UPOV

International Union for the Protection of New Varieties of Plants

E

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

BMT/20/9 Add.

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

Original: English
Date: September 23, 2021

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

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 on "Developing a strategy to apply SNP molecular markers in the framework of winter oilseed rape DUS testing", prepared by an expert from France, to be made at the twentieth session of the BMT.

[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

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

BMT/20, Alexandria, United States of America, September 22 to 24, 2021

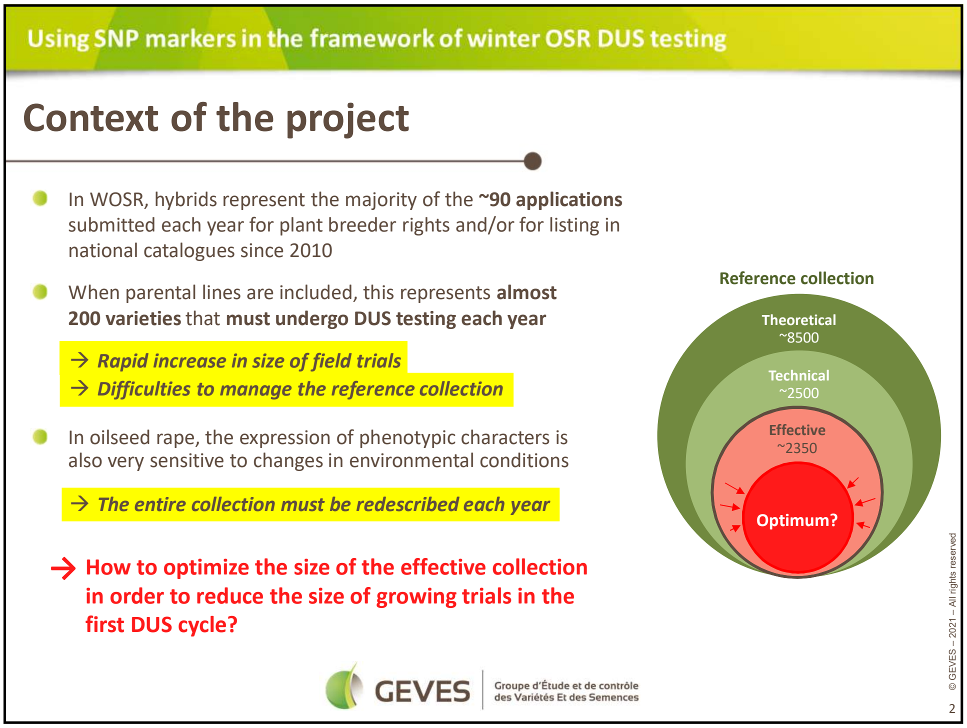
Document prepared by French experts (GEVES)

Bundessortenamt

NIAB Animal & Plant Health Agency

GEVES Expertise & Performance
Groupe d'Étude et de contrôle des Variétés Et des Semences

CPVO Community Plant Variety Office



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?**

Reference collection

Theoretical
~8500

Technical
~2500

Effective
~2350

Optimum?

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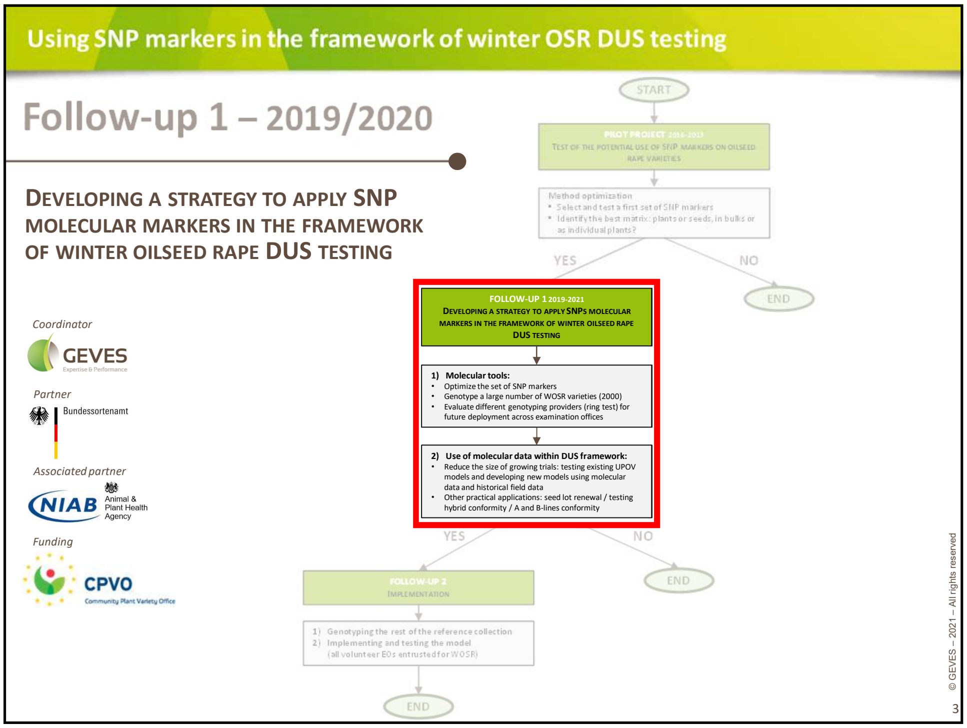

