Genetic integrity of the ITC collection : DArT genotyping

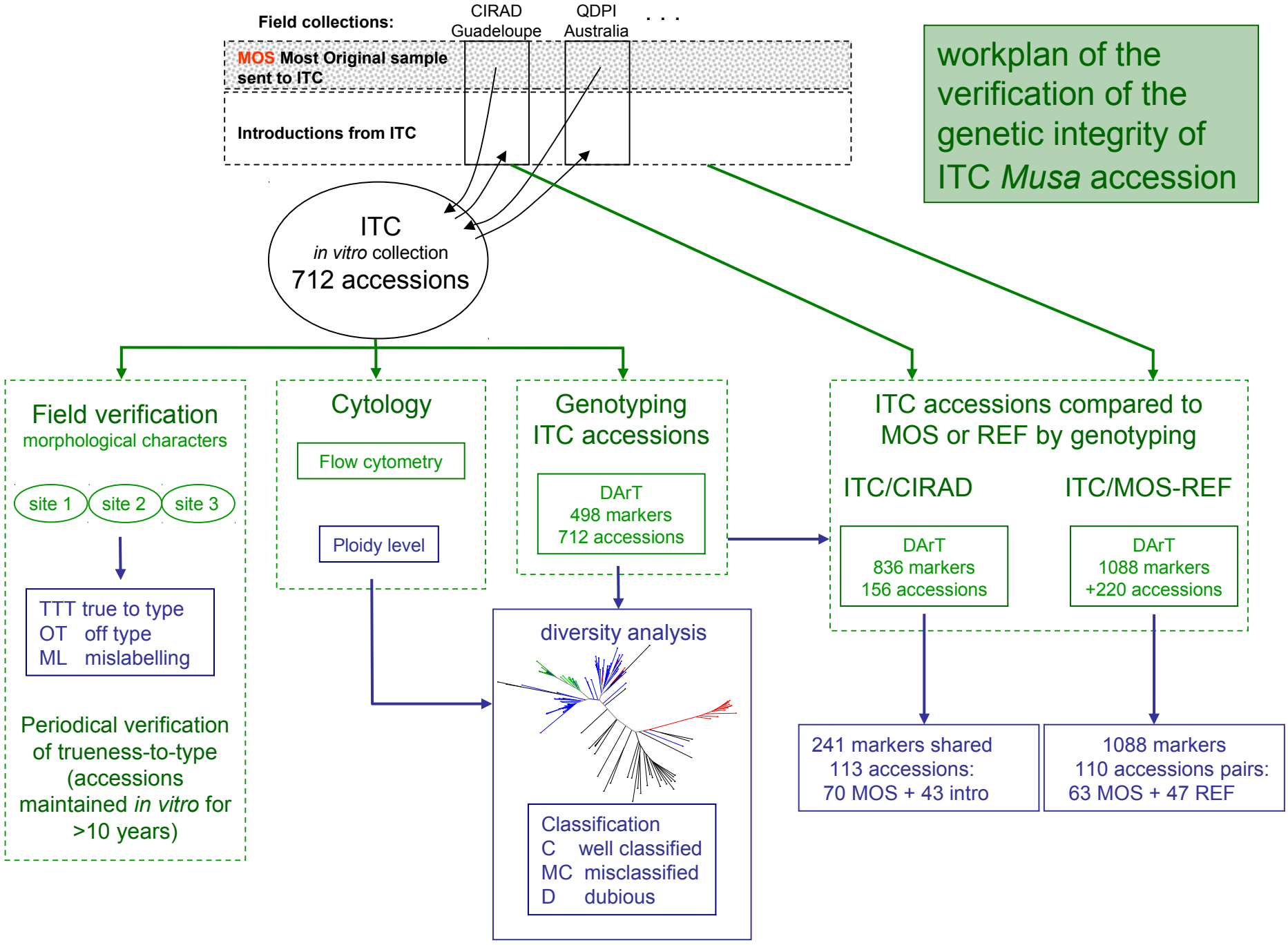
J.P. Horry, X. Perrier, N. Roux, S. Channeliere

Rationale and objectives

Objective: reducing and managing the loss of genetic integrity of conserved germplasm.

- genetic integrity : identity of the genetic composition of the sample conserved at ITC to that of the original collected, bred or improved.
- To detect loss of genetic integrity :
  - compare an (ITC) accession to its most original sample (MOS),
  - or be able to determine that the accession doesn't behave as it should.

