

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

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CPVO REPORT ON IMODDUS: LATEST DEVELOPMENTS (INVITE) AND UPDATE ON R&D PROJECTS

Document prepared by an expert from the European Union

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The annex to this document contains a copy of a presentation on “CPVO report on IMODDUS: latest developments (INVITE) and update on R&D projects”, made at the eighteenth session of the BMT.

[Annex follows]



CPVO report on IMODDUS : latest developments (INVITE) and update on R&D projects

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IMODDUS Background



- The CPVO Administrative Council adopted a R&D Strategy for the period 2015-2020.
- The strategy includes the set up of a CPVO BMT working group for the promotion of the use of bio-molecular techniques in DUS testing and variety identification.
- Its name is CPVO **IMODDUS** which stands for **I**ntegration of **M**olecular **D**ata into **D**US testing.
- IMODDUS works as a think-tank on developing a strategy on how to integrate BMT into DUS testing.
- IMODDUS has a **practical approach** which aims to assist the CPVO identifying and assessing R&D project proposals for species where BMT could improve the cost efficiency of DUS testing.



IMODDUS Meetings



- First meeting: April 2016
- Second meeting: January 2017
- Third meeting: April 2018

- No meeting in 2019 → INVITE consortium



INVITE



- call for tender SFS-29 of Horizon 2020 programme on “Innovation in plant variety testing” in 2017.

- “INnovations in plant VarIety Testing in Europe to foster the introduction of new varieties better adapted to varying biotic and abiotic conditions and to more sustainable crop management practices”
 - Duration : 60 months
 - Budget : 8 Million euros
 - Aim : improving both efficiency of variety testing and availability of information to stakeholders on variety performance under a range of production conditions and biotic and abiotic stresses.



INVITE – CONCEPT and OBJECTIVES

- Based from main end-user requests (EOs, CPVO, breeders, ...)
- New tools, new procedures, optimized networks, common DB
- balance between research and application in variety testing (TRL 2 to 7)
- Get benefits from existing knowledge/projects
- ↗ synergies between DUS and VCU
- Dissemination to a wide range of stakeholders



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INVITE – SPECIES

7 "model" crops :

maize, wheat, rye grass, sunflower, potato, tomato, apple



+ 3 "application" crops :

lucerne, soybean, rapeseed



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INVITE - Technical approaches

High throughput phenotyping tools to speed up observations and provide detailed and accurate phenotypic data.



Genomic data for :

- new sets of molecular markers to improve the management of reference collections for DUS testing (reduce the number of reference varieties used in the field when a candidate variety is tested).
- new markers linked to a range of phenotypic traits to accelerate the assessment of certain DUS characteristics."

Modelling to make prediction of variety behaviour in various environments.



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INVITE – CONSORTIUM (29 partners)

INVITE
CPVO Network of EOs

Non-profit

- 1 – INRA
- 2 – UH
- 3 – FIBL
- 4 – WU
- 5 – DLO
- 6 – Teagasc
- 7 – IRTA
- 8 – JHI
- 9 – Agroscope
- 10 – RCL
- 11 – CREA
- 12 – CRA-W
- 13 – CPVO
- 14 – Naktuinbouw
- 15 – BSA
- 16 – GEVES
- 17 – NIAB
- 18 – AGES
- 19 – UKZUZ
- 20 – SASA
- 21 – NEBIH
- 22 – ACTA

Industry

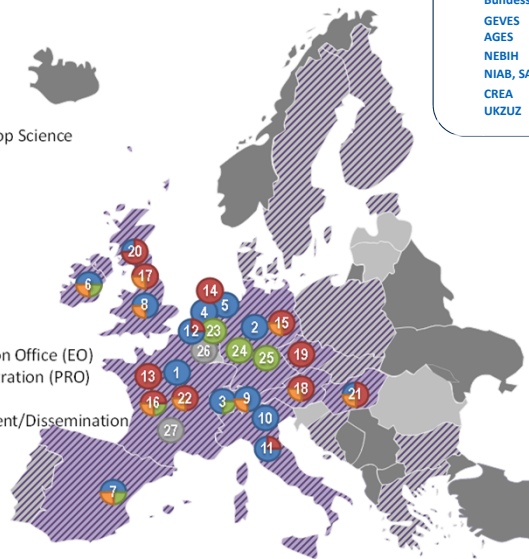
- 23 – ESA
- 24 – NPZ
- 25 – Bayer Crop Science
- 26 – ARCADIA
- 27 – IT

