

Status of Molecular Marker – Assisted Selection (MAS) Technology in Clonally Propagated Crops

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Outline

- ❖ **Introduction**
- ❖ **MAS Technologies in Cassava breeding**
- ❖ **MAS Technologies in Banana breeding**
- ❖ **Development of markers for MAS in
Coconut and Yam breeding**
- ❖ **Future Perspectives**

Breeding Strategy for Clonally Propagated Crops

- ❖ Clonal propagation requires breeding methods that takes advantage of both additive and non-additive variance
- ❖ Large effective population sizes required to retain favorable dominant alleles and epistatic loci combination
- ❖ Managing such large number of progenies are beyond most breeding programs and requires MAS to reduce in a logical manner

Ideal MAS Technologies for Clonally Propagated Crops

- ❖ Markers explaining a large percentage of phenotypic variance associated with genes controlling traits with low heritability
- ❖ Markers that can be easily assayed in rudimentary molecular biology labs and adaptable to 'high-through put'
- ❖ Markers where GXE and GXG plays a minor role
- ❖ Markers for high heritability traits where the cost of marker assay is lower and faster than field-based phenotyping

Marker Technologies for MAS: Quantitative Trait Loci (QTL) Mapping

$$y_y = \mu + [G_i] + E_j + I_y + \epsilon$$

Y = Phenotypic value of individual y

μ = Mean phenotype of the population

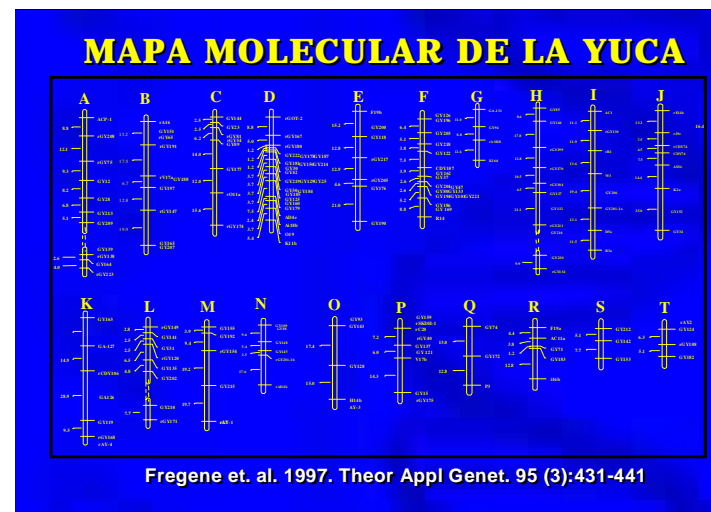
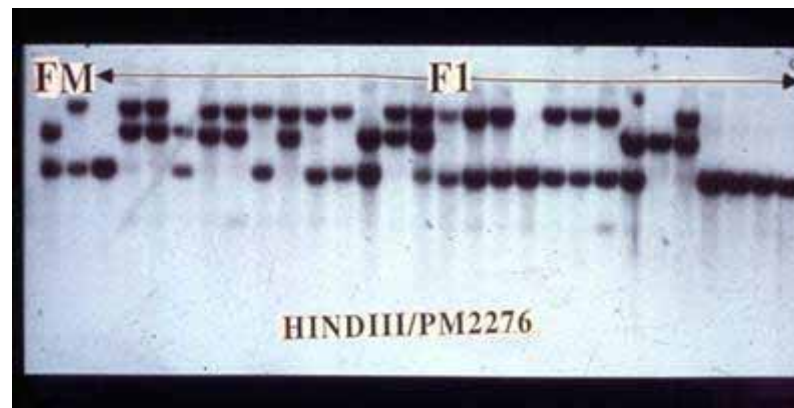
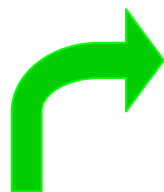
G and E = the net effects due to an individual having genotype i and environment j

