UPOV

International Union for the Protection of New Varieties of Plants

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NEW DEVELOPMENTS IN BIOCHEMICAL AND MOLECULAR TECHNIQUES
CPVO REPORT ON IMODDUS: UPDATE ON R&D PROJECTS CO-FUNDED BY CPVO

Document prepared by an expert from the European Union

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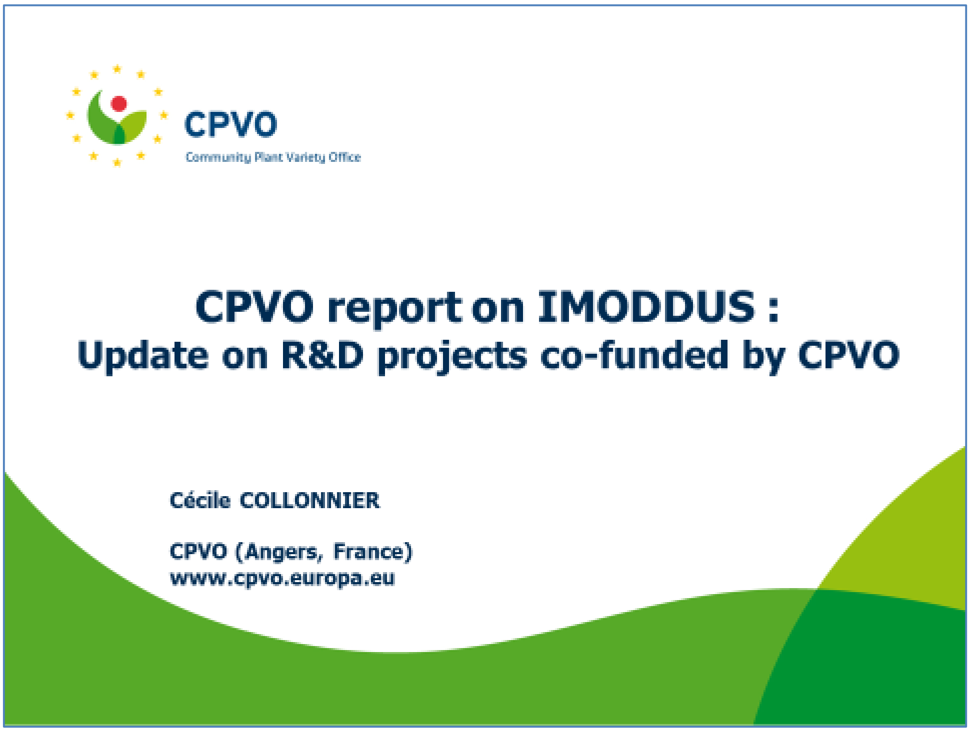
The presentation prepared by an expert from the Community Plant Variety Office (CPVO) entitled “CPVO report on IMODDUS: Update on R&D projects co-funded by CPVO” is reproduced in the Annex to this document.

[Annex follows]

CPVO REPORT ON IMODDUS: UPDATE ON R&D PROJECTS CO-FUNDED BY CPVO

Presentation prepared by an expert from the European Union



CPVO
Community Plant Variety Office

CPVO report on IMODDUS :
Update on R&D projects co-funded by CPVO

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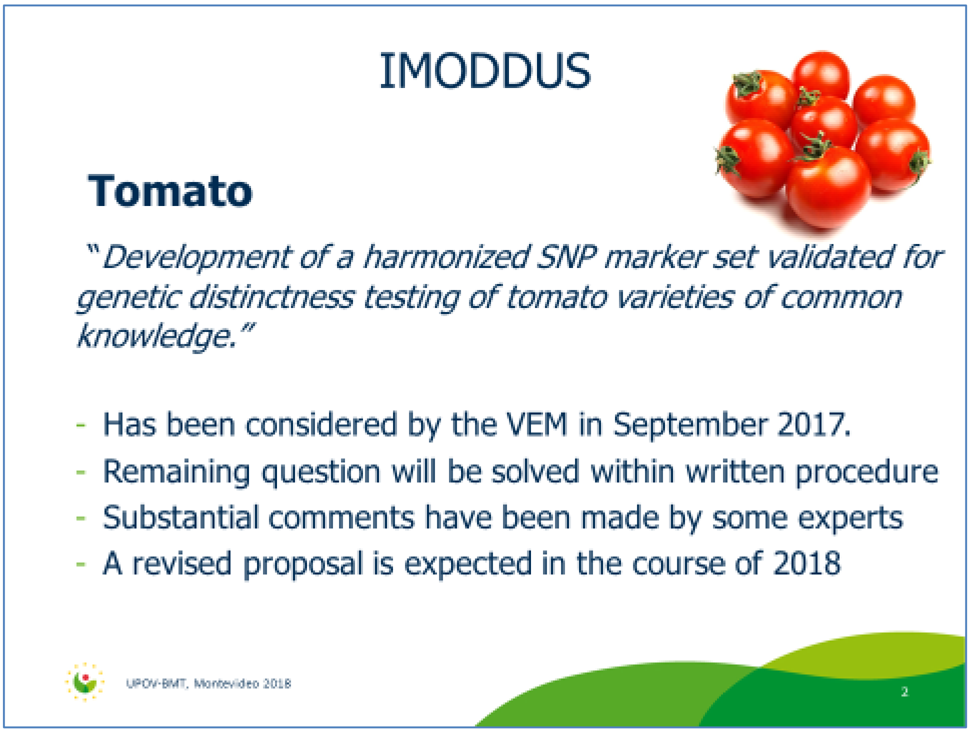

IMODDUS

Tomato

"Development of a harmonized SNP marker set validated for genetic distinctness testing of tomato varieties of common knowledge."

