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 ↑

![Graph showing quantites (mT) from 1961 to 2009 for Bananes, Plantains, and Total. The quantities increase steadily over the years.]

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
Principals of Banana Breeding (1/3)

Principal landraces/ main features:

- Edibility (seedless, parthenocarpy)
- Triploidy
- Highly heterozygous (sub- & interspecificity)
- Vegetatively propagated
Constraints to breeding:

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

**Wild types**

- AA \( w \)
- BB
- SS
- TT

**Intra-subspecific and interspecific crosses**

Domestication for parthenocarpy and sterility

**Edible diploids**

- AA \( cv \)
- AB
- AS
- AT
- BT

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

Natural hybridization & selection

Musa acuminata microcarpa

Musa textilis

Musa schizocarpa

Musa balbisiana
Banana evolution

From: X. Perrier, 2013

- Interspecific crosses
- Intrasubspecific crosses
- Edible diploids
- Cultiwilds
- Triploid landraces
- Triploidisation by 2N gametes
- fixation and selection by vegetative propagation

« Value »

Diploid wild types

Time
Evolutionary history of banana
The first sub-speciations

- M. balbisiana
- burma/siamea
- malaccensis
- microcarpa?
- errans
- zebrina
- banksii
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

Emergence of the first Edible Diploids

Example: Mlalis
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

Questions:
ª Genome mosaics ?
ª How many recombination cycles between CWR and ED ?
ª Evidences for high linkage desequilibrium ?

Curk et al., 2012. International Citrus Congress Valencia
Reconstructive breeding in Musa

**Synthesis of triploid hybrids**

- **AAw** × **AAcv** → **AAcv** → chromosome doubling (colchicine) → **AAAAAcv**
- **AAcv** × **AAcv** → **ABcv** → chromosome doubling (colchicine) → **AABBcv**
- **AAcv** × **BB** → **ABcv** → **AABBcv**
- **AA** × **AAAAAcv** → **AAAcv**
- **BB** × **AAAAAcv** → **AABcv**
- **AA** × **AABBcv** → **AABcv**
- **BB** × **AABBcv** → **ABBcv**

**Selections**

- **AAAAAcv** → **AAAcv**
- **AAAAAcv** → **AABcv**
- **AABBcv** → **AABcv**
- **AABBcv** → **ABBcv**

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)

Parent A
ED
2n = 2x

No reduction (FDR/SDR ?)

Diploid gamete
n = 2x

Parent B
CWR/ED
2n = 2x

Haploid Gamete
n = x

Natural crosses
(plant material translocations; backyards ? …)

Edible Triploids
2n = 2x + x = 3x
(human selection)

NB: Some rare indirect pathways of triploid synthesis may have occurred
Diploid ancestors of triploid export bananas

22 SSR/131 AA cv

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

NORMAL MEIOSIS
1st meiotic division (Reductional)

1st Division Restitution (FDR)

2nd meiotic division (Equational)

2nd Division Restitution (SDR)

Tetrade step

Non Sister Chromatids

Sister Chromatids

acuminata/balbisiana chromosome pairing in interspecific varieties (GISH)

Pelipita - A B B (GISH): 8 A chromosomes; 25 B chromosomes

Genome A
Genome B
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?
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

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 resistance, quality traits, ...)
- early genomic selection (allele constitution, etc...) : fine tuning
Breeding for AAA hybrids

CV Rose, $2X$
CV Rose, $4X$ (male)

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

Seedy banana
Heterozygosity and cross potential in AAA hybrid families

Relation between bunch weight and fruit diameter

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)
AAB and ABB breeding

Kunnan T
AABBcv
(fertile)

X

M. a. malaccensis, AAw

→

Synthetic AAB hybrid

X

M. balbisiana, BBw

→

Synthetic ABB hybrid
**M. a. malaccensis x Kunnan 4X**

Proportion of parthenocarpic hybrids

- Parthenocarpic and non-parthenocarpic hybrids
- High variability expressed for bunch weight
- Evidence for heterosis effects
Regulation of expression in interspecific hybrids

**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
Deciphering genetic diversity in vegetatively propagated relatives

Genetic/phenotypic diversity in Mlalis

Mlali Pima Moja (« one hand only »)
Postbreeding improvement: induced somaclonal variations, GMO’s, etc...

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

Application at post-breeding level...

Triploid development

...or at parental level

Key: cv = cultivar; w = wild species
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, ...

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