

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

BMT/20/7

**Twentieth Session
Alexandria, United States of America, September 22 to 24, 2021**

Original: English
Date: August 11, 2021

UPDATE ON IMODDUS ACTIVITIES

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 “Update on IMODDUS”, prepared by an expert from the European Union, to be made at the twentieth session of the BMT

[Annex follows]



UPOV-BMT20 (2021)

Update on IMODDUS activities

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IMODDUS Background



- **IMODDUS** = **I**ntegration of **MO**lecular **D**ata into **DUS** testing.
- CPVO BMT working group for the promotion of the use of bio-molecular techniques in DUS testing and variety identification.
- Set up by CPVO Administrative Council in the framework of its R&D Strategy in 2016.
- IMODDUS works as a **think-tank** for the development of strategies supporting the integration of BMT into DUS testing.
- IMODDUS has a **practical approach** which aims at assisting the CPVO in identifying and assessing R&D projects proposals for species where BMT could improve the **quality** and potentially the **cost efficiency** of DUS testing.



IMODDUS Activities



Meetings of the WG

- April 2016
- January 2017
- April 2018
- No meeting in 2019 → INVITE consortium
- Meeting cancelled in 2020 due to Covid19
- **4th meeting on line in Feb 2021**

(Anne Weitz departure, coordination taken over by Cécile Collonnier)

A few highlights :



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R&I in the context of the Green Deal: how are SDG requirements reflected in breeding programs?

All of the 17 SDGs appear in one or more of the six headline ambitions of President von der Leyen's Political Guidelines:



- Many breeding programmes can contribute to the SDGs, depends on the species concerned (Euroseeds, CIOFORA, Plantum)
- SDGs could be considered as evaluation criteria for CPVO R&D projects but not limiting



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BMT approach for soybean DUS proposed by American breeders

ORIGINAL RESEARCH ARTICLE

Crop Science

Crop Breeding & Genetics

Single nucleotide polymorphisms facilitate distinctness-uniformity-stability testing of soybean cultivars for plant variety protection

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- SNP sets and threshold identified based on the use of varieties having previously met DUS eligibility
- More information still needed regarding the concrete way to support the decision making process :
 - How to implement UPOV model 2 in a context where variety descriptions are provided by applicants...
 - How to use genotypic differences between varieties that are not sufficiently distinct phenotypically...

→ additional characteristics (like isozymes) ?
(Cf. study on PRG at NAKT)

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Update on on-going molecular activities

WP3: Genotyping tools to characterize varieties and their performance

WP1: finding new types of markers

T1.4 Exploring the use of epigenetic marks APPLE

variety identification (INRAE)
(follow-up of CPVO project on apple mutants : 7 genotypes in 7 locations in the EU)

and adaptation to environment (IRTA) (3 varieties in 2 locations)

- **T3.1: Genome wide marker sets for distinguishing heterogeneous and open pollinated varieties**
PRG (TEAGASC, INRAE, Agroscope, GEVES, CPVO, BioSS)
- **T3.2: Genome wide marker sets for line and hybrid varieties**
WHEAT, MAIZE, SOYBEAN (UHOH, NIAB, INRAE, GEVES, CPVO)
- **T3.3: Identification of specific markers for DUS related traits and development of genotyping assays using these markers**
POTATO, TOMATO, APPLE (NIAB, INRA, GEVES, CPVO, WU)
(scab, powdery mildew)
- **T3.4: GWAS mapping and genomic prediction of DUS traits**
WHEAT, MAIZE, PRG, SOYBEAN (UHOH, NIAB, INRA, BioSS)
- **T3.5: Improved models for marker-based evaluation of D and U**
WHEAT, MAIZE, PRG, SOYBEAN (UHOH, BioSS)
(genetic U based on genetic variance)

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• **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
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)			

(on going validation of the correlation between GAIA and genetic distances estimated by allelic frequencies)



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)

Presentation of the value molecular distinctness = vmD Concept

Received: 14 December 2019 | Revised: 9 April 2020 | Accepted: 31 May 2020
DOI: 10.1111/ghs.12492

Grass and Forage Sciences | WILEY

REVIEW ARTICLE

A proposal for enhanced EU herbage VCU and DUS testing procedures

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vmD can be used to compare a candidate and its blocking registered variety when there is clear proof that the candidate's end-use value (VCU) is superior to those of the registered variety.