- Has been considered by the VEM in September 2017.
- Remaining question will be solved within written procedure
- Substantial comments have been made by some experts
- A revised proposal is expected in the course of 2018

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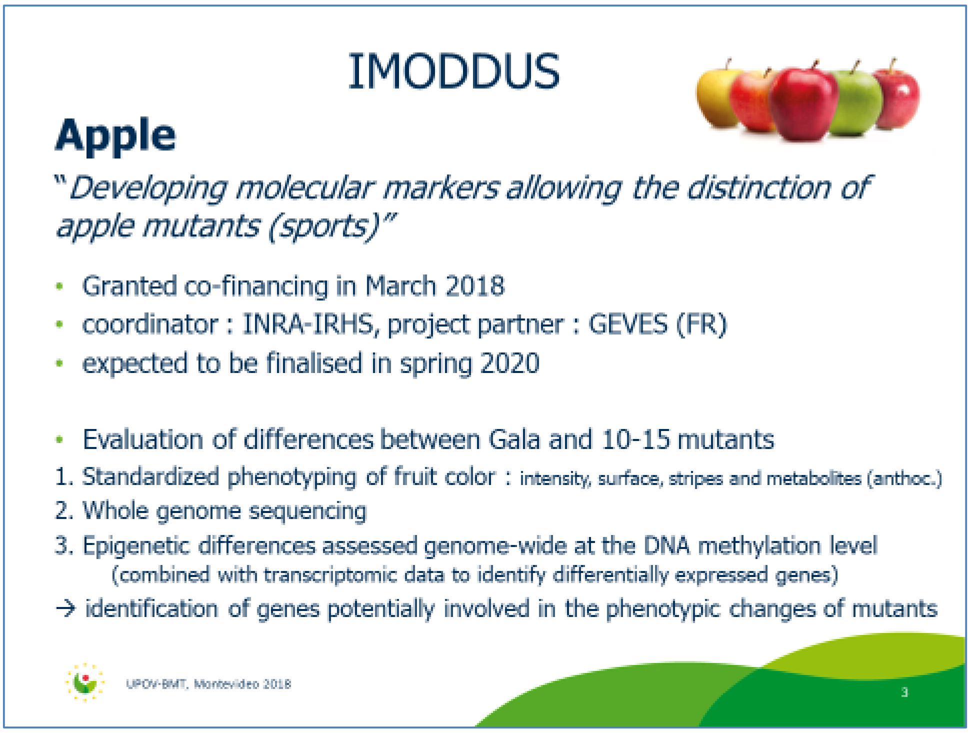


IMODDUS

Apple

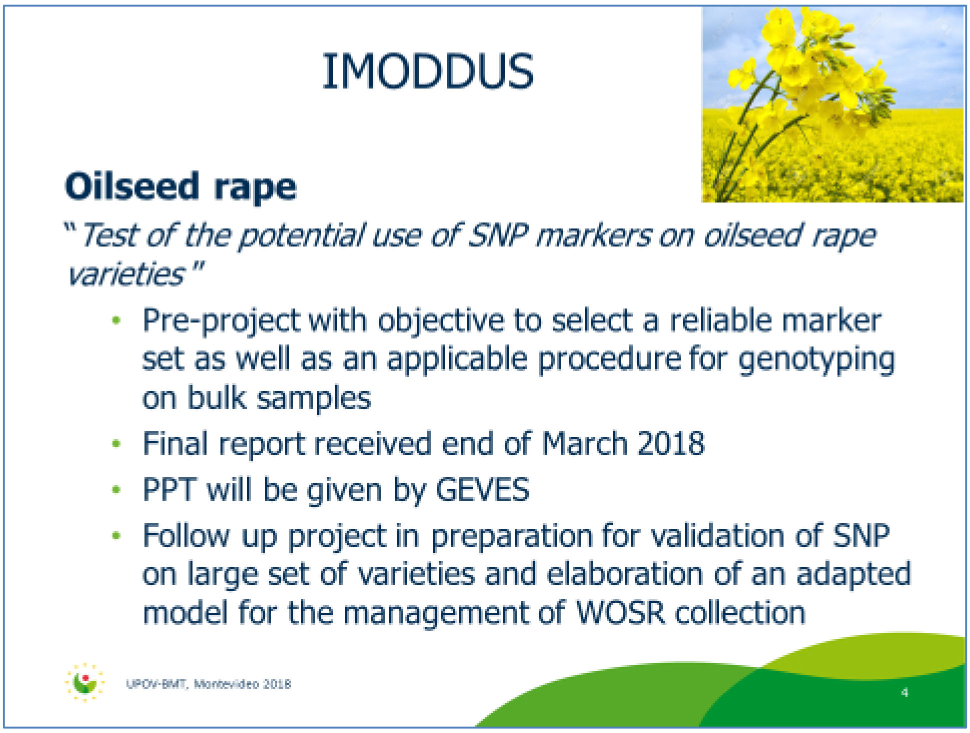
"Developing molecular markers allowing the distinction of apple mutants (sports)"

- Granted co-financing in March 2018
- coordinator : INRA-IRHS, project partner : GEVES (FR)
- expected to be finalised in spring 2020

- Evaluation of differences between Gala and 10-15 mutants

1. Standardized phenotyping of fruit color : intensity, surface, stripes and metabolites (anthoc.)
2. Whole genome sequencing
3. Epigenetic differences assessed genome-wide at the DNA methylation level
   (combined with transcriptomic data to identify differentially expressed genes)

→ identification of genes potentially involved in the phenotypic changes of mutants