I = the interaction between i and j

ϵ = a random contribution to the phenotype

Marker Technologies for MAS: Quantitative Trait Loci (QTL) Mapping

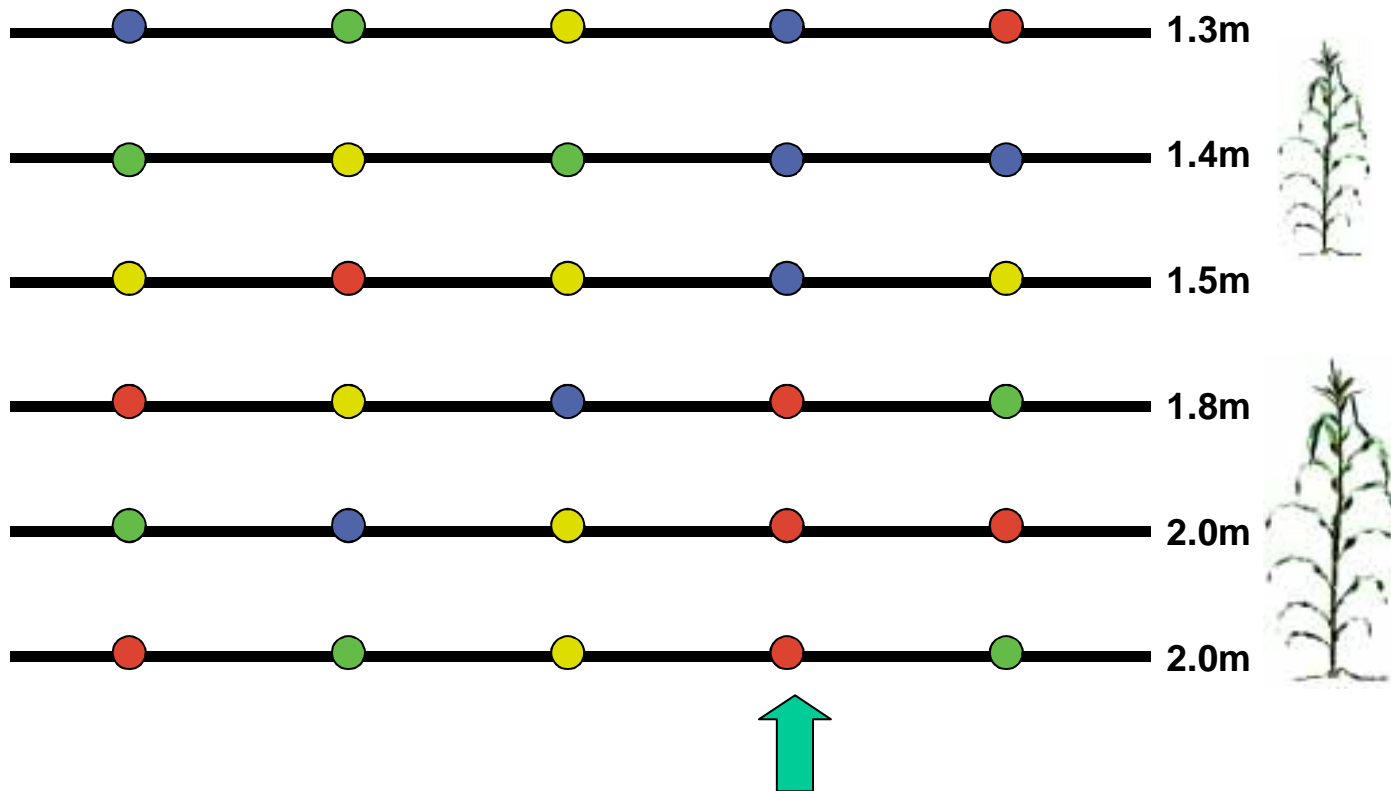
612 mapped markers: 202 SSRs, 307 RFLPs, 100 RAPDs, 3 isozymes
>4000 unmapped markers: 1050 SSRs, 3100 RFLPs, ESTs



Funding: Rockefeller Foundation

Association Tests

- Evaluate whether nucleotide polymorphisms associate with phenotype
- Natural populations
- Exploit extensive recombination



Breeding Objectives in Cassava: Centralized Breeding

- ❖ High Dry Matter Yield
- ❖ Resistance to Pests and Diseases
- ❖ Dry Matter Content
- ❖ Harvest Index
- ❖ Plant Architecture
- ❖ High Value Traits (protein, starch types, beta-carotene)

Can We Increase the Productivity of Small Cassava Farmers?

- ❖ Many cassava farmers in Africa continue to grow pest and disease susceptible varieties with low yield but having culinary, processing, or agronomic traits
- ❖ Adoption of varieties from centralized breeding programs have been low in Africa with the exception of a few countries where there is a large market for a single processed product
- ❖ A combination of MAS and PPB was proposed as a way around this problem