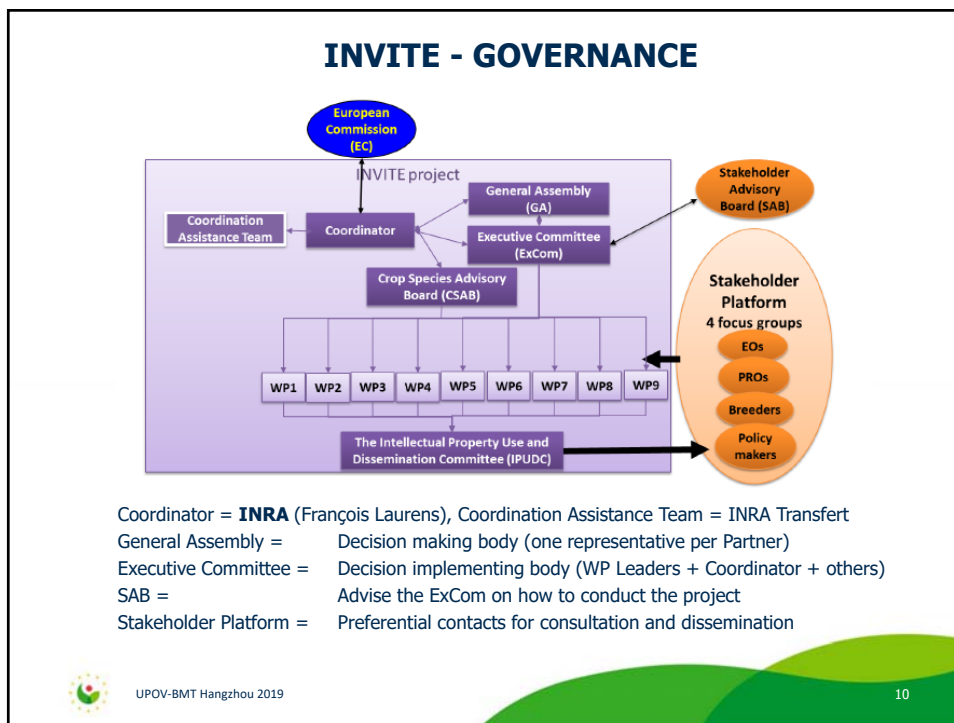
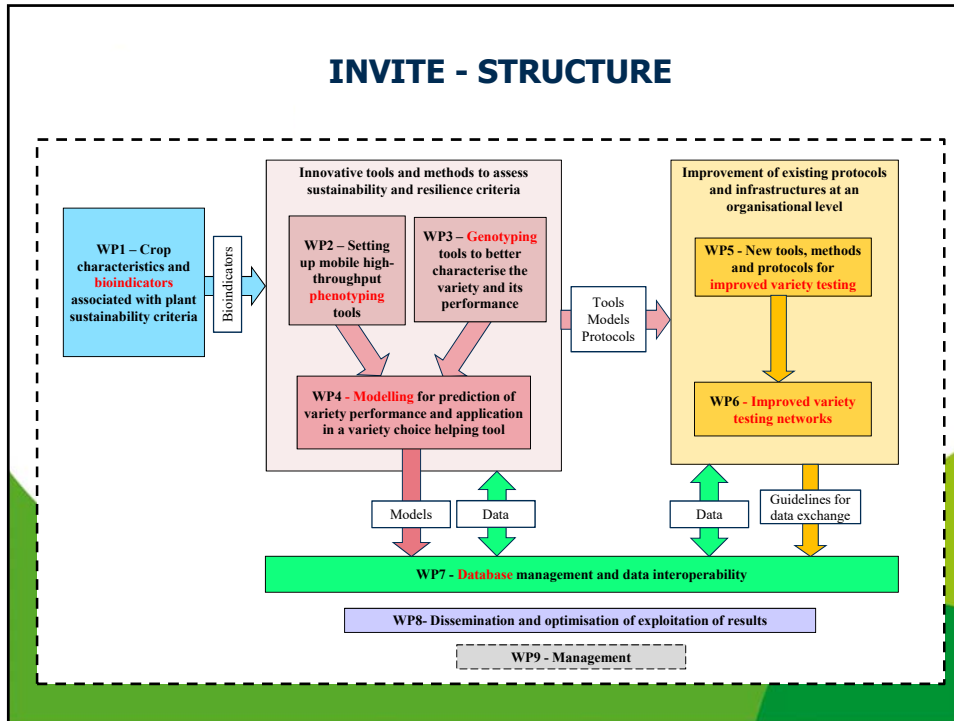
Activities