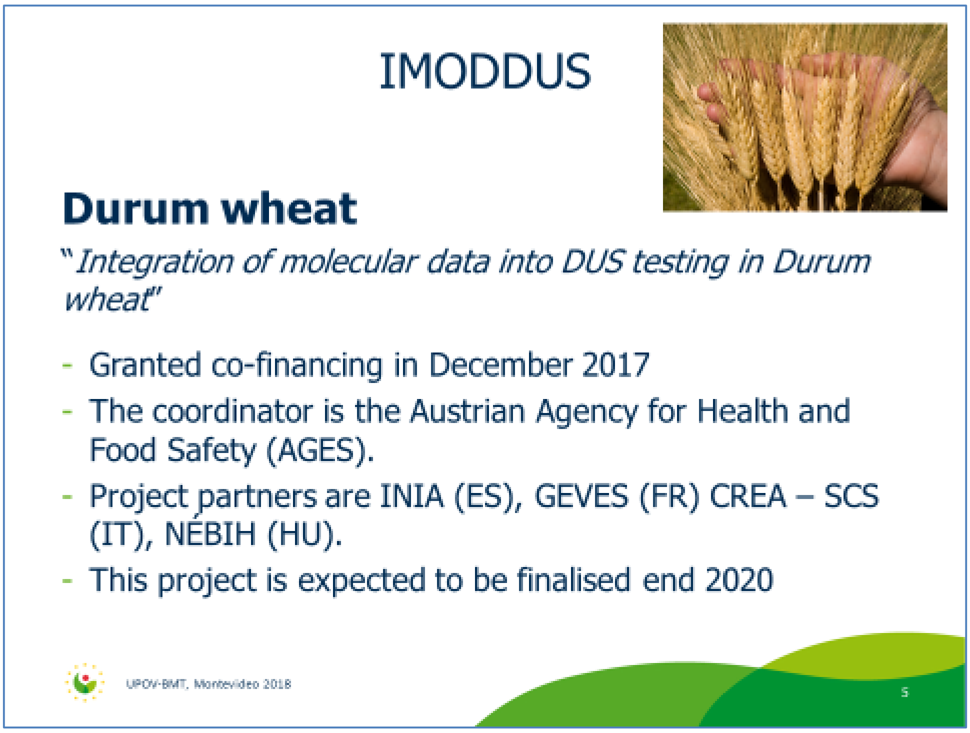


IMODDUS

Oilseed rape

"Test of the potential use of SNP markers on oilseed rape varieties"

- Pre-project with objective to select a reliable marker set as well as an applicable procedure for genotyping on bulk samples
- Final report received end of March 2018
- PPT will be given by GEVES
- Follow up project in preparation for validation of SNP on large set of varieties and elaboration of an adapted model for the management of WOSR collection



IMODDUS

Durum wheat

"Integration of molecular data into DUS testing in Durum wheat"

- Granted co-financing in December 2017
- The coordinator is the Austrian Agency for Health and Food Safety (AGES).
- Project partners are INIA (ES), GEVES (FR) CREA – SCS (IT), NÉBIH (HU).
- This project is expected to be finalised end 2020

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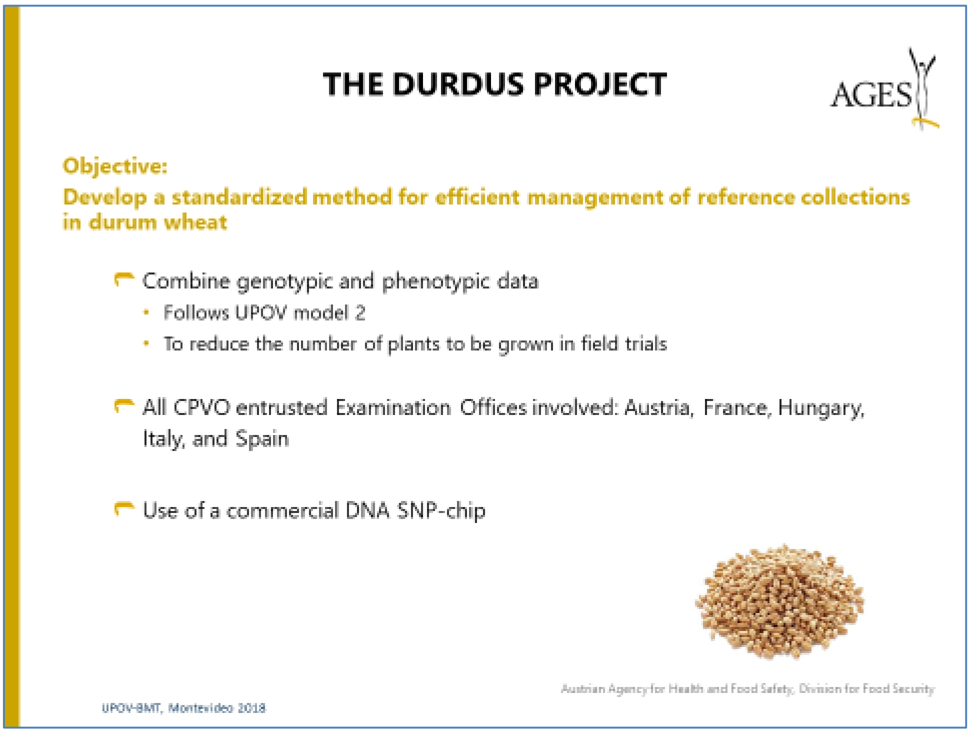

THE DURDUS PROJECT

Objective:
Develop a standardized method for efficient management of reference collections in durum wheat

- Combine genotypic and phenotypic data
  - Follows UPOV model 2
  - To reduce the number of plants to be grown in field trials
- All CPVO entrusted Examination Offices involved: Austria, France, Hungary, Italy, and Spain
- Use of a commercial DNA SNP-chip

