UPOV

International Union for the Protection of New Varieties of Plants

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Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

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IMODDUS PROPOSAL: DEVELOPING A TOOLBOX TO DISTINGUISH APPLE MUTANTS FOR DUS TESTING

Document prepared by an expert from the European Union

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation on "Imoddus proposal: Developing a toolbox to distinguish apple mutants for DUS testing", prepared by an expert from the European Union, to be made at the sixteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

IMODDUS PROPOSAL: DEVELOPING A TOOLBOX TO DISTINGUISH APPLE MUTANTS FOR DUS TESTING

Presentation prepared by an expert from the European Union

Imoddus proposal: Developing a toolbox to distinguish apple mutants for DUS testing

Etienne Bucher, Hélène Muranty, Charles-Eric Durel, Laurence Feugey and FrançoisLaurens

IRHS INRA angers

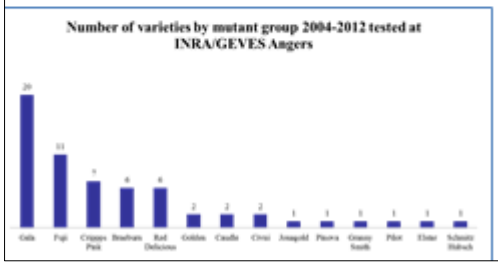
Imoddus meeting, 26th April 2016

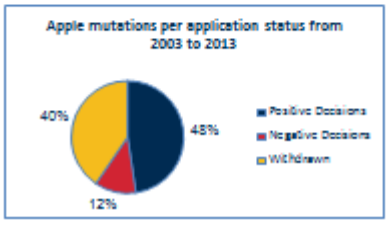
Main characteristics of DUS-CPOV testing in apple

- "DUS testing of fruit species is long and expensive compared to other crop sectors"
- Apple varieties = seedlings and mutants

# of CPVO applications for apple varieties 2004-2012

	seedlings	mutants
Total	101	74
INRA/Geves Angers	63	64





Characteristics of apple mutants

- Spontaneous or induced
- Mainly colour but also tree architecture, fruit size, ripening time
- Today's economically most important trait = colour

Gala mutants (INRA-Geves- Angers)

Issues to distinguish mutants in currentDUS tests

- Very tiny differences => Difficulty to demonstrate
- Uniformity ?
- Stability: Dependant to the environment

Mutant of Cripps Pink

Issues to distinguish mutants in currentDUS tests

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Consequences for DUS testing:

- Complex experimental designs : 10 trees/mutant (5/seedling) + ~1-5 control varieties (x 4 trees)
- Long duration of the DUS examination: 4 to 6 years for mutants (2-3y for seedlings)

⇒ Higher cost
- for the applicant/mutant (cost> 5-9000€/seedling)
- for the testing sites

- Concerns and Claims from applicants

↗ New Protocols but ↗ costs
↗New tools

Genotyping tools and knowledge on apple genetics/genomics

2010: Apple genome

Genetic/genomic tools...

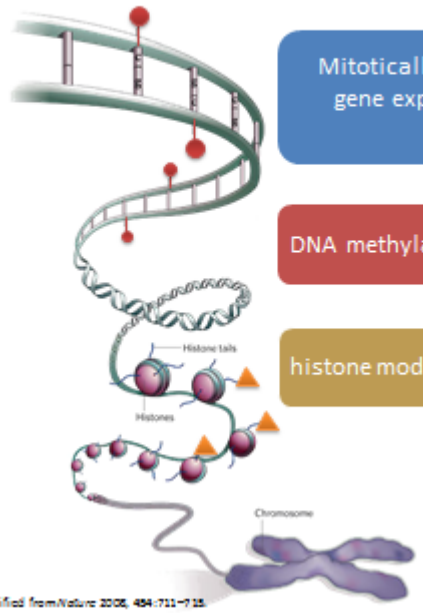
SSR | SNP chips ...>9K, 20K, 487K | cDNA chips, RNAseq

FruitBreedomics

- Variety identification
- Structure of the genetic diversity
- Q genetics (QTL, PBA, GWAS)
- Functional genomics