Using SNP markers in the framework of winter OSR DUS testing

Follow-up 1 – 2019/2020

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

START

PILOT PROJECT 2016-2017
TEST OF THE POTENTIAL USE OF SNP MARKERS ON OILSEED RAPE VARIETIES

Method optimization
- Select and test a first set of SNP markers
- Identify the best matrix: plants or seeds, in bulks or as individual plants?

YES

NO

END

FOLLOW-UP 1 2019-2021
DEVELOPING A STRATEGY TO APPLY SNPS MOLECULAR MARKERS IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING

1) Molecular tools:
- Optimize the set of SNP markers
- Genotype a large number of WOSR varieties (2000)
- Evaluate different genotyping providers (ring test) for future deployment across examination offices

2) Use of molecular data within DUS framework:
- Reduce the size of growing trials: testing existing UPOV models and developing new models using molecular data and historical field data
- Other practical applications: seed lot renewal / testing hybrid conformity / A and B-lines conformity

YES

NO

END

FOLLOW-UP 2
IMPLEMENTATION

1) Genotyping the rest of the reference collection
2) Implementing and testing the model (all volunteer EOs entrusted for WOSR)

END

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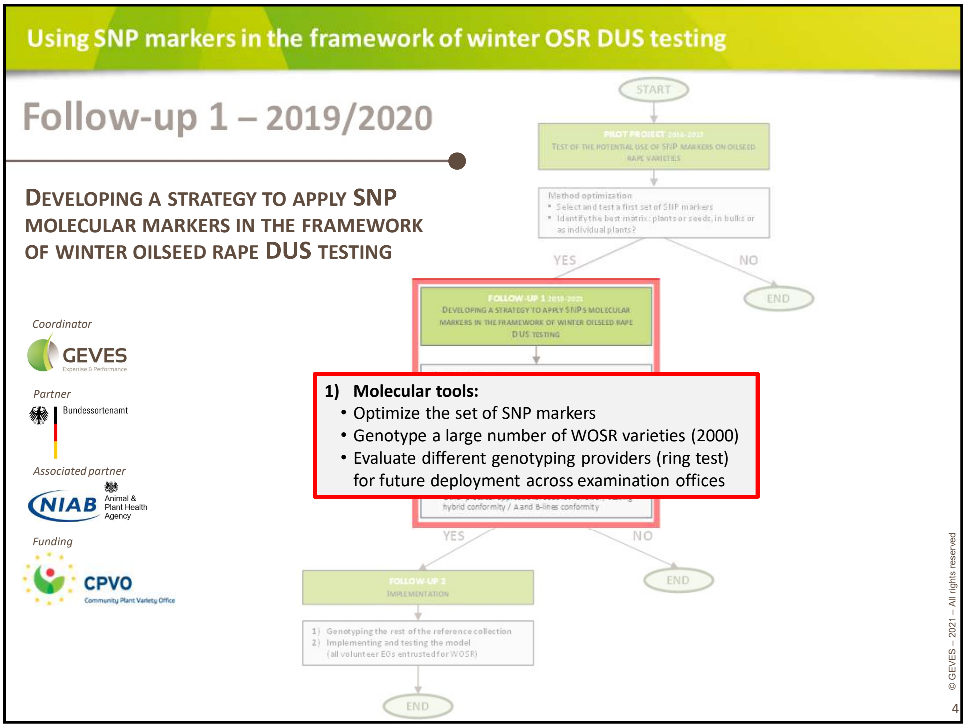