Austrian Agency for Health and Food Safety, Division for Food Security

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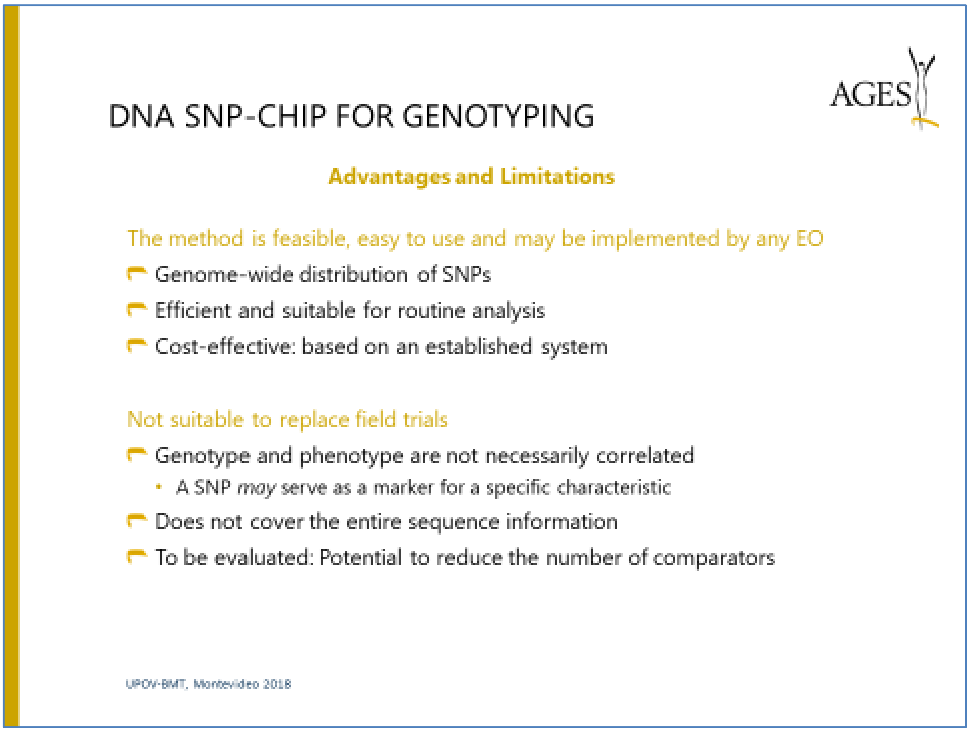

DNA SNP-CHIP FOR GENOTYPING

Advantages and Limitations

The method is feasible, easy to use and may be implemented by any EO

- Genome-wide distribution of SNPs
- Efficient and suitable for routine analysis
- Cost-effective: based on an established system

Not suitable to replace field trials

- Genotype and phenotype are not necessarily correlated
  - A SNP *may* serve as a marker for a specific characteristic
- Does not cover the entire sequence information
- To be evaluated: Potential to reduce the number of comparators

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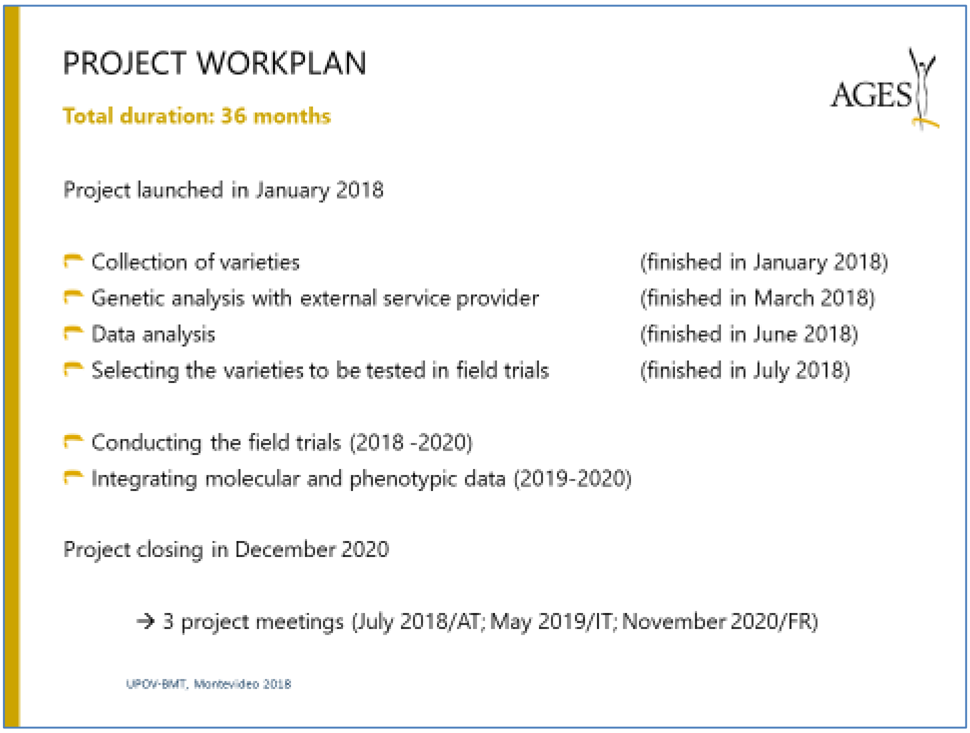

PROJECT WORKPLAN

Total duration: 36 months

Project launched in January 2018

Collection of varieties	(finished in January 2018)
Genetic analysis with external service provider	(finished in March 2018)
Data analysis	(finished in June 2018)
Selecting the varieties to be tested in field trials	(finished in July 2018)

- Conducting the field trials (2018 -2020)
- Integrating molecular and phenotypic data (2019-2020)

Project closing in December 2020

→ 3 project meetings (July 2018/AT; May 2019/IT; November 2020/FR)

