UPOV

International Union for the Protection of New Varieties of Plants

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**Technical Working Party for Vegetables**
Sixtieth Session
Pacific Grove, United States of America, May 18 to 21, 2026

**Technical Working Party on Testing Methods and Techniques**
Fourth Session
Cambridge, United Kingdom, June 2 to 5, 2026

**TWV/60/7-TWM/4/6**

**Original:** English
**Date:** May 7, 2026

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# PROGRESS IN THE USE OF MOLECULAR MARKERS TO SUPPORT TOMATO DUS TESTING AT NAKTUINBOUW

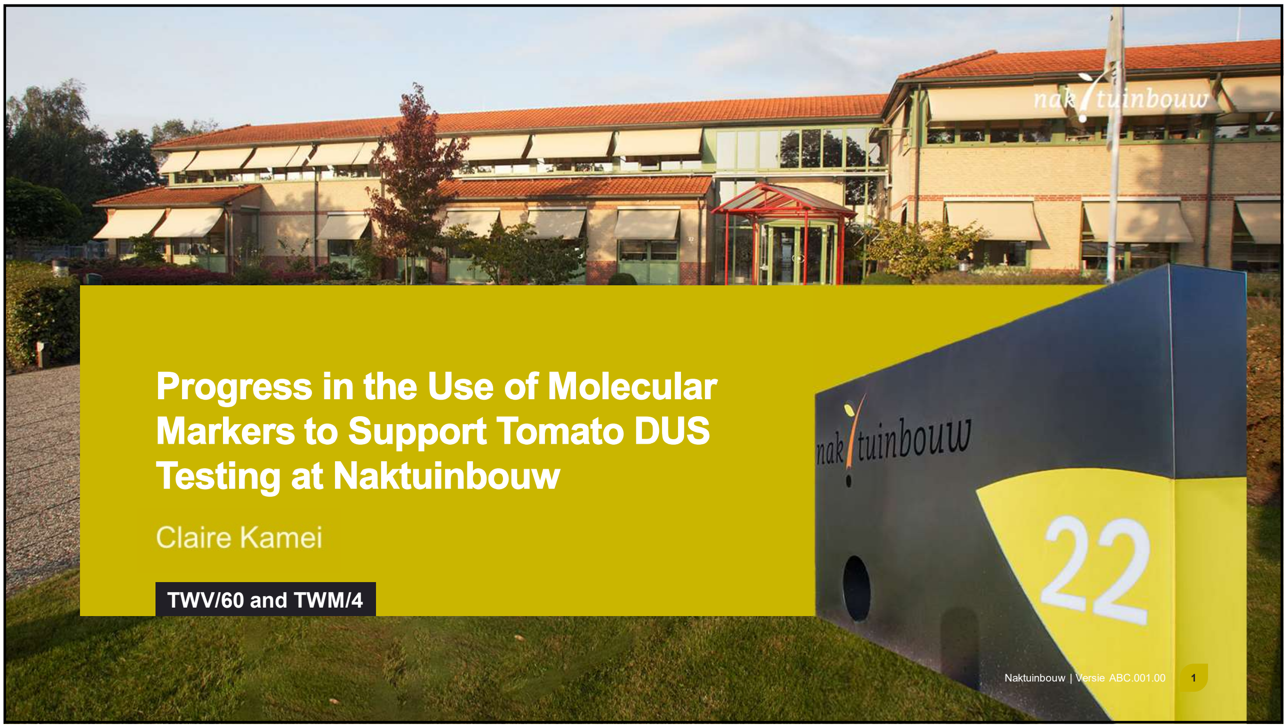
*Document prepared by an expert from the Netherlands (Kingdom of)*

1. The annex to this document contains a copy of a presentation “Progress in the use of molecular markers to support tomato DUS testing at Naktuinbouw”, to be made by an expert from the Netherlands (Kingdom of), at the sixtieth session of the TWV and at the fourth session of the TWM.

2. This presentation provides an update on recent progress at Naktuinbouw in the use of molecular markers in the context of tomato DUS testing. It focuses on our contribution to the CPVO Working Group on disease resistance in tomato, the development and implementation of an international SNP database, and the establishment of a DNA-based approach to support varietal trueness control in maintenance samples. Together, these activities illustrate how DNA techniques can support the work of DUS experts by increasing efficiency and helping to make more effective use of greenhouse space, time, and resources, while phenotypic assessment remains central to DUS testing.

