UPOV

International Union for the Protection of New Varieties of Plants

E

Technical Working Party for Agricultural Crops

Forty-Ninth Session
Saskatoon, Canada, June 22 to 26, 2020

TWA/49/5

Original: English
Date: June 11, 2020

USE OF MOLECULAR TECHNIQUES IN DUS EXAMINATION

Document prepared by the Office of the Union

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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”, prepared by an expert from France, to be considered by the forty-ninth session of the Technical Working Party for Agricultural Crops (TWA).

[Annex follows]

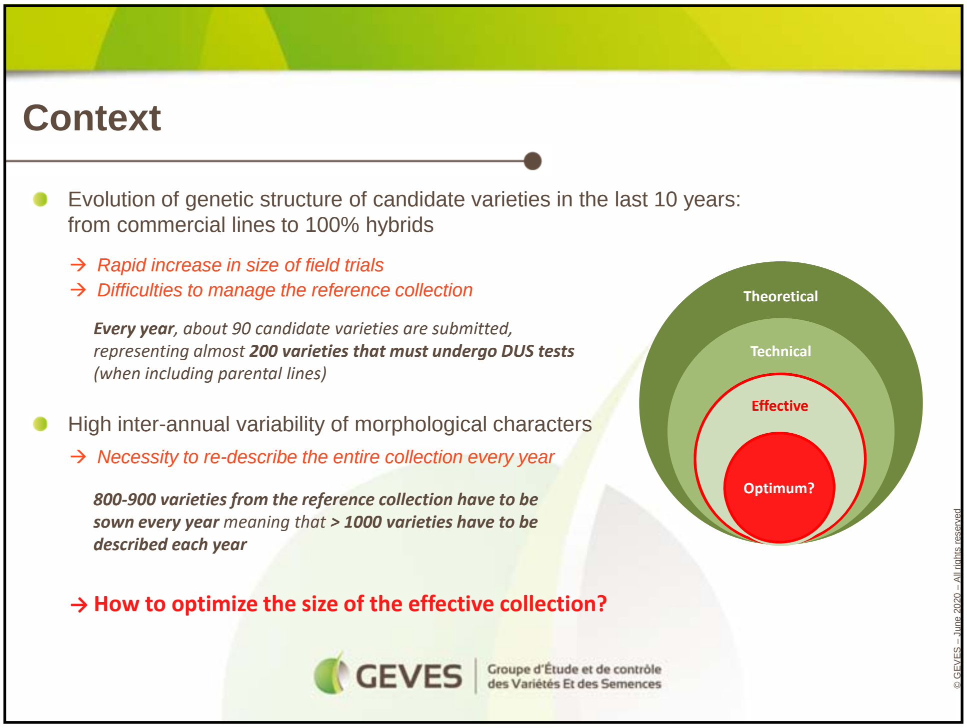
ANNEX

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

Anne-Lise Corbel
UPOV, June 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 sown every year** meaning that **> 1000 varieties have to be described each year

→ How to optimize the size of the effective collection?

Theoretical
Technical
Effective
Optimum?

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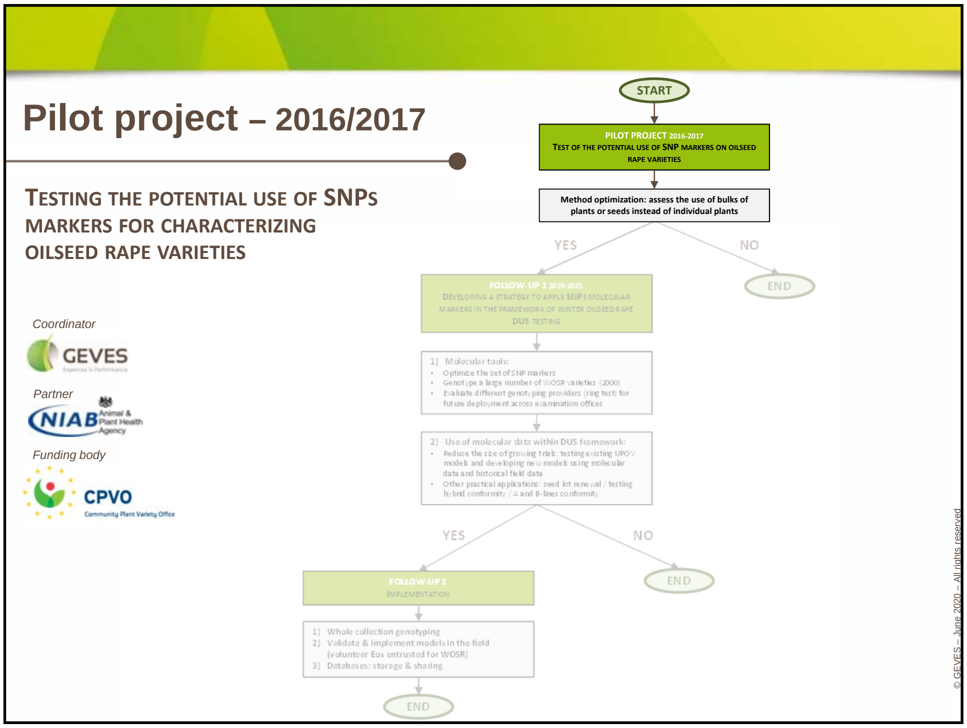

