

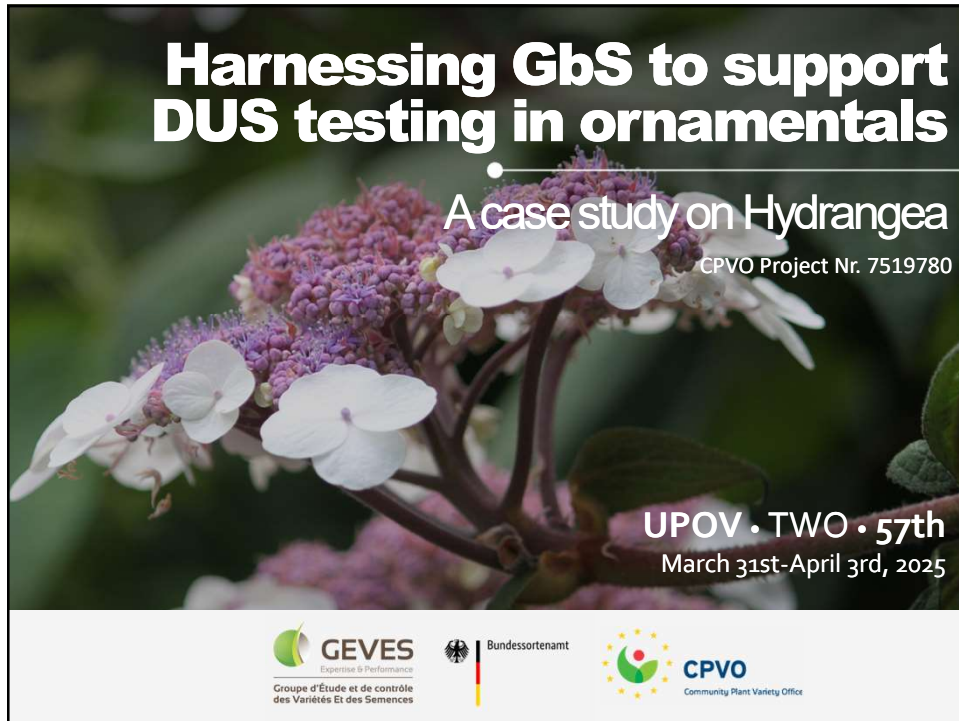
**Technical Working Party for Ornamental Plants and Forest Trees****TWO/57/5 Add.****Fifty-Seventh Session****Roelofarendsveen, Kingdom of the Netherlands,  
March 31 to April 3, 2025****Original:** English**Date:** April 1, 2025

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**ADDENDUM TO:  
HARNESSING MOLECULAR DATA TO SUPPORT DUS TESTING IN ORNAMENTALS: A CASE-STUDY  
ON *HYDRANGEA****Document prepared by experts from France**Disclaimer: this document does not represent UPOV policies or guidance*

The annex to this document contains a copy of a presentation “Harnessing GbS to support DUS testing in ornamentals: a case-study on *Hydrangea*”, made by an expert from France, at the fifty-seventh session of the TWO.

[Annex follows]