Bioversity has adopted a workplan to identify accessions that have eventually undergone a genetic change.


Field collections:
CIRAD Guadeloupe
QDPI Australia
. . .
MOS Most Original sample sent to ITC
Introductions from ITC
workplan of the verification of the genetic integrity of ITC *Musa* accession
ITC
in vitro collection
712 accessions
Field verification
morphological characters
site 1
site 2
site 3
TTT true to type
OT off type
ML mislabelling
Periodical verification of trueness-to-type (accessions maintained *in vitro* for >10 years)
Cytology
Flow cytometry
Ploidy level
Genotyping ITC accessions
DArT
498 markers
712 accessions
diversity analysis
Classification
C well classified
MC misclassified
D dubious
ITC accessions compared to MOS or REF by genotyping
ITC/CIRAD
ITC/MOS-REF
DArT
836 markers
156 accessions
DArT
1088 markers
+220 accessions
241 markers shared
113 accessions:
70 MOS + 43 intro
1088 markers
110 accessions pairs:
63 MOS + 47 REF

Diversity Arrays Technology (DArT)

Theor Appl Genet (2009) 119:1093–1103
DOI 10.1007/s00122-009-1111-5

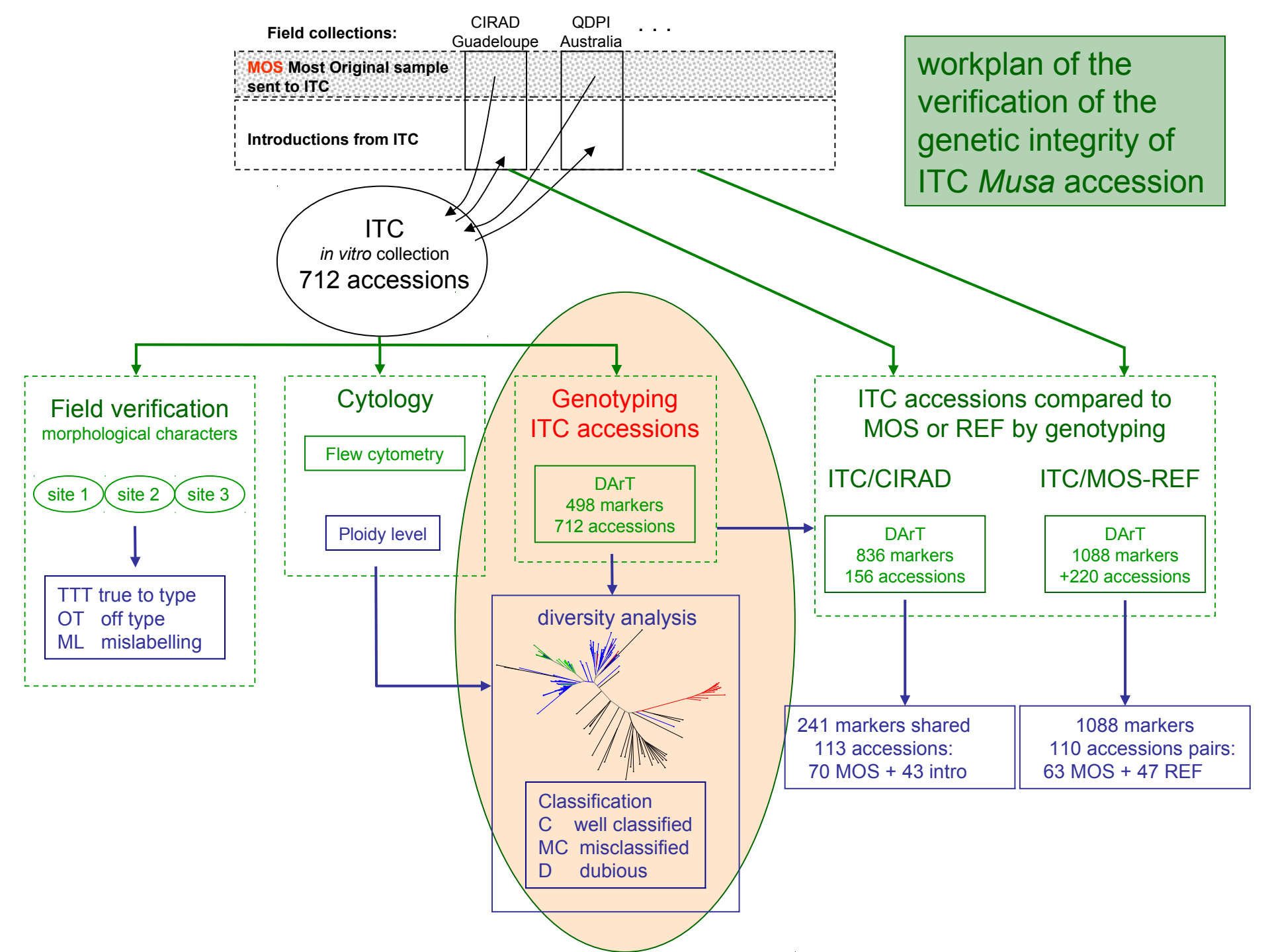
ORIGINAL PAPER

Development and assessment of Diversity Arrays Technology for high-throughput DNA analyses in *Musa*

