Advances in Genomics: application to banana breeding

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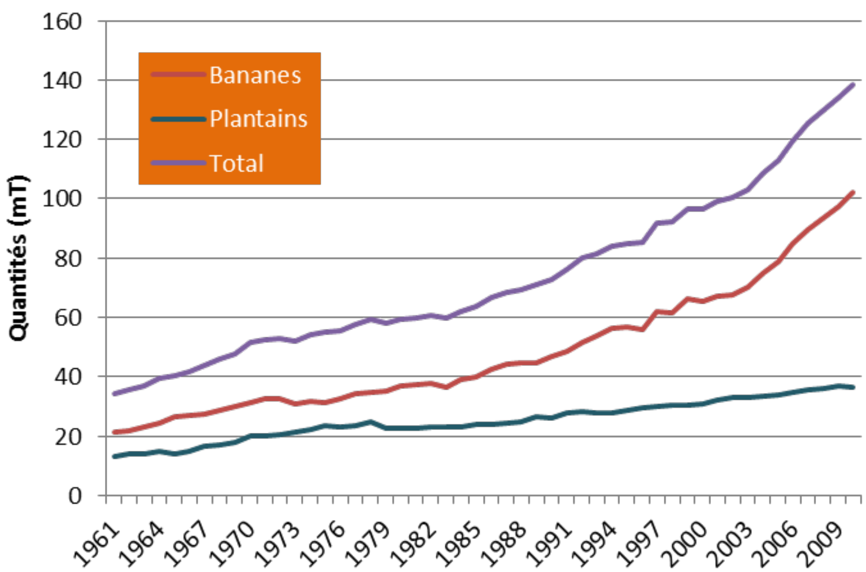
Current cropping system

Narrow genetic diversity in edible triploids (AAA, AAB, ABB)

Fragile:
- diseases
- climate changes
- access to inputs

Markets:
- popular fruit/vegetable
- world production ↗



Objectives for Banana Breeding

Resistant varieties close to natural varieties

- Cercosporiosis , FOCs, in priority
- Same attributes of quality and phenology
- Productivity: equivalent or higher
- Reduced production costs

→ agronomical and economical sustainability

Demand for new Musa products ?

- Unknown fresh fruits/legumes
- Processing
- Varietal mix

Principles of Banana Breeding (1/3)

Principal landraces/ main features:

- Edibility (seedless, parthenocarpy)
- Triploidy
- Highly heterozygous (sub- & interspecificity)
- Vegetatively propagated

Principles of Banana Breeding (2/3)

Constraints to breeding :

- High gamete sterility
- Strong gamete desequilibrium
- Little knowledge on genetics and heredity
- Few recombination events
- New hybrids: primeval products

Principles of Banana Breeding (3/3)

« Reconstructive breeding »
for dessert banana (CIRAD)

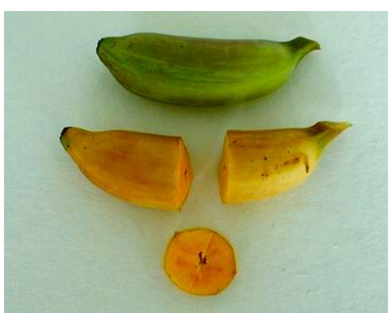
- Synthesis of triploids from diploid germplasm
- Based on Musa evolution knowledge
- Maximizing heterozygosity
- Crosses between edible varieties and disease resistant fertile clones
- Parental combinations evaluated on progenies

Musa balbisiana

Musa acuminata microcarpa

Musa textilis

Musa schizocarpa



Evolution and domestication of bananas

Wild types: AA w | BB | SS | TT

Natural hybridization & selection

Intra-subspecific and interspecific crosses
Domestication for parthenocarpy and stérility

Edible diploids: AA cv | AB | AS | AT | BT

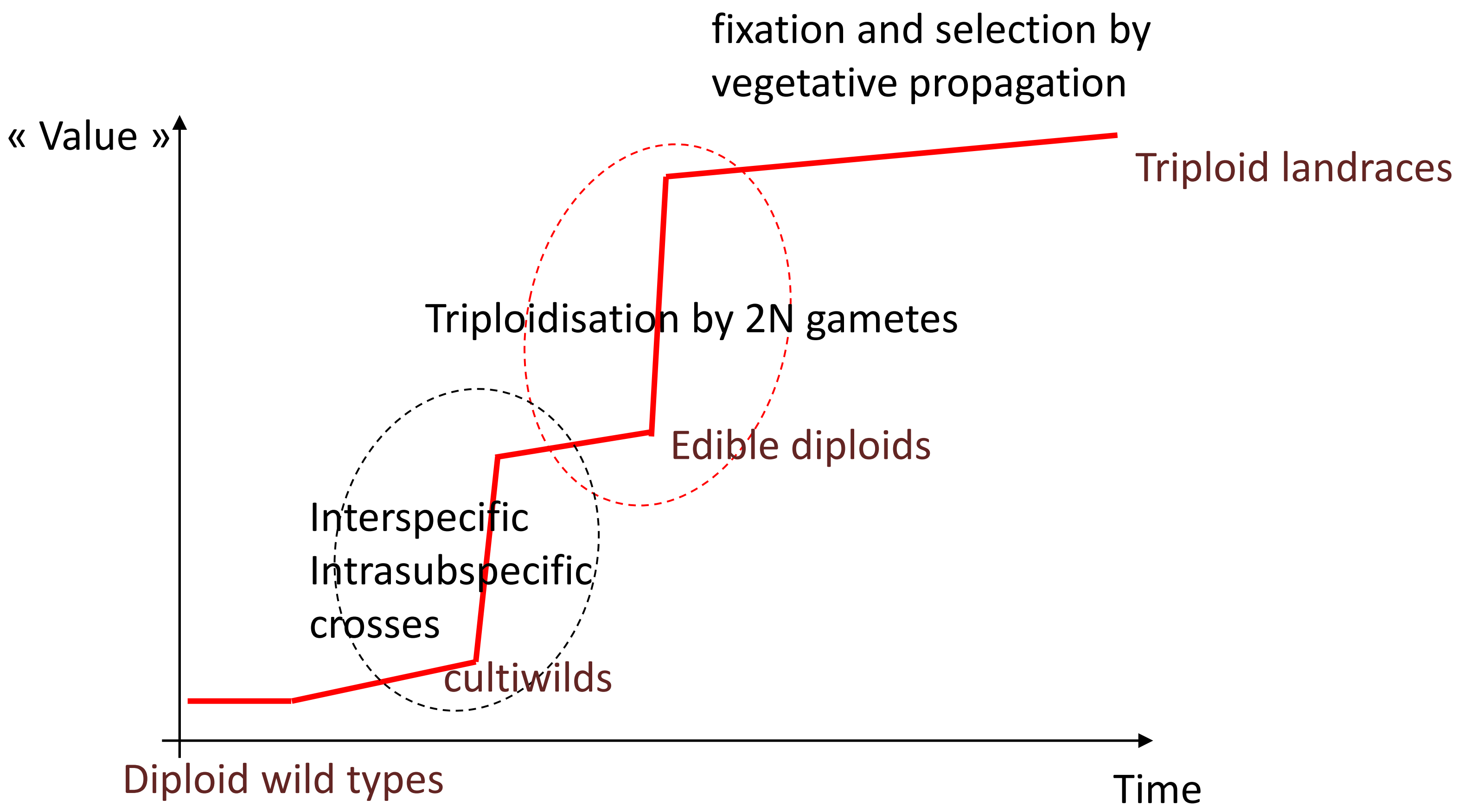
Natural hybridization & selection

Production of N and 2N gametes
Crosses between edible diploids and wilds or ED/ED