Breeding Objectives in Cassava: Decentralized Breeding

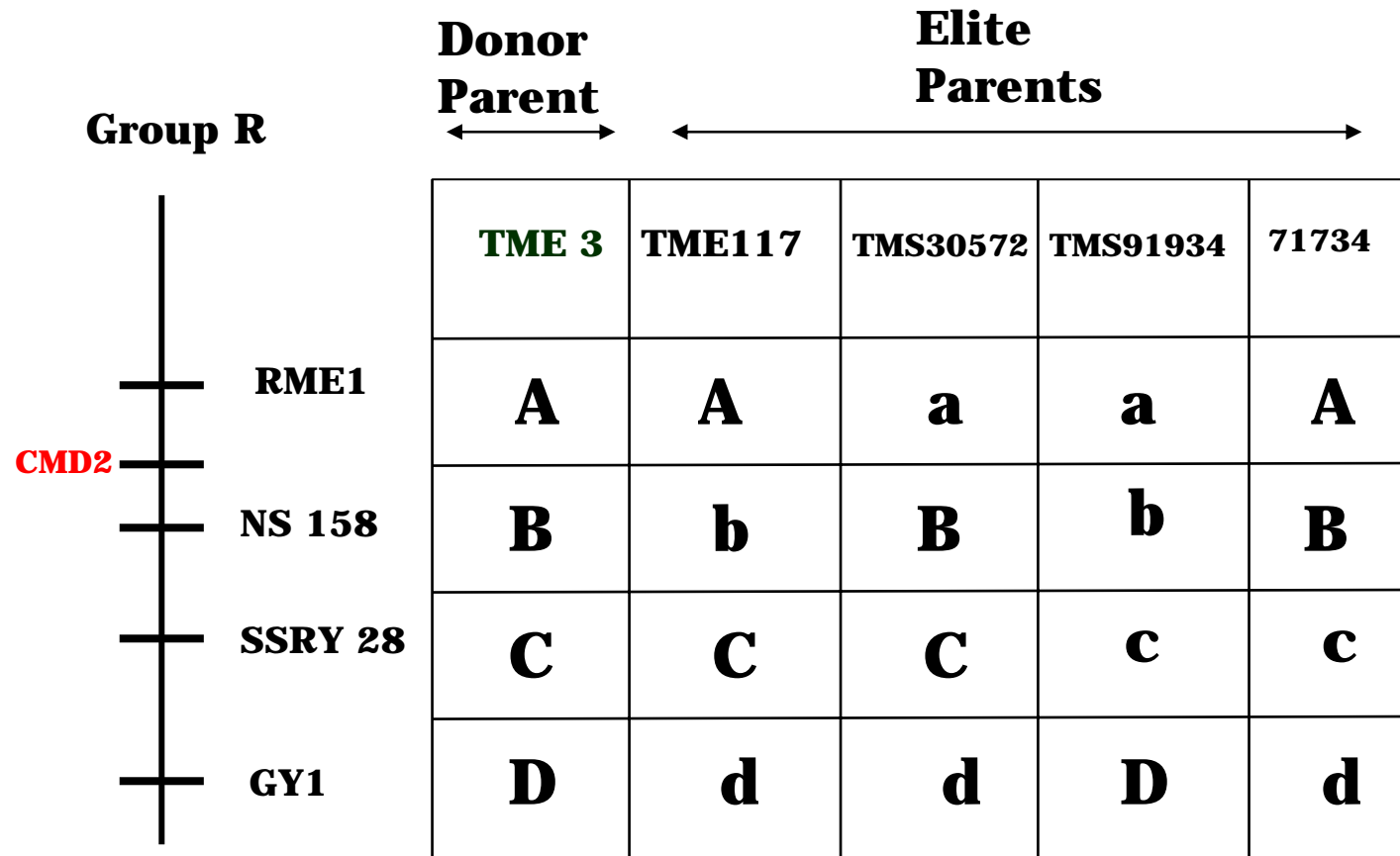
- ❖ Early bulking
- ❖ Culinary and storage quality
- ❖ Adaptation to agro-ecological niches
- ❖ Pest and disease resistance
- ❖ Production of Planting materials
- ❖ High dry matter yield
- ❖ Plant architecture

The Cassava Mosaic Disease (CMD)

- ❖ A viral disease endemic in SS-Africa and India
- ❖ Not recorded in the Americas
- ❖ Prevents germplasm shipment from the crop's center of diversity to Africa and India
- ❖ Need to breed for resistance at CIAT