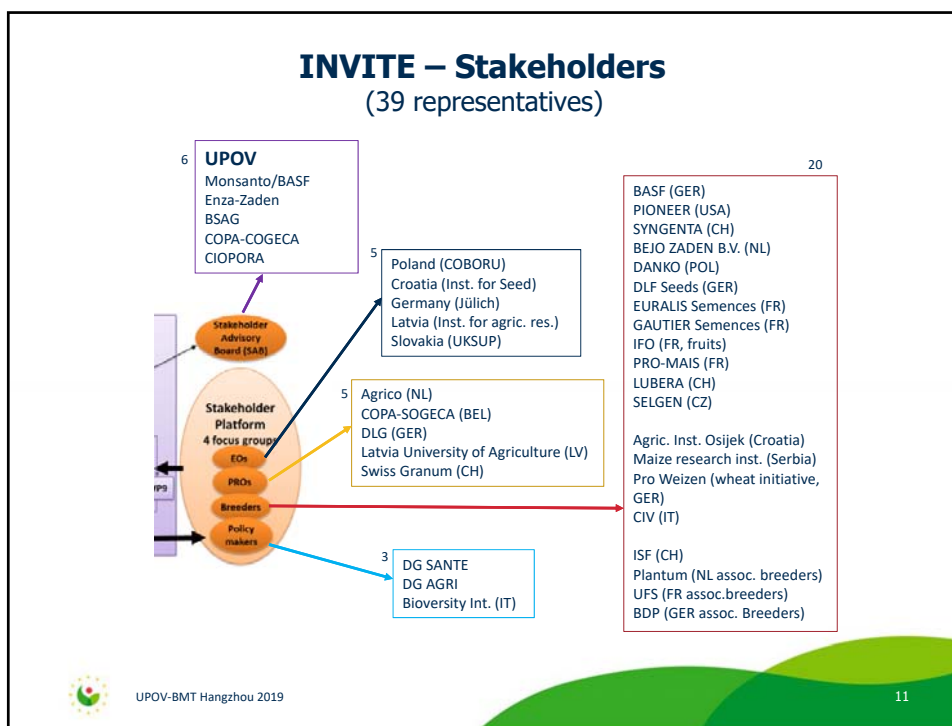
- - Research
- - Examination Office (EO)
- - Post-Registration (PRO)
- - Breeding
- - Management/Dissemination

EOs

CPVO	EU
CRA-W, ILVO	BE
Naktuinbouw	NL
Bundessortenamt	GER
GEVES	FR
AGES	AU
NEBIH	HU
NIAB, SASA	UK
CREA	IT
UKZUZ	CZ







WP3: Genotyping tools to characterize varieties and their performance

Karl Schmid (University of Hohenheim, Germany)

- **Task 3.1: Genome wide marker sets for distinguishing heterogeneous and open pollinated varieties PRG (TEAGASC, INRA, Agroscope, GEVES, CPVO, BioSS)**
- **Task 3.2: Genome wide marker sets for line and hybrid varieties WHEAT, MAIZE, SOYBEAN (UHOH, NIAB, INRA, GEVES, CPVO)**
- **Task 3.3: Identification of specific markers for DUS related traits and development of genotyping assays using these markers POTATO, TOMATO, APPLE (NIAB, INRA, GEVES, CPVO, WU)**
- **Task 3.4: GWAS mapping and genomic prediction of DUS traits WHEAT, MAIZE, PRG, SOYBEAN (UHOH, NIAB, INRA, BioSS)**
- **Task 3.5: Improved models for marker-based evaluation of D and U WHEAT, MAIZE, PRG, SOYBEAN (UHOH, BioSS)**

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WP5: Integration of new methods and tools in advanced variety testing protocols and demonstration in field trials

Aurélia GOULEAU (GEVES, France)
Cécile COLLONNIER (CPVO)

- **Task 5.1: Demonstration in field test conditions of the applicability of phenotypic tools**

- **Task 5.2: New procedures using molecular tools for optimization of DUS testing management of reference collection and direct evaluation of specific traits.**