Triploid landraces: AAA | AAB | ABB | AAS | AAT

Natural somaclonal variations & selection

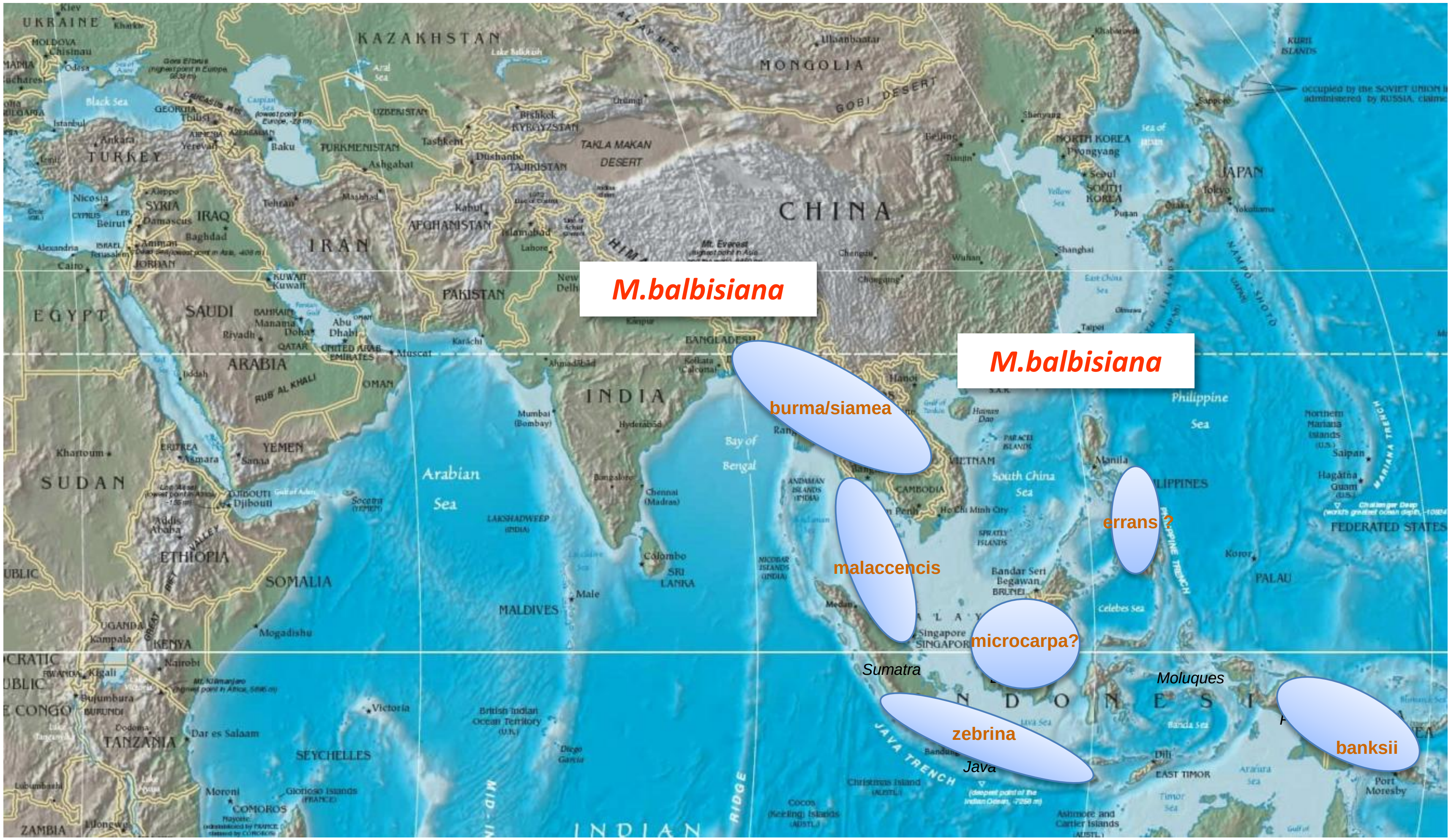
Banana evolution



From: X. Perrier, 2013

Evolutionary history of banana

The first sub-speciations



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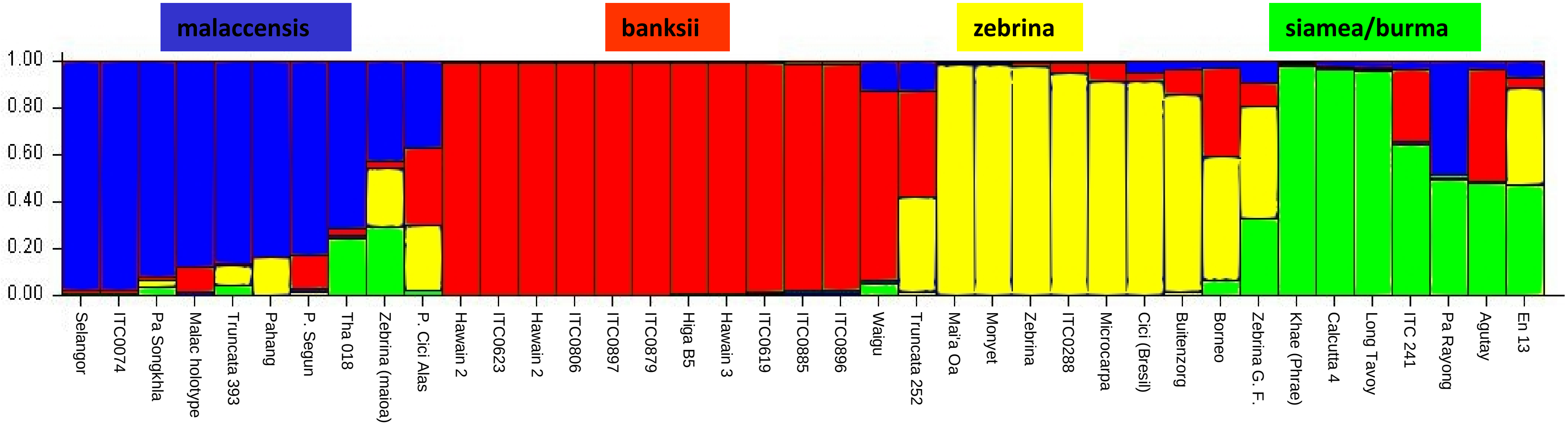
Wild : M. acuminata malaccensis (AA), 2X, Malaysia

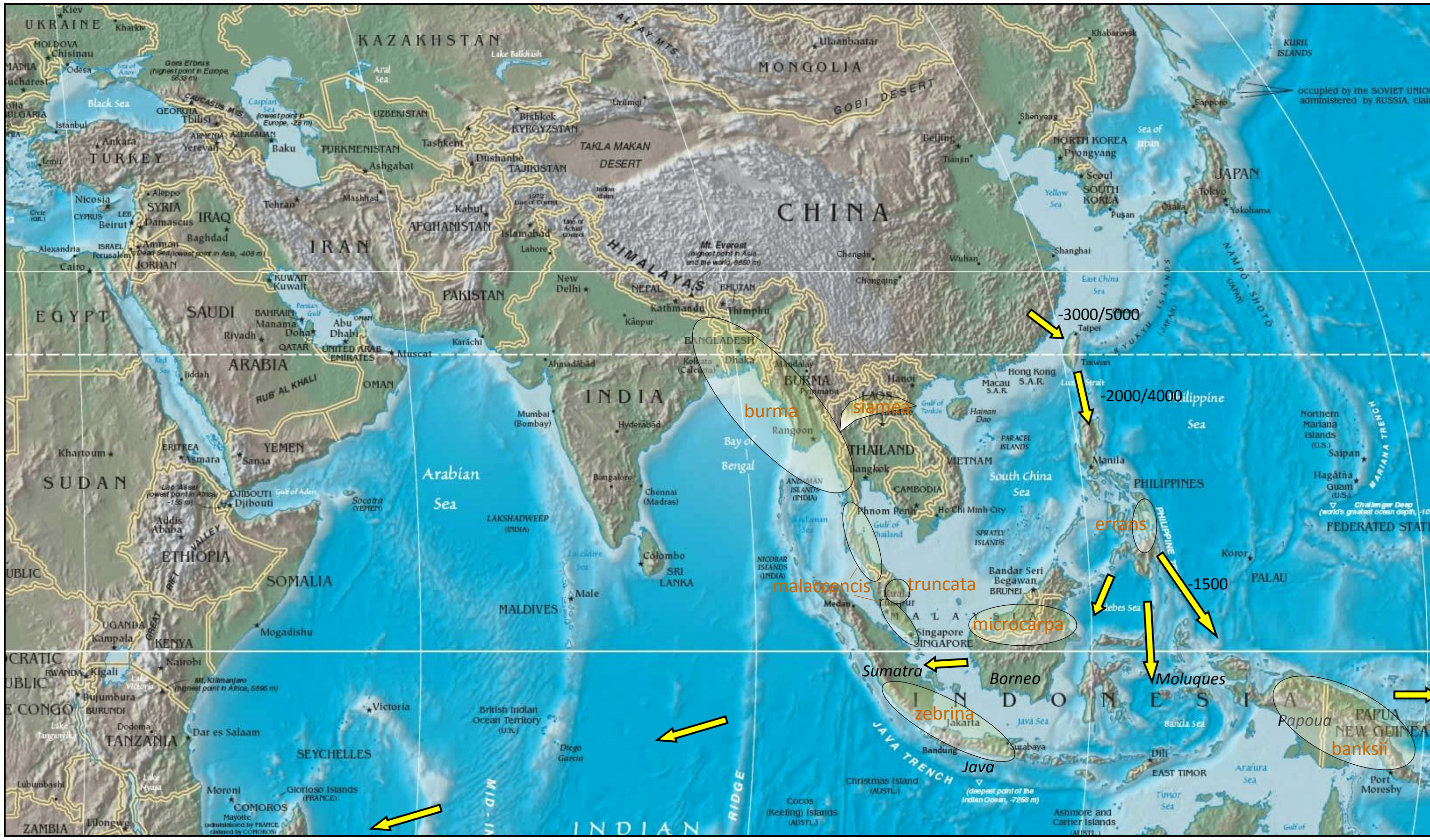
Cultiwild : M. Yunnanensis (YY), 2X, Yunnan, China (M. Hakkinen et al.)