[Annex follows]

ANNEX



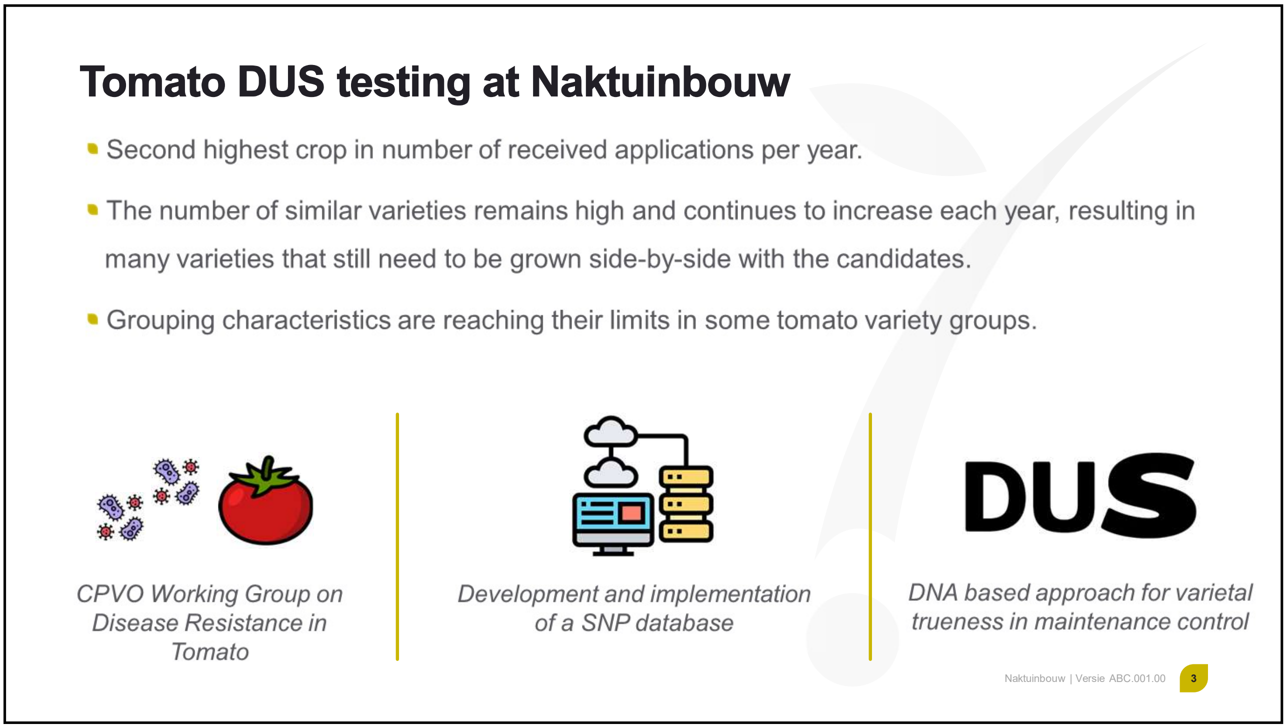
# Progress in the Use of Molecular Markers to Support Tomato DUS Testing at Naktuinbouw