- **Task 5.3: Development and proposals of new protocols to integrate the sustainability and resilience criteria for specific traits linked to biotic and abiotic stresses through multi criteria evaluation under conventional and organic cropping**



- **Task 5.2: New procedures using molecular tools for optimization of DUS testing management of reference collection and direct evaluation of specific traits.**

Related WP: WP1, WP3	Wheat	Rye-grass	potato	apple	tomato	Soy bean	lucerne
Management of reference collection	GEVES, IRTA, CREA, AGES, NIAB M24-M36 WP3 → set of molecular markers (M24)	BIOS, ILVO, NIAB, NAKT, TEAGASC M24-M36 WP3 → set of molecular markers (M24)					GEVES, INRA, CREA M24-M48 Eucleg → ?
Certification	GEVES, ACTA M24-M36 WP3 → set of molecular markers (M24)						
Molecular markers linked to traits of interest			SASA, NAKT: disease M36-M48 WP3 → Gene specific markers (M36)	ACTA, IRTA: disease M36-M48 WP3 → Gene specific markers (M36)	GEVES, CREA NAKT: disease M18-M36	CREA, : drought M1-M30	
Epigenetic markers				ACTA, GEVES: color M46-M52 WP1 → Epigenetic bioindicators (M54)			

Reflexion on the integration of molecular markers and on the use of statistical approaches to describe varieties and to address uniformity issues for variety testing M40-M52 WP3 → Novel marker-based method (M48)



INVITE - Latest developments

- May-June 2019: signature of the Consortium agreement and Grant agreement
- 01 July 2019 : pre-financing of the project (35% of the budget)
- 08 – 10 July 2019 : Kick-off meeting in Angers (France)
- INVITE was invited by the Commission to collaborate with a sister project called **Innovar** which will focus on the development of a machine learning approach based on genomic, phenotypic and agronomic data for the optimization of variety testing in wheat.
 - memorandum of understanding
 - INNOVAR's KoM in 16-18 Oct 2019 in Belfast (Ireland).
- CPVO tasks until 2024 :
 - manage access to data issues
 - follow activities of all WPs
 - co-lead WP5 (tools)
 - active role in WP6 (networks), WP7 (DB), WP8 (dissemination)



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Tomato

"International validation of a SNP set to determine genetic distances for the management of tomato reference collection"

- Granted co-financing in February 2019.
- coordinated by Naktuinbouw and partners are all EU entrusted examination offices for Tomato: GEVES; COBORU; NÉBIH; INIA; DGAV and CREA.
- DUS Testing Center of MOA, China and the Korean Seed & Variety Service (KSVS), Republic of Korea are involved and participate in the project on their own funding. Discussion on going for inclusion of Japan.
- European Seed Association is also partner to the project.
- Results expected to be available in 2021.
- If successful : → follow-up project for the implementation of markers.



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Oilseed rape

"Developing a strategy to apply SNP molecular markers in the framework of winter oilseed rape DUS testing."

- Granted co-financing in February 2019.
- coordinated by GEVES (FR), Bundessortenamt (GER) as project partner.
- = follow up project of an earlier project which validated a set of SNP markers as a tool for the management of the reference collection (concluded in 2018).
- aims at developing a method to use genetic data in the two different testing systems GAIA in France and COY in Germany in the framework of existing or new UPOV models.
- Before the end of the project, all EU entrusted examination offices will be invited to discuss the outcome of the project results.
- Expected to be finalized in 2021.



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Apple

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

- Granted co-financing in March 2018
 - coordinator : INRA-IRHS (FR), 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

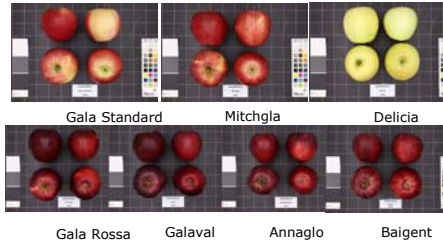


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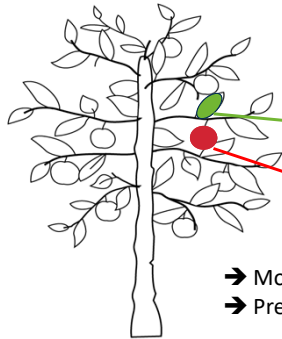
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"Developing molecular markers allowing the distinction of apple mutants"

