A SIMPLE AND ROBUST APPROACH FOR GENOTYPING IN MUSACEAE

Pavla Christelová, Eva Hřibová, Jana Čížková, Jaroslav Doležel

[1] Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic
[2] Commodities for Livelihoods Programme, Bioversity International, Montpellier, France

Genotyping using SSR markers

- SSR markers have been successfully applied in molecular genotyping
- The use of SSR markers opens a possibility for automation and multiplexing which significantly increases the throughput of genotyping
- Standardized SSR genotyping platform enables to analyze large sets of accessions as well as a few individuals
- Alternative methods - DArTs and GBS (Genotyping By Sequencing) are suitable for analysis of large set of accessions

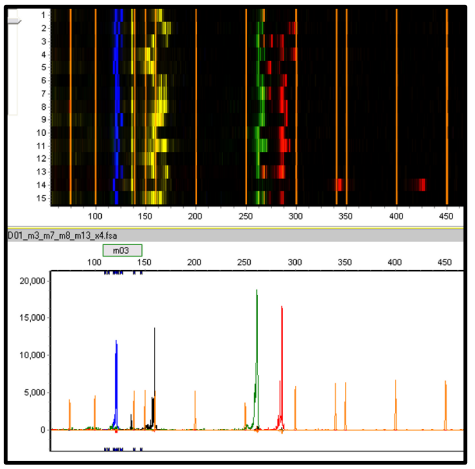
We have used 19 SSR markers selected out of the initial 22 marker set (http://www.musagenomics.org/cetest_firstpage1/genomic_dna.html), for their clear reproducible amplification pattern (Crouch *et al.* 1998; Lagoda *et al.* 1998; Hippolyte *et al.* 2010)

Experimental design

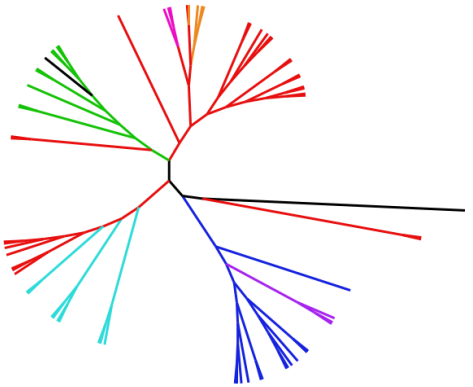
- Ploidy level estimation using flow cytometry
- Genomic DNA isolation
- PCR with fluorescently labeled primers and capillary electrophoresis analysis of resulting fragments (ABI 3730xl)
- Call for alleles and cluster analysis

- Analysis of ITS sequence region, if needed

3730*xl* DNA analyzer



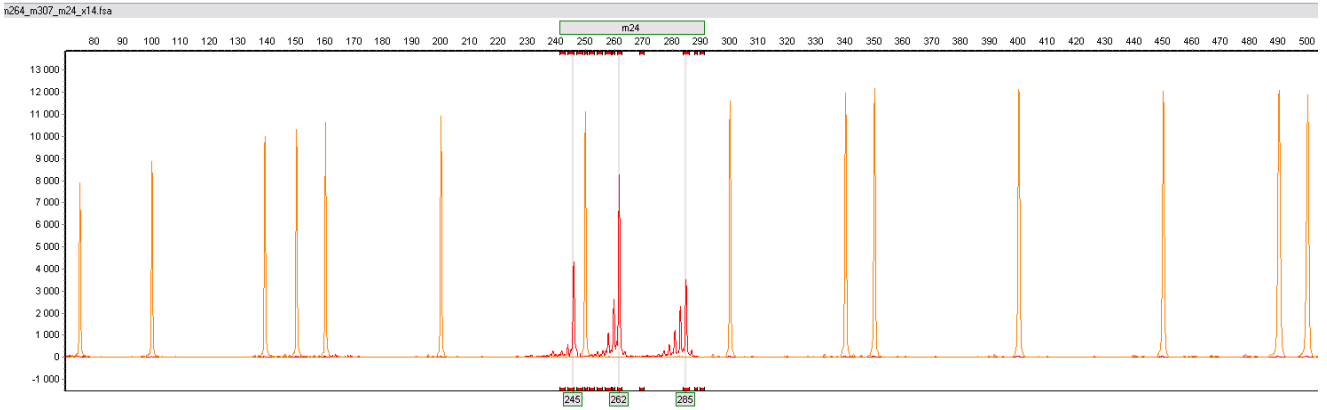
Call for alleles using GeneMarker (Softgenetics) followed by manual check



Cluster analysis

Advantages of the fragment analysis

Precise estimation of allele size, high resolution



SSR profile of a variety Sport of Silk (AAB) for marker mMaCIR24 (red peaks)

High-throughput, possible automation, possible multiplexing (5-dye set)

Multiplexed SSR profile of variety Mbwazirume (AAA) for SSR markers
mMaCIR152 (green)
mMaCIR195 (blue)
Ma1_32 (black)
mMaCIR260 (red)