Claire Kamei

**TWV/60 and TWM/4**

## Tomato DUS testing at Naktuinbouw

*Autumn trial 2025 – cherry and cocktail tomato types*



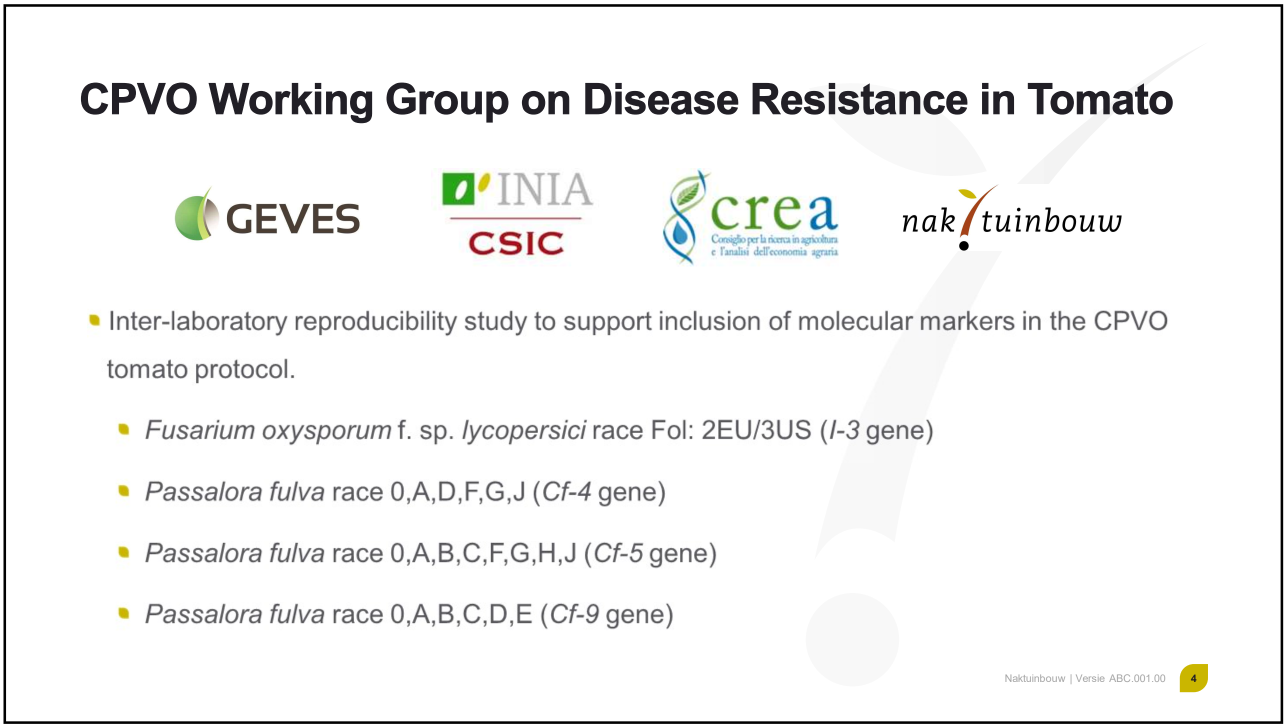
## Tomato DUS testing at Naktuinbouw

- Second highest crop in number of received applications per year.
- The number of similar varieties remains high and continues to increase each year, resulting in many varieties that still need to be grown side-by-side with the candidates.
- Grouping characteristics are reaching their limits in some tomato variety groups.

*CPVO Working Group on Disease Resistance in Tomato*

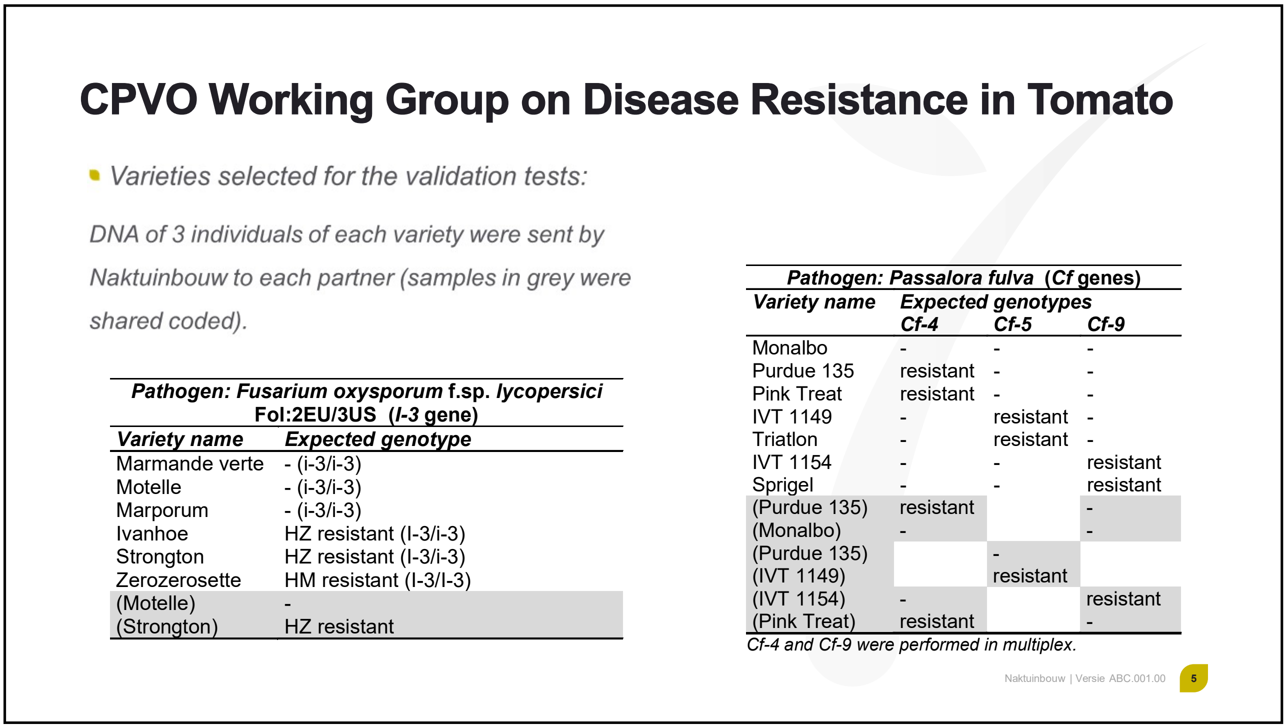
*Development and implementation of a SNP database*

