

Technical Working Party for Agricultural Crops**TWA/51/4 Add.****Fifty-First Session
Cambridge, United Kingdom, May 23 to 27, 2022****Original:** English
Date: May 10, 2022

**ADDENDUM TO
PRESENTATION ON THE USE OF MOLECULAR TECHNIQUES IN DUS EXAMINATION***Document prepared by an expert from France**Disclaimer: this document does not represent UPOV policies or guidance*

The annex to this document contains a copy of a presentation “Developing a strategy to apply SNP molecular markers in the framework of winter oilseed rape DUS testing”, to be made by an expert from France, at the fifty-first session of the TWA.

[Annex follows]

Project Nr. 7515058 - 2019/2021

**DEVELOPING A STRATEGY TO APPLY SNP MOLECULAR MARKERS
IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING**

Co-funded by the Community Plant Variety Office (CPVO) Research and Development Section
June 2019 – September 2021

TWA 2022



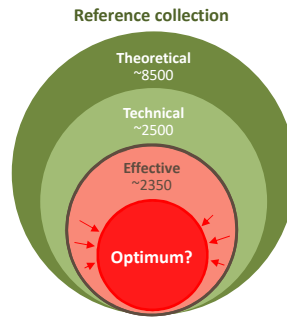
Project structure

- 1) Context and objectives of the project
- 2) Genotyping the reference collection
- 3) Presentation of the new method
- 4) Feasibility study (ring test)
- 5) Conclusions, perspectives

Using SNP markers in the framework of winter OSR DUS testing

Context of the project

- In WOSR, hybrids represent the majority of the **~90 applications** submitted each year for plant breeder rights and/or for listing in national catalogues since 2010
- When parental lines are included, this represents **almost 200 varieties that must undergo DUS testing each year**
 - **Rapid increase in size of field trials**
 - **Difficulties to manage the reference collection**
- In oilseed rape, the expression of phenotypic characters is also very sensitive to changes in environmental conditions
 - **The entire collection must be redescribed each year**



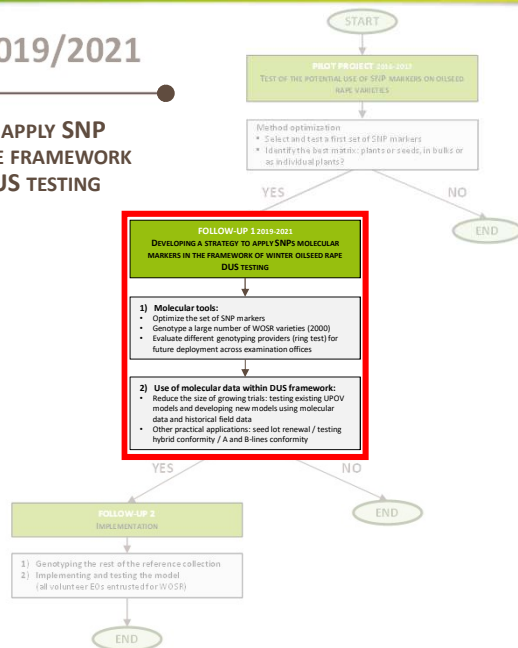
→ **How to optimize the size of the effective collection in order to reduce the size of growing trials in the first DUS cycle?**