Pilot project – 2016/2017

TESTING THE POTENTIAL USE OF SNPs MARKERS FOR CHARACTERIZING OILSEED RAPE VARIETIES

Coordinator
GEVES

Partner
NIAB

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

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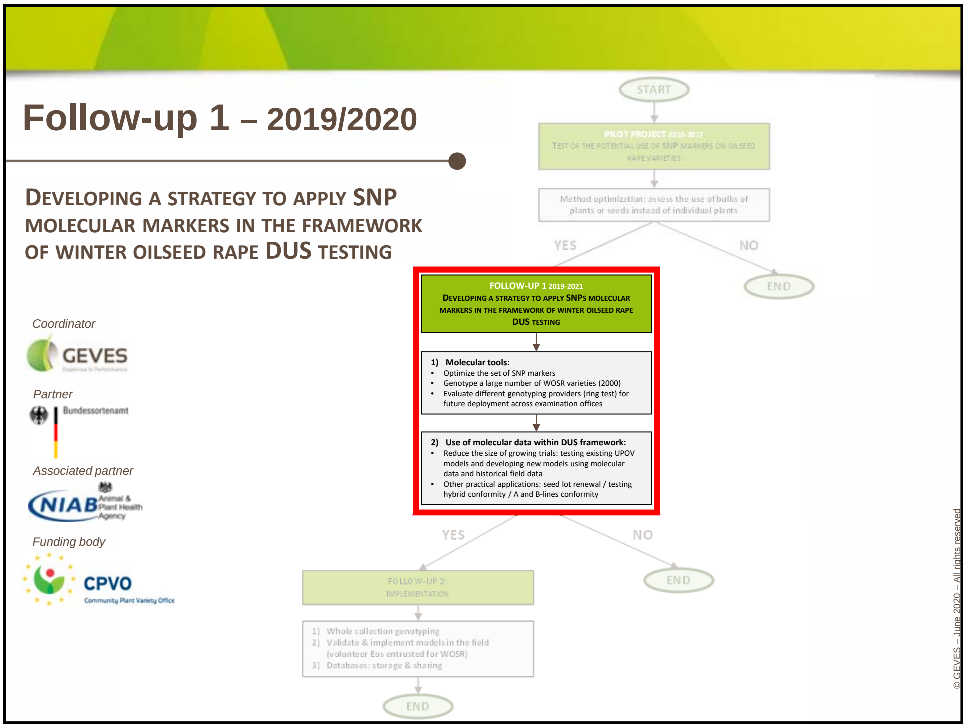