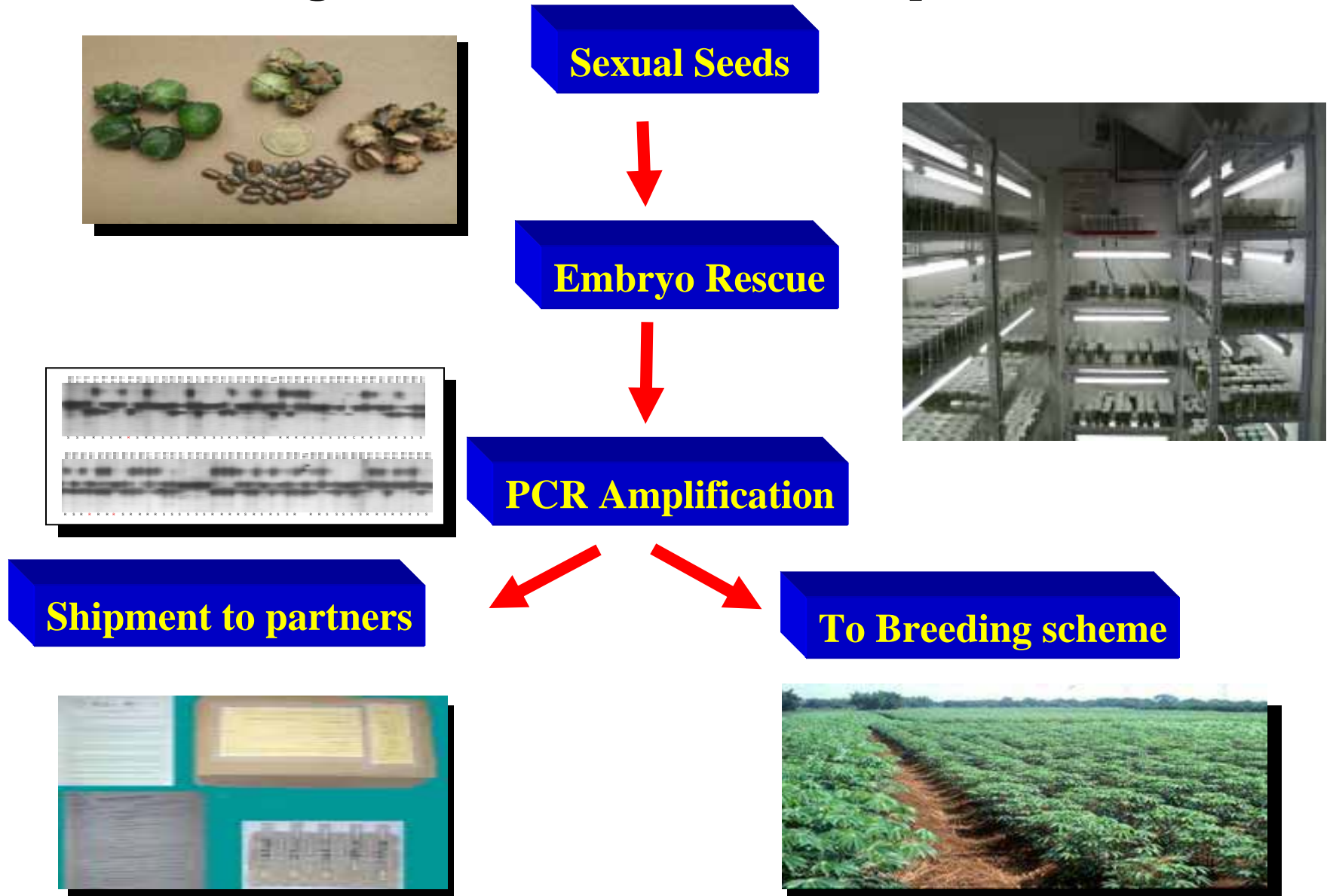


Concept of Molecular Marker Haplotypes



Marker Technologies for Centralized Cassava Breeding

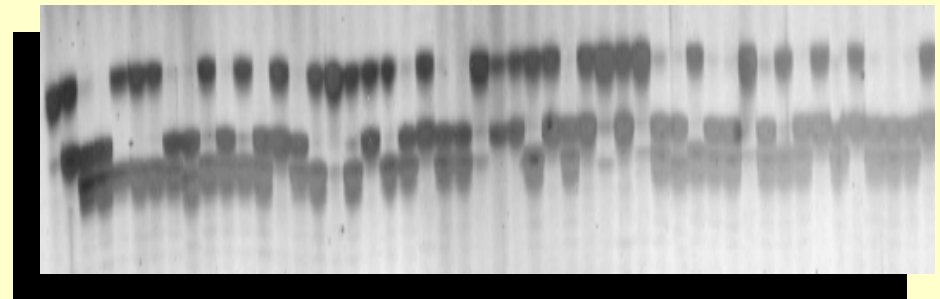
Progenies of TME3 x CIAT Elite parents



MAS for CMD Resistance: Summary

- ❖ 1500 seeds were processed in 2002
- ❖ 2400 seeds were processed in 2003
- ❖ 5,000 seeds were processed in 2004
- ❖ Cost of marker analysis is US\$0.5/genotype
- ❖ Markers are predominantly SSR markers and are

FORMATO EVALUACIÓN PARA MAS.				EVALUACIÓN:	CMD	PLACA No9			
ITEM	CODIGO	MADRE	PADRE	NÚMERO DE FRASCOS CC	EVALUACIÓN SSRY 158	EVALUACIÓN SCAR RME 1	POSO No.	PARA CAMPO/ENVIÓ	CÓDIGO TEJIDO
1	CR52A-32	C-243	SM1219-9		-	S	1	NO	998
2	CR52A-37	C-243	SM1219-9		R	R	5	YES	1003
3	CR52A-38	C-243	SM1219-9		R	S	6	NO	1004
4	cr52a-39	C-243	SM1219-9		R	R	7	YES	1005
5	CR52A-40	C-243	SM1219-9		R	R	8	YES	1006
6	CR52A-41	C-243	SM1219-9		R	R	9	YES	1007
7	CR52A-43	C-243	SM1219-9		R	R	11	YES	1009
8	CR-52B-1	SM1219-9	C-243			S	12	NO	1010
9	CR53-2	C-243	MCOL 2206		R	S	13	NO	1012
10	CR53-3	C-243	MCOL 2206		R	R	14	YES	1013
11	CR53-4	C-243	MCOL 2206		S	R	15	NO	1014



**NS 158 in
acrylamide
gel**

Facilities for MAS in Cassava: Molecular Marker Analysis

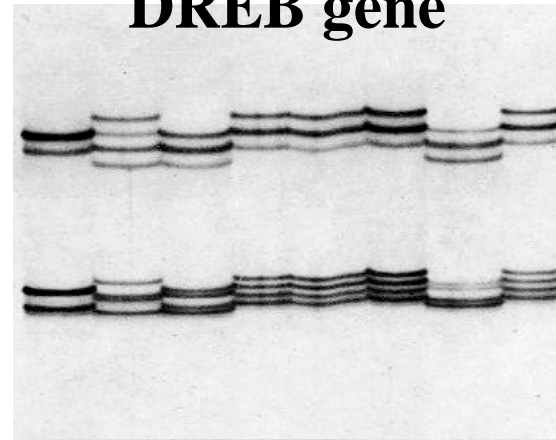
- ❖ Simple equipment and consumables for DNA isolation from dried leaves
- ❖ PCR machine and consumables
- ❖ PAGE gel rigs and power packs
- ❖ Tanks and consumables for silver staining



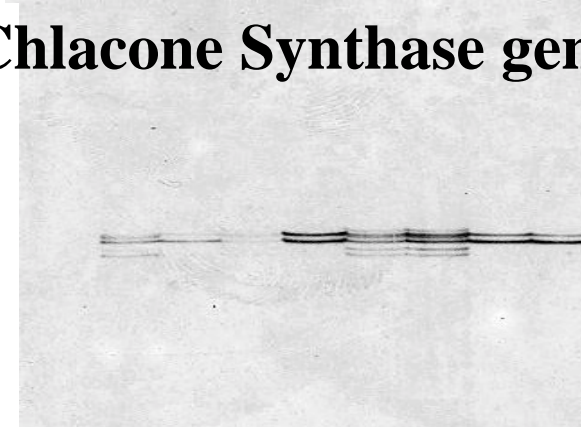
MAS for Introgressing Natural Mutant GBSS Alleles