IRHS INRA angers

Definition of epigenetics



Mitotically or meiotically heritable variations in gene expression that cannot be explained by changes in DNA sequence

DNA methylation

histone modifications (acetylation, methylation…)

modified from Nature 2008, 454:711–715

Molecular tools for DUS/CPVO tests

2010: Apple genome

Genetic/genomic tools…	
SSR	SNP chips …>9K, 20K, 487K / cDNA chips, RNAseq

DUS

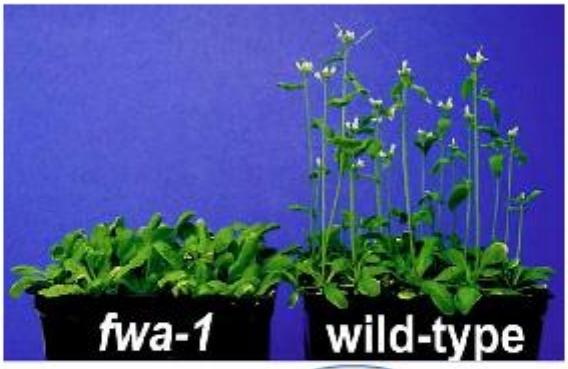
Seedlings → +++

Mutants → ---

Current genetic tools not efficient

Epigenetic causes

DNA methylation influences plant phenotypes



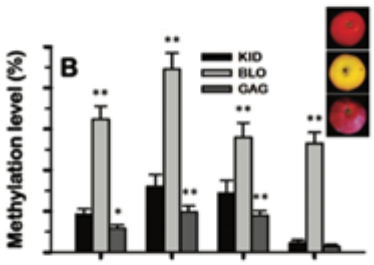
TE derived sequences

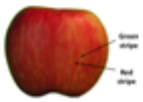
heritable epiallelesare the most important onesfor breeding

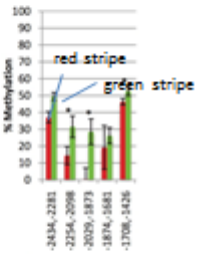
Modified from Jacobsen lab

DNA methylation influences apple fruit color

DNA methylation analysisat the *MdMYB10* promoter





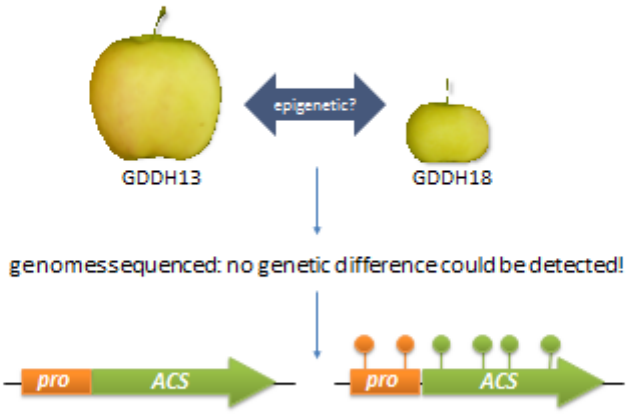


higher levelsof DNA methylation reduces *MdMYB10* expression and thus anthocyanin production