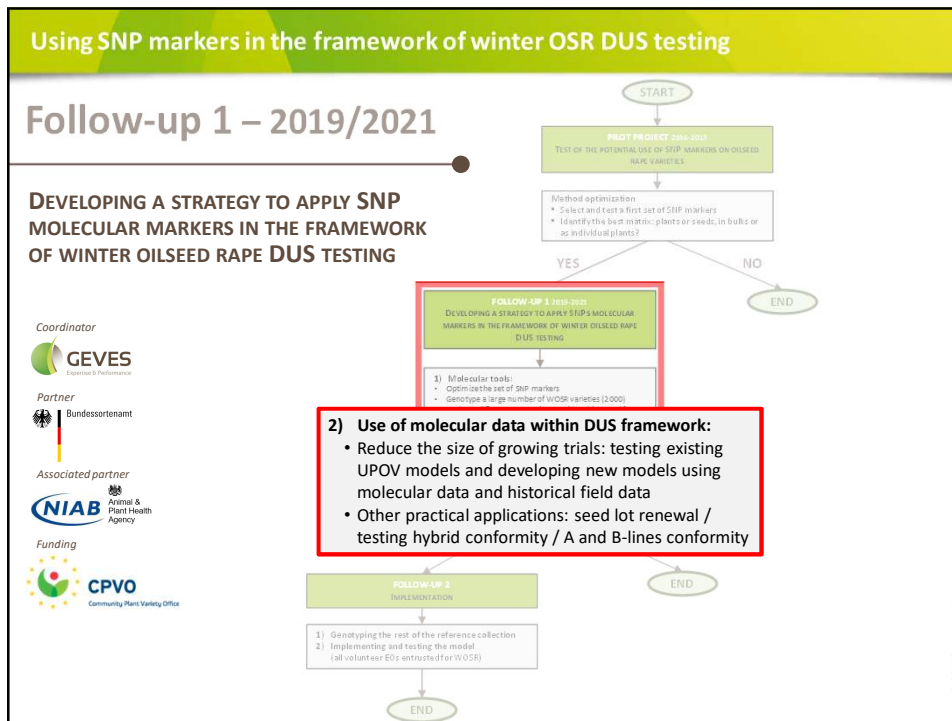
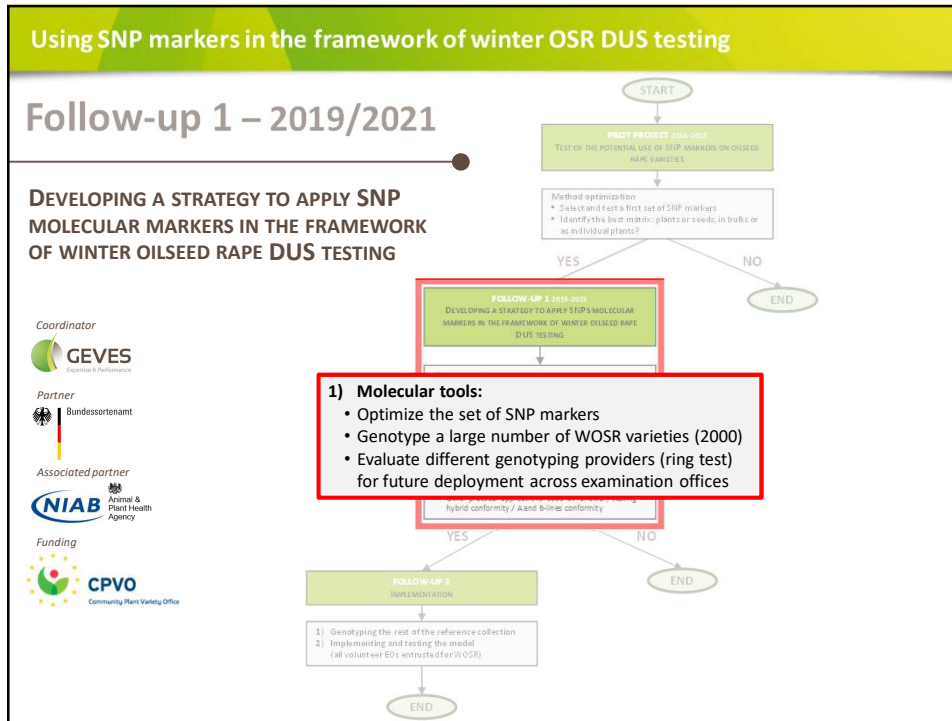
Using SNP markers in the framework of winter OSR DUS testing

Follow-up 1 – 2019/2021

DEVELOPING A STRATEGY TO APPLY SNP MOLECULAR MARKERS IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING

- Coordinator**
- GEVES**
Expertise & Performance
- Partner**
- Bundessortenamt**
- Associated partner**
- NIAB**
Animal & Plant Health Agency
- Funding**
- CPVO**
Community Plant Variety Office





Project structure

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Using SNP markers in the framework of winter OSR DUS testing

Milestone 1

Selection of varieties

- **Consensus list** between French and German collections (1531 varieties in common), *incl.*

