MOLECULAR AND CYTOGENETIC CHARACTERIZATION OF WILD MUSA SPECIES NEWLY INTRODUCED TO ITC COLLECTION



Čížková J¹, Hřibová E¹, Christelová P¹, Van den Houwe I², Roux N³, Doležel J¹

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1 Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic 2 Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Belgium 3 Bioversity International, Montpellier, France

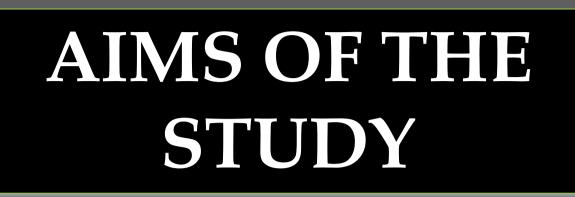




INTRODUCTION

- Bananas (*Musa* spp.) are a major food crop and an important export commodity in many countries of humid tropics
- The production of banana is threatened by various diseases and adverse environmental conditions

TAXONOMY OF THE FAMILY MUSACEAE



• Contribution to characterization of the genetic diversity of wild *Musa* species

- The preservation and characterization of banana diversity is essential for the purposes of crop improvement
- World's largest banana collection is maintained at the Bioversity International Transit Centre (ITC) in Belgium
- This reference collection is continuously extended by edible cultivars and wild species
- Efficient collection and conservation of *Musa* genetic diversity depends on unambiguous sample identification

Family: Musaceae

- Genus: Musa
 - **Section:** Eumusa (2n = 2x = 22)
 - Section: Rhodochlamys (2n = 2x = 22)
 - Section: Australimusa (2n = 2x = 20)
 - **Section:** Callimusa (2n = 2x = 18, 20)
- Genus: Ensete
 - Genus: Musella

Taxonomy of the family Musaceae is based on morphological characters and basic chromosome number

- Determination of chromosome number, nuclear DNA content and genomic distribution of rRNA genes to shed light on the genome structure of evaluated accessions
- Verification of the classification of evaluated accessions based on SSR analysis
- Verification of the putative hybrid origin of evaluated *Musa* accessions

RESULTS

SSR ANALYSIS

Standardized SSR genotyping platform (Christelová *et al.*, 2011, AoB PLANTS, doi: 10.1093/aobpla/plr024) was used to analyze genetic diversity of studied accessions

NUCLEAR DNA CONTENT

Accession name	ITC code	Section	Chromosome number	2C nuclear DNA content [pg]
M. itinerans var. xishuangbannaensis	1526	Eumusa	22	1.311
<i>M. itinerans</i> var. <i>itinerans</i>	1571	Eumusa	22	1.217
M. yunnanensis	1573	Eumusa	22	1.306
M. rubinea	1518	Rhodochlamys	22	1.310
M. x fennicae (M. siamensis x M. rosea)	1522	Rhodochlamys	22	1.261
M. siamensis	1534	Rhodochlamys	22	1.280
M. mannii	1574	Rhodochlamys	22	1.282
M. laterita	1575	Rhodochlamys	22	1.315
M. rubra	1590	Rhodochlamys	22	1.306
M. rosea x siamensis	1592	Rhodochlamys	22	1.279
<i>M. rosea</i> (hybrid)	1598	Rhodochlamys	22	1.285
<i>M.</i> cf. <i>uranoscopos</i>	1532	Australimusa	20	1.442
M. violascens	1514	Callimusa	20	1.428
M. lutea	1515	Callimusa	20	1.432
M. beccarii var. beccarii	1516	Callimusa	18	1.537
M. campestris var. sarawakensis	1517	Callimusa	20	1.417
M. monticola	1528	Callimusa	20	1.390
M. beccarii var. hottana	1529	Callimusa	18	1.673
M. borneensis	1531	Callimusa	20	1.772
M. campestris var. limbangensis	1535	Callimusa	20	1.454
M. barioensis	1568	Callimusa	20	1.480

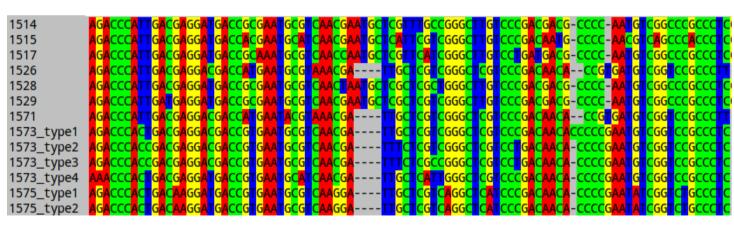
ANALYSIS OF ITS REGION

Clone based sequencing strategy was used to analyze the ITS1-5.8S-ITS2 sequence region of 45S rDNA locus