El-Sharkawy *et al.* 2015; Telias *et al.* 2011

DNA methylation influences apple fruit size

GDDH: Golden Delicious doubled haploid

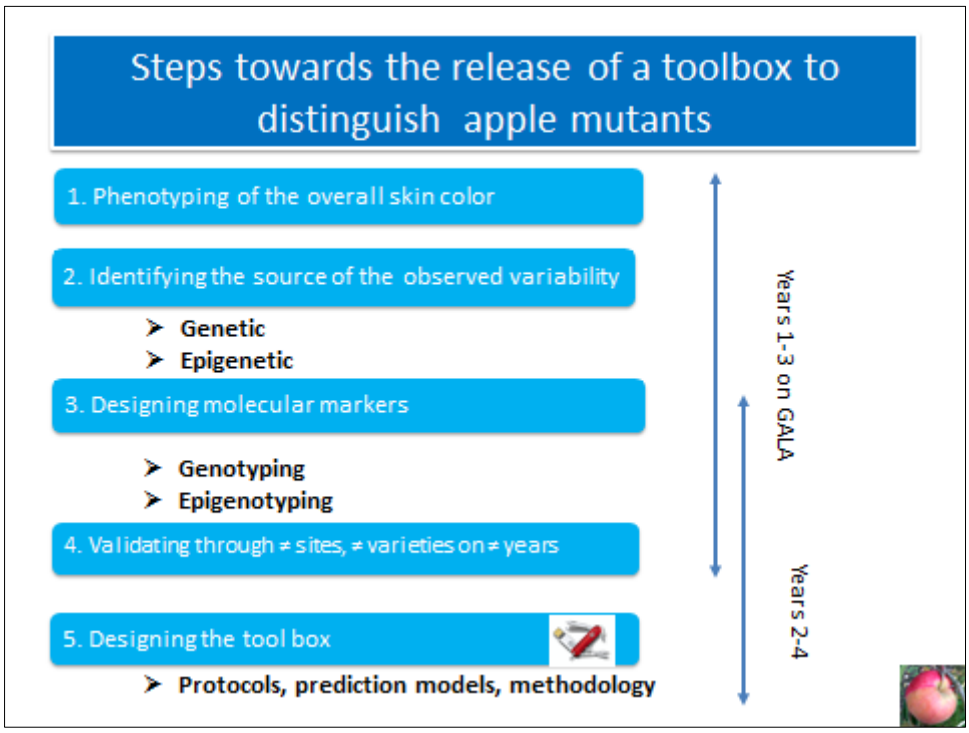


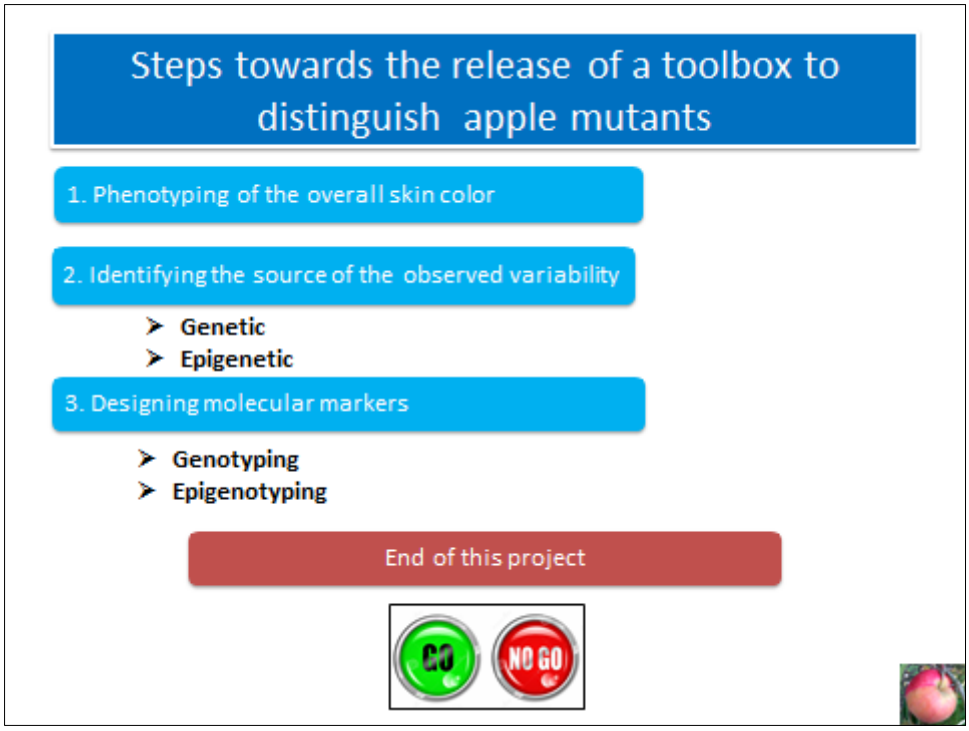
genomessequenced: no genetic difference could be detected!

epigenetic difference: ACS controls ethylene production and cell division

Aim of this project:

To develop markers and set up a toolbox to distinguish apple mutants


Steps towards the release of a toolbox to distinguish apple mutants
1. Phenotyping of the overall skin color
2. Identifying the source of the observed variability
Genetic
Epigenetic
3. Designing molecular markers
Genotyping
Epigenotyping
4. Validating through ≠ sites, ≠ varieties on ≠ years
5. Designing the tool box
Protocols, prediction models, methodology
Years 1-3 on GALA
Years 2-4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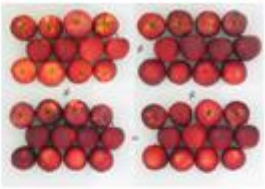
1. Phenotyping of the overall skin color
2. Identifying the source of the observed variability
Genetic
Epigenetic
3. Designing molecular markers
Genotyping
Epigenotyping
End of this project
GO
NO GO

1. Detailed phenotyping of the varieties

- Current tools



Photos: R. Guisnel, VaDiPom, INRA

QualiPom team

- visual digital analysis

QualiPom team

2a. Identifying the source of the observed variability: The genetic hypothesis

New very high quality Golden Delicious reference genome (available)

High quality **Gala** genome

Years 1-2

genomes of 10 selected **Gala** mutants

EpiCenter team

ResPom team

identification of genetic differences

indels, SNPs, copy number variations, transposable elements

2b. Identifying the source of the observed variability
The epigenetic hypothesis

genomesof 10 selected Gala mutants

Years 1-3

whole genome bisulfite sequencing

EpiCenter team

identification of epigenetic differences
(differentially methylated regions- DMRs)

3. Designing molecular markers

Years 2-3

Genome

genetics

transcriptome

Epigenome

epigenetics

genes of interest

EpiCenter team

QualiPom team

ResPom team

classic genetic markers

epigenetic markers
(pyrosequencing)

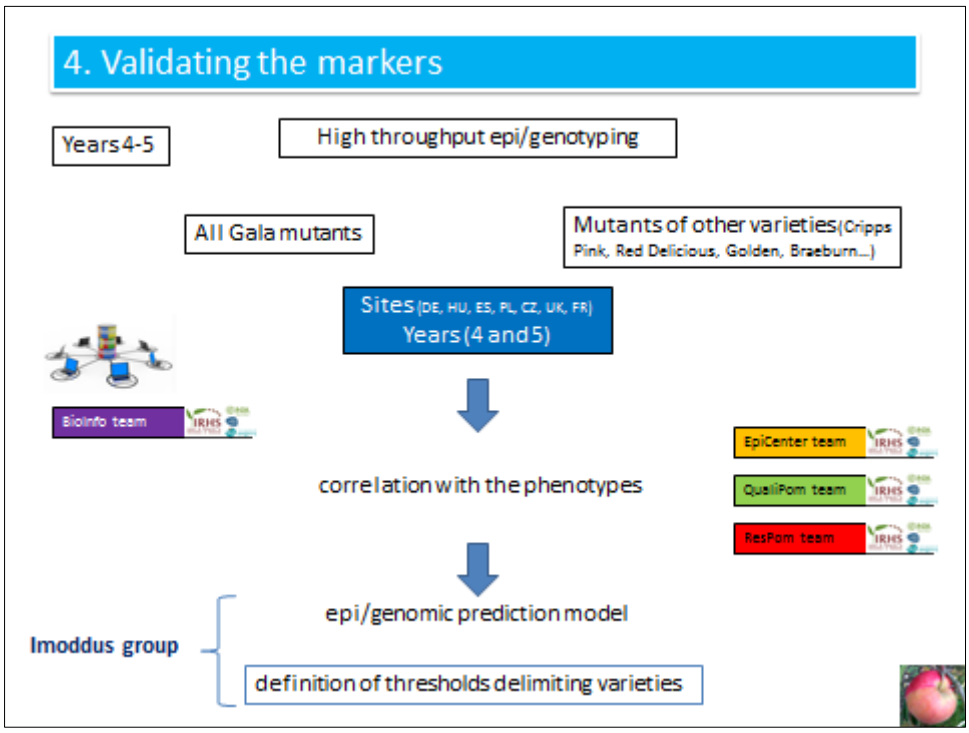
Duration: 2 years (2 harvest seasons)
Estimated budget: 250-300K€