*DNA based approach for varietal trueness in maintenance control*



## CPVO Working Group on Disease Resistance in Tomato

- Inter-laboratory reproducibility study to support inclusion of molecular markers in the CPVO tomato protocol.
  - *Fusarium oxysporum* f. sp. *lycopersici* race Fol: 2EU/3US (*I-3* gene)
  - *Passalora fulva* race 0,A,D,F,G,J (*Cf-4* gene)
  - *Passalora fulva* race 0,A,B,C,F,G,H,J (*Cf-5* gene)
  - *Passalora fulva* race 0,A,B,C,D,E (*Cf-9* gene)



# CPVO Working Group on Disease Resistance in Tomato

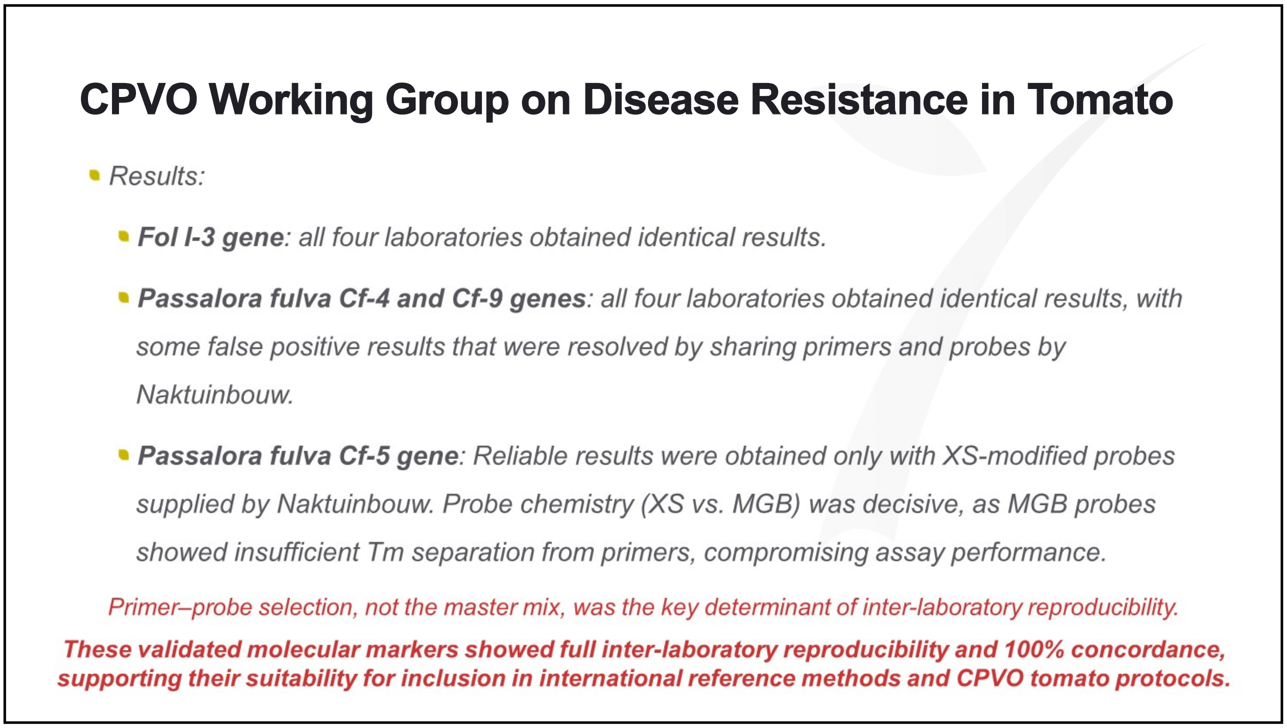
- *Varieties selected for the validation tests:*

*DNA of 3 individuals of each variety were sent by Naktuinbouw to each partner (samples in grey were shared coded).*

| ***Pathogen: Fusarium oxysporum* f.sp. *lycopersici* Fol:2EU/3US (*I-3* gene)** | |
|---|---|
| ***Variety name*** | ***Expected genotype*** |
| Marmande verte | - (i-3/i-3) |
| Motelle | - (i-3/i-3) |
| Marporum | - (i-3/i-3) |
| Ivanhoe | HZ resistant (I-3/i-3) |
| Strongton | HZ resistant (I-3/i-3) |
| Zerozerosette | HM resistant (I-3/I-3) |
| (Motelle) | - |
| (Strongton) | HZ resistant |