# Harnessing GbS to support DUS testing in ornamentals

A case study on Hydrangea

CPVO Project Nr. 7519780

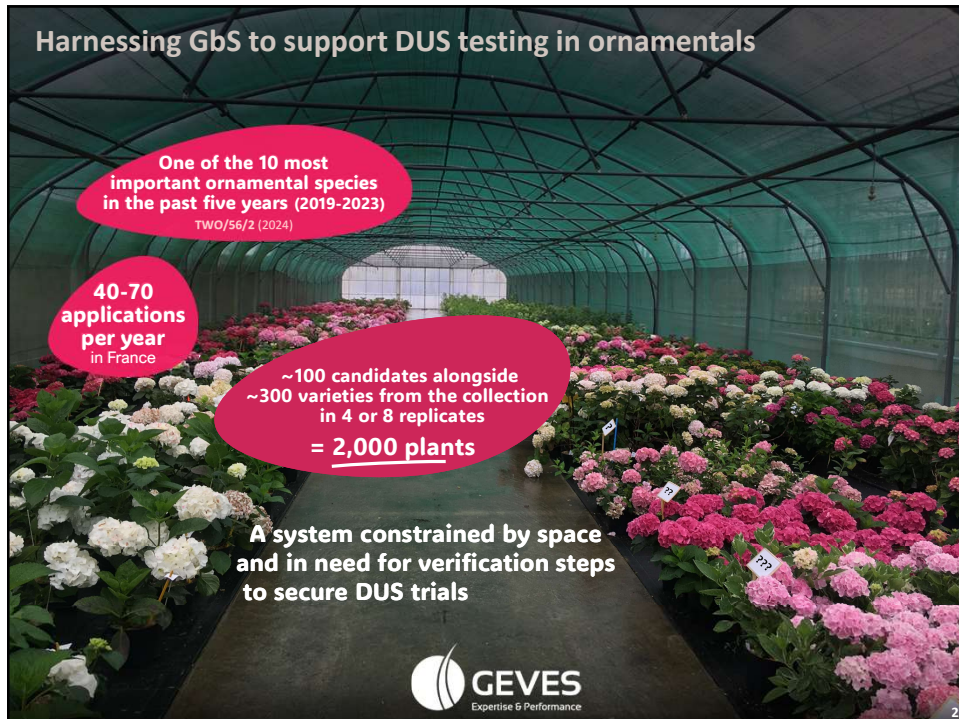
UPOV • TWO • 57th  
March 31st-April 3rd, 2025

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**Bundessortenamt**

**CPVO**  
Community Plant Variety Office

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## Harnessing GbS to support DUS testing in ornamentals

One of the 10 most important ornamental species in the past five years (2019-2023)  
TWO/56/2 (2024)

40-70 applications per year in France

~100 candidates alongside  
~300 varieties from the collection  
in 4 or 8 replicates  
= 2,000 plants

A system constrained by space  
and in need of verification steps  
to secure DUS trials

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## Harnessing GbS to support DUS testing in ornamentals

### Two main objectives

- 1 To **genotype the French national reference collection of Hydrangeas**, an *in vivo* collection of over 1,100 accessions maintained at the Gaston-Allard Arboretum in Angers

#### An innovative approach:

- ★ Using **high-throughput sequencing technologies** to identify, screen and select a genome-wide panel of SNP markers
- ★ Combining **'neutral' genetic markers and markers** linked to ornamental traits to characterize varieties in collection

- 2 To **design an optimal approach to integrate molecular analyses in routine DUS examinations** of Hydrangeas  
with the purpose of (1) **securing DUS tests** and  
(2) **guiding the selection of similar varieties** from the collection to optimize examinations



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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 1

#### Genotyping the national reference collection of Hydrangeas

- 1 Identify a **genome-wide panel of SNPs** for Hydrangeas
- 2 Generate a **comprehensive database of molecular data**



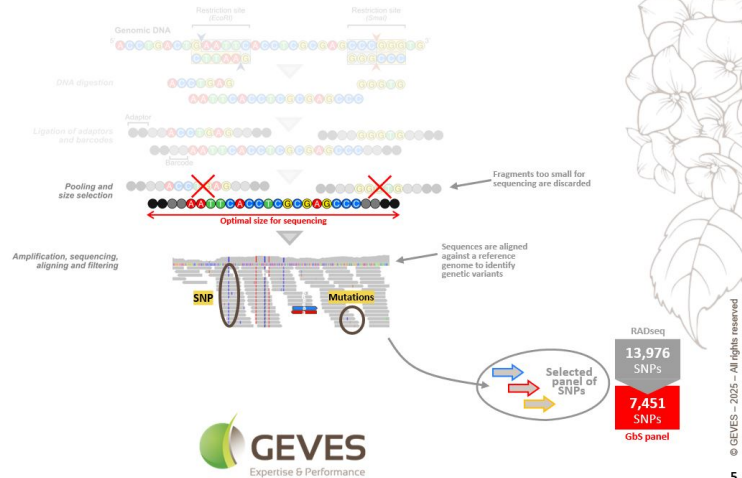
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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 1

**Step 1** Identify a **genome-wide panel of SNPs** for Hydrangeas  
using **Restriction-Associated DNA Sequencing (RADseq)**