Structure software 22 SSRs/39 AAw

K=4



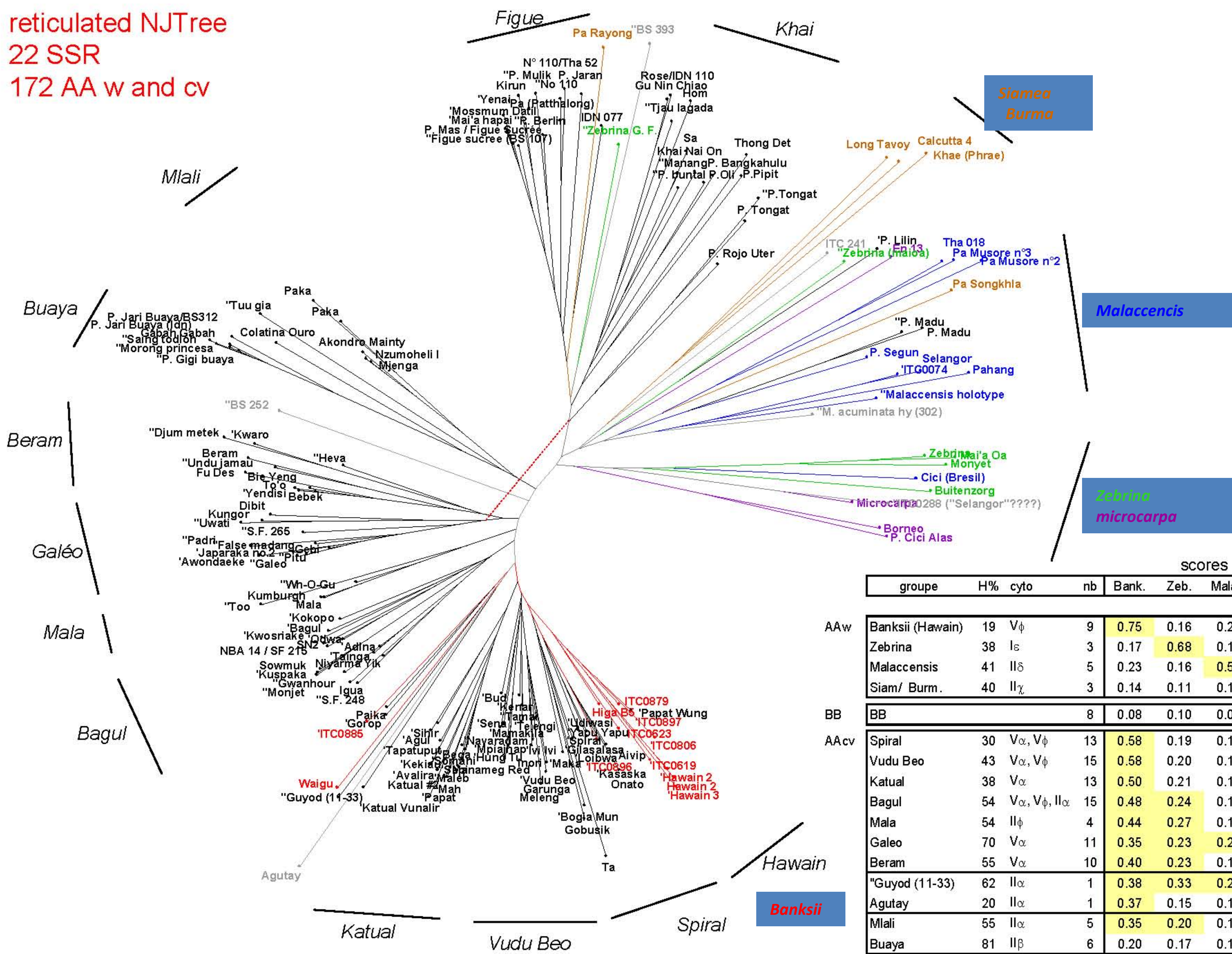
Austronesian migrations vectors of domestication



Evolutionary history of banana

Diffusion and hybridizations in contact areas

Example : Mlalis



					scores			
	groupe	H%	cyto	nb	Bank.	Zeb.	Malac.	Burm.
AAw	Banksii (Hawain)	19	Vφ	9	0.75	0.16	0.22	0.13
	Zebrina	38	Iε	3	0.17	0.68	0.14	0.10
	Malaccensis	41	IIδ	5	0.23	0.16	0.50	0.14
	Siam/ Burm.	40	IIχ	3	0.14	0.11	0.13	0.45
BB	BB			8	0.08	0.10	0.05	0.09
AAcv	Spiral	30	Vα, Vφ	13	0.58	0.19	0.19	0.12
	Vudu Beo	43	Vα, Vφ	15	0.58	0.20	0.18	0.11
	Katual	38	Vα	13	0.50	0.21	0.16	0.12
	Bagul	54	Vα, Vφ, IIα	15	0.48	0.24	0.19	0.12
	Mala	54	IIφ	4	0.44	0.27	0.17	0.09
	Galeo	70	Vα	11	0.35	0.23	0.23	0.10
	Beram	55	Vα	10	0.40	0.23	0.18	0.12
	"Guyod (11-33)	62	IIα	1	0.38	0.33	0.22	0.10
	Agutay	20	IIα	1	0.37	0.15	0.16	0.14
	Mlali	55	IIα	5	0.35	0.20	0.18	0.14
	Buaya	81	IIβ	6	0.20	0.17	0.16	0.12
	Figue	59	IIα	15	0.16	0.25	0.20	0.13
	Khai	65	IIα, IIδ	12	0.17	0.17	0.24	0.14

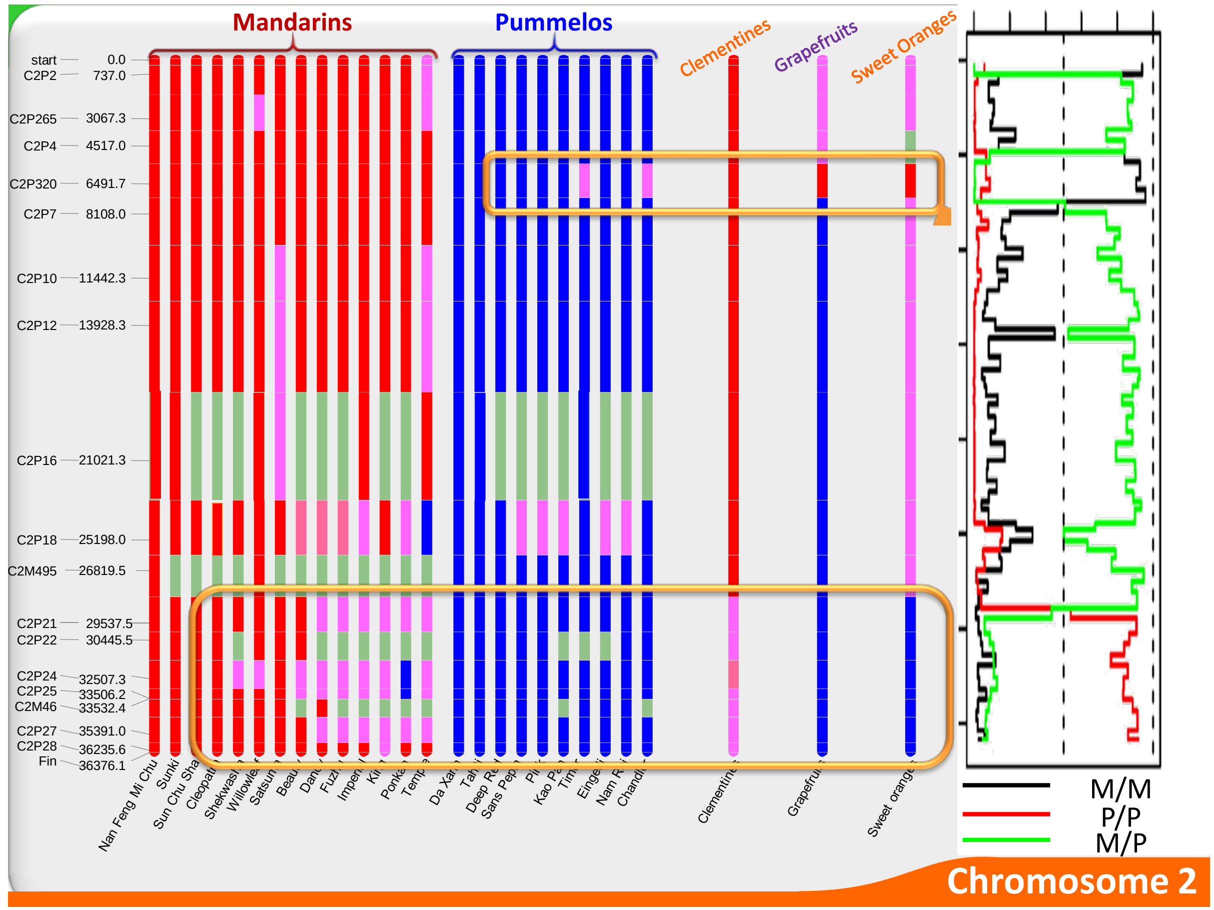
Genetic diversity and structuration of diploid AA bananas

Deciphering the genome-wide mosaic structure of chromosomes
Multilocus haplotyping for CWR/ED

- Haplotypic analysis of amplicons
- Numerous amplified sites
- Numerous génotypes
- Parallele sequencing

Phylogenetic assignements to segments of chromosomes

Phylogenetic reconstruction of chromosomes by genotyping for CWR and ED



Curk et al., 2012. International Citrus Congress Valencia

Questions:

ª Genome mosaics ?