Ange-Marie Risterucci · Isabelle Hippolyte · Xavier Perrier · Ling Xia · Vanessa Caig · Margaret Evers · Eric Huttner · Andrzej Kilian · Jean-Christophe Glaszmann

GCP: 168 accessions from IITA and CIRAD analysed with 836 DArTs markers :

- «DArTs can be used for genome wide analyses»,
- Despite the dominant nature of DArT markers, they can be used to «compare different genomes at a large number of loci in a single assay»,
- «The analysis cluster genotypes consistently with the accepted classification knowledge».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Analysis of 712 ITC accessions with DArTs

- 498 DArT markers.
- The phylogenetic tree produced by analyzing the DArT markers show the separation of accessions in species / groups and eventually subspecies/ subgroups, confirming the separation from morphological observations and previous molecular markers (RFLP, SSR).
- DArT markers are able to spot accessions which are not grouping with what was expected. These are clearly misclassified accessions.
- In many cases DArT analysis allowed to complement a classification (eg. the subgroup of a poorly identified accession can be identified).

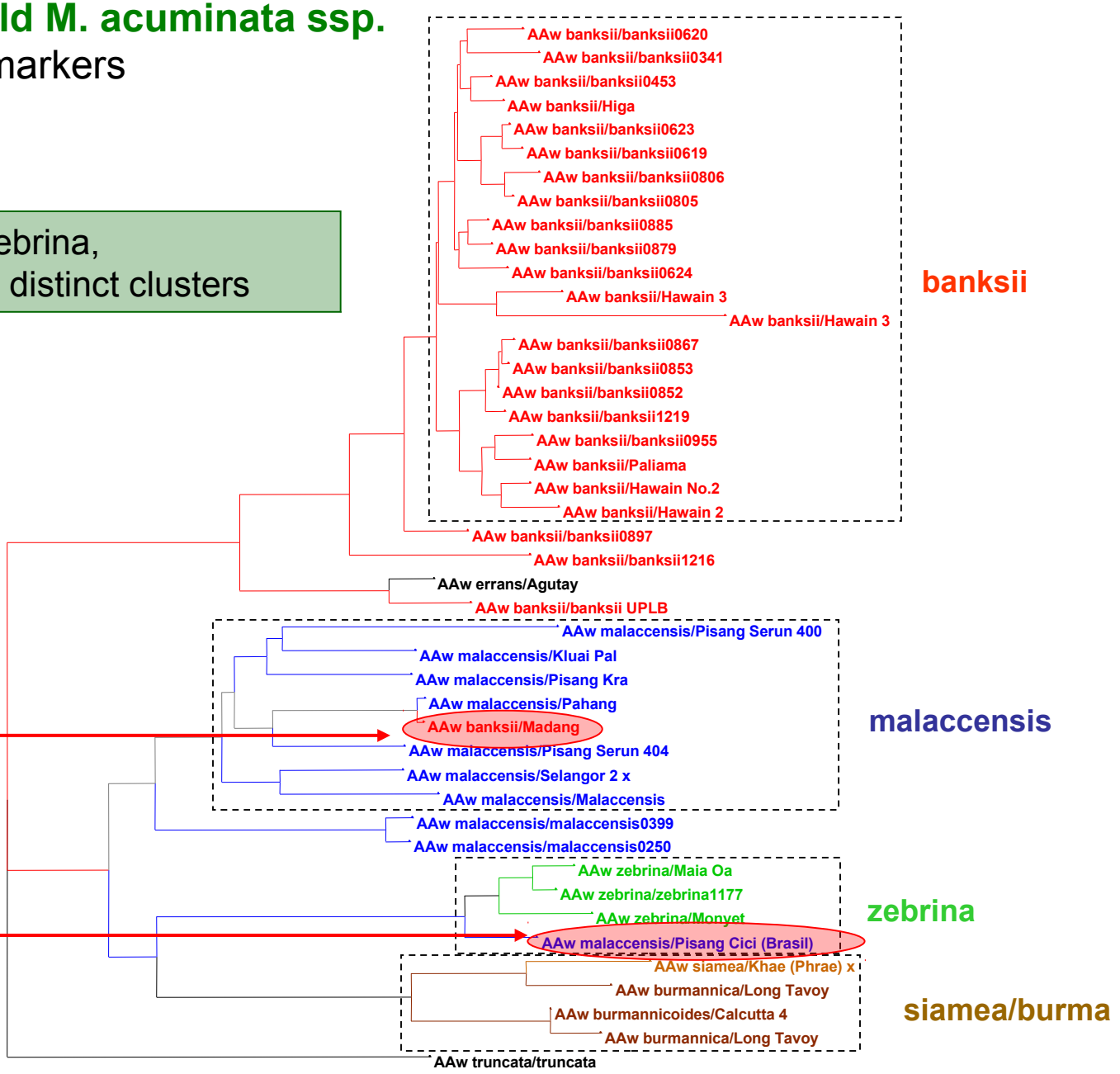
NJ tree analysis wild M. acuminata ssp.