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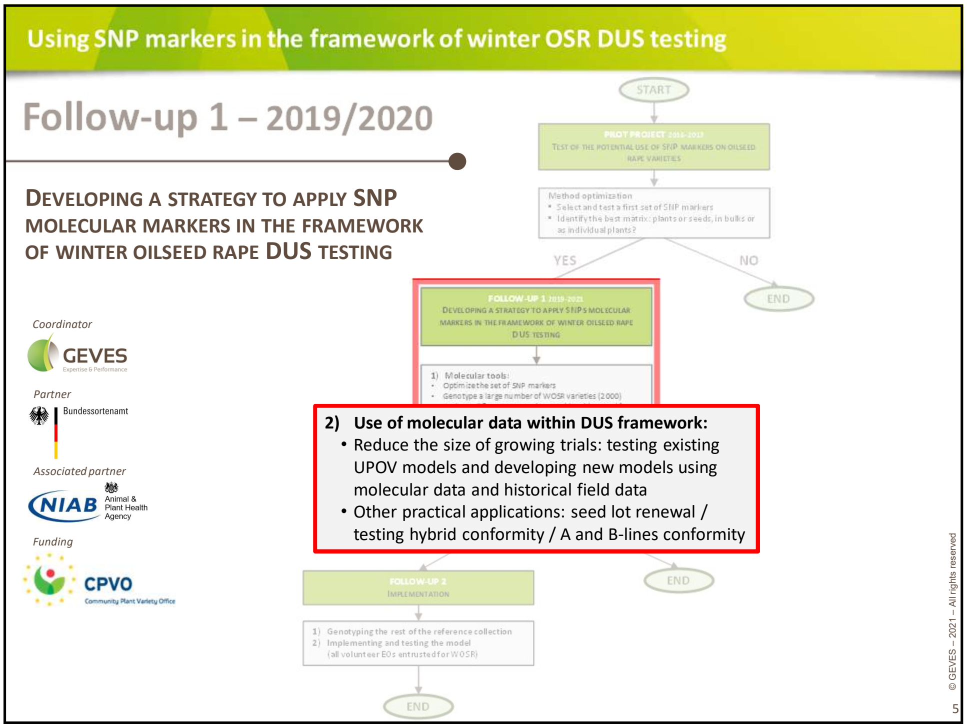

Using SNP markers in the framework of winter OSR DUS testing

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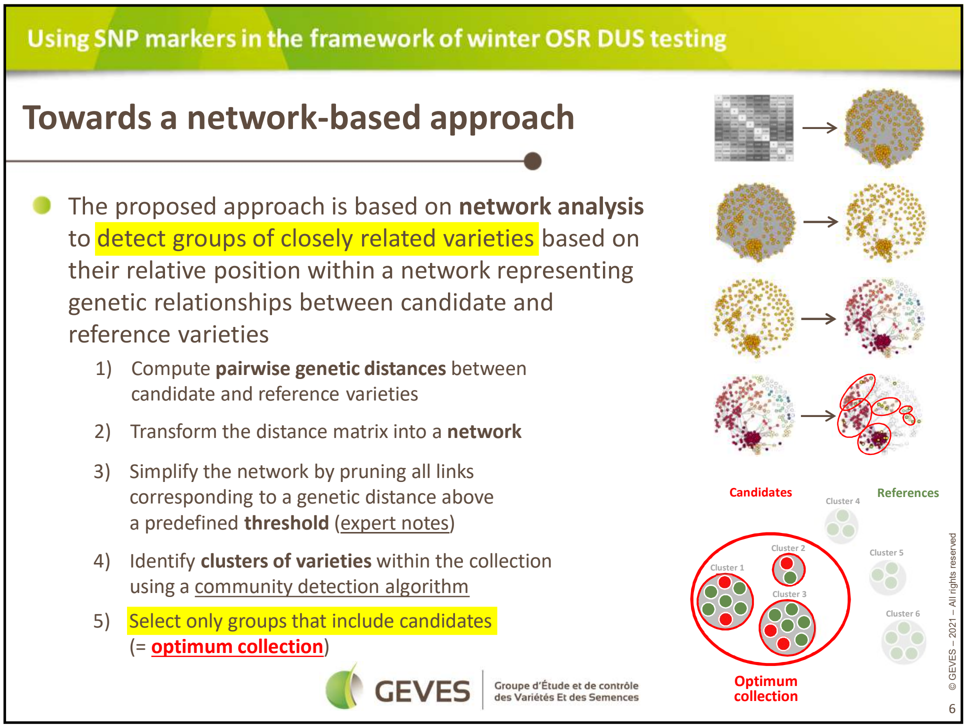

Using SNP markers in the framework of winter OSR DUS testing

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

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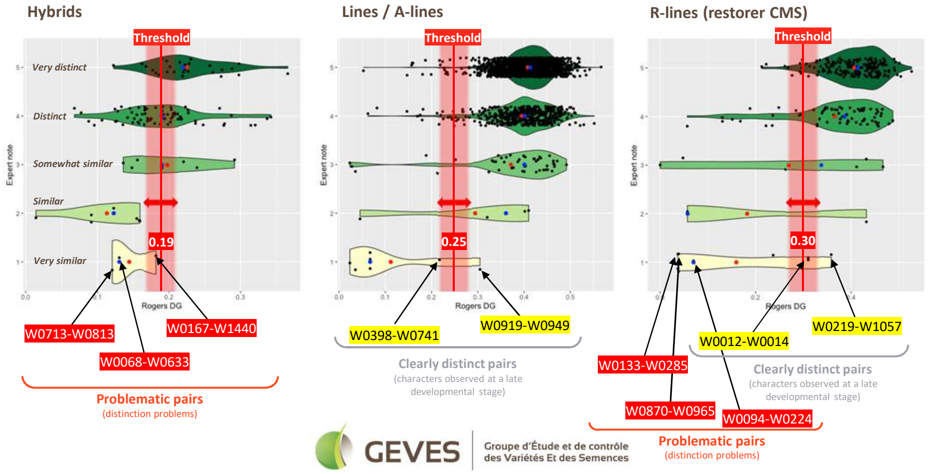
Using SNP markers in the framework of winter OSR DUS testing