Newly characterized *Musa* accessions are labeled in colors in the tree bellow

BL16_1308/blind 16/ AA cv Pisang Sipulu ITC 1308/AA Ref32 ITC 0312/AA cv P. Jari Buaya ref32/AA BL11 1034/blind 11/ AB cv Kunnan ITC 1034/AB - BL3 0245/blind 3/ AB cv Safet Velchi ITC 0245/AB Ref23 ITC 0245/AB cv Safet Velchi ref23/AB ITC 1571/Musa itinerans var itinerans/itinerans ITC 0246/M. balbisiana Cameroun ITC 0246/BE BL1 0080/blind 1/ M. balbisiana ITC 0080/BB Ref39 ITC 0247/M. balbisiana Honduras ref39/BB Ref4 KUWU/M. balbisiana PKW ref4/BB - ITC_1120/M. balbisiana Tani ITC 1120/BB Ref21_ITC_1120/M. balbisiana Tani ref21/BB Ref5_ITC_1156/M. balbisiana P. Batu ref5/BB ITC 0545/M. balbisiana ITC 0545/BB ITC 0094/M. balbisiana /10852/ ITC 0094/BB — ITC_0564/M. balbisiana Butuhan ITC 0564/BB - ITC_1074/BBxT Butuhan ITC 1074/BT ITC_0248/M. balbisiana Singapuri ITC 0248/BB Ref40 BVEL/M. balbisiana Lal Velchi ref40/BB - ITC_0072/M. acuminata Tavoy ITC 0072/AA - Ref22 ITC 0283/M. acuminata burmannica Long Tavoy ref22/AA ITC 0672/M. acuminata siamea ITC 0672/AA Ref8_ITC_0249/M. acuminata burmannicoides Calcutta4 ref8/AA - ITC_0093/M. acuminata Long Tavoy ITC 0093/AA - ITC 0249/M. acuminata burmannicoides Calcutta4 ITC 0249/AA Ref38_ITC_1187/AA cv Tomolo ref38/AA ITC_0250/M. acuminata malaccensis ITC 0250/AA Ref52 ITC 1511/M. acuminata malaccensis DH Pahang ref52/AA ITC_1573/M. yunnanensis/yunnanensis - ITC_1330/M. ornata ITC 1330/ornata - ITC 1598/Musa rosea-hybrid/Rhodochlamys/hybrid — ITC_1572/M. rosea x M. ornata/hybrid - ITC_0011/M. velutina ITC 0011/velutina - ITC_0638/M. velutina ITC 0638/velutina Ref16 ITC 0660/M. acuminata siamea Khae/Phrae ref16/AA - BL6_0370/blind 6/ M. ornata ITC 0370/ornata ITC 0370/M. ornata ITC 0370/ornata - ITC 0528/M. ornata Kluai Bou ITC 0528/ornata - Ref51 ITC 0637/M. ornata ref51/ornata - ITC 1574/M. mannii/Rhodochlamys/manii — ITC_0543/M. mannii ITC 0543/manii - ITC 1411/M. mannii ITC 1411/manii ITC_1570/Musa ornata Royal Purple/ornata — ITC 1590/Musa rubra/rubra — ITC 1575/Musa laterita/laterita — ITC 1575B/Musa laterita December2011/Rhodochlamys — ITC 1534/Musa siamensis/siamensis ITC 1534B/Musa siamensis December2011/siamensis ITC 1591/Musa rosea/rosea — ITC_1591B/Musa rosea December2011/Rhodochlamys - ITC_1522/M. x fennicae/M. siamensis x M. rosea/hybrid ITC 1592/Musa rosea x siamensis/hybrid ITC_1532/Musa cf uranoscopos/uranoscopos — ITC_1517/Musa campestris var Sarawakensis/campestris - ITC 1531/Musa borneensis/borneensis ITC 0287/M. coccinea ITC 0287/coccinea ITC 1515/M. lutea/lutea — ITC_1568/Musa barionensis/barionensis - ITC 1535/M. campestris var. Limbangensis/campestris ITC_1535B/Musa campestris var Limbangensis December2011/campestris TC 1516B/Musa beccarii December2011/Callimusa ITC_1070/M. beccarii ITC 1070 TC 1516/M. beccarii/beccarii