- All CPVR varieties (737 varieties), *and*
- All varieties listed in countries entrusted by CPVO for WOSR (CZ, DE, DK, ES, FR, GB*, PL, SK)
- Priority given to varieties (lines and hybrids + parental lines) for which morphological data were available in both countries
 - **638 restored hybrids** (and their parental lines), *incl.*
23 semi-dwarf, 32 IMI, 20 high oleic/low linolenic acid (HOLL), 3 high erucic acid
 - **22 three-way hybrids** (and their parental lines)
 - **18 sterile hybrids** (and their parental lines)
 - **1290 lines**, *incl.*
35 dwarf, 20 HOLL, 13 high erucic acid, 8 IMI (imidazolinone herbicide tolerant)

Additionally:

- Candidate varieties that failed DUS tests (distinctness, uniformity...)
- Spring OSR and forage varieties (as outgroups)

1968 winter OSR varieties
incl. 23 varieties from pre-project

+

21 'problematic' varieties
6 with problems of distinctness
15 with problems of uniformity

+

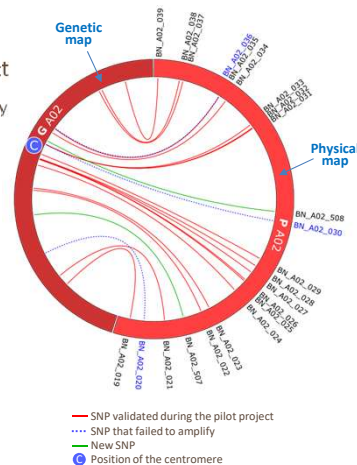
41 spring OSR varieties
= 20% of the SOSR reference collection
+ 1 forage variety

Using SNP markers in the framework of winter OSR DUS testing

Milestone 1

Optimization of the SNP set

- First set of **452 SNPs** validated during the pilot project
 - SNPs selected from the 60K Illumina Infinium™ array developed by Clarke *et al.* (2016) using genotyping data from INRAE (French National Research Institute for Agriculture & Environment)
 - ✓ **Non-multilocus** (sub-genome specific)
 - ✓ **High PIC** (Polymorphism Information Content)
 - ✓ **Homogenous genome coverage**
- **8 monomorphic SNPs** from pre-project for the 23 varieties tested
- New set of **48 SNPs** selected to replace markers that failed to amplify or showed little/no polymorphism
 - ✓ **New primer design based on reference genome**
 - ✓ **Same selection criteria**
 - ✓ **Position on physical and genetic map of the chromosomes**



= 508 SNPs in total

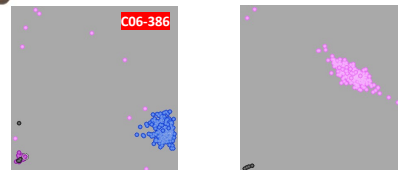
Clarke WE *et al.* (2016) A high-density SNP genotyping array for *Brassica napus* and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. *Theor. Appl. Genet.* **229**, 1887–1899.

Using SNP markers in the framework of winter OSR DUS testing

Milestone 1

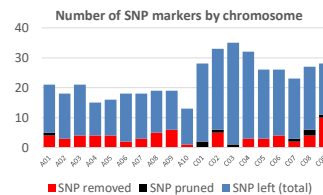
Optimization of the SNP set

- Filtering criteria:
 - ✓ **Monomorphic or failing to converge -77 SNPs**
 - ✓ **Percentage of missing data (< 15%) -5 SNPs**
 - ✓ **Hybrid conformity (< 30% mismatch) -42 SNPs**
 - ✓ **Percentage of heterozygosity (parental lines) (< 30%) -3 SNPs**
 - ✓ **Controls, replicates and pseudo-replicates (< 30%) -12 SNPs**
 - ✓ **LD-pruning (linkage disequilibrium) ($r^2 > 0.95$) -9 SNPs**



- Final decision whether to keep or discard problematic SNP markers based on all criteria taken together, taking care that it does not result in significant gaps in genome coverage