44 accessions 468 markers

banksii, malaccensis, zebrina, siamea/burma ssp form distinct clusters

wrong accession held at ITC

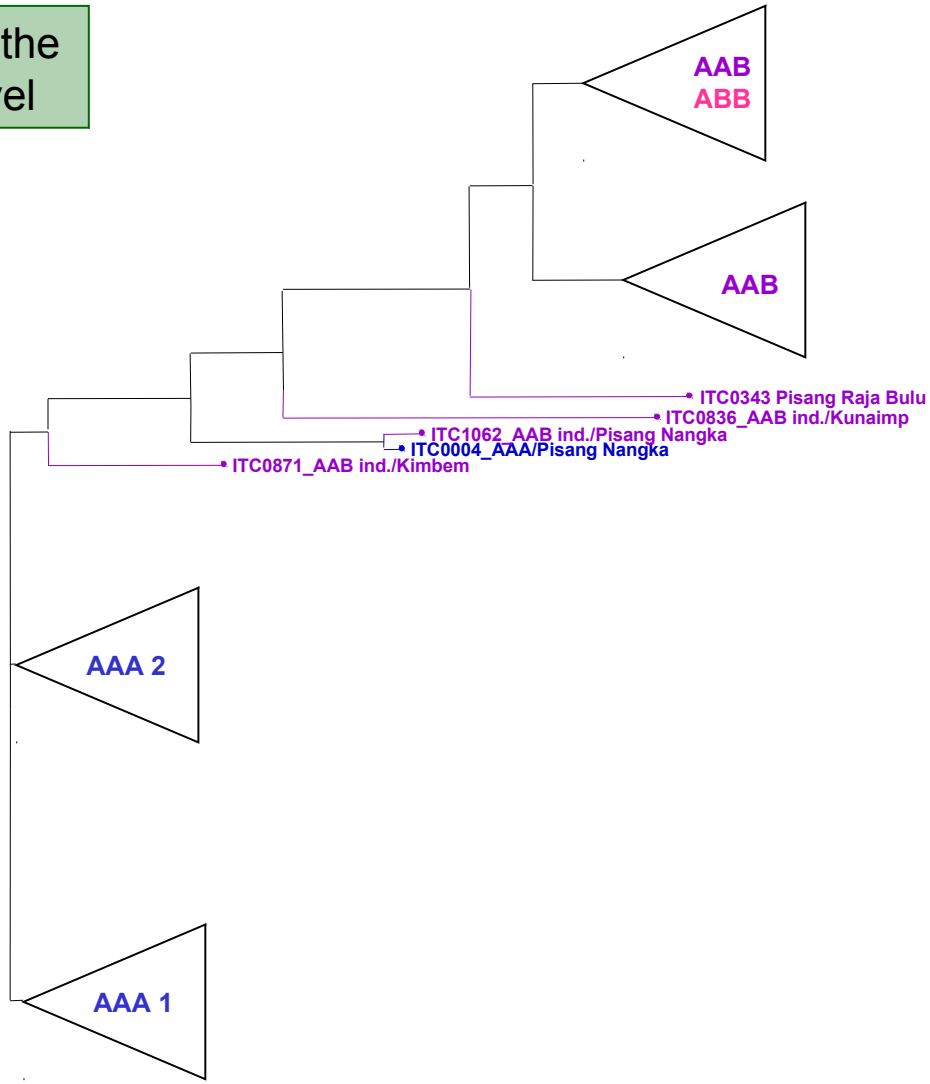
accession misclassified in the field genebank