ª How many recombination cycles between CWR and ED ?

ª Evidences for high linkage desequilibrium ?

Reconstructive breeding in Musa

AAw x AACv
AAcv x AAcv → AAcv —chromosome doubling (colchicine)→ AAAAcv (1)

AAcv x BB → ABcv —chromosome doubling (colchicine)→ AABBcv (2)

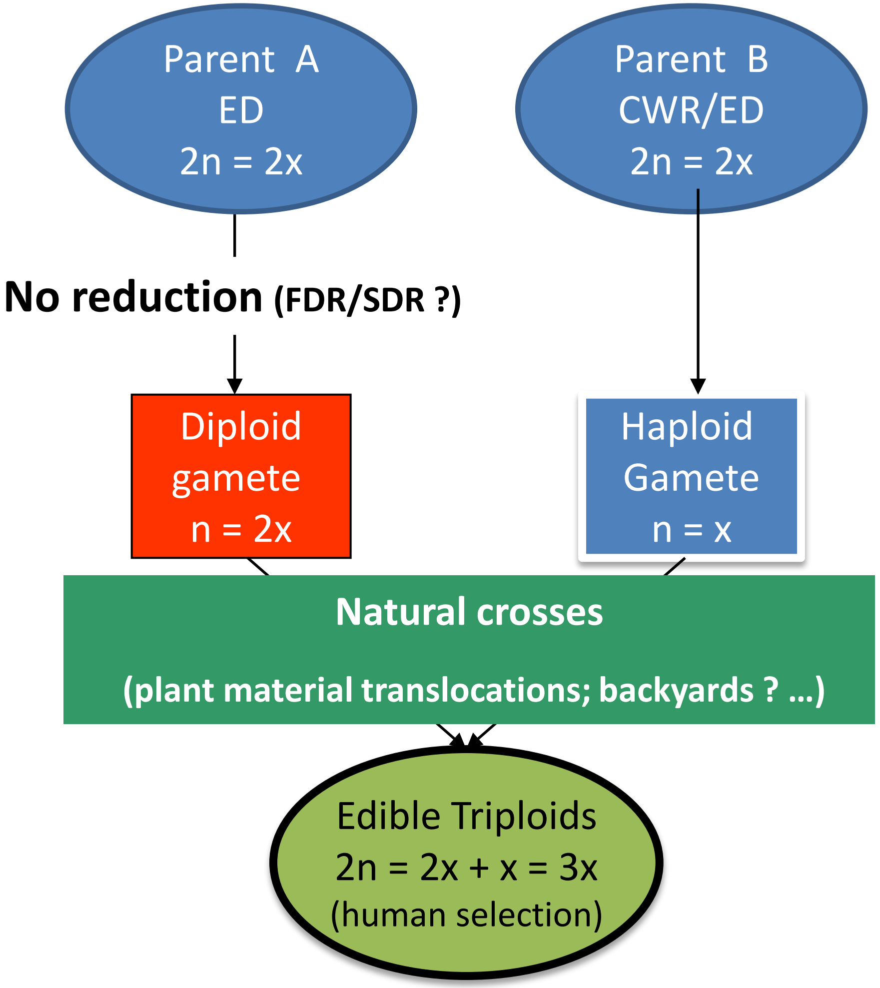
Synthesis of triploid hybrids

					Selections
AA	x	AAAAcv	AAAcv	(3,7)	FLHORBANs & CIRAD925
BB	x	AAAAcv	AABcv	(3, 5)	IRFA909, IRFA910, IRFA914
AA	x	AABBcv	AABcv	(3, 4)	CIRADBAN L9, CIRADBAN X17
BB	x	AABBcv	ABBcv		Under selection

Key : cv = cultivar ; w = wild species ; (1) = wide range of doubled-diploids; (2) = neo-allotetraploid (ex: Kunnan T)
(3) = from 200 to 500 individuals by progeny; "L9" selected from this cross
(4) = 98% of the progeny are triploid ; (5) = IRFA 909, 910 & 914 were selected from this cross

Natural occurrence of triploids

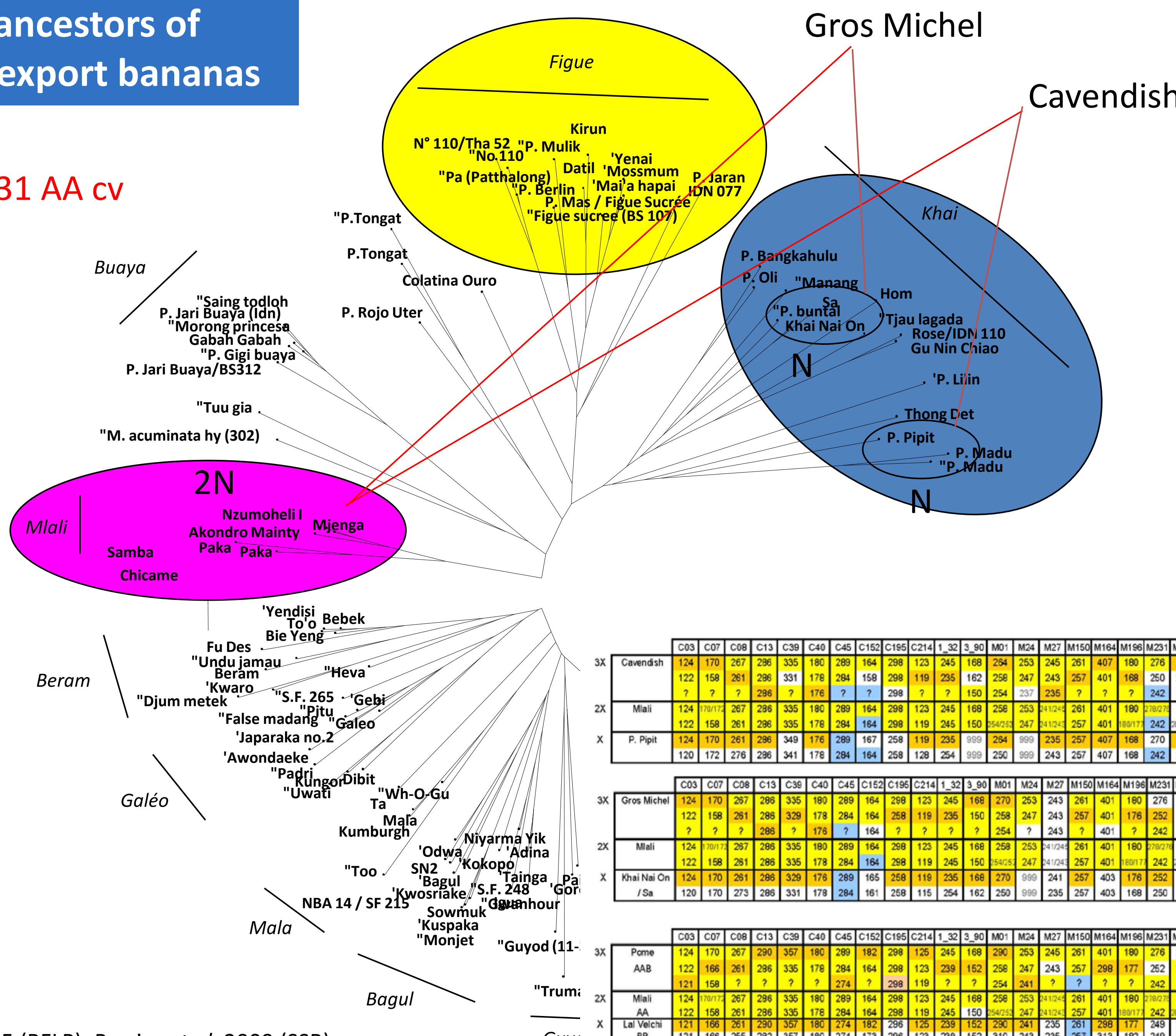
(main hypothesis: direct pathway)



