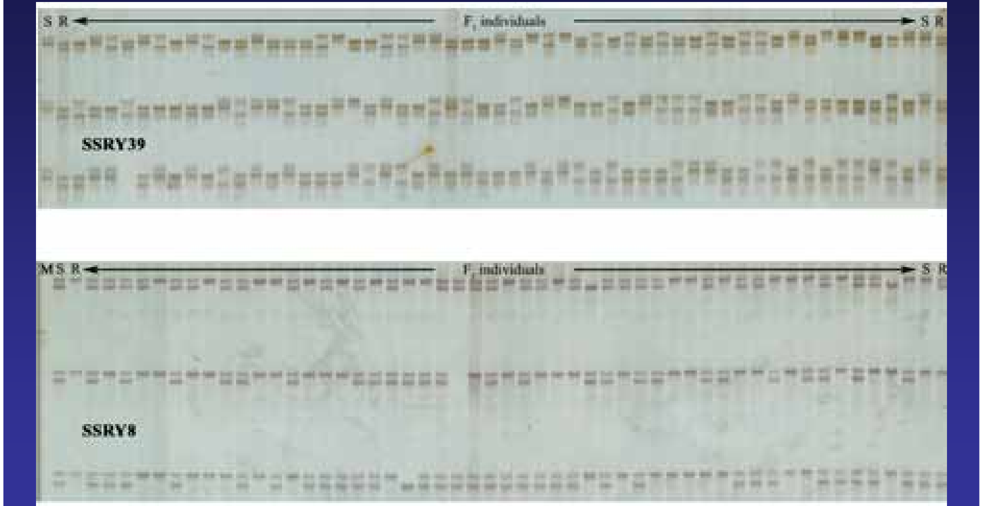


Plate 9. Segregation pattern of SSRY40 and SSRY13 primers.

Genotyping with SSR primers

S- Susceptible parent R – Resistant parent



M : Marker   S : CO2   R : MNga-1

Plate 8. Segregation pattern of SSRY39 and SSRY8 primers.

Genotypic scoring

- Genotyping done as described by Wu *et al*. (1992)
- Single-dose Restriction Fragment – (SDFR)

DNA markers present in one parent and absent in another parent and segregate in a 1:1 ratio in progeny

Genotypic scoring - SDFR

SSRY28

S	R	F_1s			
▬		▬	▬		
	▬		▬	▬	
▬	▬	▬		▬	▬

S - Susceptible parent   R - Resistant Parent

QTL mapping

QTL – Quantitative Trait Loci

- To identify SSR markers (genotypic data) associated with CMD resistance (phenotypic data)
- Single marker analysis – one way ANOVA
- A significant F-test indicates association of marker locus with phenotype

Associated linkage map-G

- SSRY28[a]
- SSRY235[d]
- SSRY44[b]
- NS136[d]

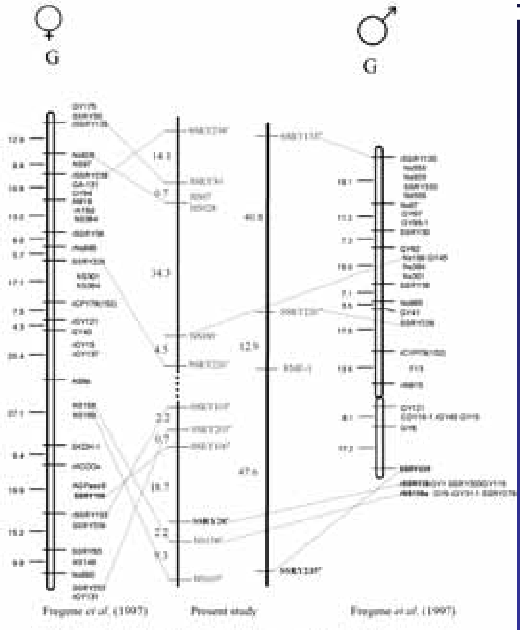


Figure 3. Combined linkage map of linkage group #5, #11 and #12 along with linkage group G of Fregene *et al.* (1997).

CMD associated linkage map

- SSRY28[a]
- SSRY235[d]

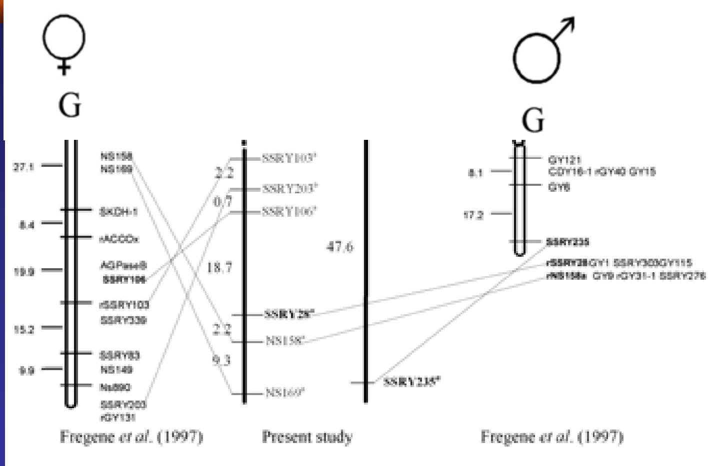


Figure 3. Combined linkage map of linkage group #5, #11 and #12 along with linkage group G of Fregene *et al.* (1997).