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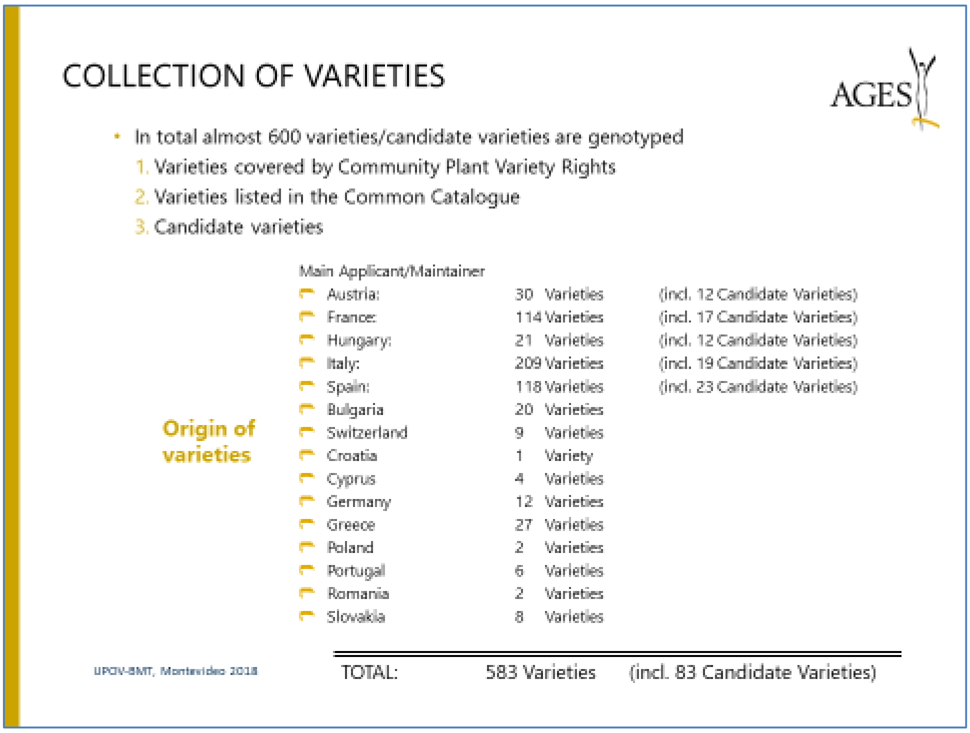
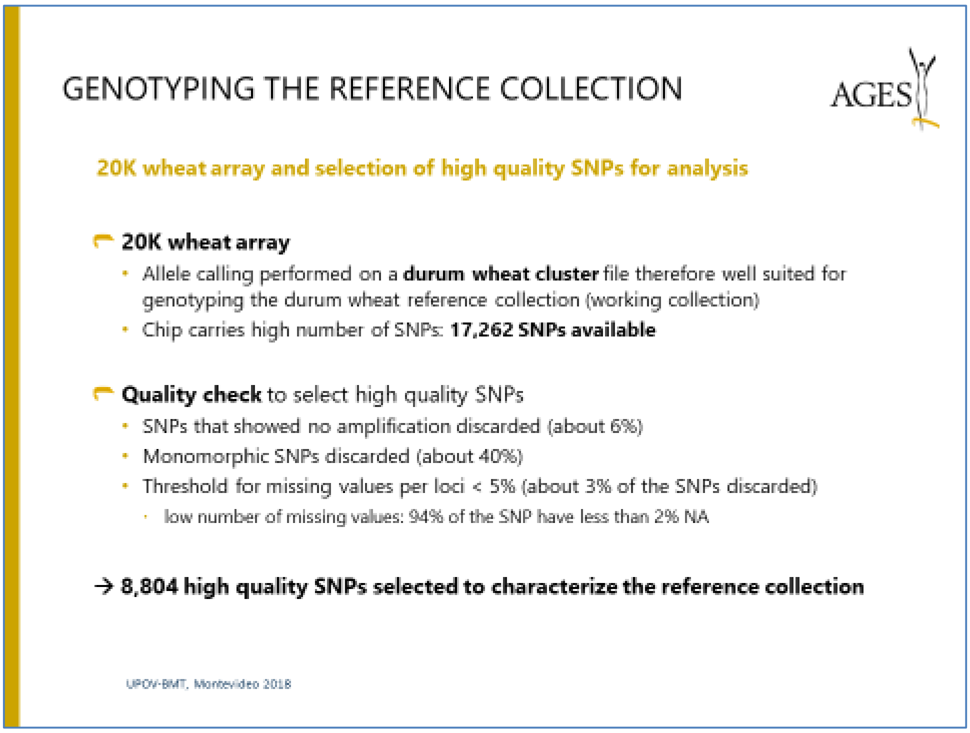


COLLECTION OF VARIETIES

- In total almost 600 varieties/candidate varieties are genotyped
  1. Varieties covered by Community Plant Variety Rights
  2. Varieties listed in the Common Catalogue
  3. Candidate varieties

Origin of varieties

Main Applicant/Maintainer		
Austria:	30 Varieties	(incl. 12 Candidate Varieties)
France:	114 Varieties	(incl. 17 Candidate Varieties)
Hungary:	21 Varieties	(incl. 12 Candidate Varieties)
Italy:	209 Varieties	(incl. 19 Candidate Varieties)
Spain:	118 Varieties	(incl. 23 Candidate Varieties)
Bulgaria	20 Varieties	
Switzerland	9 Varieties	
Croatia	1 Variety	
Cyprus	4 Varieties	
Germany	12 Varieties	
Greece	27 Varieties	
Poland	2 Varieties	
Portugal	6 Varieties	
Romania	2 Varieties	
Slovakia	8 Varieties	
TOTAL:	583 Varieties	(incl. 83 Candidate Varieties)

