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/19/11

Nineteenth Session
Alexandria, United States of America, September 23 to 25, 2020

Original: English
Date: August 31, 2020

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 nineteenth session of the BMT.

[Annex follows]

Follow-up 1 - 2019/2021

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

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

Marc DELÊTRE

UPOV, BMT/19, 23-25 September 2020

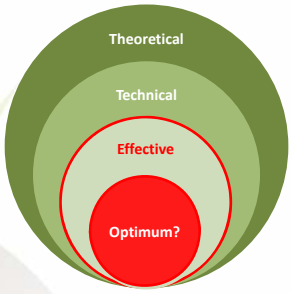
Context

- Evolution of genetic structure of candidate varieties in the last 10 years: from commercial lines to 100% hybrids
  - → *Rapid increase in size of field trials*
  - → *Difficulties to manage the reference collection*

  Every year, about 90 candidate varieties are submitted, representing almost **200 varieties that must undergo DUS tests** (when including parental lines)

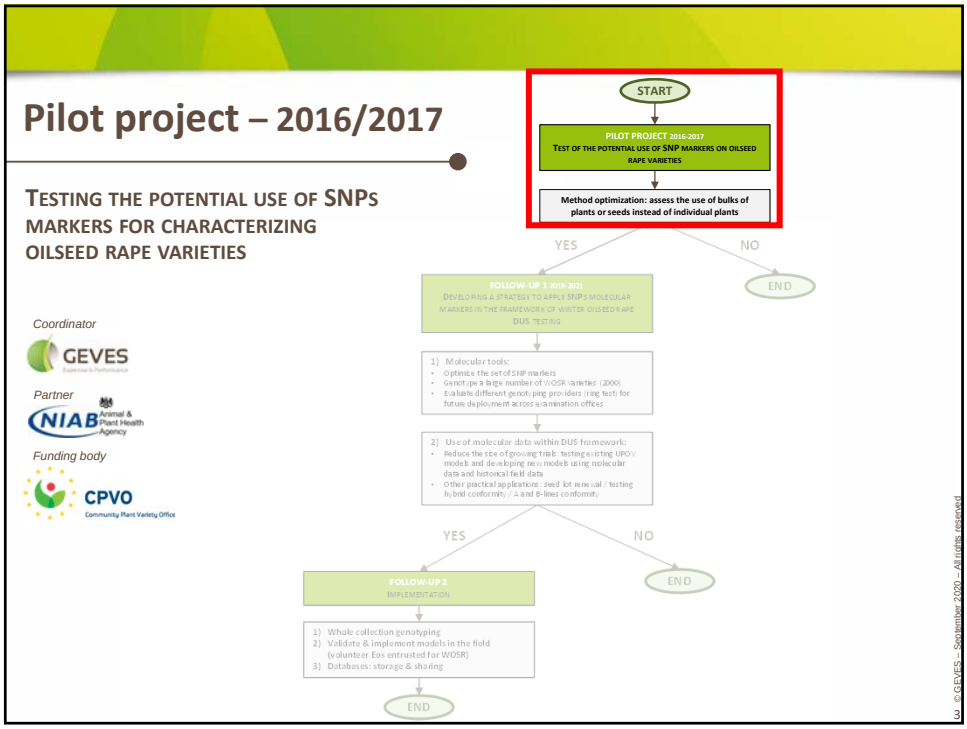
- High inter-annual variability of morphological characters
  - → *Necessity to re-describe the entire collection every year*