Results

AoB PLANTS http://aobplants.oxfordjournals.org/

Open access – Research article

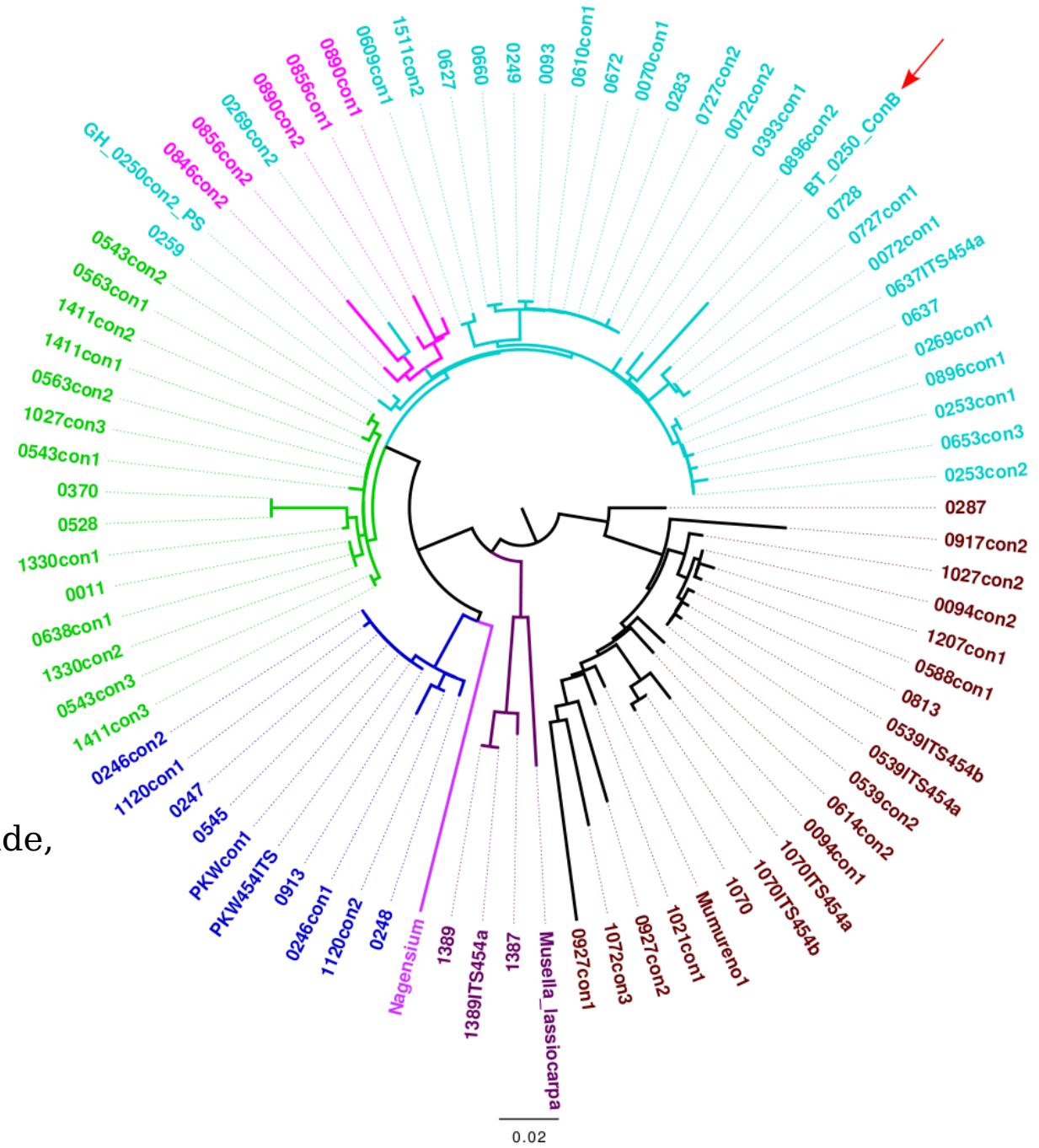
THIS ARTICLE IS PART OF A SPECIAL ISSUE ENTITLED 'MOLECULAR TECHNOLOGIES TO IMPROVE VEGETATIVELY PROPAGATED BANANA AND CASSAVA'

A platform for efficient genotyping in *Musa* using microsatellite markers

Pavla Christelová[1], Miroslav Valárik[1], Eva Hřibová[1], Ines Van den houwe[2], Stéphanie Channelière[3], Nicolas Roux[3] and Jaroslav Doležel[1*]

Example of ITS analysis

- incongruence in the blind test results of the ITC 0250 accessions
- ITS analysis confirmed that the blind sample no. 4 *M. acuminata* ssp. *malaccensis* ITC 0250 is not the same genotype as the *M. acuminata* ssp. *malaccensis* ITC 0250, that we obtained from the Genbank and stored in our greenhouse



brown - Australimusa/Callimusa clade,
green – Rhodochlamys species
dark blue – BB genotypes
light blue – AA genotypes
pink – M. schizocarpa entries

Analysis of SSRs - conclusions

- SSR genotyping is a powerful tool for molecular characterization of *Musa* germplasm
- **SSR genotyping is suitable for classification of the unknown samples**
- A prior knowledge of ploidy level of an unknown sample is important prior to analysis - can be determined by flow cytometry
- In case of uncertain results, ITS sequence analysis can be employed to unravel the identity of an unknown sample
- **A centralized database of molecular profiles keeps growing with every new sample, resulting in stepwise improvement in the grouping**
- To facilitate building of the database of electrophoretic profiles, guarantee standard genotyping conditions and reproducibility of results, the genotyping should be centralized