= 360 SNPs left



Project structure

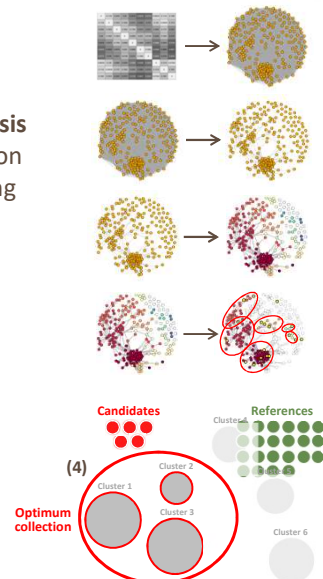
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Towards a network-based approach

- The proposed approach is based on **network analysis** to **detect groups of closely related varieties** based on their relative position within a network representing genetic relationships between candidate and reference varieties

- 1) Compute **pairwise genetic distances** between candidate and reference varieties
- 2) Transform the distance matrix into a **network**
- 3) Simplify the network by pruning all links corresponding to a genetic distance above a predefined **threshold** (expert notes)
- 4) Identify **clusters of varieties** within the collection using a **community detection algorithm**
- 5) **Select only groups that include candidates** (= **optimum collection**)



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Using SNP markers in the framework of winter OSR DUS testing

Defining a genetic threshold

Not an expert note system
as described in TGP/15/3

● Expert notes

The scale is based on a global appreciation of the degree of morphological similarity/difference between varieties *a posteriori*, using recordings of their individual characteristics during the second year of evaluation (GAIA system)

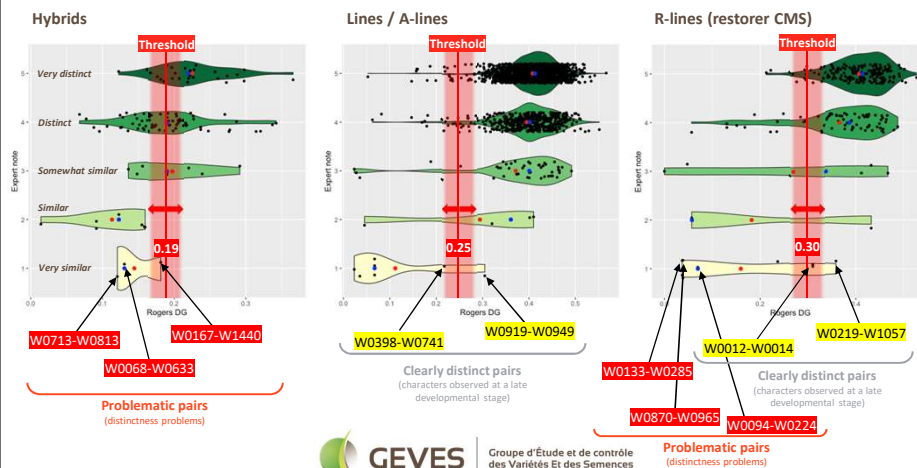
Varieties declared distinct at leaf stage before the first commission are considered <u>very distinct</u>	Note = 5
Varieties declared distinct during at leaf stage (first commission) are considered <u>distinct</u>	Note = 4
Varieties declared distinct during at leaf stage (2nd commission) are considered <u>somewhat similar</u>	Note = 3
Varieties declared distinct at flowering stage are considered <u>similar</u>	Note = 2
Varieties not declared distinct after the flowering stage are considered <u>very similar</u>	Note = 1