| ***Pathogen: Passalora fulva* (*Cf* genes)** | | | |
|---|---|---|---|
| ***Variety name*** | ***Expected genotypes*** | | |
| | ***Cf-4*** | ***Cf-5*** | ***Cf-9*** |
| Monalbo | - | - | - |
| Purdue 135 | resistant | - | - |
| Pink Treat | resistant | - | - |
| IVT 1149 | - | resistant | - |
| Triatlon | - | resistant | - |
| IVT 1154 | - | - | resistant |
| Sprigel | - | - | resistant |
| (Purdue 135) | resistant | | - |
| (Monalbo) | - | | - |
| (Purdue 135) | | - | |
| (IVT 1149) | | resistant | |
| (IVT 1154) | - | | resistant |
| (Pink Treat) | resistant | | - |

*Cf-4 and Cf-9 were performed in multiplex.*

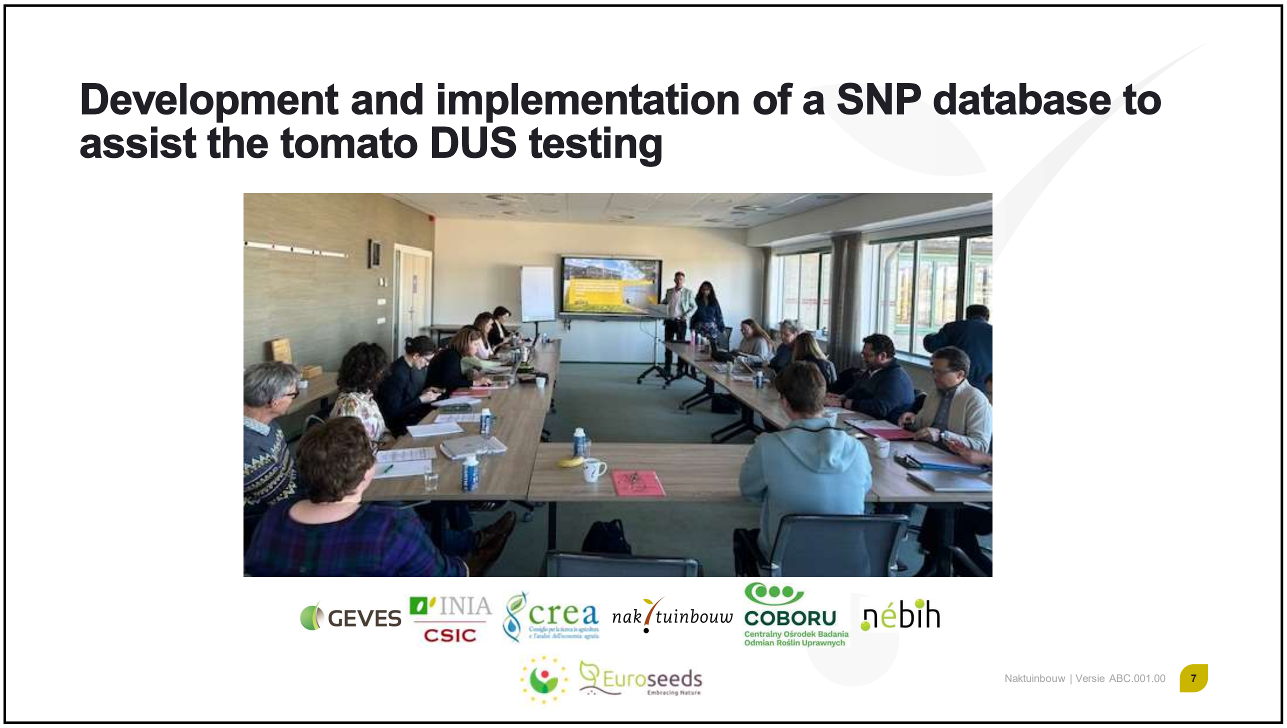


# CPVO Working Group on Disease Resistance in Tomato

- *Results:*
  - ***Fol I-3 gene****: all four laboratories obtained identical results.*
  - ***Passalora fulva Cf-4 and Cf-9 genes****: all four laboratories obtained identical results, with some false positive results that were resolved by sharing primers and probes by Naktuinbouw.*
  - ***Passalora fulva Cf-5 gene****: Reliable results were obtained only with XS-modified probes supplied by Naktuinbouw. Probe chemistry (XS vs. MGB) was decisive, as MGB probes showed insufficient Tm separation from primers, compromising assay performance.*