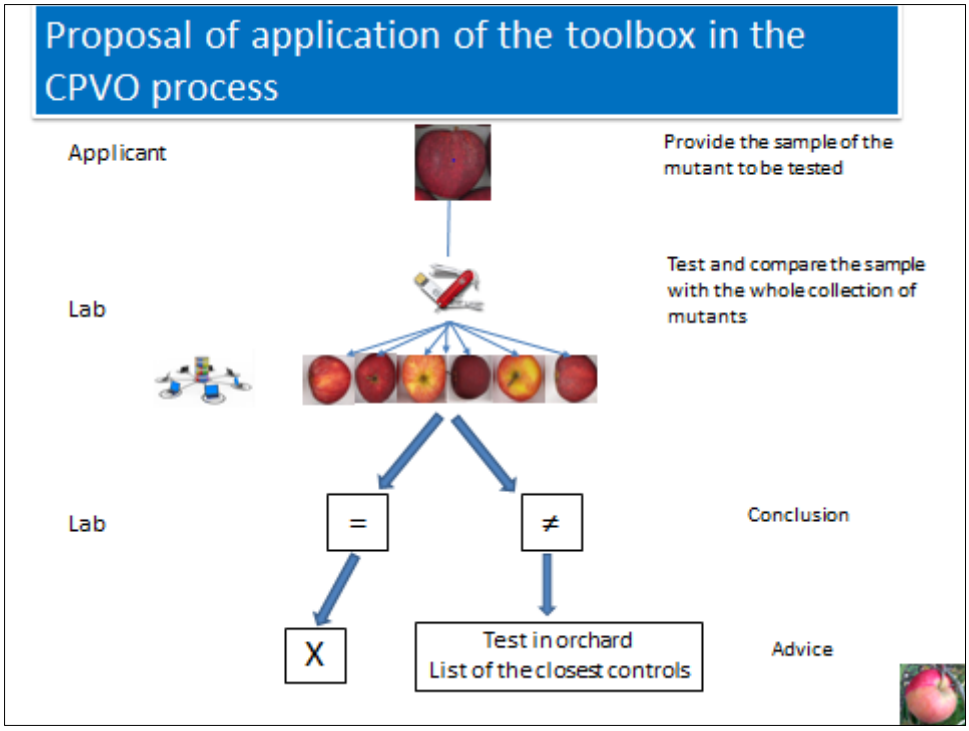
Steps towards the release of a toolbox to distinguish apple mutants

1. Phenotyping of the overall skin color

2. Identifying the source of the observed variability
   - Genetic
   - Epigenetic

3. Designing molecular markers
   - Genotyping
   - Epigenotyping


4. Validating the markers
Years 4-5
High throughput epi/genotyping
All Gala mutants
Mutants of other varieties (Cripps Pink, Red Delicious, Golden, Braeburn…)
Sites (DE, HU, ES, PL, CZ, UK, FR)
Years (4 and 5)
BioInfo team
correlation with the phenotypes
EpiCenter team
QualiPom team
ResPom team
epi/genomic prediction model
Imoddus group
definition of thresholds delimiting varieties


Proposal of application of the toolbox in the CPVO process
Applicant
Provide the sample of the mutant to be tested
Lab
Test and compare the sample with the whole collection of mutants
Lab
=
≠
Conclusion
X
Test in orchard
List of the closest controls
Advice

Conclusion

➔Brand new approach which combines the latest high-throughput genetic/epigenetic/genomic methodologies to distinguish apple mutants

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➔ Take advantage of the skills and knowledge of the participants of the project

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➔Brand new approach which combines the latest high-throughput genetic/epigenetic/genomic methodologies to distinguish apple mutants

➔ Take advantage of the skills and knowledge of the participants of the project

=> scientific output

=> practical impact for CPVO testing :

↘ costs, ↗ efficiency

Thanks for your attention!

[End of Annex and of document]