- ❖ Development of waxy starch in cassava
- ❖ Development SSCP-SNP markers
- ❖ Association mapping using 'Structure' to determine genes linked to DMC

DREB gene



Chalcone Synthase gene



MASCas: Network of Cassava MAS Projects

Address  http://www.ciat.cgiar.org/mascas/index.htm Go Links >>

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MASCas

Introduction to the Molecular Marker

Protocols for Marker Assisted Selection in Cassava

Molecular Tools Available to MASCas

Genetic Stock for Gene Mapping

Partners

Cassava Links

MASCas

Molecular Marker Assisted Selection in Cassava Breeding

This project is funded by:
[Rockefeller Foundation](#); [CIAT](#); [FAO](#); [Generation Challenge Program \(GCP\)](#).

Project leader: [Dr. Martin Fregene](#).

Site maintained by: [Ing. Fernando Rojas](#).

For further information contact: [Martin Fregene](#).

Last update: 14 January 2005

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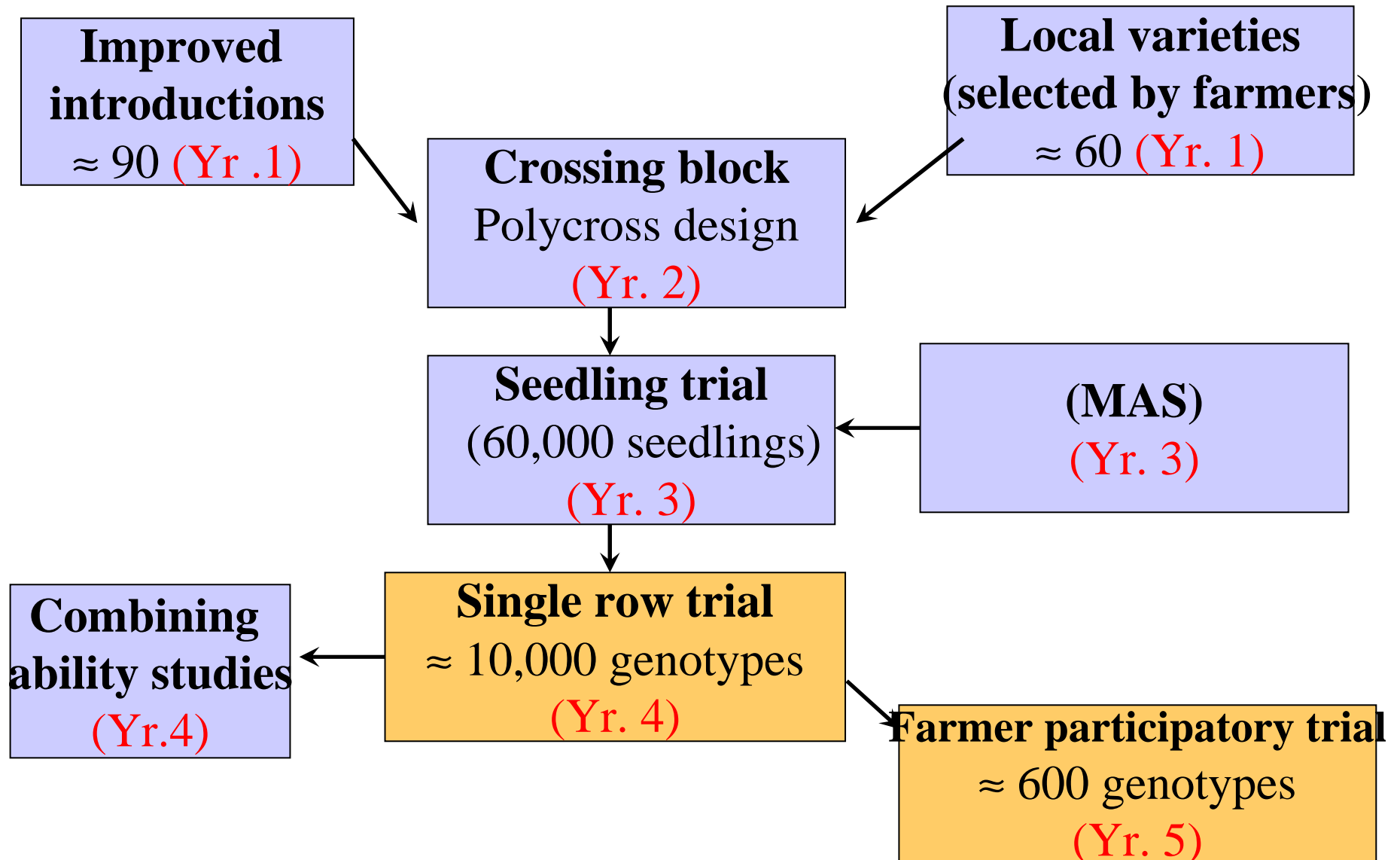
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Cassava is a crop that has largely been left behind by scientific plant breeding partly due to its status as an orphan crop with limited resources for research and development, its highly heterozygous nature, and its long reproductive cycle.