NB: Some rare indirect pathways of triploid synthesis may have occured

Diploid ancestors of triploid export bananas

22 SSR/131 AA cv

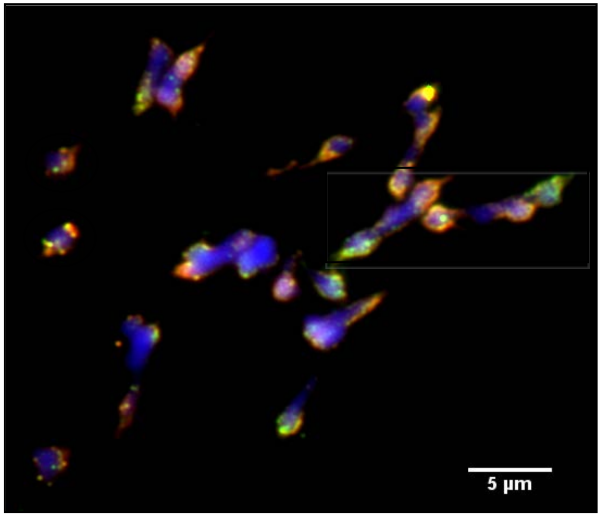


		C03	C07	C08	C13	C39	C40	C45	C152	C195	C214	1_32	3_90	M01	M24	M27	M150	M164	M196	M231	M260	M264	M307	
3X	Cavendish	124	170	267	286	335	180	289	164	298	123	245	168	264	253	245	261	407	180	276	212	258	164	
		122	158	261	286	331	178	284	158	298	119	235	162	258	247	243	257	401	168	250	212	250	162	
		?	?	?	286	?	176	?	?	298	?	?	150	254	237	235	?	?	?	242	212	?	?	
2X	Mlali	124	170/172	267	286	335	180	289	164	298	123	245	168	258	253	241/245	261	401	180	278/278	210	258	164	21/22
		122	158	261	286	335	178	284	164	298	119	245	150	254/252	247	241/243	257	401	180/177	242	206/210	258	164	
X	P. Pipit	124	170	261	286	349	176	289	167	258	119	235	999	264	999	235	257	407	168	270	212	250	162	18/20
		120	172	276	286	341	178	284	164	258	128	254	999	250	999	243	257	407	168	242	208	264	160	

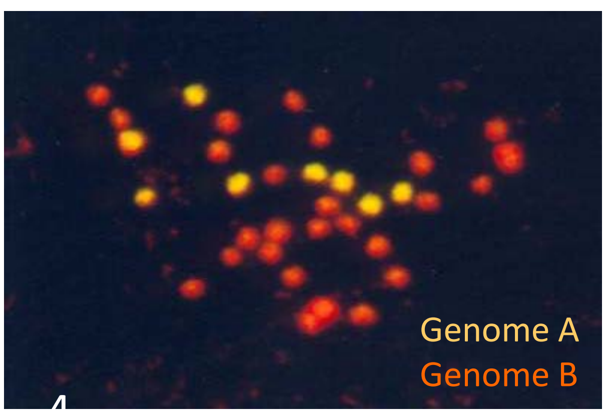
		C03	C07	C08	C13	C39	C40	C45	C152	C195	C214	1_32	3_90	M01	M24	M27	M150	M164	M196	M231	M260	M264	M307	
3X	Gros Michel	124	170	267	286	335	180	289	164	298	123	245	168	270	253	243	261	401	180	276	212	266	164	
		122	158	261	286	329	178	284	164	258	119	235	150	258	247	243	257	401	176	252	212	258	162	
		?	?	?	286	?	176	?	164	?	?	?	?	254	?	243	?	401	?	242	212	?	?	
2X	Mlali	124	170/172	267	286	335	180	289	164	298	123	245	168	258	253	241/245	261	401	180	278/278	210	258	164	21/22
		122	158	261	286	335	178	284	164	298	119	245	150	254/252	247	241/243	257	401	180/177	242	206/210	258	164	
X	Khai Nai On	124	170	261	286	329	176	289	165	258	119	235	168	270	999	241	257	403	176	252	210	266	162	17/21
	/ Sa	120	170	273	286	331	178	284	161	258	115	254	162	250	999	235	257	403	168	250	210	252	162	

		C03	C07	C08	C13	C39	C40	C45	C152	C195	C214	1_32	3_90	M01	M24	M27	M150	M164	M196	M231	M260	M264	M307	
3X	Pome	124	170	267	290	357	180	289	182	298	125	245	168	290	253	245	261	401	180	276	225	258	168	
	AAB	122	166	261	286	335	178	284	164	298	123	239	152	258	247	243	257	298	177	252	210	242	164	
		121	158	?	?	?	?	274	?	298	119	?	?	254	241	?	?	?	?	242	?	?	?	
2X	Mlali	124	170/172	267	286	335	180	289	164	298	123	245	168	258	253	241/245	261	401	180	278/278	210	258	164	21/22
	AA	122	158	261	286	335	178	284	164	298	119	245	150	254/252	247	241/243	257	401	180/177	242	206/210	258	164	
X	Lal Velchi	121	166	261	290	357	180	274	182	296	125	239	152	290	241	235	261	298	177	249	212	242	168	18/22
	BB	121	166	255	282	357	180	274	173	296	123	239	152	310	243	235	257	313	182	249	212	242	168	

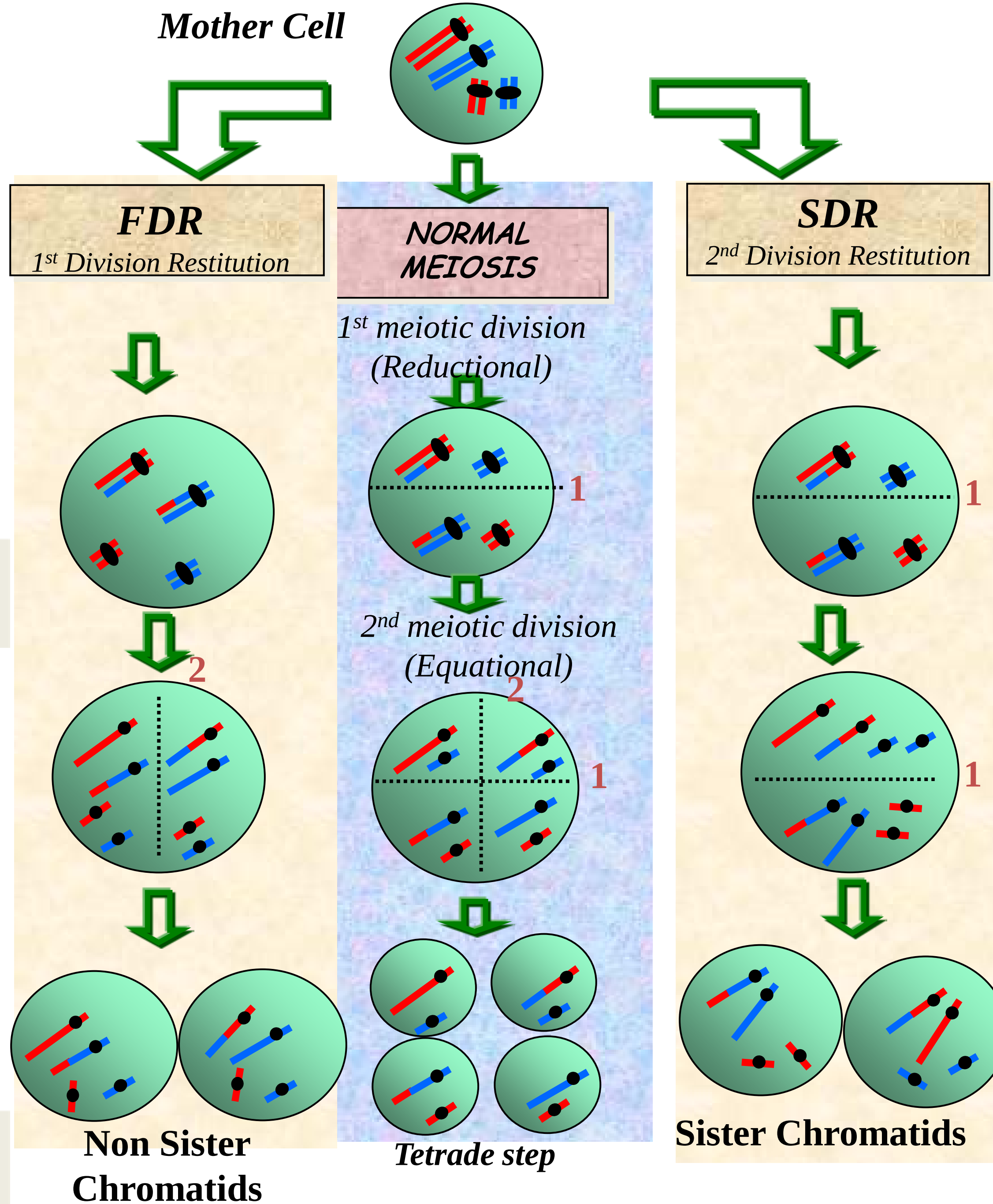
Raboin *et al*, 2005 (RFLP); Perrier *et al*, 2009 (SSR)



acuminata/balbisiana chromosome pairing in interspecific varieties (GISH)