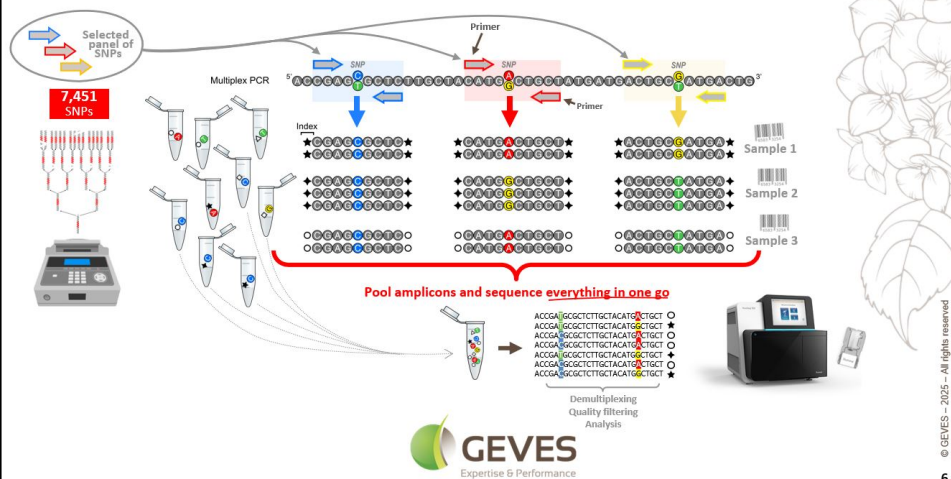


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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 1

**Step 2** **Genotyping the collection**  
using **targeted Genotyping-by-Sequencing**



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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 2


#### Integrating molecular analyses in routine DUS examinations


1 Identify a genome-wide panel of SNPs for Hydrangeas

2 Generate a comprehensive database of molecular data

1 Secure DUS trials

2 Optimize DUS trials





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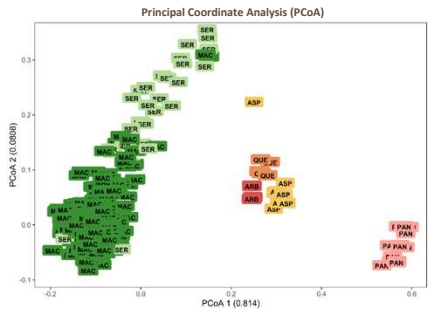
## Harnessing GbS to support DUS testing in ornamentals

### Milestone 2

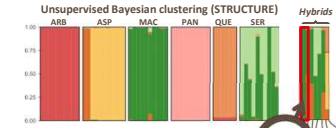
#### Objective 1 Using SNPs to **secure DUS examinations**

★ **Checking hybrid status**


Principal Coordinate Analysis (PCoA)



Unsupervised Bayesian clustering (STRUCTURE)



Hybrids




Molecular analyses do not verify the applicant's declaration that the candidate is a cross between *Macrophylla* and *Arborescens*

Phylogenetic tree showing relationships between *H. chinensis*, *H. luteovirens*, *H. scandens*, *H. macrophylla*, *H. macrophylla* ssp. *serrata*, *H. quercifolia*, *H. arborescens* ssp. *radiana*, *H. arborescens*, *H. sargentiana*, *H. aspera*, *H. longipes*, *H. involucrata*, *H. serratifolia*, *H. seemannii*, *H. integrifolia*, *H. anomala*, *H. petiolaris*, *H. amplex*, *H. paniculata*, and *H. heteromalla*.

Counts for specific clusters: 708, 71, 16, 30, 27, 117.

With only 20 SNPs it is possible to assign a species to a variety with high accuracy, and check for signs of genetic admixture in declared interspecific hybrids