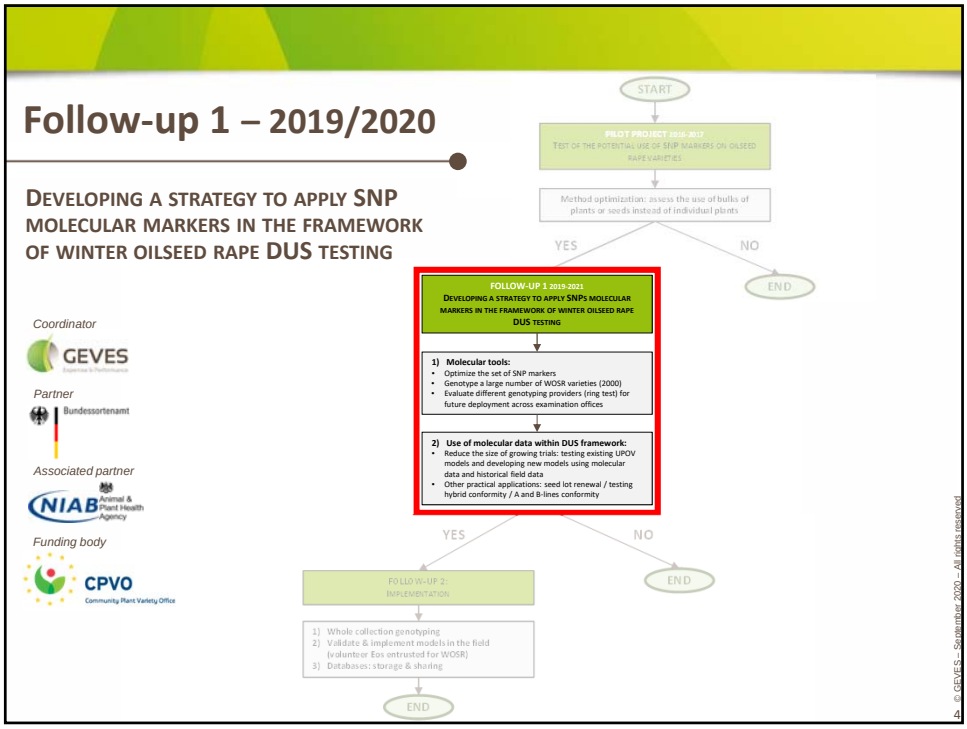
  800-900 varieties from the reference collection have to be planted every year meaning that **> 1000 varieties have to be described each year**