Great interest but limits to evaluate:

- Value criteria may vary between MS.
- Even with similar vmD rules, MS may still produce different DUS decisions due to different environmental conditions.
- Necessary to have statistical means of comparing candidate and registered variety if not tested together...

→ **Approach will be presented to the CPVO Agriculture Experts Group Meeting in 2021** for discussion and potential set up of specific studies (on molecular threshold, vmD rules, legal implications....)



IMODDUS Activities (2/2)



Reviewing of R&D proposals in 2021

- **Harnessing molecular data to support DUS testing in ornamentals: a case-study on Hydrangea**
(variety identification and management of reference collection)
- **Updating DUS resistance tests according to pests' evolution:**
 - Setting up resistance tests to ToBRFV for tomato and pepper
 - Improvement of resistance test melon/*Aphis gossypii*



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On going IMODDUS projects



Tomato

"International harmonisation and validation of a SNP for the management of tomato reference collection"

- Granted co-financing in February 2019.
- coordinated by Naktuinbouw; partners are all EU entrusted examination offices for Tomato: GEVES; COBORU; NÉBIH; INIA; DGAV and CREA.
- The Beijing Sub-Center for DUS testing (CAAS) in China, the Korean Seed & Variety Service from the Republic of Korea and the Center for Seeds and Seedlings, NARO (NCSS) in Japan participate on their own funding.
- Euroseeds is also partner to the project.
- Results expected to be available in 2021.
- If successful → follow-up project for the complete characterization of the collection and the application of the markers to its management following UPOV model (e.g. French bean approach)



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On going IMODDUS projects

Hemp

"Development of a SNP marker set in Cannabis to support DUS testing"



- Context: importing plant material for DUS testing of pharmaceutical varieties is particularly demanding in terms of time and money, which creates reluctance of breeders to submit reference varieties.
- Aim : identifying a SNP marker set for Cannabis to help identify precisely what reference varieties are needed for the testing (French bean model) and limit the request for materials.
- Coordinator : Naktuinbouw (NL), partner : NEBIH (HUN)
- The project started in 2020 and will last 24 months.
- If successful → follow-up project for the complete characterization of the collection and potential application of the markers following UPOV model (e.g. French bean approach)



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On going IMODDUS projects

Oilseed rape

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



- Granted co-financing in March 2019.
- Coordinated by GEVES (FR), Bundessortenamt (GER) as project partner.
- Follow up project of an earlier project which validated a set of SNP markers for KasPAR assay on bulk samples 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.
- All EU entrusted examination offices will be invited to discuss the outcome of the project and prepare a 2nd follow-up to extend the genotyping to the whole collection.
- Expected to be finalized end 2021.



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On going IMODDUS projects



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 October 2021 (18 months extension due to Covid19)

 - Evaluation of differences between 7 Gala mutants (2 trees on 2 years) :
 1. Standardized phenotyping of fruit color on the basis of hyperspectral images recorded automatically from conveyer machines: intensity, surface, stripes and metabolites (anthoc.)
 2. Whole genome sequencing to detect genetic differences (SNPs, TEs, indels, copy number variations)
 3. Epigenetic differences assessed genome-wide at the DNA methylation level (DMRs)
 4. Transcriptomic data (RNA sequencing on apple skin) to identify differentially expressed genes
- identification of genes potentially involved in the phenotypic changes of mutants.



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On going IMODDUS projects



Durum wheat (DurdusTools)

*"Integration of molecular data into DUS testing in Durum wheat - development of a **common online molecular database and a genetic distance calculation tool**"*

- Aim: Efficient management of reference collections
- Follow-up project of the Durdus project which allowed the definition of a genotyping protocol (microarray), the genotyping of a large part of the collection and a preliminary genetic threshold.
- Granted co-financing in Oct 2020
- Coordinator : Austrian Agency for Health and Food Safety (AGES)
- Project partners are INIA (ES), CREA-SCS (IT), NÉBIH (HU)
- Duration : 24 months (Jan 2021 – Jan 2023)



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Thanks for your attention !



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[End of Annex and of document]