Pelipita – ABB (GISH):
8 A chromosomes; 25 B chromosomes

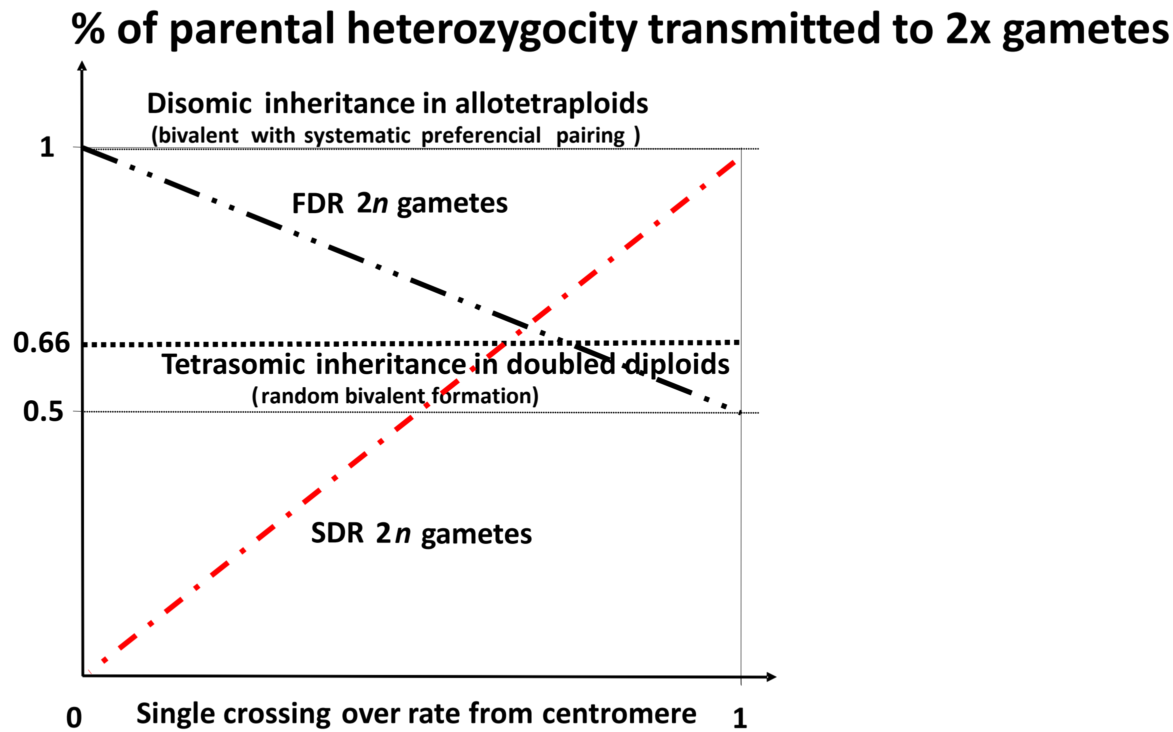


Heterozygocity transmission by 2x gametes

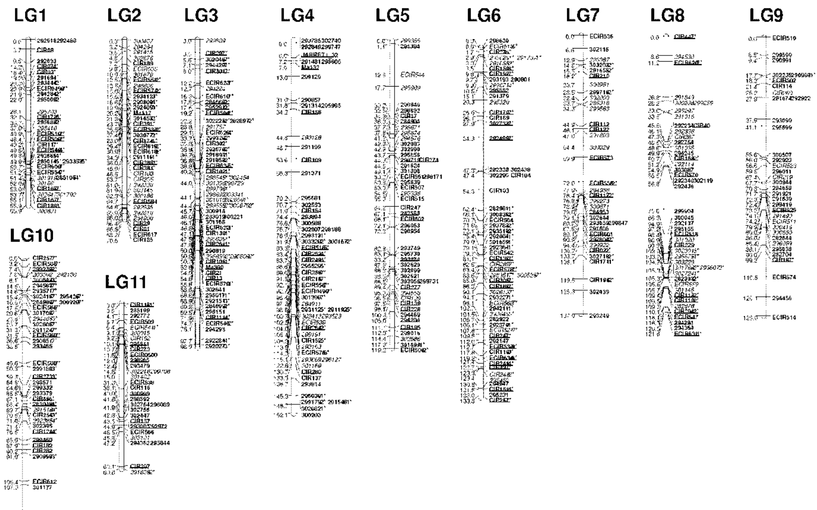
For banana breeding:

Parental heterozygocity and pathways of 2x gamete formation are two key points of the genetic structure of triploid progenies.

How to maximize heterozygocity in triploid progenies ?

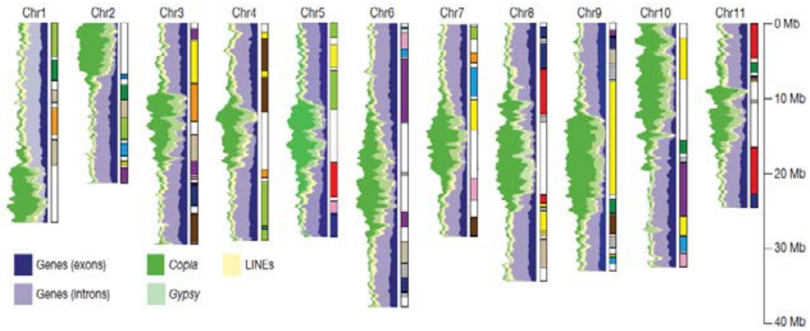


Genetical and physical maps



A saturated SSR/DArT linkage map of *Musa acuminata* addressing genome rearrangements among bananas

Hippolyte *et al. BMC Plant Biology* 2010, **10**:65
http://www.biomedcentral.com/1471-2229/10/65



The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants

Angélique D'Hont[1]*, France Denoeud[2,3,4]*, Jean-Marc Aury[2], Franc-Christophe Baurens[1], Françoise Carreel[1,5], Olivier Garsmeur[1], Benjamin Noel[2], Stéphanie Bocs[1], Gaëtan Droc[1], Mathieu Rouard[6], Corinne Da Silva[2], Kamel Jabbari[2,3,4], Céline Cardi[1], Julie Poulain[2], Marlène Souquet[1], Karine Labadie[2], Cyril Jourda[1], Juliette Lengellé[1], Marguerite Rodier-Goud[1], Adriana Alberti[2], Maria Bernard[2], Margot Correa[2], Saravanaraj Ayyampalayam[7], Michael R. Mckain[7], Jim Leebens-Mack[7], Diane Burgess[8], Mike Freeling[8], Didier Mbéguié-A-Mbéguié[9], Matthieu Chabannes[5], Thomas Wicker[10], Olivier Panaud[11], Jose Barbosa[11], Eva Hribova[12], Pat Heslop-Harrison[13], Rémy Habas[5], Ronan Rivallan[1], Philippe Francois[1], Claire Poiron[1], Andrzej Kilian[14], Dheema Burthia[1], Christophe Jenny[1], Frédéric Bakry[1], Spencer Brown[15], Valentin Guignon[1,6], Gert Kema[16], Miguel Dita[19], Cees Waalwijk[16], Steeve Joseph[1], Anne Dievart[1], Olivier Jaillon[2,3,4], Julie Leclercq[1], Xavier Argout[1], Eric Lyons[17], Ana Almeida[8], Mouna Jeridi[1], Jaroslav Dolezel[12], Nicolas Roux[6], Ange-Marie Risterucci[1], Jean Weissenbach[2,3,4], Manuel Ruiz[1], Jean-Christophe Glaszmann[1], Francis Quétier[18], Nabila Yahiaoui[1] & Patrick Wincker[2,3,4]

Evidence for high segregating distortions by gamete abortion (lethal genes, structural rearrangements) and aneuploidy

Genomics opens the floor to:

- *detailed genetic structure of X and 2X recombined gametes and patterns of inheritance*
- *detailed QTL and association mapping analysis (disease ressitance, quality traits, …)*
- *early genomic selection (allele constitution, etc…) : <u>fine tuning</u>*

Breeding for AAA hybrids

Seedy banana

CV Rose, **2X**

CV Rose, **4X** (male)

X

M. acuminata malaccensis, AAw, **2X**

(female, seedy)

AAA hybrid family

- 99,5 % of triploids:
(32 to 34 chromosomes)