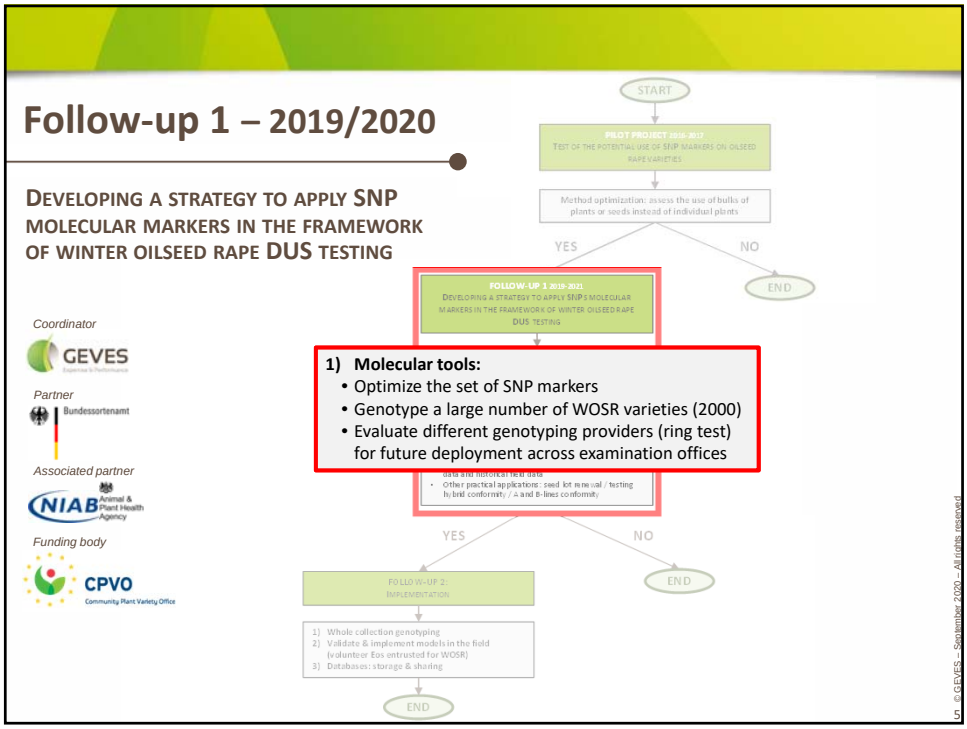


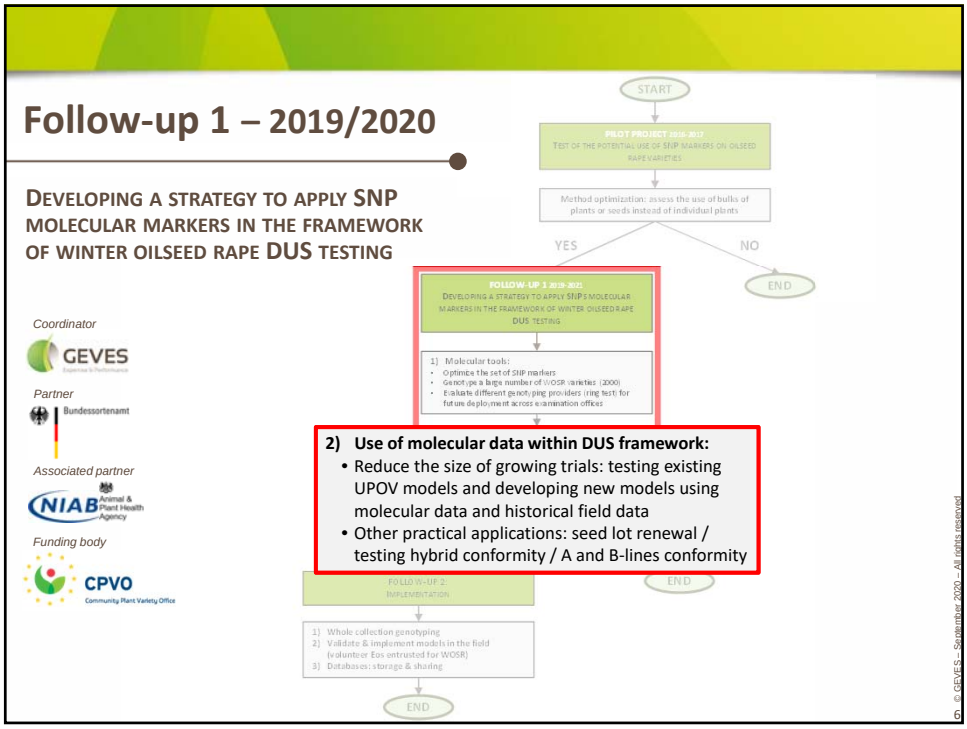
→ How to optimize the size of the effective collection?

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences


Pilot project – 2016/2017
TESTING THE POTENTIAL USE OF SNPS MARKERS FOR CHARACTERIZING OILSEED RAPE VARIETIES
Coordinator
GEVES
Partner
NIAB
Animal & Plant Health Agency
Funding body
CPVO
Community Plant Variety Office
START
PILOT PROJECT 2016-2017
TEST OF THE POTENTIAL USE OF SNP MARKERS ON OILSEED RAPE VARIETIES
Method optimization: assess the use of bulks of plants or seeds instead of 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) Whole collection genotyping
2) Validate & implement models in the field (volunteer Eos entrusted for WOSR)
3) Databases: storage & sharing
END
© GEVES - September 2020 - All rights reserved