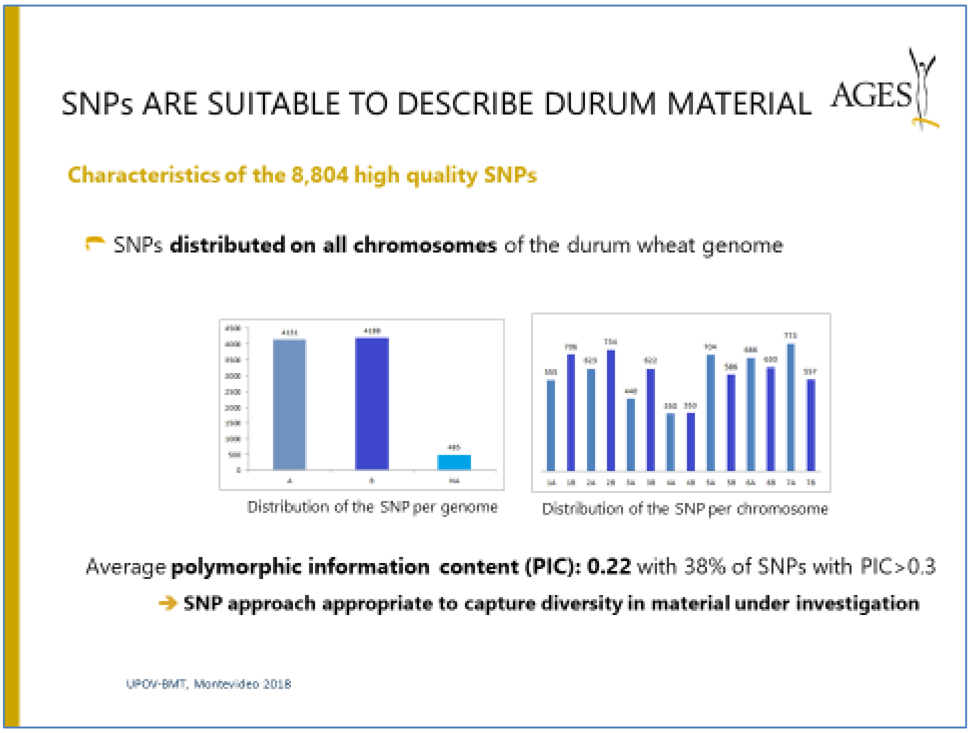


GENOTYPING THE REFERENCE COLLECTION

20K wheat array and selection of high quality SNPs for analysis

- **20K wheat array**
  - Allele calling performed on a **durum wheat cluster** file therefore well suited for genotyping the durum wheat reference collection (working collection)
  - Chip carries high number of SNPs: **17,262 SNPs available**
- **Quality check** to select high quality SNPs
  - SNPs that showed no amplification discarded (about 6%)
  - Monomorphic SNPs discarded (about 40%)
  - Threshold for missing values per loci < 5% (about 3% of the SNPs discarded)
    - low number of missing values: 94% of the SNP have less than 2% NA

→ 8,804 high quality SNPs selected to characterize the reference collection



SNPs ARE SUITABLE TO DESCRIBE DURUM MATERIAL

Characteristics of the 8,804 high quality SNPs

- SNPs **distributed on all chromosomes** of the durum wheat genome

Distribution of the SNP per genome

Distribution of the SNP per chromosome

Average **polymorphic information content (PIC): 0.22** with 38% of SNPs with PIC>0.3

➔ **SNP approach appropriate to capture diversity in material under investigation**

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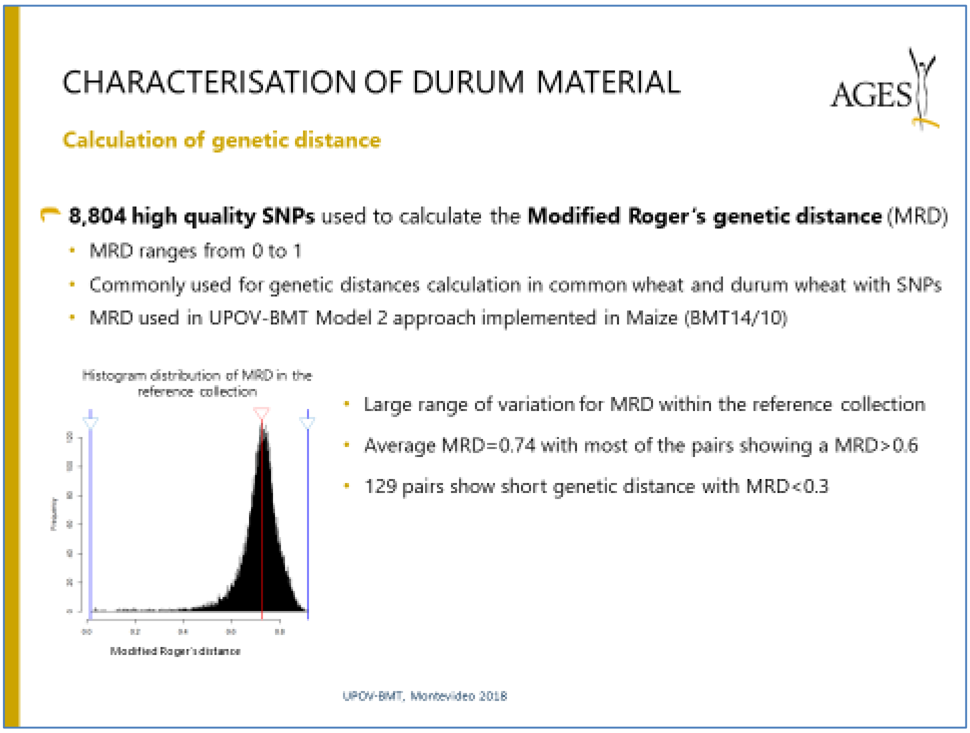