7 varieties of Gala mutants
Two trees
Two years



On the same branch:



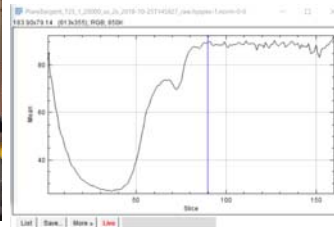
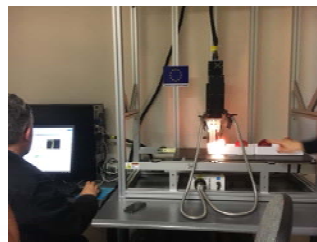
WGBS => Epigenetic differences (DMRs)
WGS => Genetic differences (SNPs, TEs, indels, copy number variations)
RNA sequencing (apple skin) => Gene expression differences

- Most of the data produced...
- Preliminary results are promising, **bioinformatic** analyses ongoing.

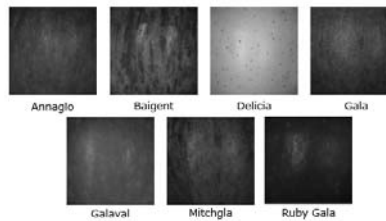


"Developing molecular markers allowing the distinction of apple mutants"

➤ **Phenotyping** by hyperspectral camera to detect stripes (preliminary results)



ImHorPhen tea



IMODDUS



Durum wheat

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

- Aim: Efficient management of reference collections to better select the variety pairs to be grown in field trials for comparison
UPOV Model 2 "Combining Phenotypic and Molecular Distances in the Management of Variety Collections"
- 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)
→ All CPVO entrusted Examination Offices.
- This project is expected to be finalised end 2020



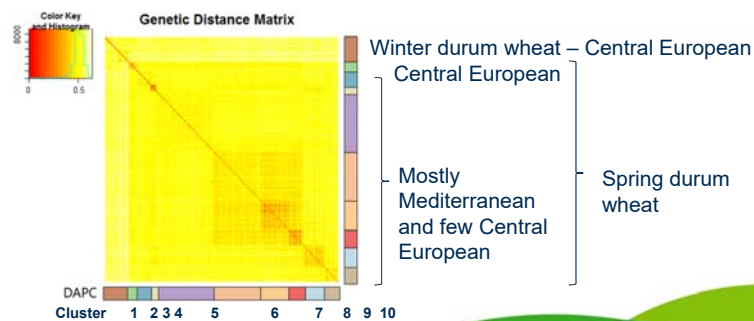
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THE DURDUS PROJECT



- Commercial DNA SNP-chip (20K → 25K array, Illumina, TraitGenetics)
~18000 SNPs → 8800 high quality polymorphic SNPs covering all chromosomes
- ~600 varieties genotyped (CPVR, Common Catalogue, Candidate varieties)
- Genetic distances :
 - ↳ GD calculated with **R Software** using **Modified Roger's distance**
 - ↳ GD based on **8.194 high quality SNPs**
 - ↳ GD in reference collection varies from <0.01 to 0.65



THE DURDUS PROJECT



- phenotyping at the participating EOs to define a threshold for genetic distance

Phenotypic distance between pairs grown **side-by-side**

Global note given by the EO-DUS experts after **visual appreciation**, following the scheme:

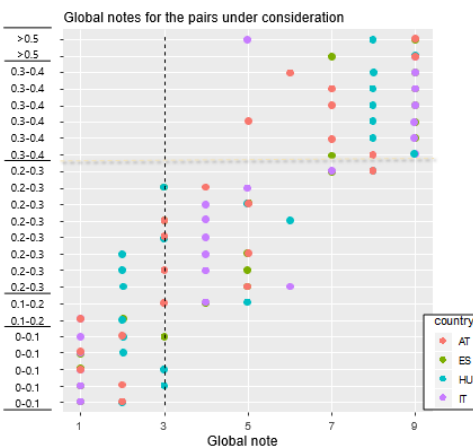
- 1 The two varieties are similar or very close
- 3 The two varieties are distinct but close
- 5 The comparison was useful, but the varieties are clearly distinct
- 7 The comparison should have been avoided because the varieties are very different
- 9 The comparison should have been avoided because the varieties are totally different



THE DURDUS PROJECT



- Definition of a preliminary GD threshold of 0.3



- Year 2 : confirm results of year 1 and propose conclusions.





[End of Annex and of document]