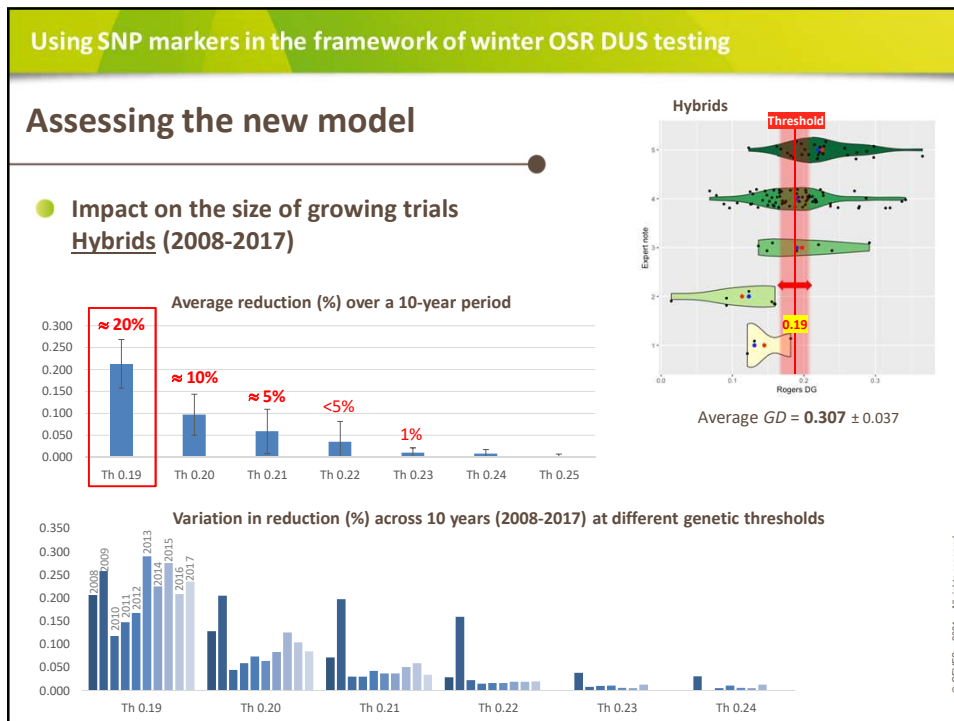
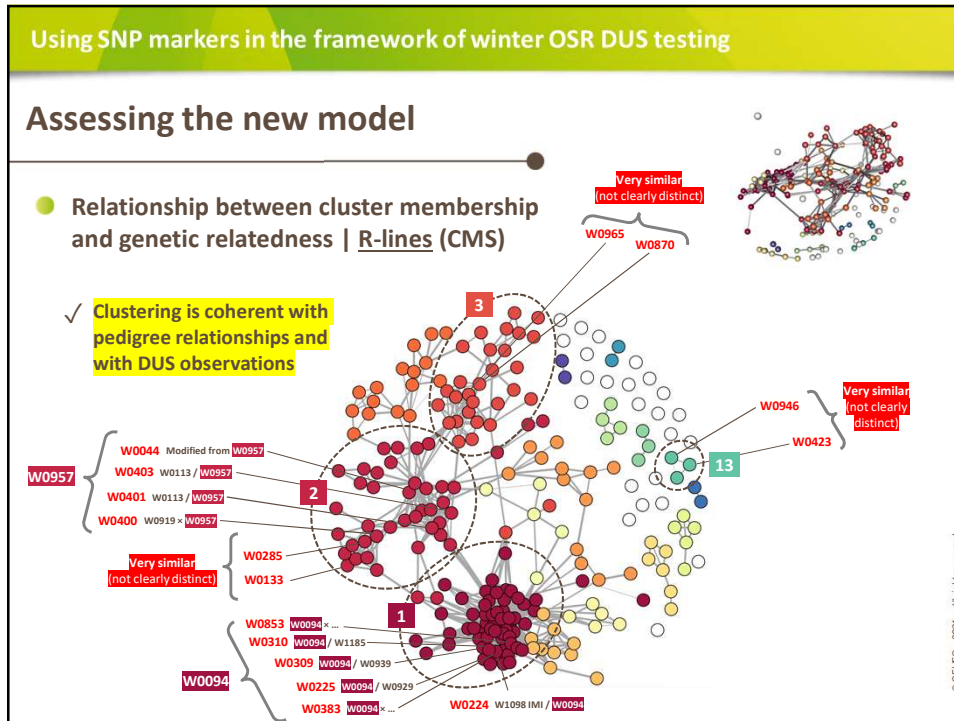
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