

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****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

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

Bundessortenamt

CPVO Community Plant Variety Office

1

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 for verification steps to secure DUS trials

GEVES Expertise & Performance

2

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



© GEVES - 2025 - All rights reserved
3



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



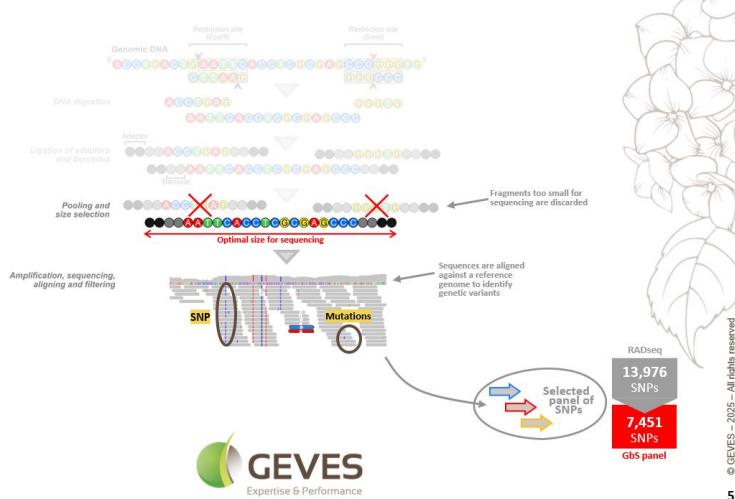
4



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)

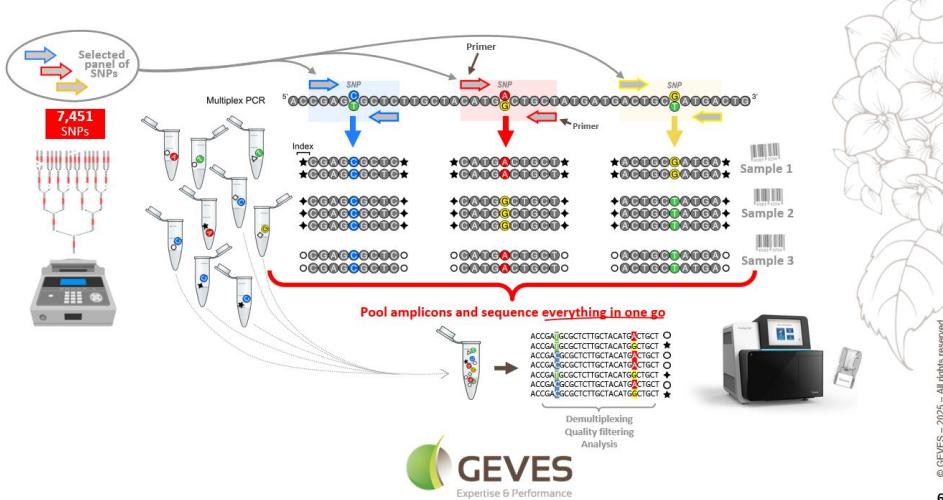


5

Harnessing GbS to support DUS testing in ornamentals

Milestone 1

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



6

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**



7

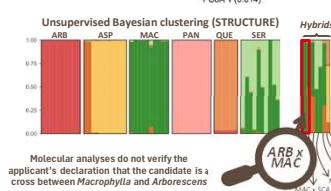
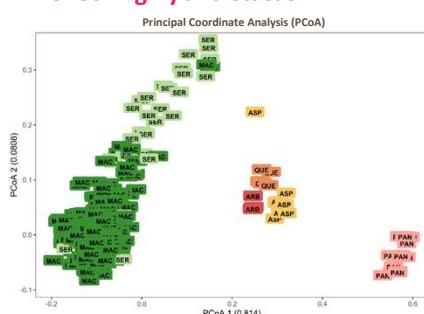
7