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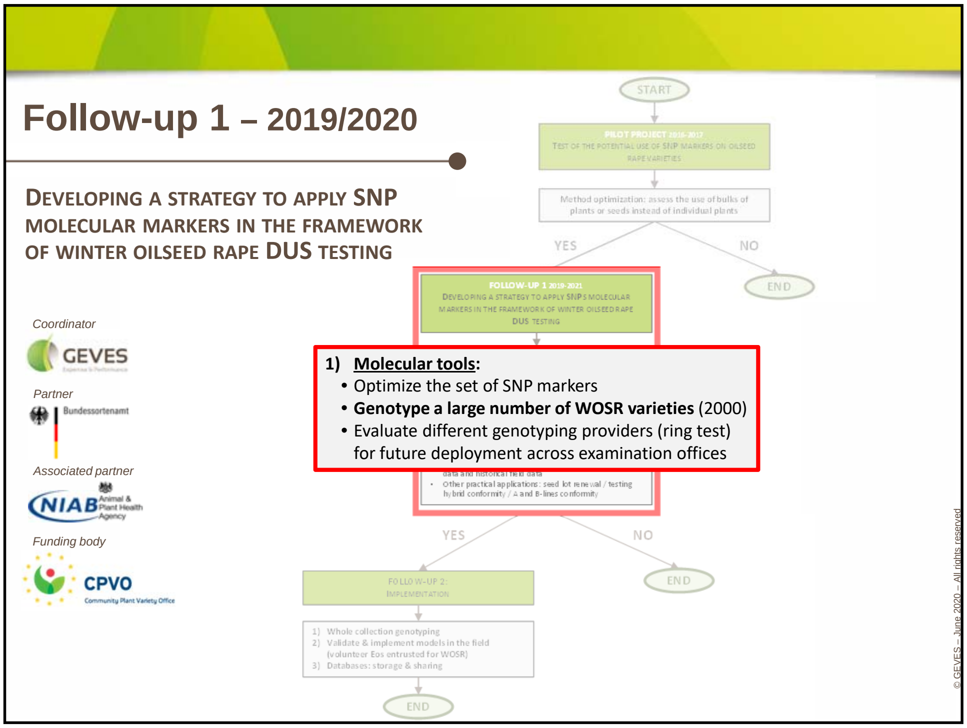

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data and historical field data
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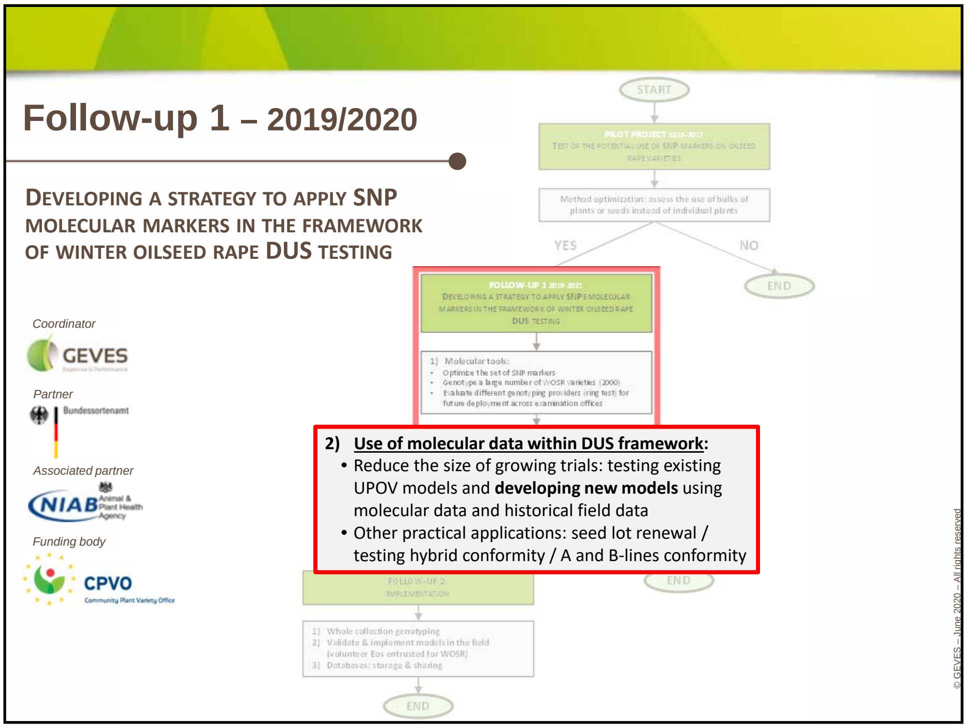

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Selection of varieties

- 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, PL, SK) + UK

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)

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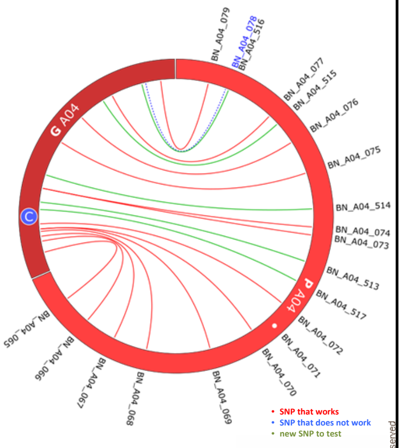
Optimization of the SNP set