Marker Technologies for Decentralized Cassava Breeding



Low-Cost Allele-Specific PCR Markers Assayable on Agarose Gels

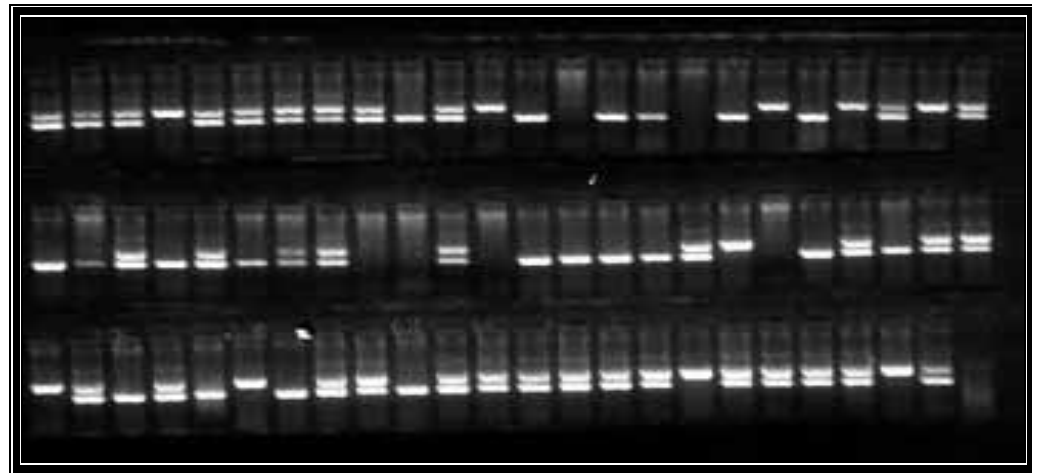
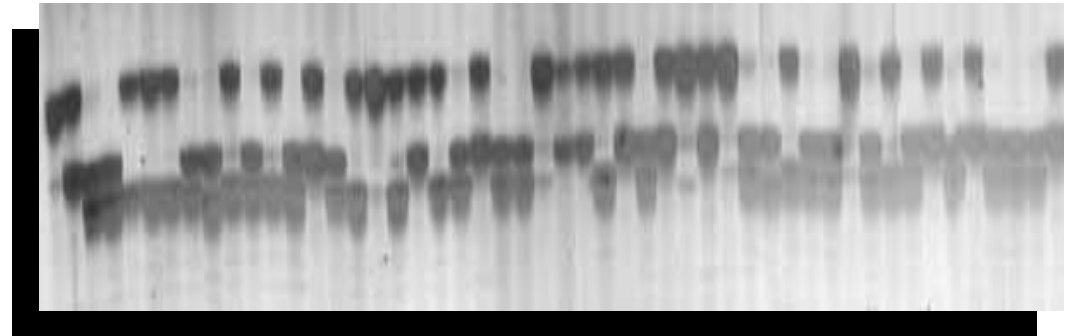
Cloning of the marker allele
associated with CMD resistance
and sequencing



Design several allele specific
primers, typically involving a
mismatch at the penultimate
or 3rd nucleotide from the 3'
end



Testing of the allele-specific
primers and selection of the most
appropriate for MAS

