A SIMPLE AND ROBUST APPROACH FOR GENOTYPING IN MUSACEAE

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Introduction

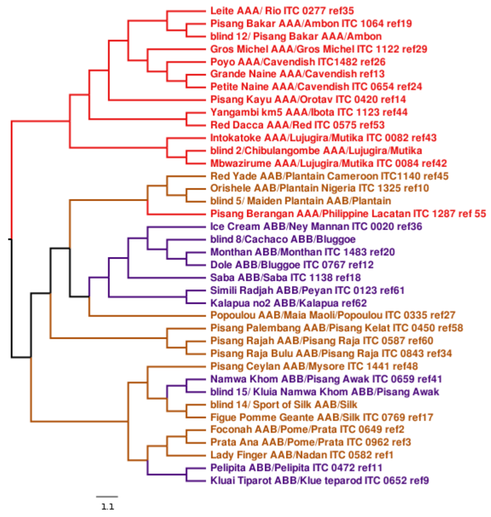
- Efficient collection and conservation of *Musa* genetic diversity depends on unambiguous sample identification
- Traditional taxonomy of *Musa* based on plant phenotype and chromosome number has been questioned
- The main problem is the characterization of *Musa* hybrids
- SSR markers have been successfully 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

Results

- 19 SSR markers were selected out of the initial 22 marker set (http://www.musagenomics.org/cetest_firstpage1/genomic_dna.html), for their clear reproducible amplification pattern
- Genotyping platform was tested and optimized on a set of 70 diploid and 38 triploid banana accessions
- Marker set provided enough polymorphism to discriminate between individual species, subspecies and subgroups of all *Musa* accessions
- Capability of identifying duplicate samples was confirmed
- Genotyping system was confirmed to be suitable for characterization of unknown accessions (based on the results of blind test)
- For more detailed information, please see Christelova et al. 2011, AoB Plants

UPGMA cluster analysis of diploid accessions based on the Nei (1973) genetic distance

UPGMA cluster analysis of triploid accessions based on the Nei (1973) genetic distance



Conclusions

- Complex and standardized platform for molecular characterization of *Musa* germplasm has been established
- The platform was tested on blind samples
- The platform is ready-to-use for the wider *Musa* research and breeders community
- This genotyping system offers a versatile tool that can accommodate all possible requirements for characterizing *Musa* diversity

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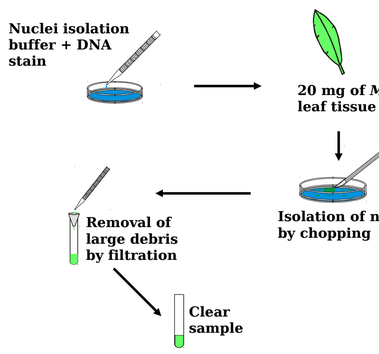
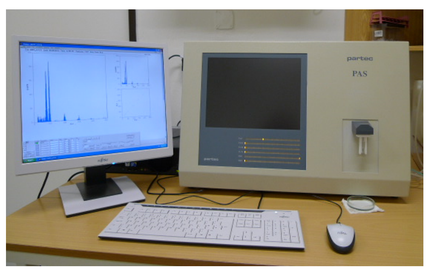
Experimental design

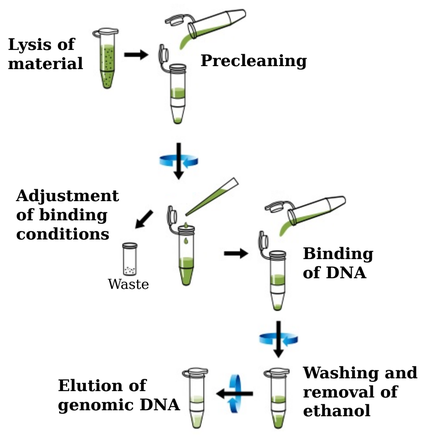
1 Ploidy level estimation using flow cytometry





Partec PAS flow cytometer

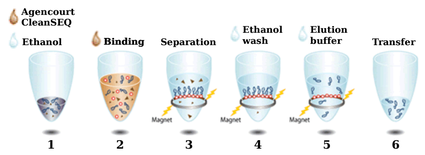
2 Genomic DNA isolation



3 PCR with fluorescently labeled primers

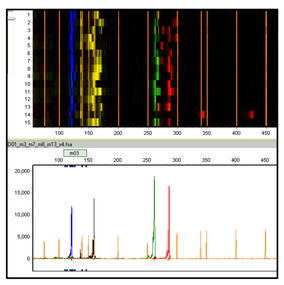
- Four different flurophores are used for the primer labeling (6-FAM, VIC, NED, PET), allowing for subsequent multiplexing
- The reaction (20 µl) contains 10 ng of genomic DNA, reaction buffer (consisting of 10mM Tris-HCl (pH 8), KCl 50 mM, 0.1% Triton-X100 and 1.5 mM MgCl2), 200 µM dNTPs (each), 1U of Taq polymerase, 8 pmol of the M13 tailed locus specific forward primer, 6 pmol of the fluorescently labeled universal M13 forward primer, 10 pmol of the locus specific reverse primer.

Purification of PCR products using paramagnetic beads (CleanSEQ, ABI)

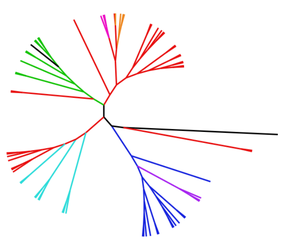


4 Alleles identification using GeneMarker and data analysis

3730*xl* DNA analyzer

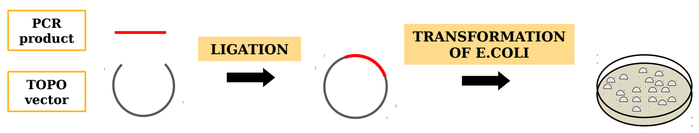


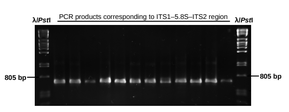
Call for alleles using GeneMarker (Softgenetics) followed by manual check



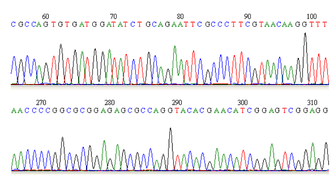
Cluster analysis

5 Analysis of ITS sequence region (if needed)





SEQUENCING



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SEQUENCING DATA ANALYSIS