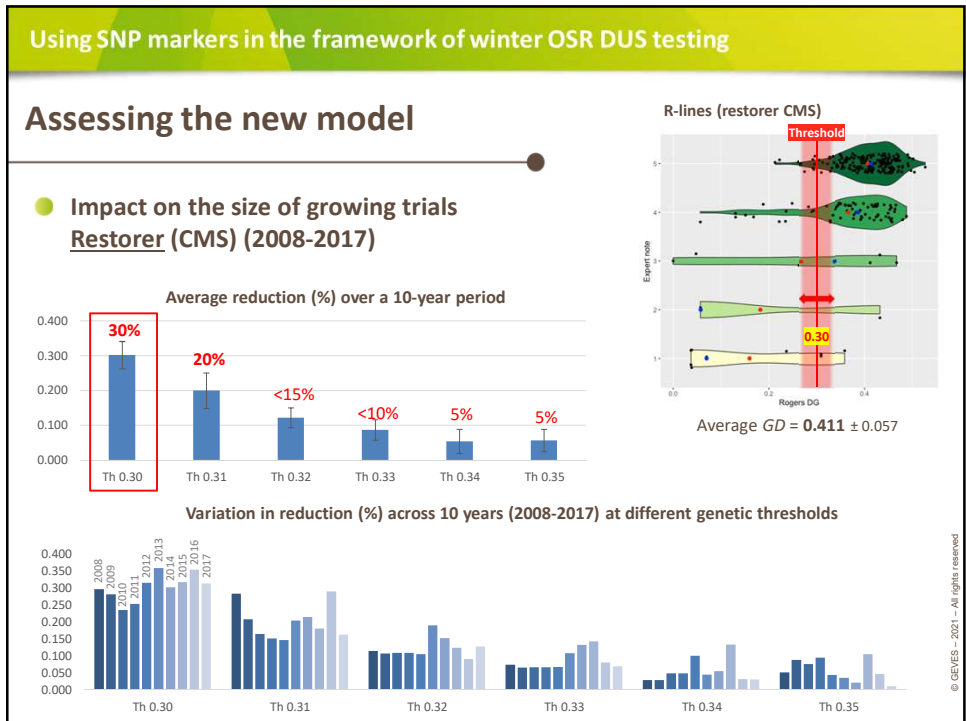
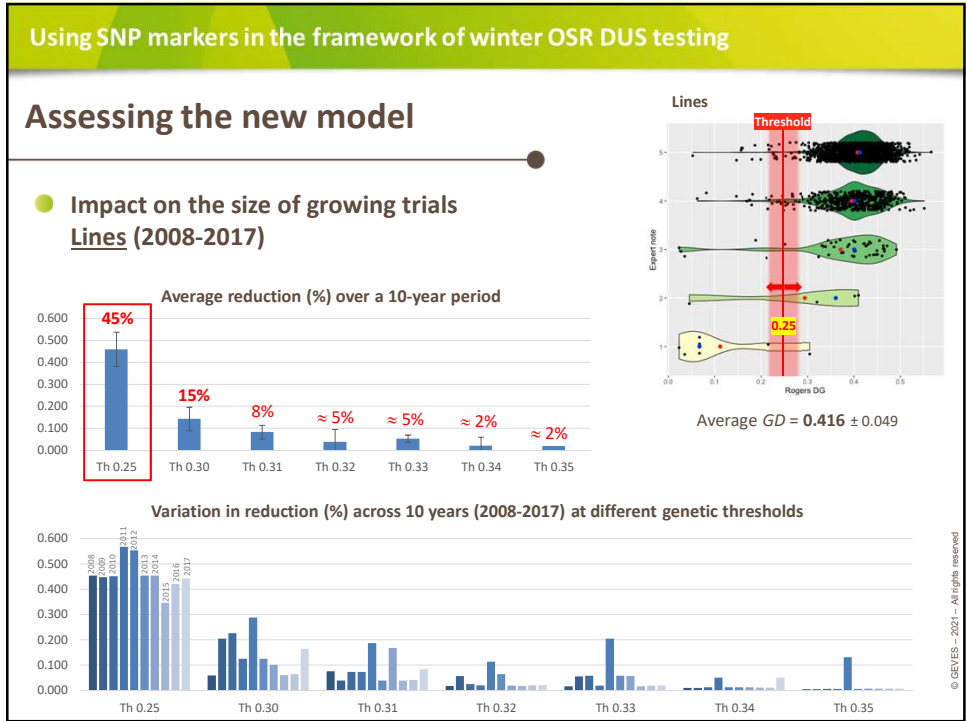
Using SNP markers in the framework of winter OSR DUS testing