Defining a genetic threshold

- **Plotting Rogers G_D *vs.* expert notes by category of variety**



Problematic pairs
(distinction problems)

Clearly distinct pairs
(characters observed at a late developmental stage)

Clearly distinct pairs
(characters observed at a late developmental stage)

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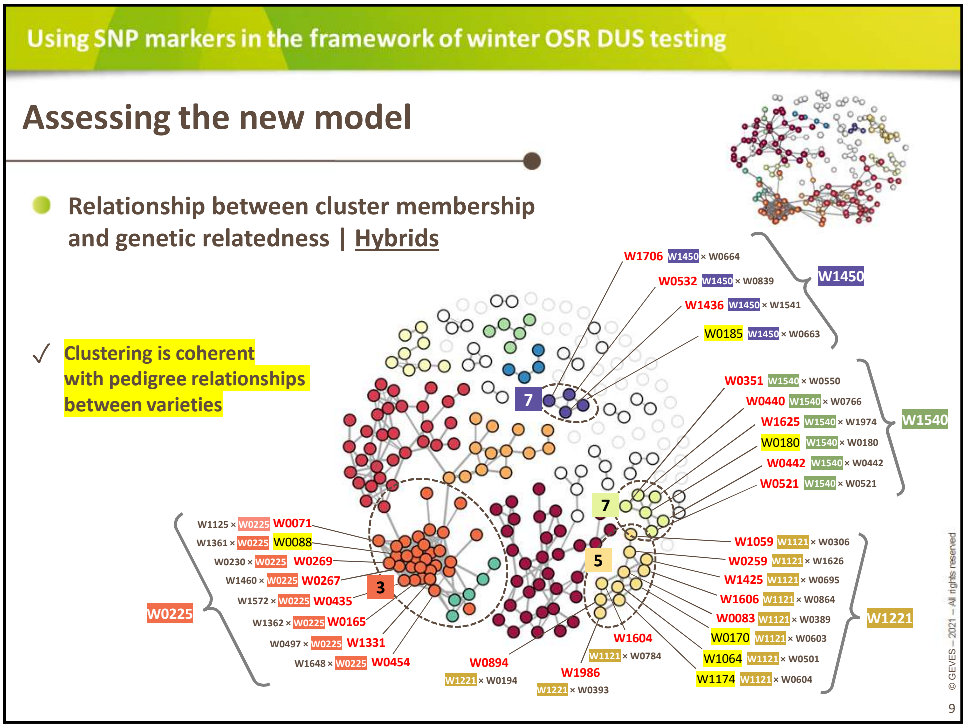

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Relationship between cluster membership and genetic relatedness | <u>Hybrids</u>

✓ Clustering is coherent with pedigree relationships between varieties

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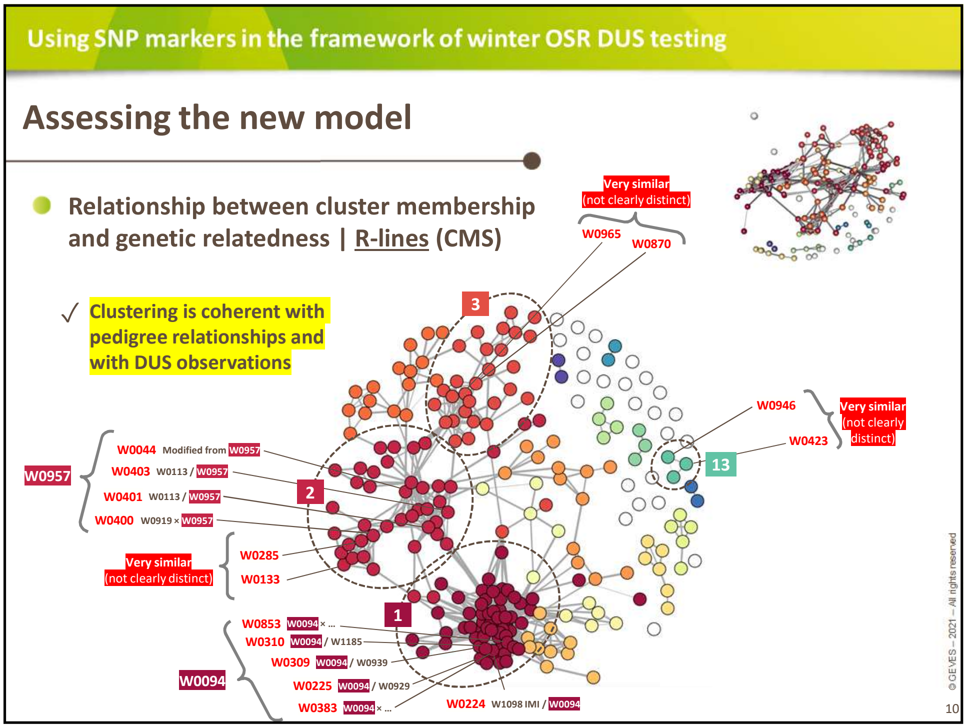