Harnessing GbS to support DUS testing in ornamentals

Milestone 2 Objective 1 Using SNPs to **secure DUS examinations**

* Checking hybrid status



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



GEVES
Expertise & Performance

© GEVES – 2025 – All rights reserved

8

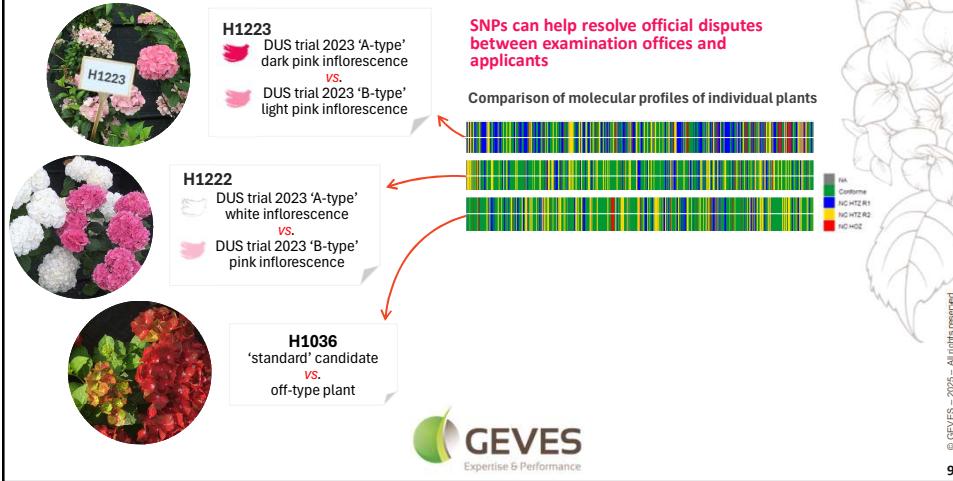
8

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



9

Harnessing GbS to support DUS testing in ornamentals

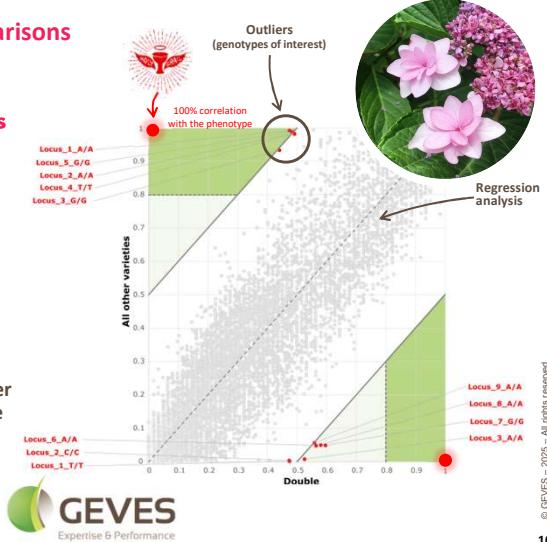
Milestone 2

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



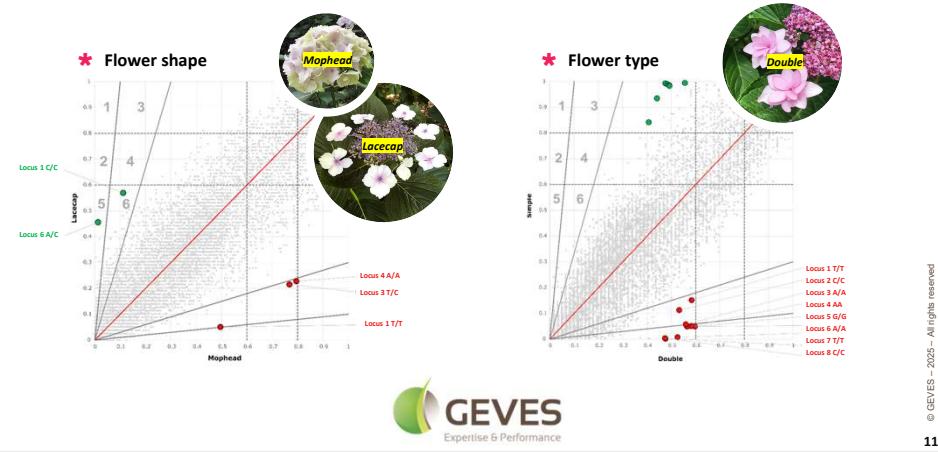
10

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



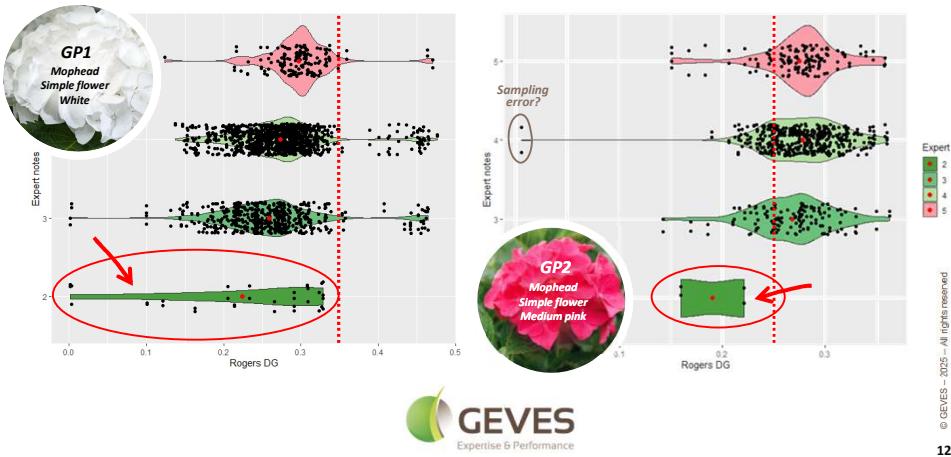
11

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



12

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

| Project_NR | Project_NR_Expert | metre_DG | SNP | Project_NR | Project_NR_Expert | metre_DG | SNP | Project_NR | Project_NR_Expert | metre_DG | SNP |
|------------|-------------------|----------|-----|------------|-------------------|----------|-----|------------|-------------------|----------|-----|
| H0064 | H0091 | 3 0.186 | 693 | H0238 | H0028 | 3 0.181 | 694 | H1075 | H0084 | 3 0.145 | 699 |
| H0064 | H0098 | 3 0.236 | 692 | H0238 | H0229 | 4 0.219 | 691 | H1075 | H014292 | 3 0.146 | 698 |
| H0064 | H0100 | 3 0.236 | 692 | H0238 | H0239 | 3 0.215 | 693 | H1075 | H01268 | 3 0.146 | 697 |
| H0064 | H004811 | 4 0.246 | 687 | H0238 | H00391 | 3 0.221 | 692 | H1075 | H0198 | 4 0.261 | 695 |
| H0064 | H004812 | 4 0.251 | 687 | H0238 | H00392 | 4 0.221 | 693 | H1075 | H0199 | 4 0.270 | 694 |
| H0064 | H004812 | 4 0.251 | 692 | H0238 | H0147 | 4 0.222 | 694 | H1075 | H0040 | 4 0.271 | 697 |
| H0064 | H00531 | 4 0.251 | 690 | H0238 | H02482 | 3 0.223 | 695 | H1075 | H0447 | 3 0.279 | 697 |