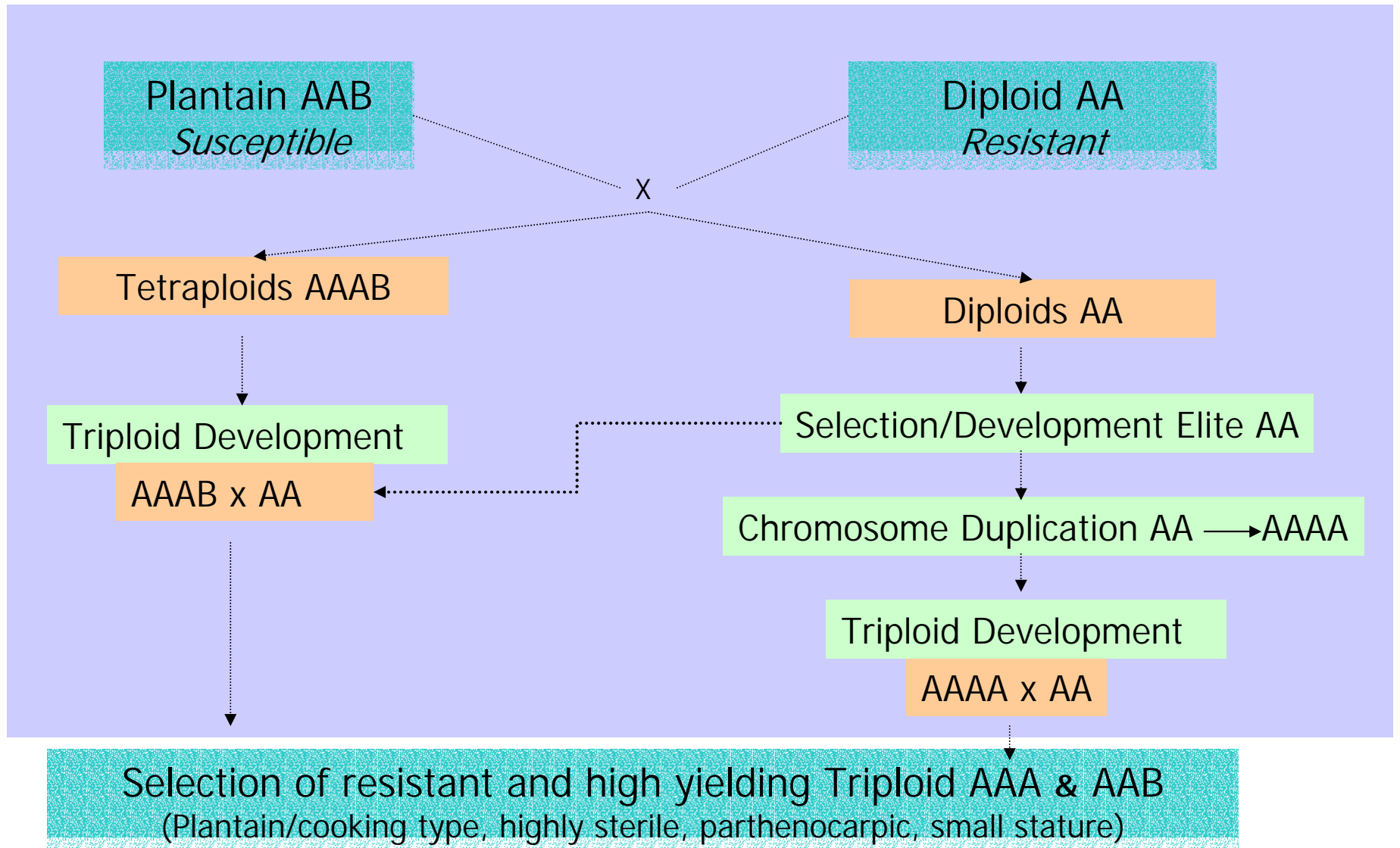




African Research Centre on Banana and Plantain



Plantain Breeding Strategy at CARBAP : Triploid Development (K. Tomekpé, 2001)



Approaches used by CARBAP to produce several dwarf plantain-type hybrids

- Sterile dwarf Plantain broadly pollinated with resistant diploids (3x/2x scheme) **using multilocational design to enhance residual fertility**
- Obtention of diverse dwarf hybrids, **more fertile than their mother** including tetraploids, diploids and triploids
- Obtention of diverse dwarf secondary triploids by crosses 4x/2x or 2x/4x



AAAB



AAB/AAA

Marker Technologies for Banana Breeding

Traits:

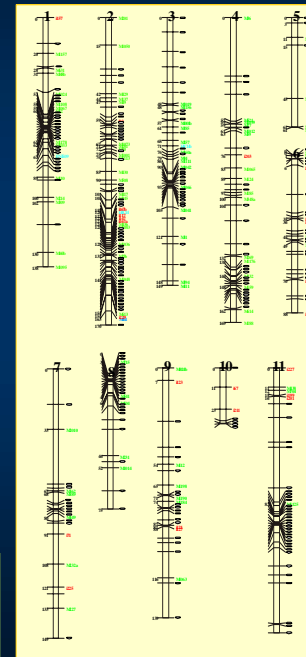
- ❖ Disease Resistance
- ❖ Parthenocarpy

Markers

- ❖ SSR Markers
- ❖ IRAPs
- ❖ COS Markers

Planning the crosses, understanding the problems...

Basic genetic map of *Musa*



Courtesy of CIRAD



ACORBAT, Oaxaca, Mexico - 2004

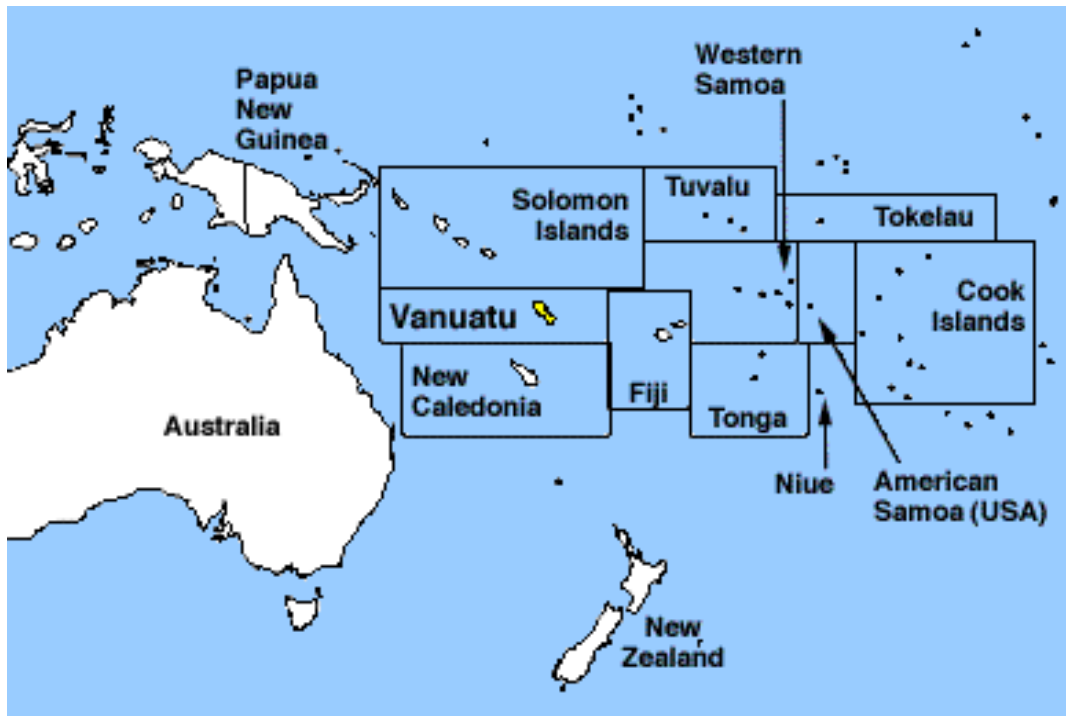
Population structure, phenotypic
information and association studies
in long generation crops