NJ tree analysis triploides

292 accessions, 498 markers

consistent clustering at the group and subgroup level



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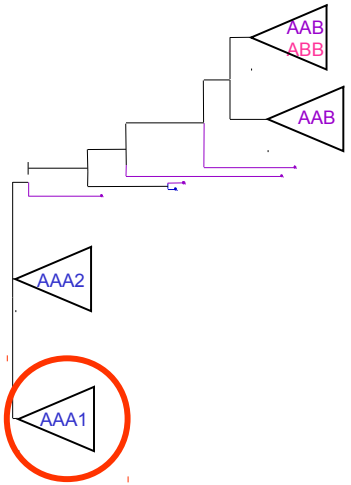


Cavendish

accessions classified “AAA undetermined” that should be reclassified as “Cavendish”

Gros Michel

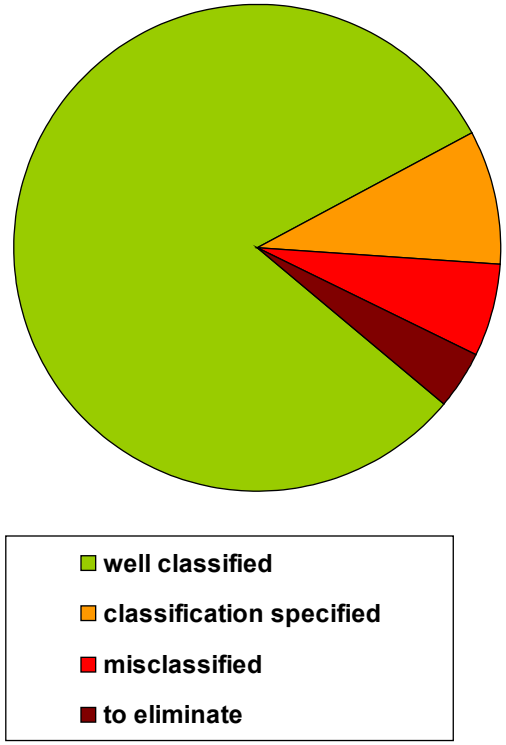
Ibota

accessions classified “AAB” to reclassify “AAA”