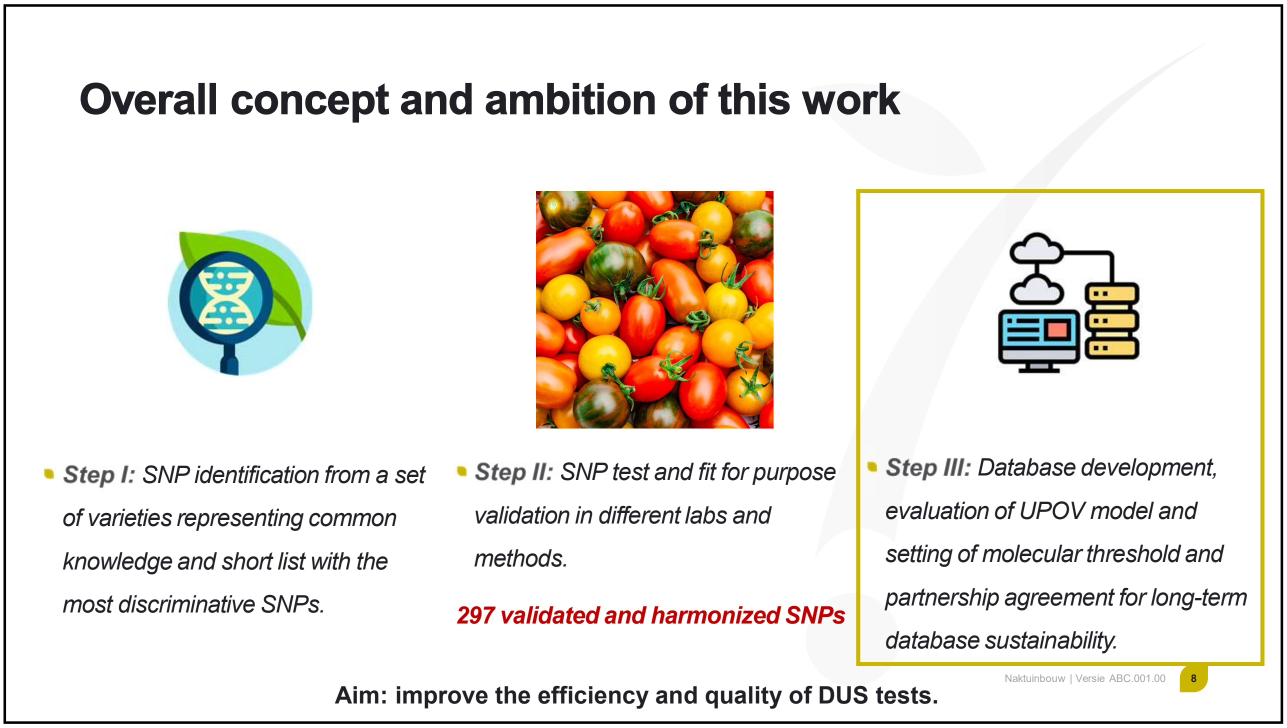
*Primer–probe selection, not the master mix, was the key determinant of inter-laboratory reproducibility.*

***These validated molecular markers showed full inter-laboratory reproducibility and 100% concordance, supporting their suitability for inclusion in international reference methods and CPVO tomato protocols.***



# Development and implementation of a SNP database to assist the tomato DUS testing

GEVES INIA CSIC crea nak tuinbouw COBORU Centralny Ośrodek Badania Odmian Roślin Uprawnych nébih Euroseeds Embracing Nature