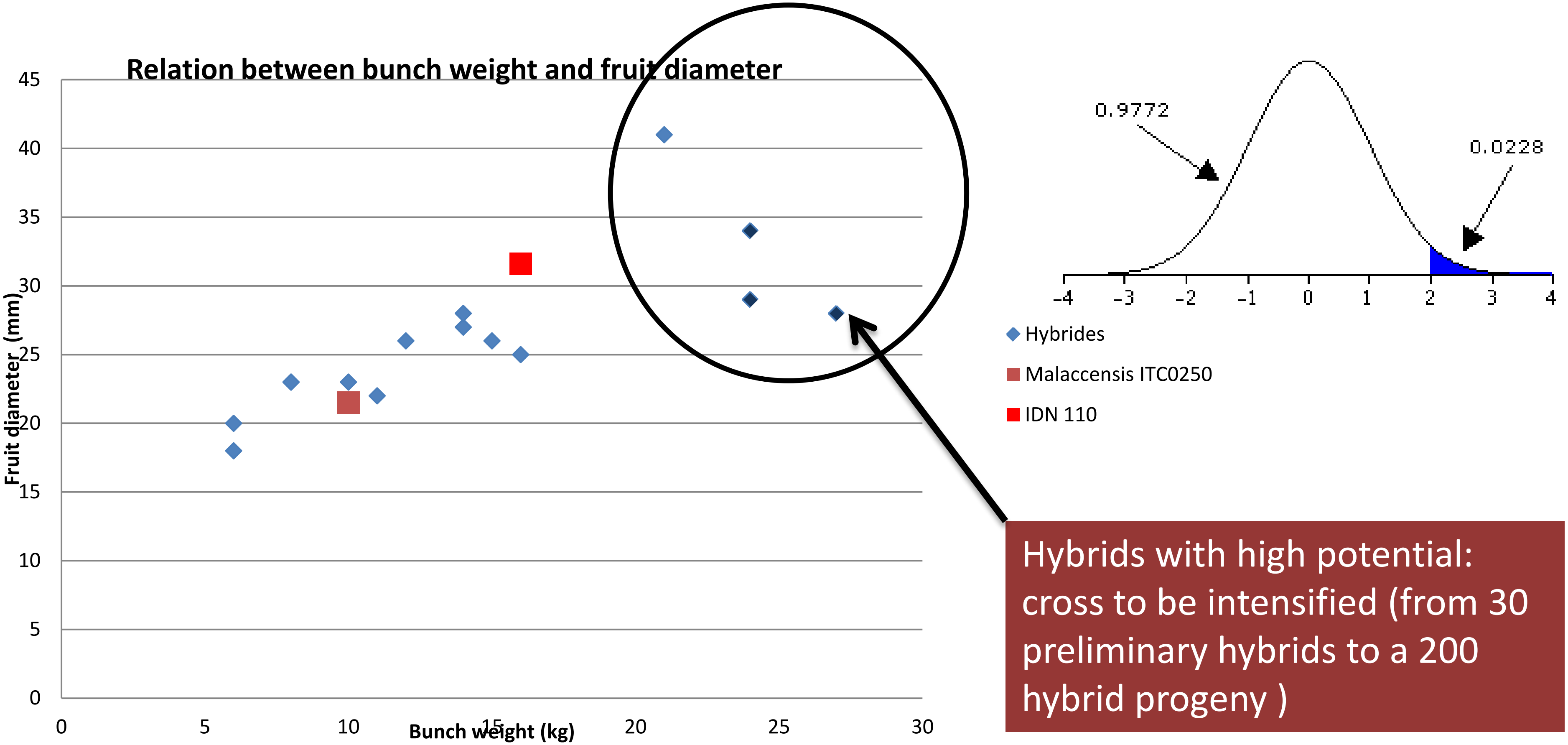
- Parthenocarpic and no parthenocarpic hybrids

-Mean value of the family tested over 30 individuals

-Selection over 200 individuals

Heterozygosity and cross potential

in AAA hybrid families



Hybrids with high potential: cross to be intensified (from 30 preliminary hybrids to a 200 hybrid progeny)

Genomics: fine tuning - research of the best allelic combinations

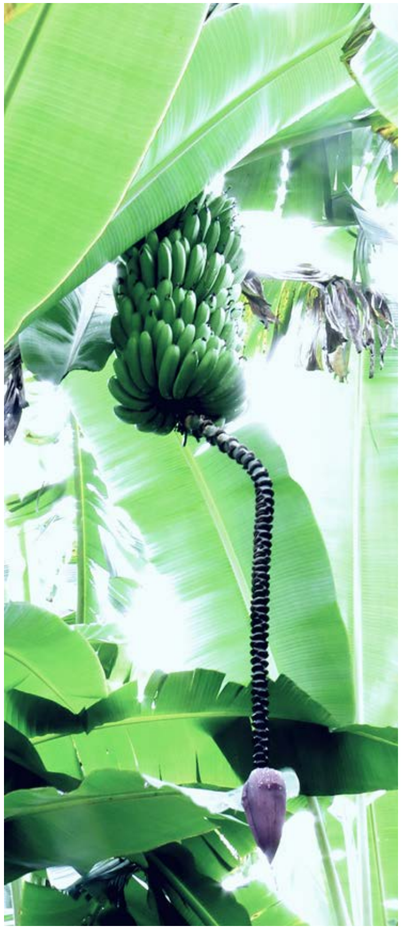
Gros Michel/Cavendish:
Natural 3 ways hybrids : banksii/zebrina/malaccensis)

Proof of the concept in Guadeloupe:
First « Cavendish like » progenies relying on
Khaï (female) x Doubled-Mlalis (male) crosses

But lack of « good Khaï » accessions:
malaccensis background, combining various resistances,
high gamete fertility and favorable agronomic features, ...

↓

Improvement of « Khaï diploid germplasm »
by continuous backcrosses and/or recurrence
with malaccensis seedy accessions
(genomic assisted selection)



'Poyo Like' hybrid

AAB and ABB breeding

Kunnan T
AABBcv
(fertile)

X

M. a. malaccensis, AAw

→

Synthetic AAB hybrid

X

M. balbisiana, BBw

→

Synthetic ABB hybrid

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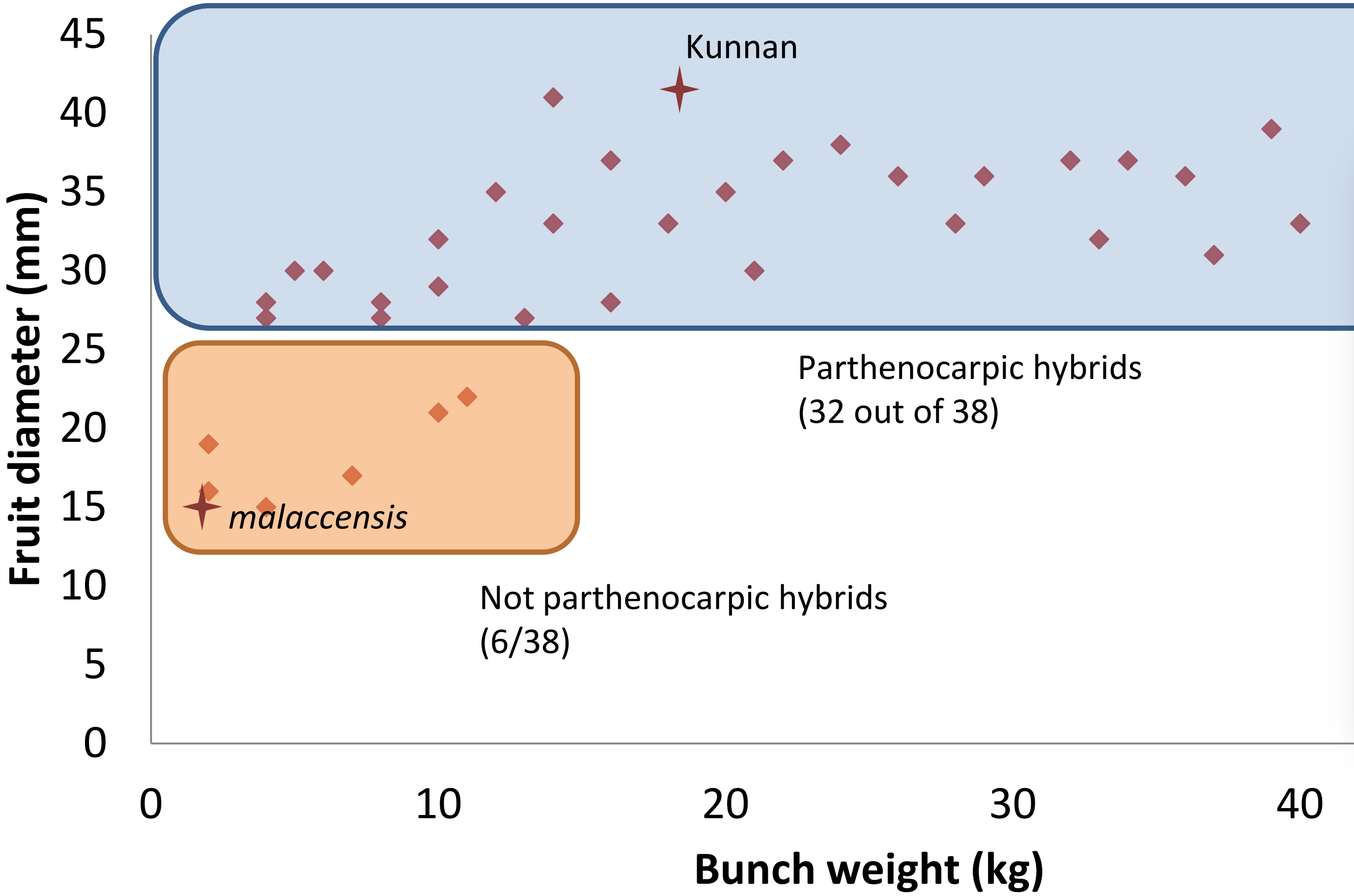
M. a. malaccensis x Kunnan 4X
Proportion of parthenocarpic hybrids