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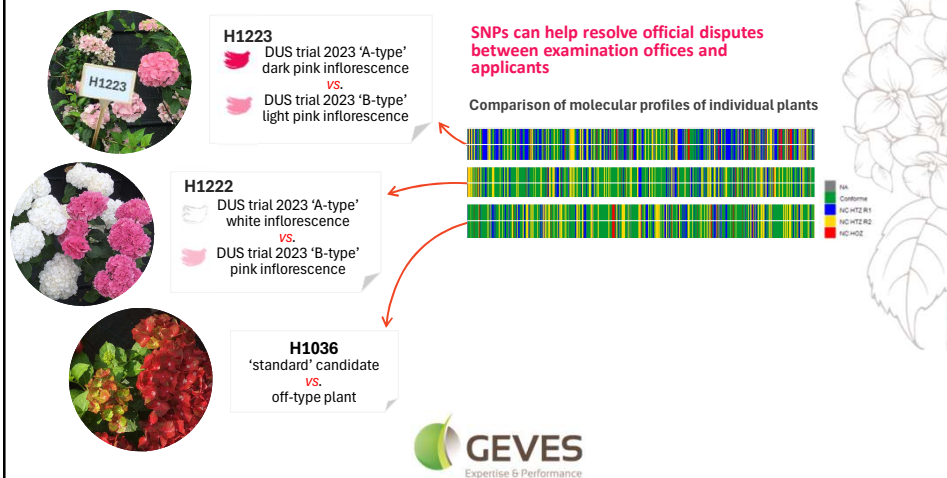


## Harnessing GbS to support DUS testing in ornamentals

### Milestone 2

#### Objective 1 Using SNPs to **secure DUS examinations**

##### ★ Spotting early on varieties with uniformity problems



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## Harnessing GbS to support DUS testing in ornamentals

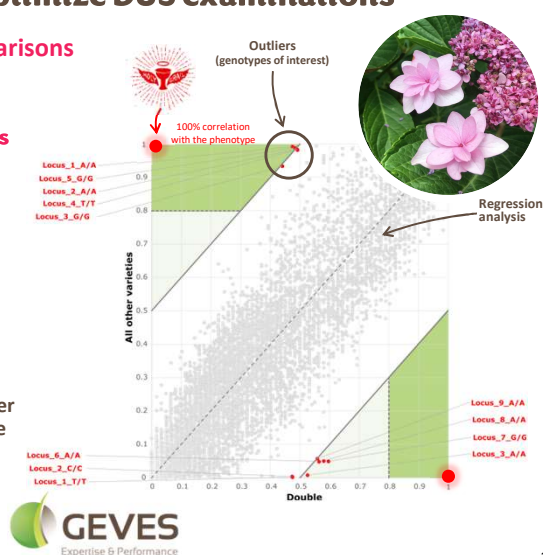
### Milestone 2

#### Objective 2 Using SNPs to **optimize DUS examinations**

##### ★ Reducing the number of comparisons using trait-linked markers

★ To be able to **predict characteristics of candidate varieties** and choose more precisely which varieties to compare with candidates would help reduce the size of DUS trials ...but trait-linked markers with 100% correlation are rare

★ Focusing on SNPs with **strong genotype / phenotype associations**, it may be possible to **use markers in combination** to infer the phenotype of candidates and guide the selection of varieties in collection with the most similar characteristics



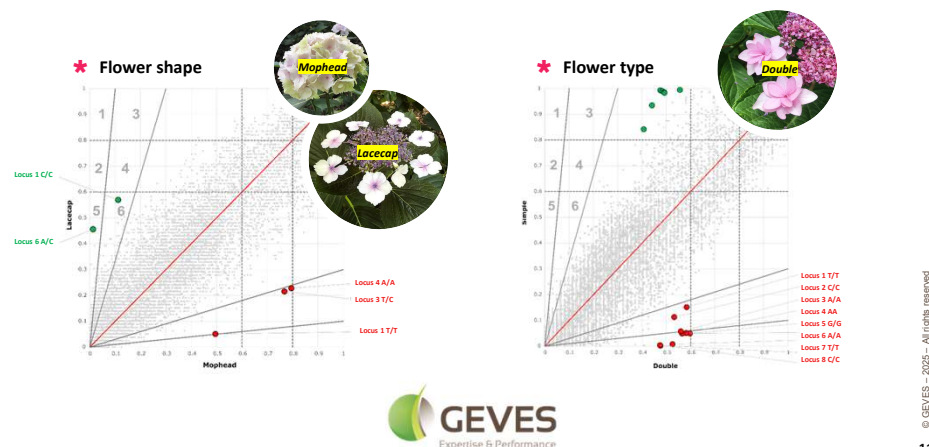
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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 2