Coconut in Vanuatu
Luc Baudouin



Location

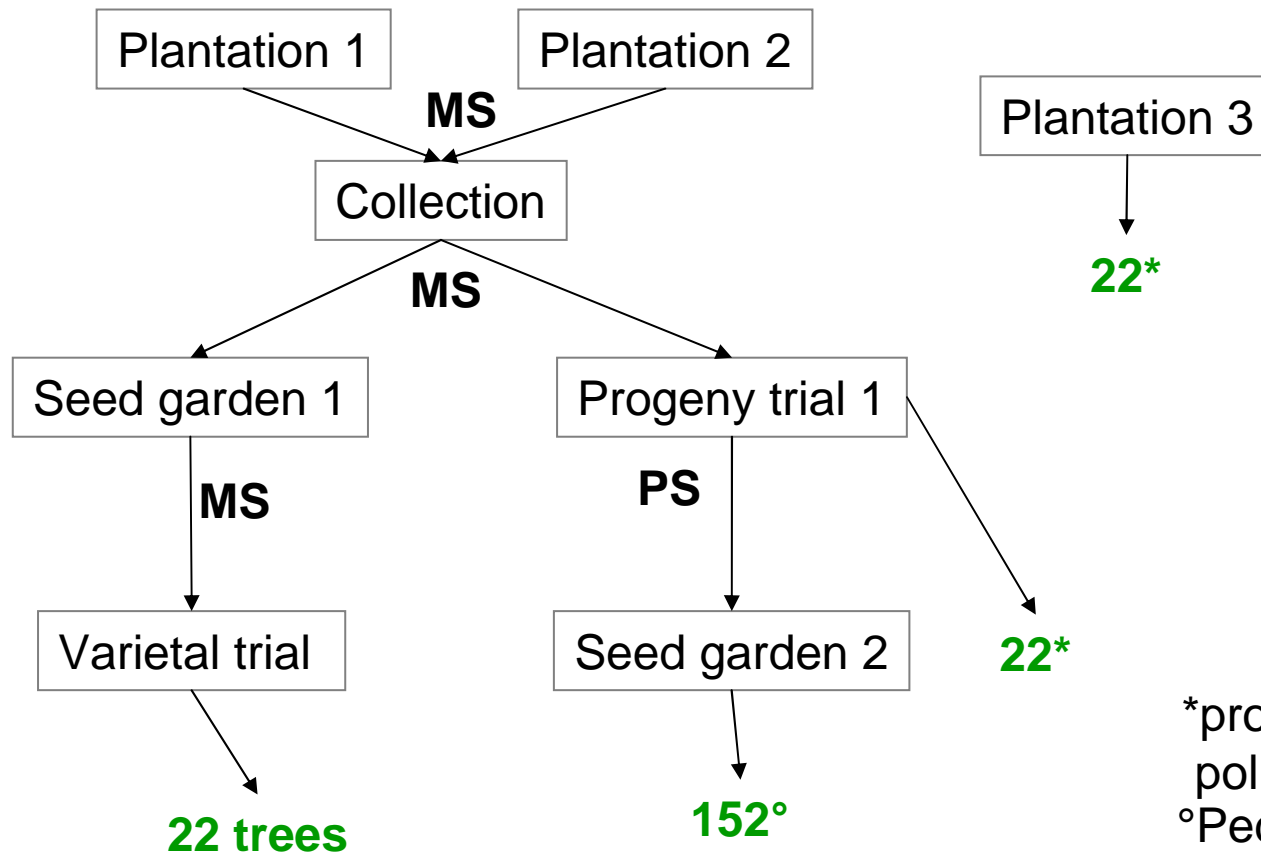
- Archipelago
- 166° E, 16°N
- About 700 km from N to south



Constraints

- Presence of a viral disease, which is lethal to almost all foreign cultivars (coconut foliar decay)
- High cost of improved seed production AND transportation.

Sampling



*progeny by open
pollination (tall trees)
°Pedigrees are partially
known

Observations

- In accordance with the “stantech” manual
- Fruit and bunch return
 - Number of bunches
 - Number of fruits
- Fruit component analysis
 - Fruit weight
 - Nut weight
 - Husk weight
 - Shell weight
 - Meat weight
 - Water weight
- Ease of removing albumen from the shell (1 to 4)
- Stem measurements
 - Stem circumference at 20cm
 - Stem circumference at 1.5m
 - Plant height
 - Internode length
- Leaf measurement
 - Petiole length
 - Petiole width
 - Petiole thickness
 - Rachis length
 - Number of leaflets
 - Leaflet width
 - Leaflet length

Plans (achieved by september)

- 200 trees (219 extracted)
 - 13 reference (COGENT) SSRs
- + 17 SSR loci on seven linkage groups
providing 15 couples of linked markers
(0 to 7.1 cM)
(ongoing)

Collaborating institutions

- Coconut Research Institute, Sri Lanka
- Vanuatu Agricultural Research and Training Center, Vanuatu
- Agropolis-CIRAD, France

- Training in Montpellier on molecular markers
 - Tiata Sileye, VARTC (three weeks in November)
 - Dr Champa Kumari Bandanarayake, CRI (three months, September to November)

- Connection with other projects
 - Genetic structure in coconut: “Tier 2 genotyping” and “LD analysis”
 - DArT development

THANK YOU