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Relationship between cluster membership and genetic relatedness | <u>R-lines</u> (CMS)

✓ Clustering is coherent with pedigree relationships and with DUS observations

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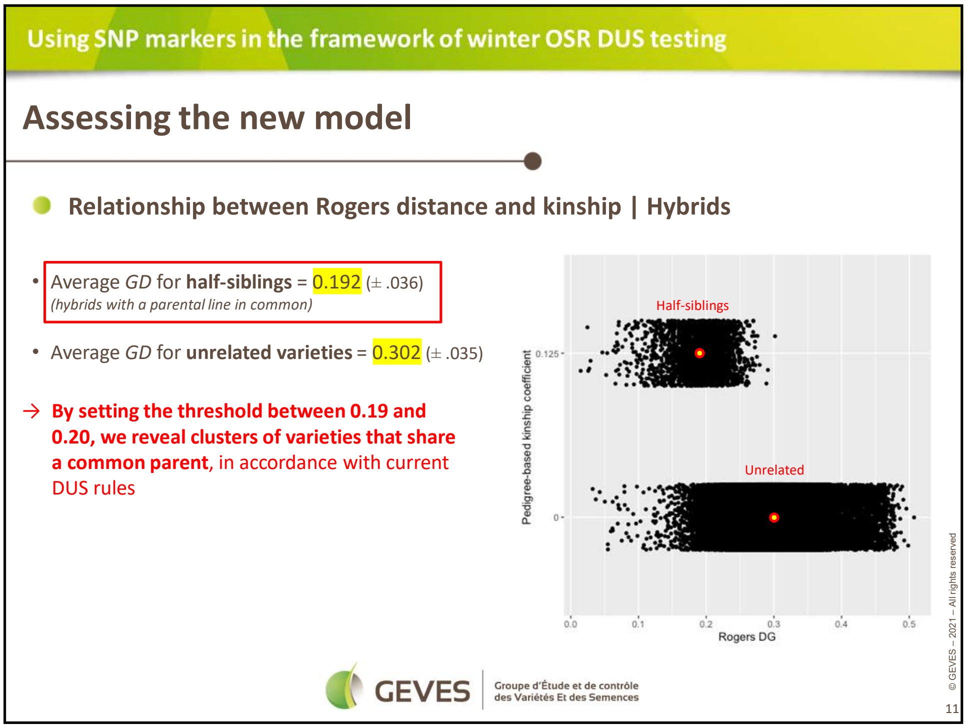

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

Relationship between Rogers distance and kinship | Hybrids

- Average *GD* for **half-siblings** = 0.192 (± .036)
  (hybrids with a parental line in common)
- Average *GD* for **unrelated varieties** = 0.302 (± .035)

→ **By setting the threshold between 0.19 and 0.20, we reveal clusters of varieties that share a common parent**, in accordance with current DUS rules

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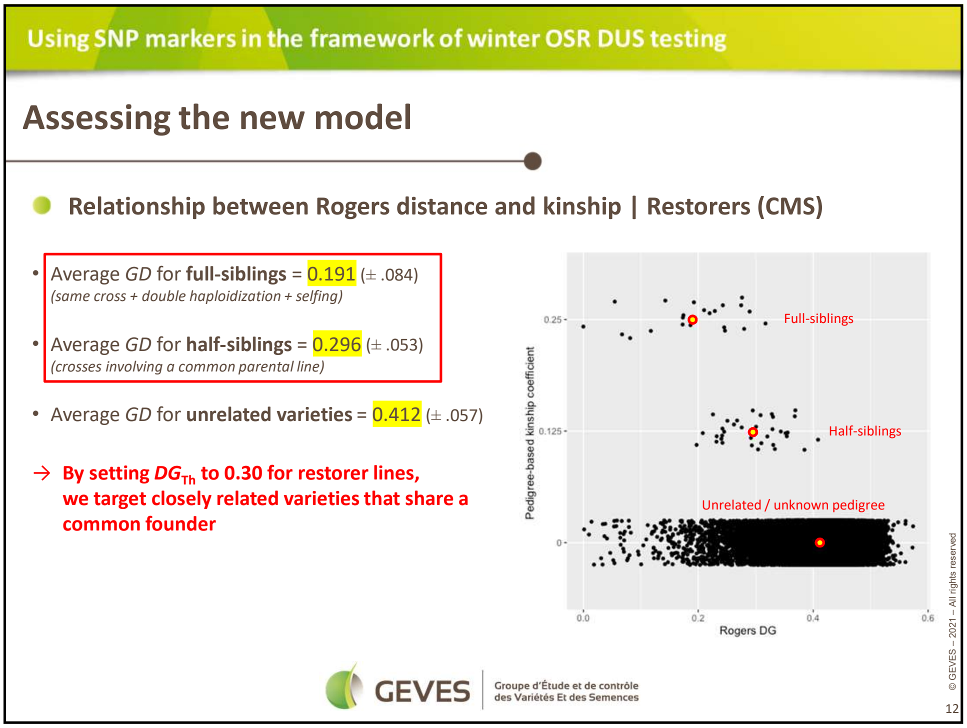