L9 selection

- Parthenocarpic and non-parthenocarpic hybrids
- High variability expressed for bunch weight
- Evidence for heterosis effects

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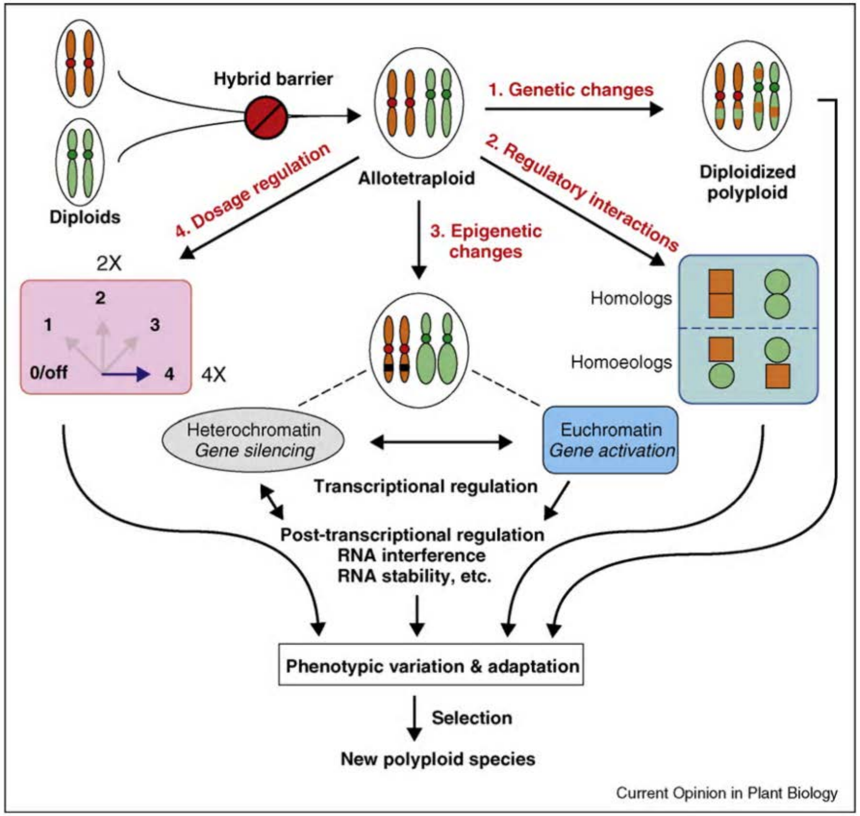
Regulation of expression in interspecific hybrids



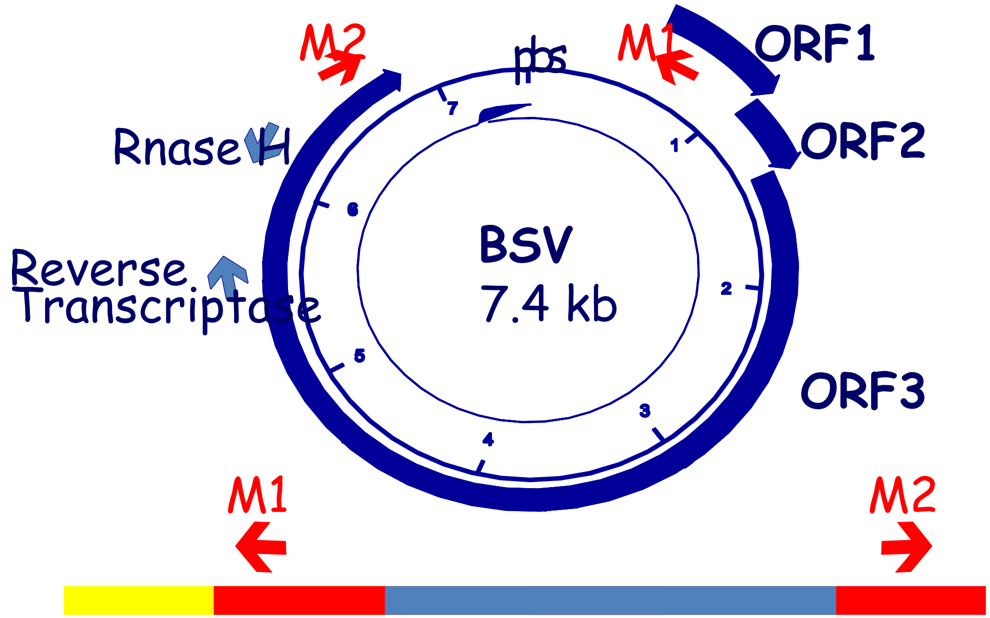
S. Jackson and Z J. Chen. Genomic and expression plasticity of polyploidy. Current Opinion in Plant Biology 2009, 13:1–7.

Neo allopolyploids:

confrontation of two differentiated and regulated genomes

- Neoregulation of gene expression
- Methylation, histones acetylation, …
- Post transcriptionnal activities (miRNA, siRNA, …)
- Reactivation of transposable elements
  → example in Musa : e-BSV

Banana Streak Virus (BSV)
(badnavirus integrated in banana genome)



- **Silent integrated sequences in *M. balbisiana* accessions:**
- activable in AAB genomes by hybridization & abiotic stresses (low temperatures, tissue culture)
- leading to lethal episomal virus particles
- Hampering the use of *M. balbisiana* accessions in breeding

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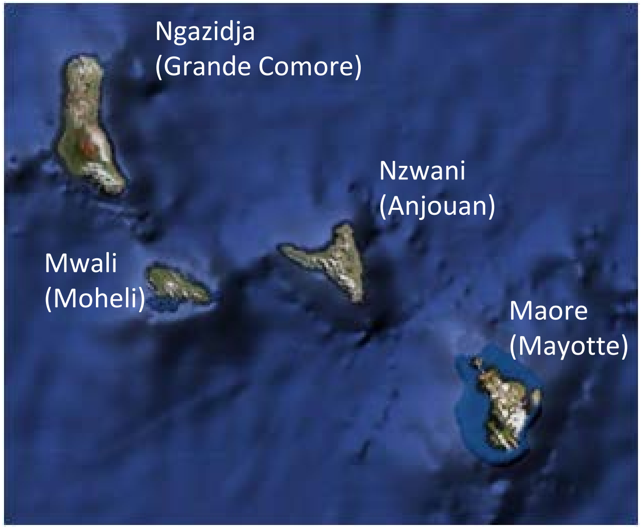
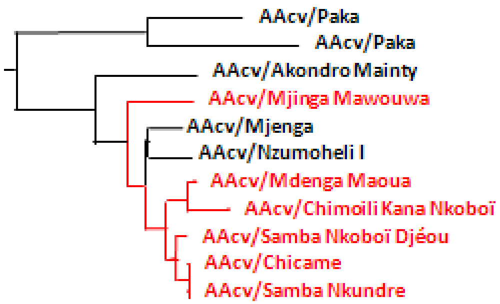
Deciphering genetic diversity in vegetatively propagated relatives



Genetic/phenotypic diversity in Mlalis

AAcv Mlali



→ From SSRs to GBS

Mlali Pima Moja (« one hand only »)

Mlali commun

Mlali Mshia Wa Komba

Postbreeding improvement: induced somaclonal variations, GMO's, etc…

Local modification of genome for a specific trait: size, bunch shape, resistance to viruses, …

AAcv x AAcv → AAcv —colchicine→ AAAAcv

AAcv x BBw → ABcv —colchicine→ AABBcv

Application at post-breeding level…

Triploid development

AAcv x AAAAcv → AAAcv

AAw x AAAAcv → AAAcv

BBw x AAAAcv → AABcv

AAcv x AABBcv → AABcv

AAw x AABBcv → AABcv

Key : cv = cultivar ; w = wild species

…or at parental level

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Banana Breeding at CIRAD: the result of a group project

Genetic Resources and Breeding: F. Bakry, J.P. Horry, Ch. Jenny, K. Tomekpe, F. Salmon, S. Ricci, …

Biomathematics: X. Perrier, …

Biomolecular Resources/Genomics: A. D'Hont, F.C. Baurens., F. Carreel, N. Yahiaoui, ….

Virology : M.L. Caruana, P.Y. Teycheney, M. Chabannes, …

Agronomy: T. Lescot, M. Dorel, ….

Post-harvest: D. Mbeguié-A-Mbeguié, O. Gibert, D. Dufour, C. Bugaud, …

Economy/competitivity: D. Loeillet, IT2, …

Hybrid distribution: VITROPIC (Y. Mathieu)

Commercialization trials of CIRAD925

Thank you for your attention

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CIRAD

FEDER / FEADER

Agropolis Fondation

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INTERREG Caraïbe