Follow-up 1 – 2019/2020
DEVELOPING A STRATEGY TO APPLY SNP MOLECULAR MARKERS IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING
Coordinator
GEVES
Partner
Bundessortenamt
Associated partner
NIAB
Animal & Plant Health Agency
Funding body
CPVO
Community Plant Variety Office
START
PILOT PROJECT 2016-2017
TEST OF THE POTENTIAL USE OF SNP MARKERS ON OILSEED RAPE VARIETIES
Method optimization: assess the use of bulks of plants or seeds instead of 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) Whole collection genotyping
2) Validate & implement models in the field (volunteer Eos entrusted for WOSR)
3) Databases: storage & sharing
END
© GEVES - September 2020 - All rights reserved


Follow-up 1 – 2019/2020
DEVELOPING A STRATEGY TO APPLY SNP MOLECULAR MARKERS IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING
Coordinator
GEVES
Partner
Bundessortenamt
Associated partner
NIAB
Animal & Plant Health Agency
Funding body
CPVO
Community Plant Variety Office
START
PILOT PROJECT 2016-2017
TEST OF THE POTENTIAL USE OF SNP MARKERS ON OILSEED RAPE VARIETIES
Method optimization: assess the use of bulks of plants or seeds instead of 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
YES
NO
END
FOLLOW-UP 2:
IMPLEMENTATION
1) Whole collection genotyping
2) Validate & implement models in the field (volunteer Eos entrusted for WOSR)
3) Databases: storage & sharing
END
© GEVES - September 2020 - All rights reserved


Follow-up 1 – 2019/2020
DEVELOPING A STRATEGY TO APPLY SNP MOLECULAR MARKERS IN THE FRAMEWORK OF WINTER OILSEED RAPE DUS TESTING
Coordinator
GEVES
Partner
Bundessortenamt
Associated partner
NIAB
Animal & Plant Health Agency
Funding body
CPVO
Community Plant Variety Office
START
PILOT PROJECT 2016-2017
TEST OF THE POTENTIAL USE OF SNP MARKERS ON OILSEED RAPE VARIETIES
Method optimization: assess the use of bulks of plants or seeds instead of 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
END
FOLLOW-UP 2:
IMPLEMENTATION
1) Whole collection genotyping
2) Validate & implement models in the field (volunteer Eos entrusted for WOSR)
3) Databases: storage & sharing
END
© GEVES - September 2020 - All rights reserved

Selection of varieties

- Priority given to varieties (lines and hybrids + parental lines) for which morphological data are available in both countries
  - → **Consensus list** between French and German reference collections, *including*
  - → All **CPVR varieties**, *and*
  - → All other **varieties listed in countries entrusted by CPVO for WOSR** (CZ, DE, DK, ES, FR, GB, PL, SK)

Additionally:

- Candidate varieties that failed DUS tests (distinction, homogeneity…)
- Spring OSR and forage varieties (as outgroups)

1986 winter OSR varieties + **27 'problematic' varieties** + **41 spring OSR varieties**

incl. **all varieties from pre-project**

6 with problems of distinction
21 with problems of homogeneity

≈ 20% of the SOSR reference collection

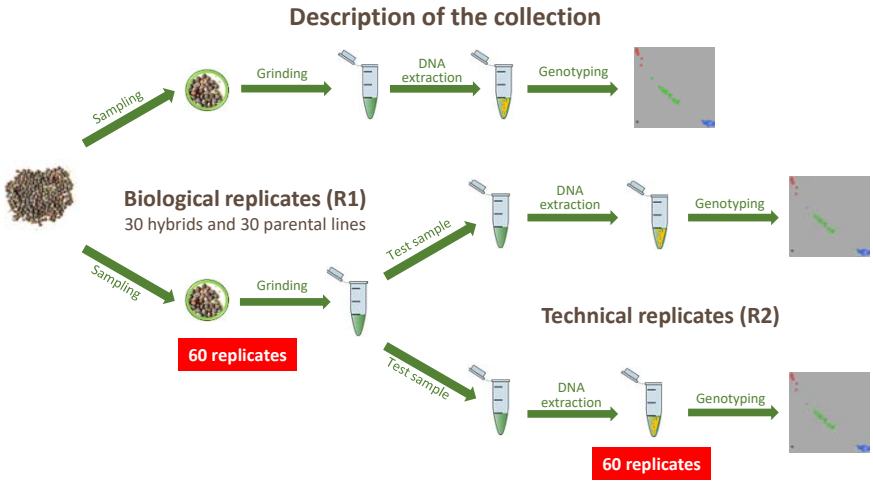
- Replicates:

180 biological / technical repeats including:

- → **Duplicated DNA templates** to check data consistency
- → **New bulks** to check data coherence
- → **Pseudo-replicates** (distinct DUS seed lots)

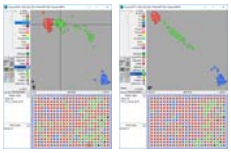
GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences

Replicates



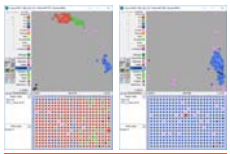
Optimization of the SNP set

- First set of **500 SNPs** tested as part of the pilot project:
  - → **Non-multilocus** (sub-genome specific)
  - → **High PIC** (Polymorphism Information Content)
  - → **Homogenous genome coverage**
  - → Suitable flanking sequence for KASPar design
  - ▪ **452 SNPs** validated
  - ▪ **48 additional markers** selected to fill in gaps in genome coverage based on physical and genetic mapping

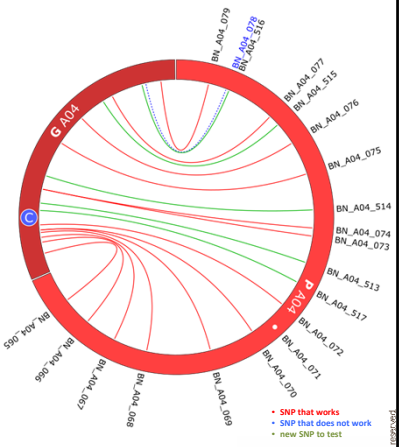


SNPs working well ☺

No clear separation Monomorphic



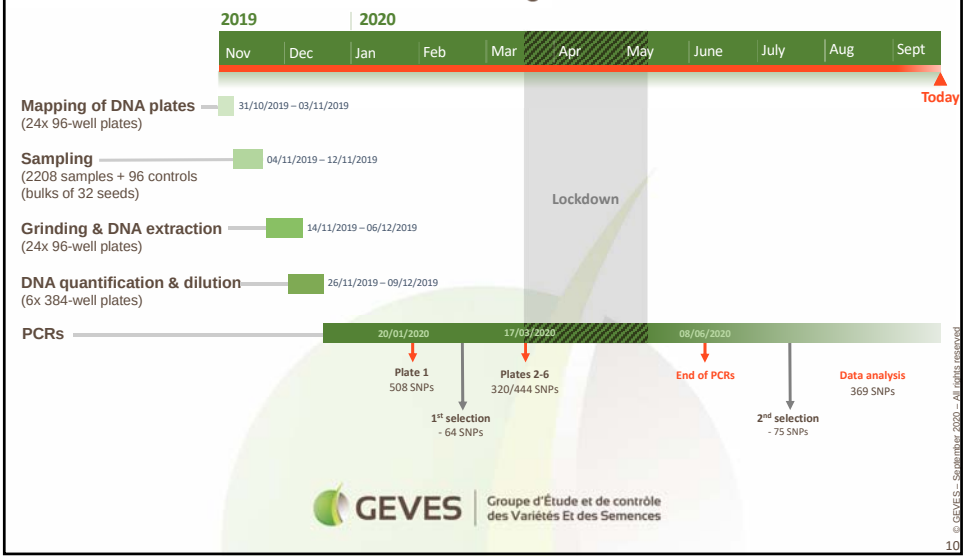
SNPs to discard ☹



Correspondance between the physical and genetic positions of SNPs along the chromosome A04 (circular map)