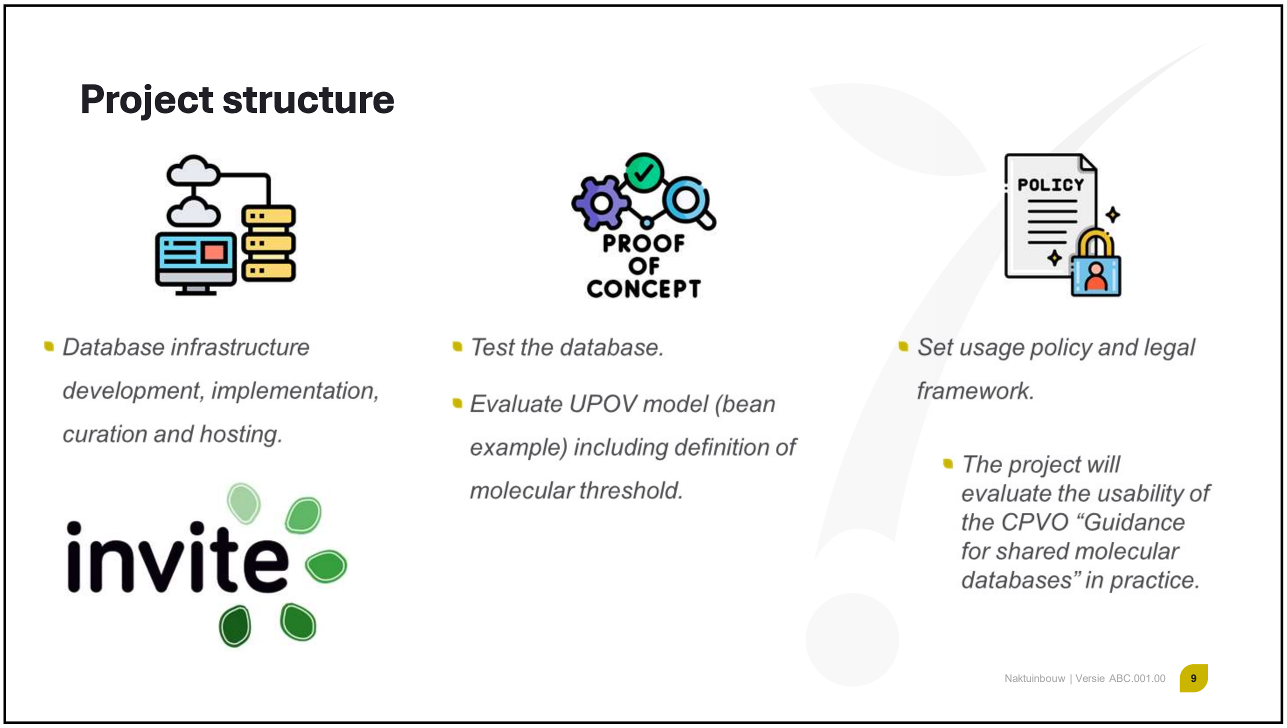
# Overall concept and ambition of this work

- **Step I:** *SNP identification from a set of varieties representing common knowledge and short list with the most discriminative SNPs.*
- **Step II:** *SNP test and fit for purpose validation in different labs and methods.*

***297 validated and harmonized SNPs***

- **Step III:** *Database development, evaluation of UPOV model and setting of molecular threshold and partnership agreement for long-term database sustainability.*

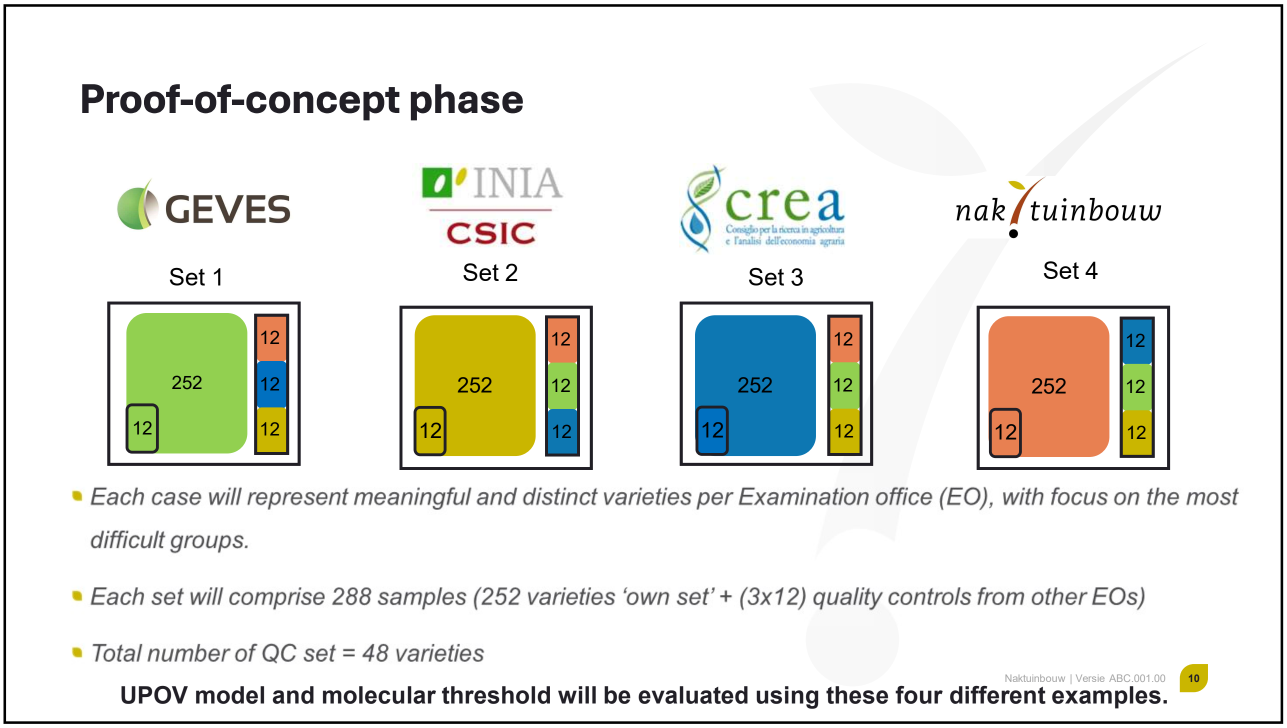
**Aim: improve the efficiency and quality of DUS tests.**