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

Relationship between Rogers distance and kinship | Restorers (CMS)

- Average *GD* for **full-siblings** = 0.191 (± .084)
  (same cross + double haploidization + selfing)
- Average *GD* for **half-siblings** = 0.296 (± .053)
  (crosses involving a common parental line)
- Average *GD* for **unrelated varieties** = 0.412 (± .057)

→ **By setting DG_{Th} to 0.30 for restorer lines, we target closely related varieties that share a common founder**

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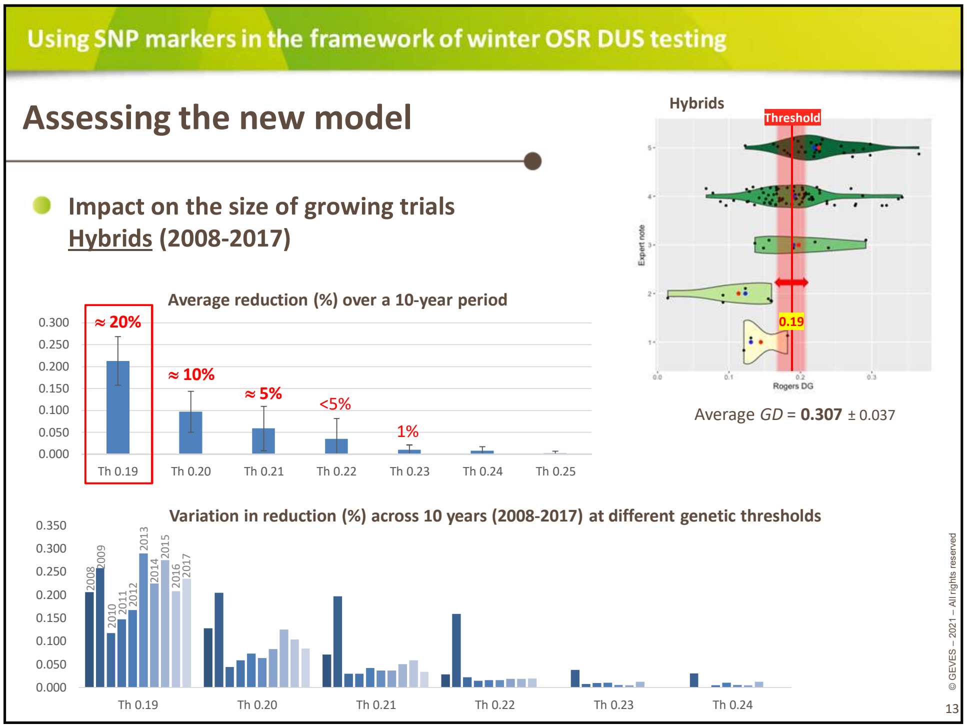

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Impact on the size of growing trials
  Hybrids (2008-2017)

Average reduction (%) over a 10-year period

Variation in reduction (%) across 10 years (2008-2017) at different genetic thresholds