9

Labwork timeline



GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

10

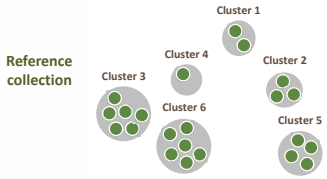
Project timeline

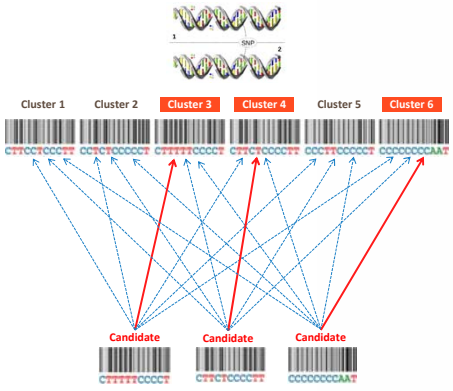
Milestones	Tasks		Sub-tasks	Project months: Year 1 05/19	06/19	07/19	08/19	09/19	10/19	11/19	12/19	01/20	02/20	03/20	04/20	05/20	Year 2 06/20	07/20	08/20	09/20	10/20	11/20	12/20	01/21	02/21	03/21	04/21	05/21
Workshop																	x										
Produce a large molecular dataset on WOSR varieties and optimize the SNP set	Task 1	Select varieties to be genotyped		x	x																						
	Task 2	Select SNP according to the genetic map		x	x																						
	Task 3	Genotyping	Sampling/grinding				x																					
			DNA extraction					x																				
			PCR					x	x	x	x	x	x	x	x													
	Task 4	Data analysis and review of the results														x	x	x									
Analyse the genetic data and test existing UPOV models and/or develop new ones	Task 1	Historical data collection		x	x	x	x																				
	Task 2	Conceptualize and assess new models	Conceptualise new models				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
			Test existing and new models															x	x	x	x	x	x	x			
Feasibility study	Task 1	Evaluation of different providers										x	x	x	x	x	x										
Final report																							x	x	x	x	x

Optimizing DUS tests in winter OSR

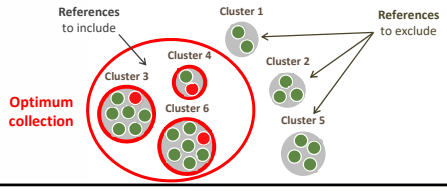
- The main objective is to develop new models using SNP markers to **reduce / optimize the size of 1st year DUS field trials**

→ *Analyze the genetic structure of the reference collection and identify clusters of varieties*





→ *Assign candidates to clusters and select reference varieties from relevant clusters*



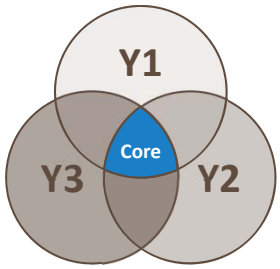
12

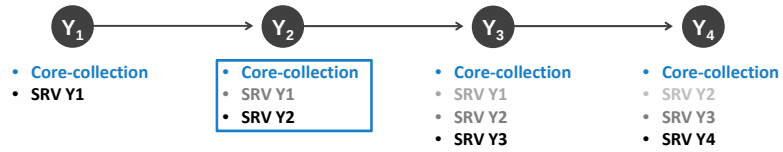
Optimizing DUS tests in winter OSR

In any given year, the reference collection will include:

- a stable **core collection** of example varieties
- the **set of reference varieties** (SRV) for the (two) previous year(s)
- a **new set of reference varieties** determined by the candidate varieties of that particular year
- new varieties listed abroad

The **core collection** is established by each EO and may be revised at the discretion of DUS experts to reflect changes in the conditions of trials





Y_1	Y_2	Y_3	Y_4
• Core-collection • SRV Y1	• Core-collection • SRV Y1 • SRV Y2	• Core-collection • SRV Y1 • SRV Y2 • SRV Y3	• Core-collection • SRV Y2 • SRV Y3 • SRV Y4

→ Rotation of varieties over a 3-year rolling period

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

13

[End of Annex and of document]