Defining a genetic threshold

● Plotting Rogers G_D vs. expert notes by category of variety







Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Testing the model using 10 years of historical field data (2008-2017)

→ **GAIA**: Will excluding part of the reference collection lead to **failing to detect distinction problems** by eliminating important varieties and overlooking decisive comparisons for establishing distinctness?

→ We compared clustering results with pairs of varieties that GAIA 'flagged' as important to compare side-by-side in the field

→ **COY-D**: Will reducing the size of the effective reference collection **impact COY-D calculations and change DUS decisions?**

→ We compared results of a COY-D analysis with all varieties vs. COY-D analysis with the reduced dataset

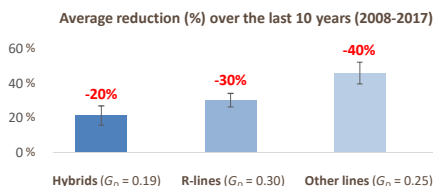
✓ Comparisons with historical data did not reveal any instance where the method excluded varieties that experts would have wanted to compare

Using SNP markers in the framework of winter OSR DUS testing

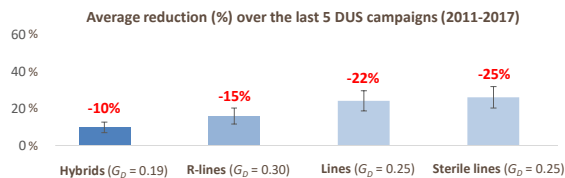
Assessing the new model

- Comparison of reductions in the GAIA / COY-D statistical framework

- **GAIA**



- **COY-D**



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Milestone 3

Ring tests: Evaluation of external providers

- The project evaluated the possibility to outsource genotyping to external providers in order to test the **transferability of the markers** set, **compare results between different technologies** (robustness, **reproducibility**), and evaluate the **reliability, flexibility** and **delays** of outsourcing molecular analyses
- Two providers:
 - **Provider A** (KASPar, **360 SNPs**) – sent January 2021
 - **Provider B** (Genotyping-by-Sequencing [NGS], **443 SNPs**) – sent February 2021
- 72 varieties (95 samples in total):
 - **23 samples** (varieties from pilot project) repeated once (46 in total)
 - **49 samples** (simple hybrids, three-way hybrids, sterile hybrids + parental lines) (many varieties for which we have also replicates and/or pseudo-replicates)
 - 5 varieties with high % of missing data (>20%)
 - 5 varieties with high % of heterozygosity (>50%)

Problematic varieties

Milestone 3

● Ring test (Feasibility study)

- ✓ Molecular analyses were successfully outsourced to external providers using distinct technologies
- ✓ Data were congruent (90-95%) between platforms
- ✗ Delays are long (~2 months) but can be negotiated/anticipated in a context of routine analyses
- ✗ One SNP could not be converted to the NGS-based technology
- ✗ Some SNPs failed to amplify after conversion
- ✗ Clear guidelines/rules must be set for homogenizing calling of genotypes with the KASP assay


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Conclusions

- Project deliverables:
 - ✓ An optimized set of 360 SNP markers for WOSR collection
 - ✓ A molecular reference library representing 80% of the reference collection (~2000 varieties)
 - ✓ A new model of molecular data integration in DUS testing for WOSR
 - ✓ Potential use for other applications (seed lot renewal / testing hybrid conformity / A and B-lines conformity)
- The proposed network-based optimization approach:
 - ✓ yields biologically meaningful clusters, coherent with pedigree relationships between varieties
 - ✓ helps reducing significantly (≈ 20-45% in theory) the size of 1st year trials
 - ✓ is compatible with all DUS systems (GAIA, COY-D)
 - ✓ is fast and easy to implement:
 - requires R and Python (open source, cross-platform)
 - minimal assumptions / parameters
 - ✗ **but requires large collections to perform well (min. 100-200 varieties: 'critical mass')**



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Perspectives

IMPLEMENTING THE MODEL (FOLLOW-UP 2)

Diffusion of the final report: End of year
Discussion between EOs: 1st semester 2022

1) Genotyping the rest of the reference collection ← About 20% of the reference collection left to genotype

2) Implementing and testing the model (all volunteer EOs entrusted for WOSR) ← Calibrating genetic thresholds by including more pairs with distinction problems

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Thank you!

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