Average *GD* = **0.307** ± 0.037

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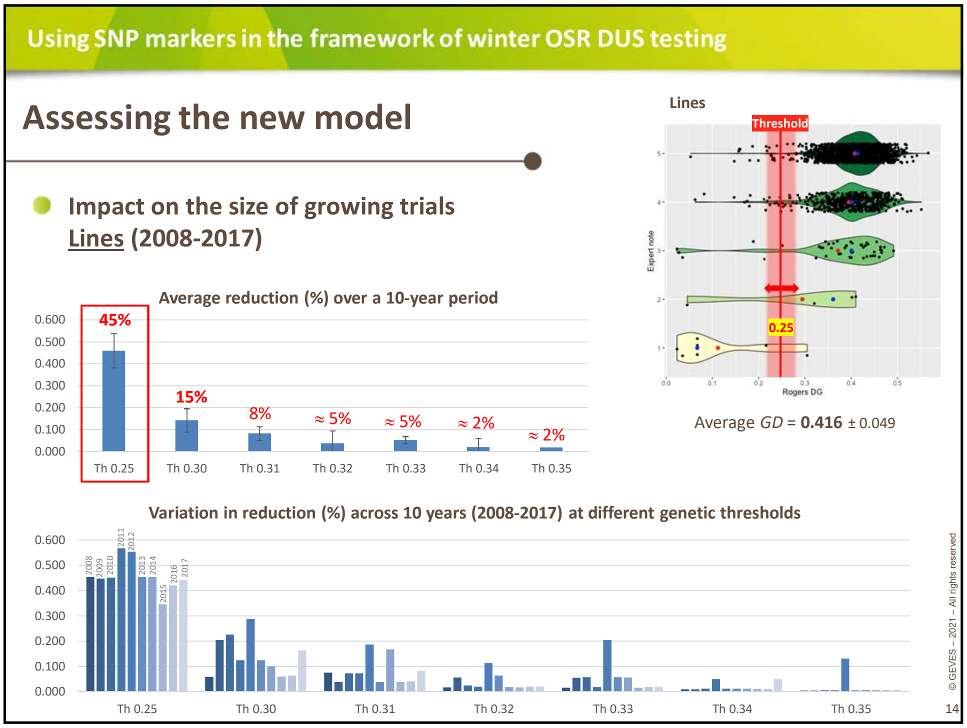

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Impact on the size of growing trials
  Lines (2008-2017)

Average reduction (%) over a 10-year period

Variation in reduction (%) across 10 years (2008-2017) at different genetic thresholds

Average *GD* = **0.416** ± 0.049

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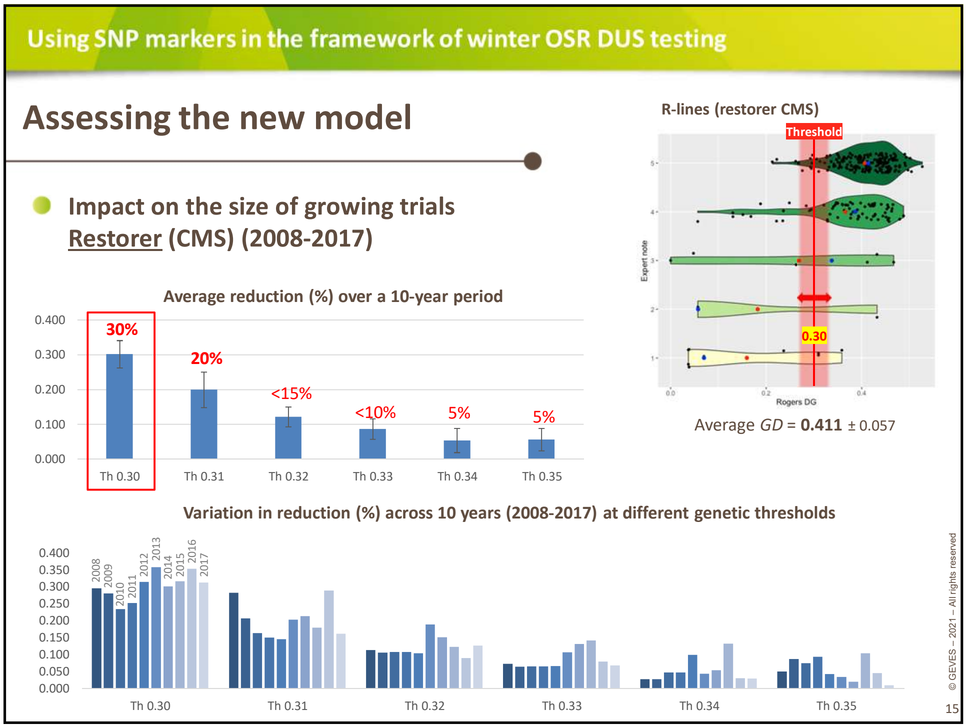

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Impact on the size of growing trials
  Restorer (CMS) (2008-2017)

Average reduction (%) over a 10-year period

R-lines (restorer CMS)

Average *GD* = **0.411** ± 0.057

Variation in reduction (%) across 10 years (2008-2017) at different genetic thresholds

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Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

- Testing the model using 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**