CHARACTERISATION OF DURUM MATERIAL

Calculation of genetic distance

- **8,804 high quality SNPs** used to calculate the **Modified Roger's genetic distance** (MRD)
  - MRD ranges from 0 to 1
  - Commonly used for genetic distances calculation in common wheat and durum wheat with SNPs
  - MRD used in UPOV-BMT Model 2 approach implemented in Maize (BMT14/10)

Histogram distribution of MRD in the reference collection

- Large range of variation for MRD within the reference collection
- Average MRD=0.74 with most of the pairs showing a MRD>0.6
- 129 pairs show short genetic distance with MRD<0.3

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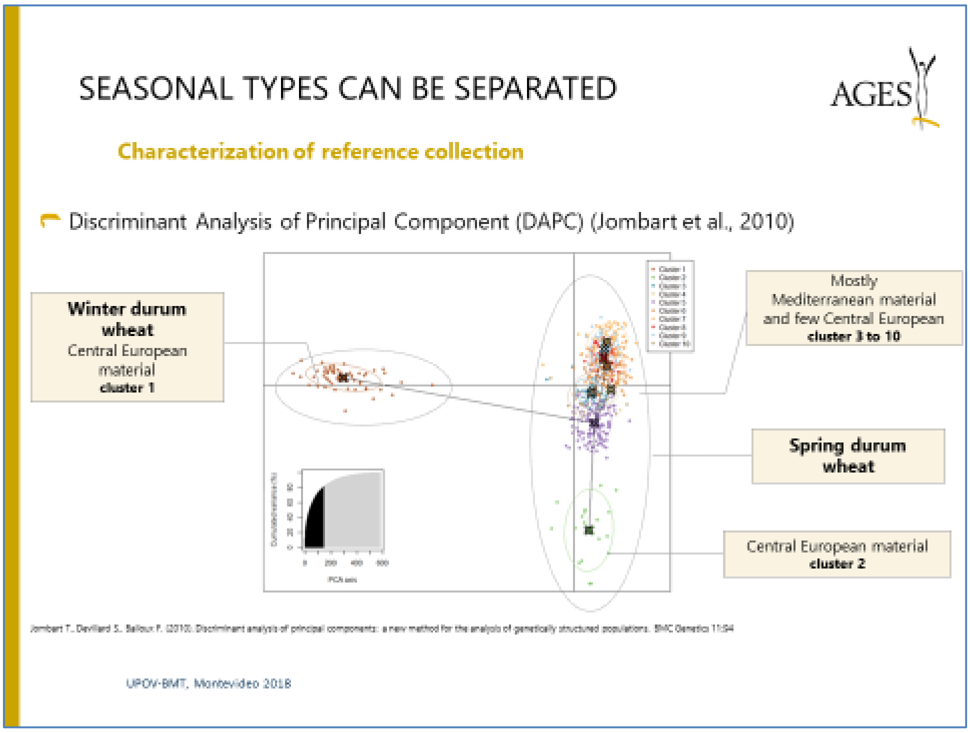

SEASONAL TYPES CAN BE SEPARATED

Characterization of reference collection

- Discriminant Analysis of Principal Component (DAPC) (Jombart et al., 2010)

Winter durum wheat
Central European material
cluster 1

Mostly Mediterranean material and few Central European
cluster 3 to 10

Spring durum wheat

Central European material
cluster 2

Jombart T., Devillard S., Balloux F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94

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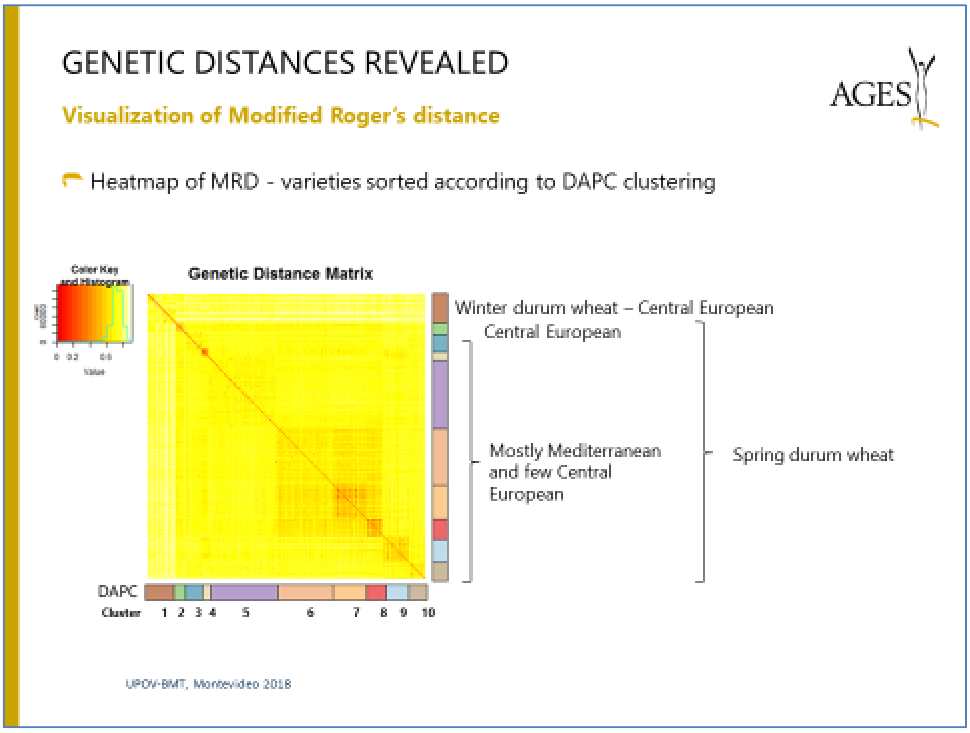