Associated linkage map-P

- SSRY44^b
- NS136^d

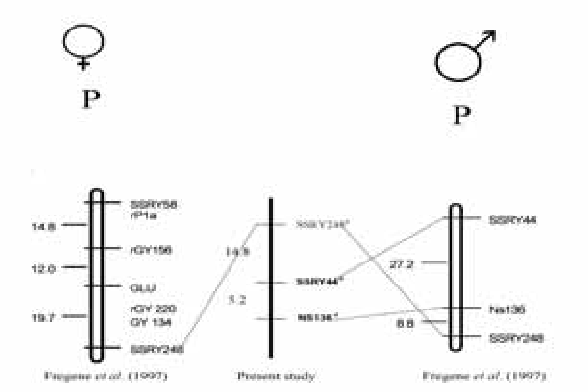
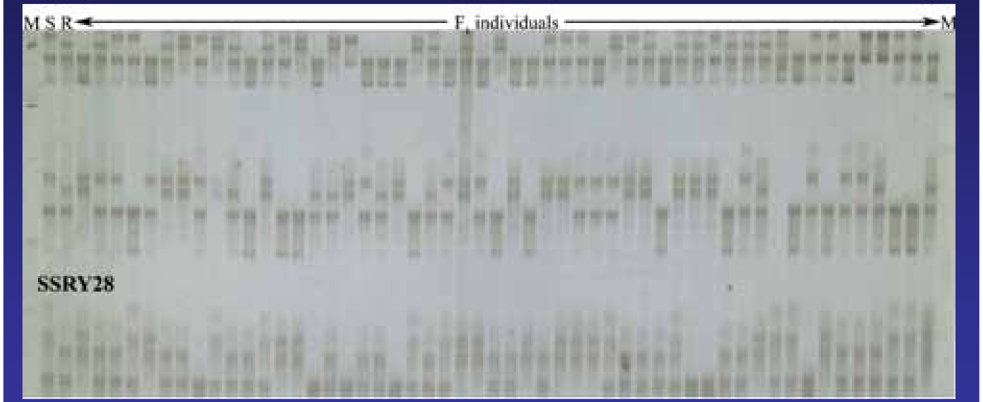


Figure 4. Linkage map of linkage group #14 along with linkage group P of Fregene *et al.* (1997).

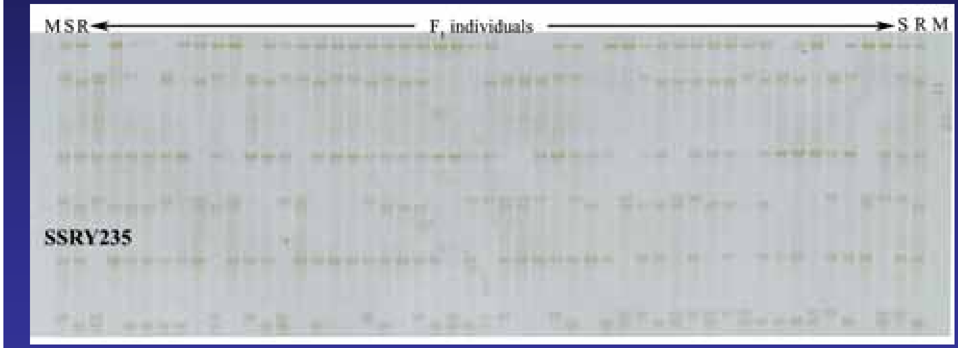
Marker associated with CMD

SSR primers - SSRY28



Marker associated with CMD

SSR primers - SSRY235

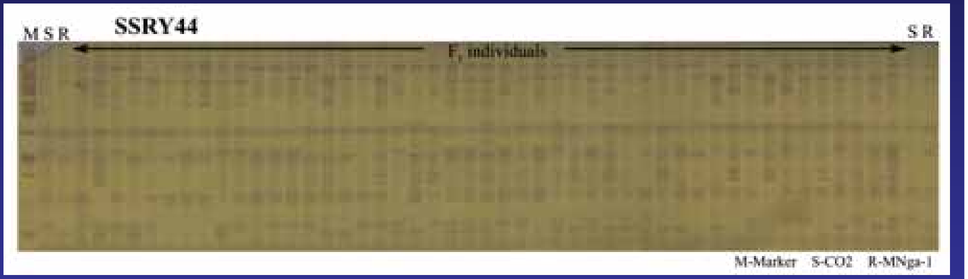


Marker associated with CMD

SSR primers – NS136

Marker associated with CMD

SSR primers – SSRY44



Conclusion

- Virus present in both parents but expressed in susceptible parent alone
- Markers SSRY28^{a}, SSRY44^{b}, NS 136^{d}and SSRY235^{d} associated with CMD resistance
- Markers are present in different linkage group – polygenic nature of inheritance
- Based on these markers resistant lines were isolated which are being field evaluated in CTCRI


THANK YOU