| H0064 | H0103 | 3 0.256 | 692 | H0238 | H0147 | 3 0.223 | 691 | H1075 | H0041 | 3 0.273 | 693 |
| H0064 | H0190 | 3 0.258 | 693 | H0238 | H04882 | 4 0.237 | 695 | H1075 | H0027 | 3 0.283 | 692 |
| H0064 | H0233 | 3 0.262 | 694 | H0238 | H0147 | 3 0.230 | 695 | H1075 | H0022 | 3 0.284 | 694 |
| H0064 | H026802 | 3 0.269 | 694 | H0238 | H014292 | 3 0.240 | 695 | H1075 | H0263 | 3 0.289 | 699 |
| H0064 | H0298 | 3 0.270 | 691 | H0238 | H0132 | 3 0.230 | 694 | H1075 | H0028 | 3 0.296 | 695 |
| H0064 | H030301 | 3 0.270 | 694 | H0238 | H0239 | 3 0.230 | 695 | H1075 | H0027 | 3 0.297 | 697 |
| H0064 | H0229 | 3 0.271 | 690 | H0238 | H0147 | 3 0.231 | 691 | H1075 | H0132 | 3 0.301 | 695 |
| H0064 | H0203 | 3 0.271 | 690 | H0238 | H0147 | 3 0.231 | 691 | H1075 | H0211 | 3 0.302 | 698 |
| H0064 | H022392 | 3 0.272 | 691 | H0238 | H04881 | 4 0.236 | 689 | H1075 | H0190 | 3 0.305 | 693 |
| H0064 | H0247 | 3 0.274 | 694 | H0238 | H0084 | NA | NA | H1075 | H0084 | 3 0.307 | 693 |
| H0064 | H0179 | 3 0.274 | 690 | H0238 | H0027 | 3 0.236 | 690 | H1075 | H0383 | NA | NA |
| H0064 | H0264 | 3 0.274 | 690 | H0238 | H0027 | 3 0.236 | 690 | H1075 | H0383 | NA | NA |
| H0064 | H0183 | NA | NA | H0238 | H0175 | 3 0.261 | 694 | H1075 | H056802 | 3 0.310 | 698 |
| H0064 | H026902 | 3 0.285 | 688 | H0238 | H0247 | 3 0.261 | 692 | H1075 | H0054 | 3 0.315 | 698 |
| H0064 | H014292 | 3 0.285 | 688 | H0238 | H001 | 3 0.275 | 691 | H1075 | H0229 | 3 0.315 | 698 |
| H0064 | H0247 | 3 0.285 | 694 | H0238 | H0147 | 3 0.273 | 692 | H1075 | H0232 | 3 0.315 | 698 |
| H0064 | H0208 | 2 0.286 | 694 | H0238 | H0147 | 3 0.273 | 691 | H1075 | H0227 | 3 0.315 | 691 |
| H0064 | H024192 | 3 0.293 | 694 | H0238 | H0147 | 4 0.272 | 693 | H1075 | H02492 | 3 0.318 | 698 |
| H0064 | H0203 | 3 0.293 | 694 | H0238 | H0147 | 4 0.272 | 693 | H1075 | H02491 | 3 0.318 | 691 |