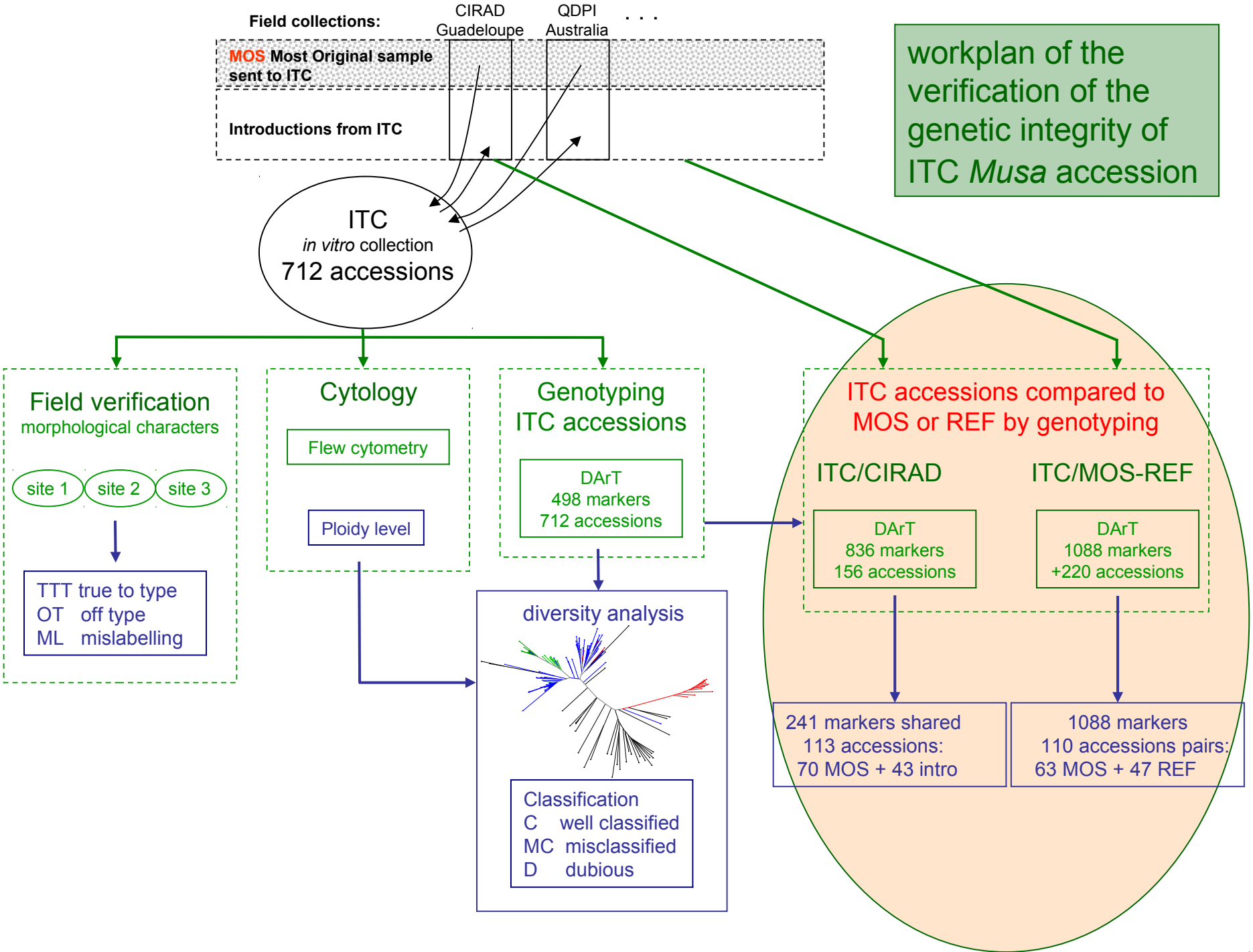
Analysis of 712 ITC accessions with DArTs

Combined with ploidy checking, the analysis of 712 ITC accessions resulted in :

- 582 are well classified (81%)
- classification of 67 accessions has been specified
- 42 (less than 6%) are truly misclassified (e.g. an accession classified AAB while it is a AAA) *Include accessions that were introduced under a false identification and errors at ITC.*
- 29 (4%) accessions to be eliminated (redundancy)