- 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



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

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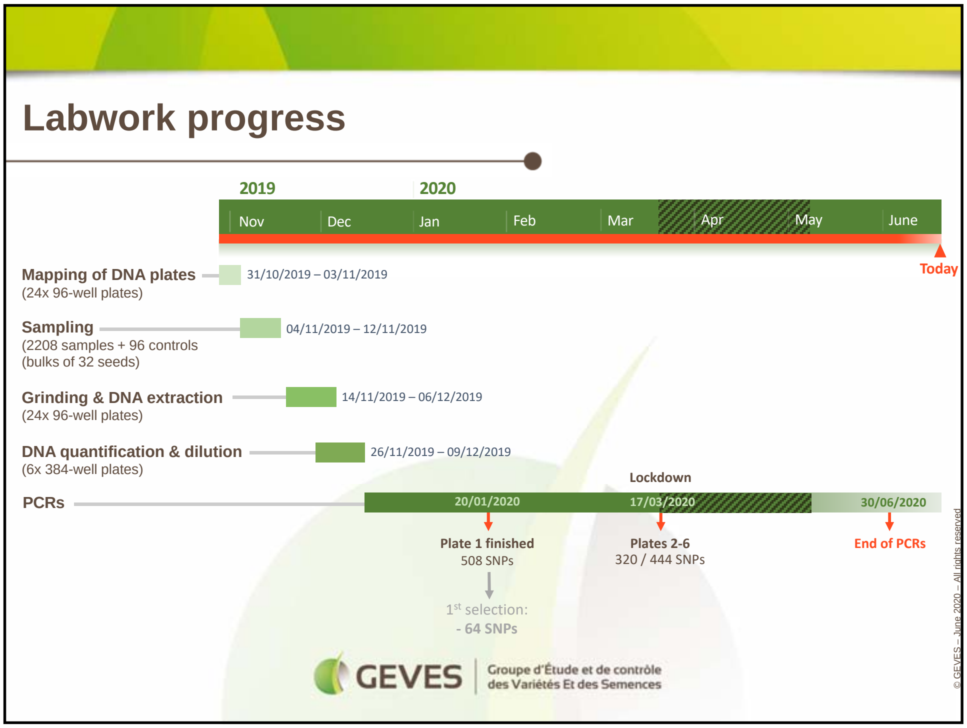