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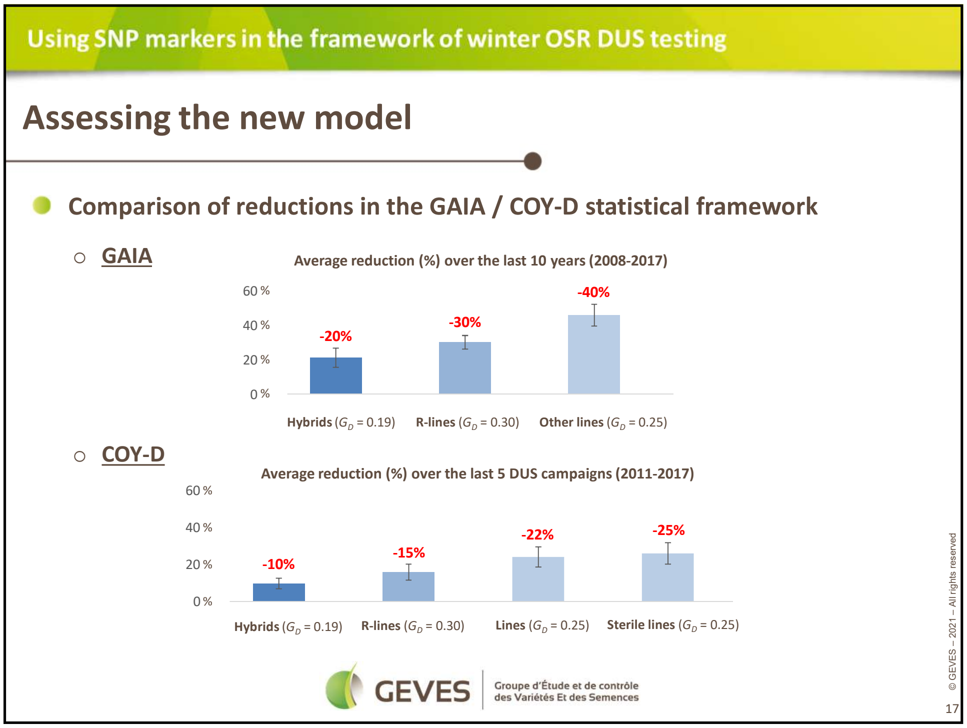

Using SNP markers in the framework of winter OSR DUS testing

Assessing the new model

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

Average reduction (%) over the last 10 years (2008-2017)

  - COY-D

Average reduction (%) over the last 5 DUS campaigns (2011-2017)

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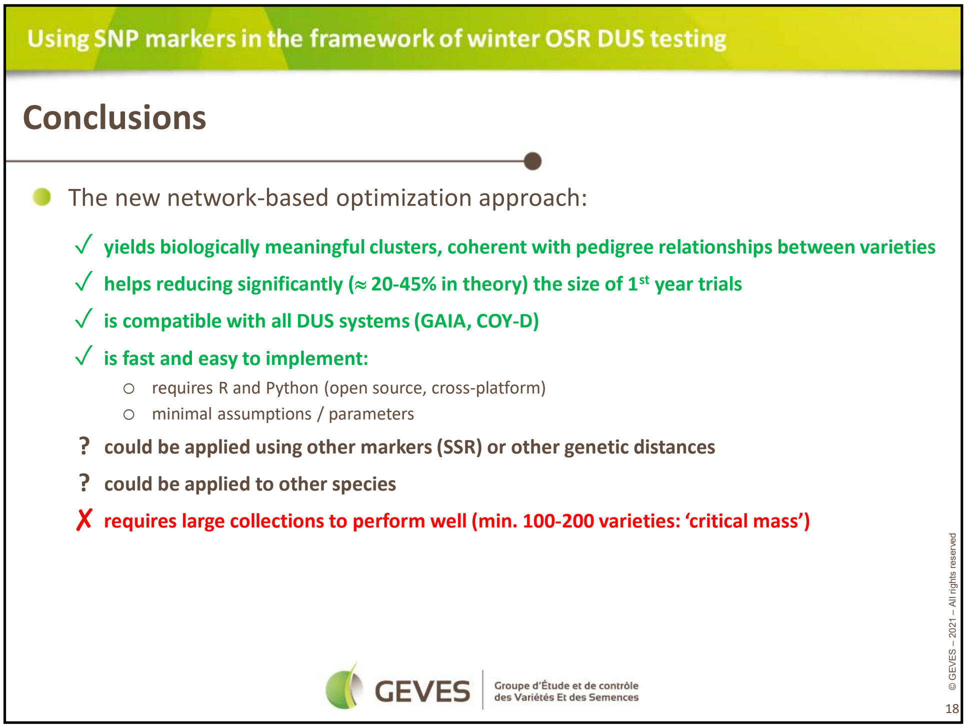

Using SNP markers in the framework of winter OSR DUS testing

Conclusions

- The new 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
  - ? **could be applied using other markers (SSR) or other genetic distances**
  - ? **could be applied to other species**
  - ✗ **requires large collections to perform well (min. 100-200 varieties: 'critical mass')**

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Thank you!

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[End of Annex and of document]