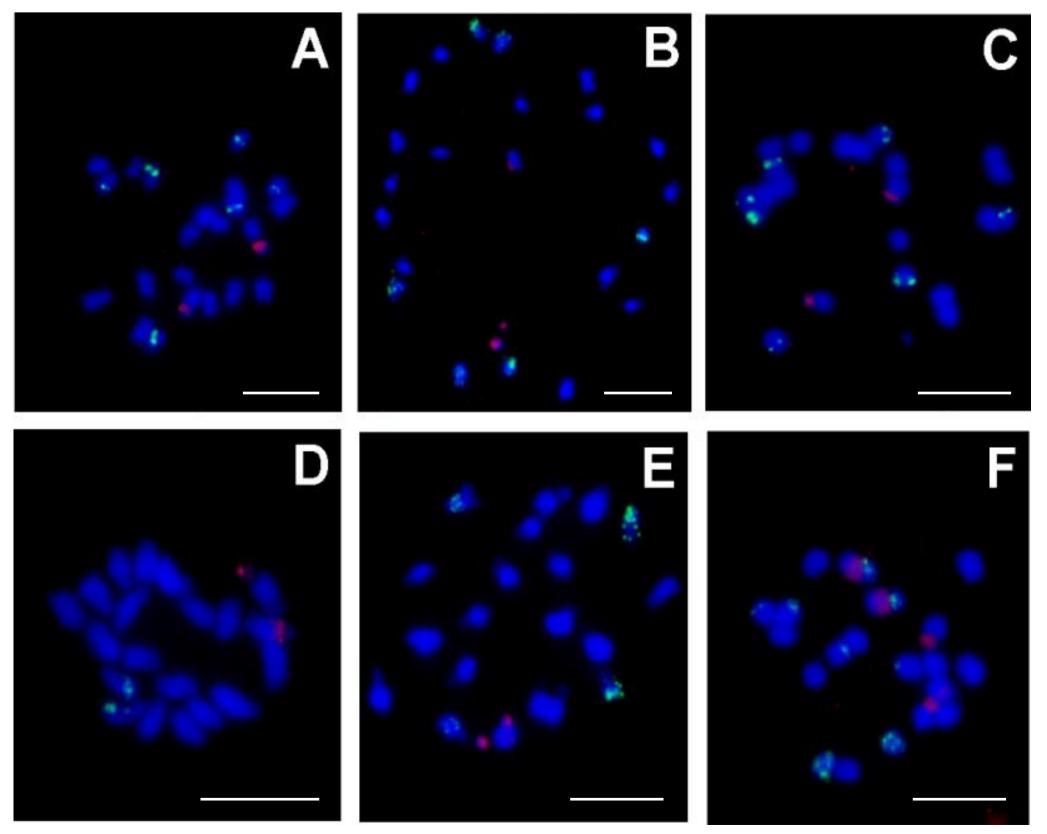
Analysis of ITS region was used with the aim to verify hybrid origin of evaluated *Musa* accessions



Multiple alignment of a part of ITS1 sequence region created using mafft

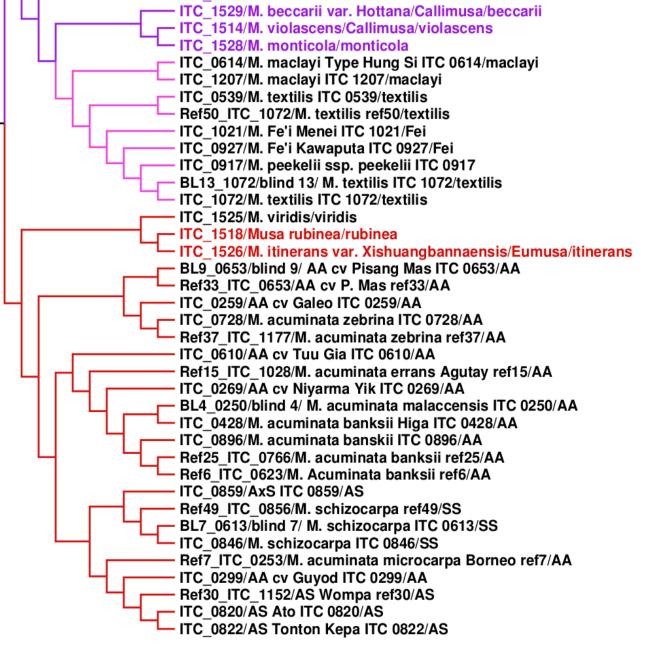
- Except for 10 accessions (ITC 1514; 1515; 1517; 1526; 1528; 1534; 1529; 1571 and 1598) all others contained three or more ITS types which support their hybrid origin
- · Hybrid origin was observed also for *M. beccarii* var. *hottana* (ITC 1529) which did not cluster together with other *beccarii* species analysed in this as well as in previous study using SSR genotyping

CYTOGENETIC MAPPING OF rDNA



CONCLUSIONS

• Nuclear DNA content, chromosome number and genomic distribution of 45S and 5S rDNA were estimated in studied accessions showing



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

Localization of 45S rDNA (red) and 5S rDNA (green) on mitotic metaphase chromosomes. (A) *M. yunnanensis,* (B) *M. rubra,* (C) *M. rosea* x *siamensis,* (D) *M. campestris* var. *sarawakensis,* (E) *M. monticola,* (F) *M. borneensis.* Bar = 5 μ m. higher variability in these characters in comparison with previous studies

- For *M. borneensis* (ITC 1531), the highest nuclear DNA content known for diploid *Musa* species was estimated
- SSR analysis did not confirm genetic origin of several accessions (ITC 1518, ITC 1532 and ITC 1573)
- Classification of these accessions should be reevaluated
- Analysis of ITS region suggested hybrid origin of most of studied accessions

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