Labwork progress

2019 2020

Nov Dec Jan Feb Mar Apr May June

Today

Mapping of DNA plates (24x 96-well plates) 31/10/2019 – 03/11/2019

Sampling (2208 samples + 96 controls (bulks of 32 seeds) 04/11/2019 – 12/11/2019

Grinding & DNA extraction (24x 96-well plates) 14/11/2019 – 06/12/2019

DNA quantification & dilution (6x 384-well plates) 26/11/2019 – 09/12/2019

PCRs

Lockdown

20/01/2020 — **Plate 1 finished** 508 SNPs → 1st selection: - 64 SNPs

17/03/2020 — **Plates 2-6** 320 / 444 SNPs

30/06/2020 — **End of PCRs**

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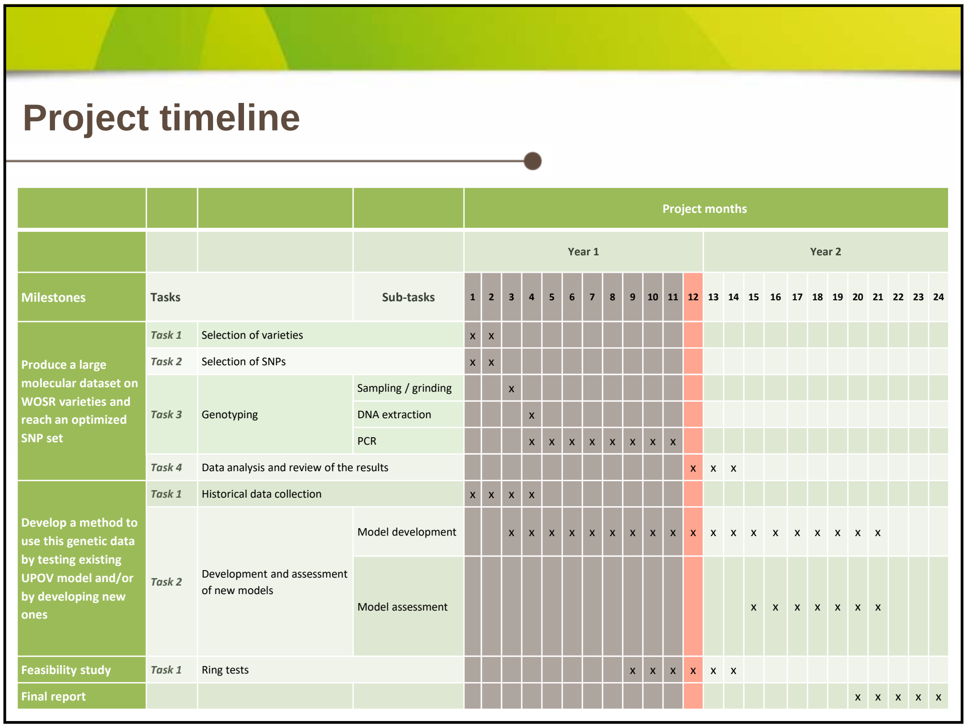
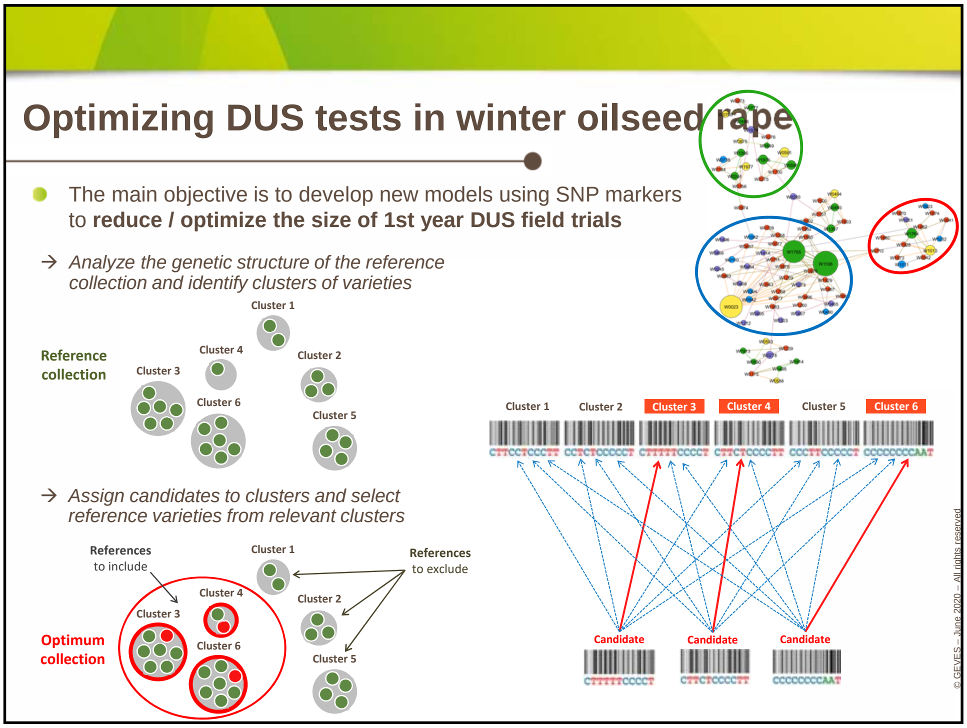


Project timeline

				Project months																							
				Year 1												Year 2											
Milestones	Tasks		Sub-tasks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Produce a large molecular dataset on WOSR varieties and reach an optimized SNP set	*Task 1*	Selection of varieties		x	x																						
	Task 2	Selection of SNPs		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										
Develop a method to use this genetic data by testing existing UPOV model and/or by developing new ones	*Task 1*	Historical data collection		x	x	x	x																				
	Task 2	Development and assessment of new models	Model development			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
			Model assessment															x	x	x	x	x	x	x			
Feasibility study	*Task 1*	Ring tests										x	x	x	x	x	x										
Final report																							x	x	x	x	x



Optimizing DUS tests in winter oilseed rape

- 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*

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[End of Annex and of document]