#### Objective 2 Using SNPs to **optimize DUS examinations**

- ★ Reducing the number of comparisons using trait-linked markers



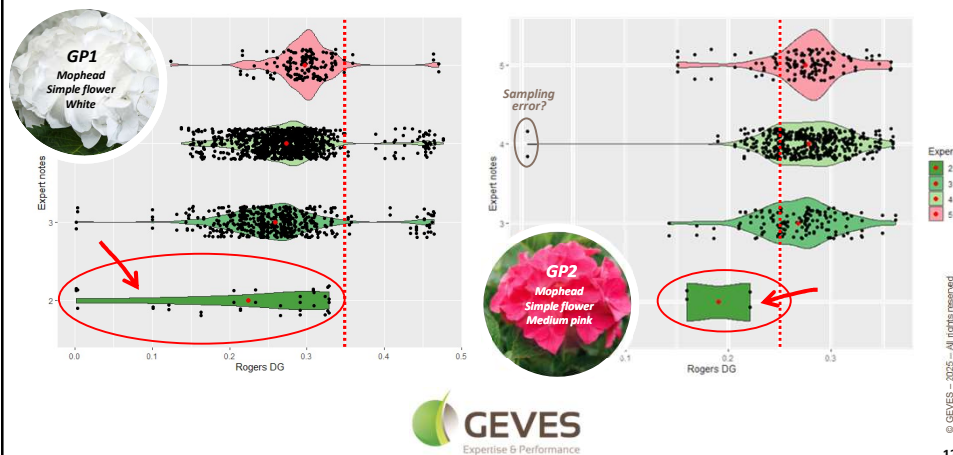
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## Harnessing GbS to support DUS testing in ornamentals

### Milestone 2

#### Objective 2 Using SNPs to **optimize DUS examinations**

- ★ Reducing the number of comparisons using diversity markers



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## Harnessing GbS to support DUS testing in ornamentals

## Milestone 2

## Objective 2 Using SNPs to **optimize DUS examinations**

- \* Reducing the number of comparisons using diversity markers

[illegible]

### No problems of distinctness, only a lack of space

- **Proposition:** remove all varieties with a pairwise GD above the median of the corresponding DUS group for each candidate individually

- ❖ in 2024, 300 varieties from the collection for 28 candidates before reduction / 216 after



-33%

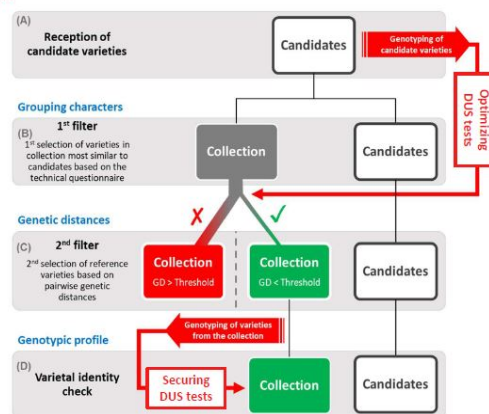


## Harnessing GbS to support DUS testing in ornamentals

## Milestone 2

## Objective 2 Using SNPs to **optimize DUS examinations**

- ## \* Implementation





## Harnessing GbS to support DUS testing in ornamentals

### Summary

- \* This project demonstrated the cost-effectiveness of opting for **high-throughput sequencing** for describing entire reference collections all in one go
- \* It may pave the way for similar projects to support DUS testing in other ornamental species for which genomic resources are not available or for which maintaining a living collection is not possible or costly

#### Main deliverables

- ✓ **7,410 SNPs** in total were identified
  - 5,649 for *H. macrophylla*
  - 5,528 for *H. serrata*
  - and between 300 and 600 for other species
- ✓ A **set of 20 SNPs** was selected for confirming species identity and checking the pedigree of declared hybrid candidate varieties
- ✓ A **set of 40 SNPs** was selected for varietal identification in *H. macrophylla*
- ✓ **Several SNPs were identified as interesting candidates to explore correlations between genotype and phenotype but work is still required to test advanced modelling approaches** such as approximate conditional phenotype analysis based on GWAS statistics



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[End of Annex and of document]