workplan of the verification of the genetic integrity of ITC *Musa* accession



Comparison of ITC and CIRAD common accessions

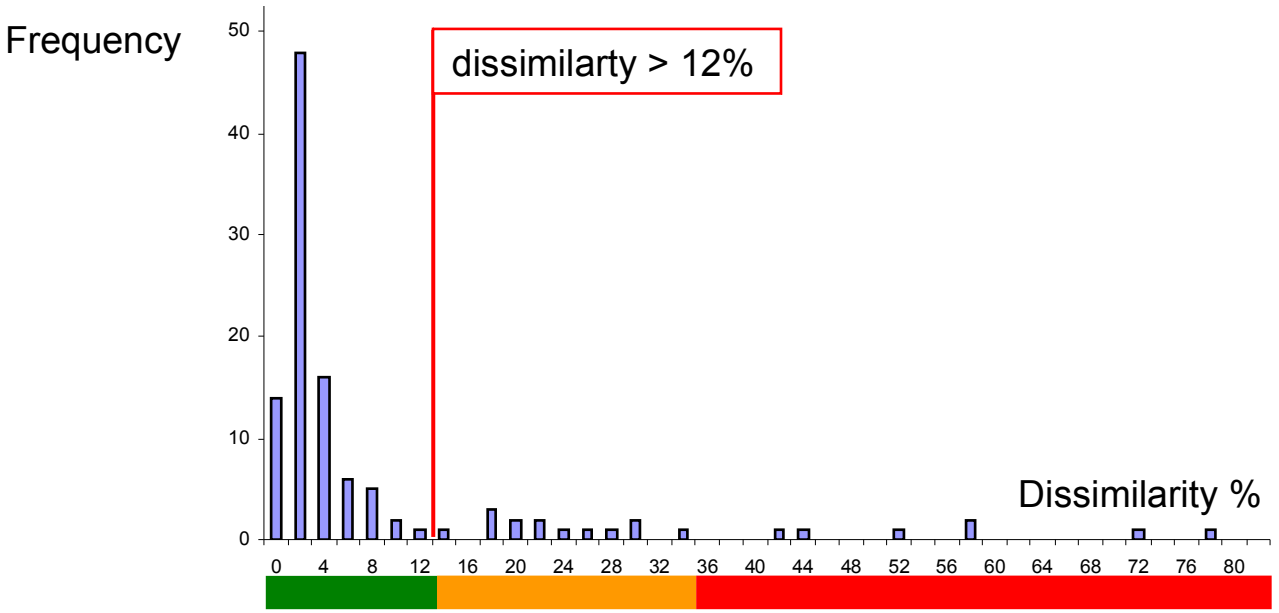
Methods

- Joint analysis of 241 DArTs markers in common on 113 genotypes in common in ITC collection and CIRAD Guadeloupe field genebank.
- Dissimilarity index calculated between each pair of accessions of the same genotype

Results

- Definition of a statistical threshold by permutation test
- Estimation of a dissimilarity between ITC and Guadeloupe accessions
- Comparison with field verification results

Comparison of ITC and CIRAD common accessions



Comparison of ITC and CIRAD common accessions

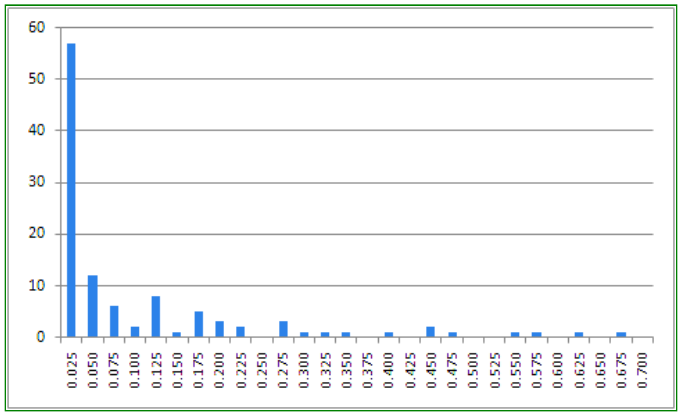
Phenotyping (fied verification)	Genotyping (DArTs) D>12%	Genotyping (DArTs) D<12%
Mislabeled	6/10	4/56
Offtypes	0/10	4/56
True to type	4/10	48/56

Comparision with field verification results

- 4 accessions out of 10 considered as mislabelled in the field are not detected by DArTS
- Offtypes are NOT detected by DArTs
- 4 accessions out of 66 considered as true to type in the field are considered different with DArTs

Comparison of ITC and MOS/REF accessions* by DArTs

AFTD
110 accession pairs
1088 DArTs
ITC (red)
63 MOS and 47 REF (green)



partner collections : CIRAD (France), FAVRI (Vietnam), FHIA (Honduras), IITA (Nigeria), NARI (Papua New Guinea)

Conclusions

Morphological and molecular characterization are complementary tools :

- DArT markers are able to detect ‘Mislabelled accessions’ if the exchange has happened between genetically distant accessions but if mislabelling occurs between two accessions from the same subgroup, our observations suggest that DArT markers would not be powerful enough to detect the error.
- DArT markers do not detect ‘Off-types’ that are due to somaclonal variations.
- Morphological observation stays the most precise way to detect any loss of genetic integrity, provided that the modification / mutation affects a visible character.

Recommendations

- Misclassification: use molecular markers and ploidy to check the classification of the accessions before being introduced in the ITC.
- Mislabelling: to regularly analyse accessions by batches, using molecular markers (SSR or DArT), which will allow to detect around half of the Mislabelled accessions.
- Off-types: so far, only the morphological observations can detect somaclonal variations.