GENETIC DISTANCES REVEALED

Visualization of Modified Roger's distance

- Heatmap of MRD - varieties sorted according to DAPC clustering

Winter durum wheat – Central European

Central European

Mostly Mediterranean and few Central European

Spring durum wheat

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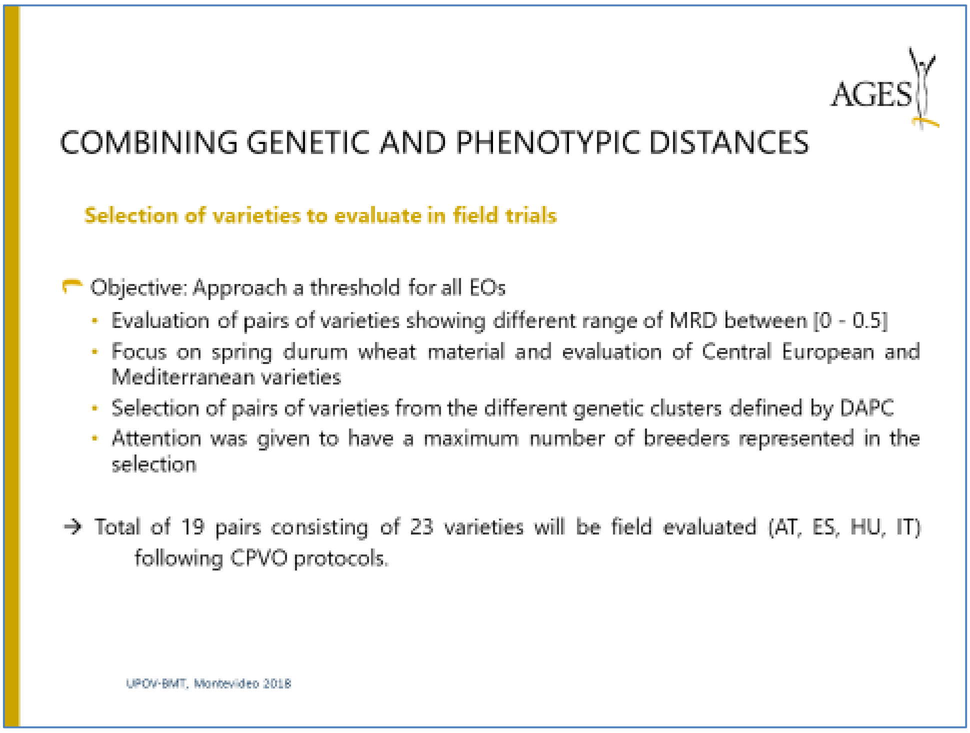
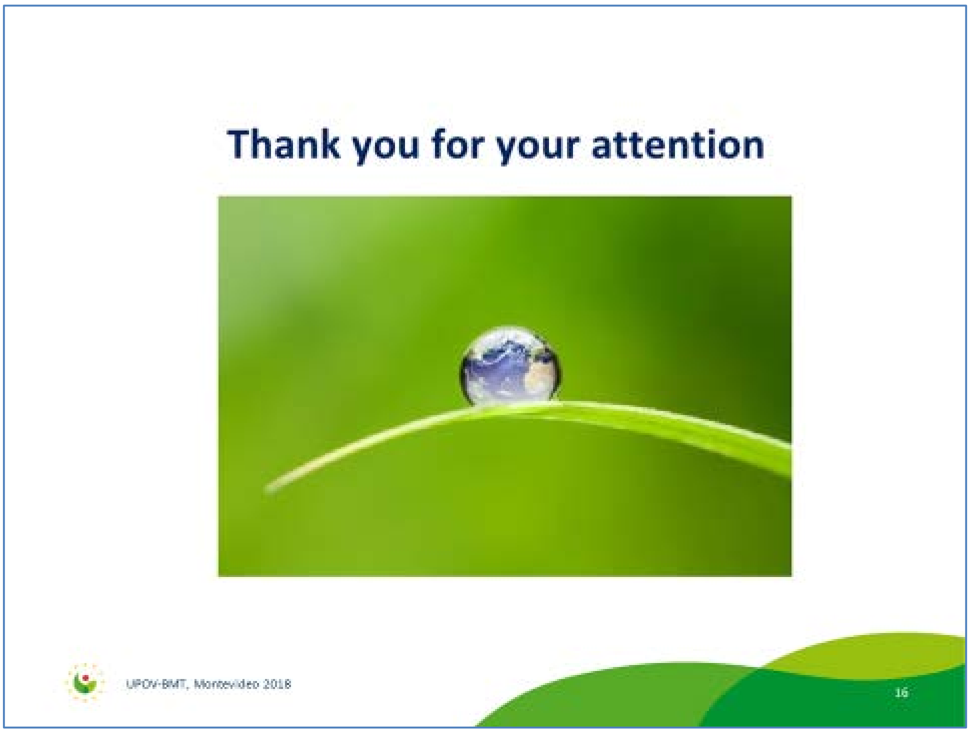


COMBINING GENETIC AND PHENOTYPIC DISTANCES

Selection of varieties to evaluate in field trials

- Objective: Approach a threshold for all EOs
  - Evaluation of pairs of varieties showing different range of MRD between [0 - 0.5]
  - Focus on spring durum wheat material and evaluation of Central European and Mediterranean varieties
  - Selection of pairs of varieties from the different genetic clusters defined by DAPC
  - Attention was given to have a maximum number of breeders represented in the selection

→ Total of 19 pairs consisting of 23 varieties will be field evaluated (AT, ES, HU, IT) following CPVO protocols.



Thank you for your attention

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