| H0064 | H0040 | 3 0.293 | 694 | H0238 | H0092 | 0.2767 | 694 | H1075 | H04898 | 3 0.324 | 698 |
| H0064 | H030301 | 3 0.294 | 694 | H0238 | H0092 | 0.2767 | 694 | H1075 | H04899 | 3 0.324 | 698 |
| H0064 | H0132 | 3 0.302 | 691 | H0238 | H0048 | 3 0.278 | 693 | H1075 | H0231 | 3 0.322 | 697 |
| H0064 | H030301 | 3 0.302 | 691 | H0238 | H0048 | 3 0.278 | 693 | H1075 | H0231 | 3 0.322 | 697 |
| H0064 | H0247 | 4 0.3074 | 693 | H0238 | H0048 | 3 0.2784 | 695 | H1075 | H0291 | 3 0.3223 | 698 |
| H0064 | H0197 | 2 0.3091 | 691 | H0238 | H0227 | 4 0.2966 | 689 | H1075 | H0217 | 4 0.3240 | 699 |
| H0064 | H0247 | 3 0.3091 | 691 | H0238 | H0227 | 4 0.2966 | 689 | H1075 | H0217 | 4 0.3240 | 699 |
| H0064 | H024792 | 3 0.3307 | 691 | H0238 | H0111R1 | 5 0.3035 | 687 | H1075 | H053031 | 4 0.3276 | 693 |
| H0064 | H0194 | 4 0.3305 | 693 | H0238 | H0133 | 5 0.3133 | 687 | H1075 | H0133 | 5 0.3387 | 551 |
| H0064 | H0193 | 0 0.3370 | 548 | H0238 | H0054 | 0 0.3165 | 693 | H1075 | H059902 | 0 0.3472 | 697 |
| H0064 | H0203 | 3 0.3370 | 693 | H0238 | H0054 | 0 0.3165 | 693 | H1075 | H0203 | 3 0.3370 | 693 |
| H0064 | H0008 | 3 0.3528 | 625 | H0238 | H0098 | 3 0.3333 | 627 | H1075 | H0098 | 3 0.3576 | 698 |
| H0064 | H0111 | 0 0.3518 | 698 | H0238 | H0211 | 0 0.3177 | 698 | H1075 | H0211 | 0 0.3576 | 698 |

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% ↘

© GEVES – 2025 – All rights reserved
13

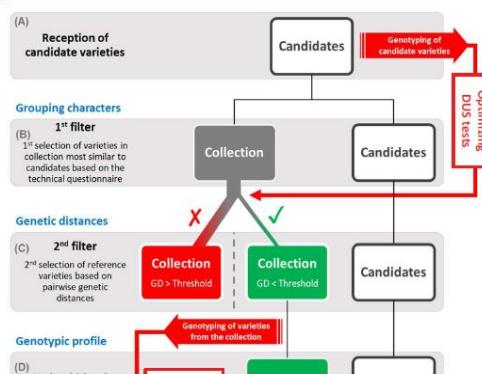
13

Harnessing GbS to support DUS testing in ornamentals

Milestone 2

Objective 2 Using SNPs to optimize DUS examinations

* Implementation



© GEVES – 2025 – All rights reserved
14

14

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



© GEVES - 2025 - All rights reserved

15

15

Thank
you!



16

[End of Annex and of document]