## Project structure

- *Database infrastructure development, implementation, curation and hosting.*
- *Test the database.*
- *Evaluate UPOV model (bean example) including definition of molecular threshold.*
- *Set usage policy and legal framework.*
- *The project will evaluate the usability of the CPVO "Guidance for shared molecular databases" in practice.*

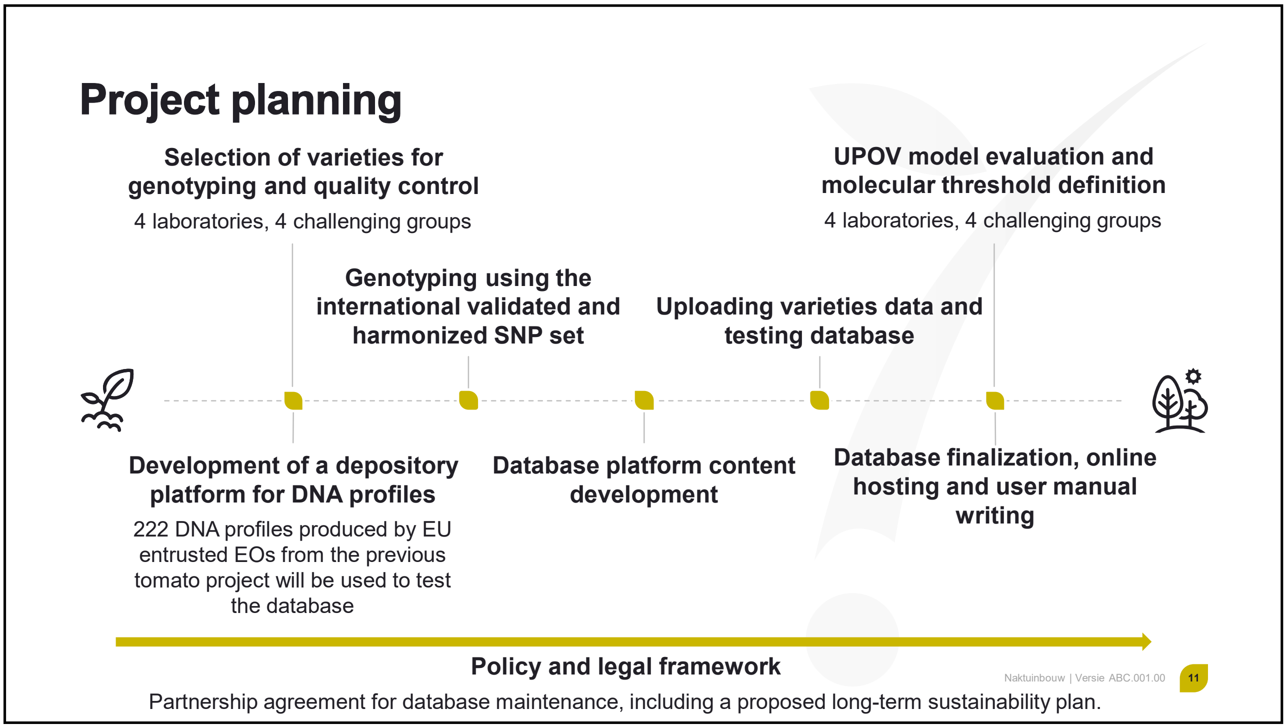
invite



## Proof-of-concept phase

- *Each case will represent meaningful and distinct varieties per Examination office (EO), with focus on the most difficult groups.*
- *Each set will comprise 288 samples (252 varieties 'own set' + (3x12) quality controls from other EOs)*
- *Total number of QC set = 48 varieties*

**UPOV model and molecular threshold will be evaluated using these four different examples.**



# Project planning

**Selection of varieties for genotyping and quality control**

4 laboratories, 4 challenging groups

**Genotyping using the international validated and harmonized SNP set**

**Uploading varieties data and testing database**

**UPOV model evaluation and molecular threshold definition**

4 laboratories, 4 challenging groups

**Development of a depository platform for DNA profiles**

222 DNA profiles produced by EU entrusted EOs from the previous tomato project will be used to test the database

**Database platform content development**

**Database finalization, online hosting and user manual writing**

**Policy and legal framework**

Partnership agreement for database maintenance, including a proposed long-term sustainability plan.

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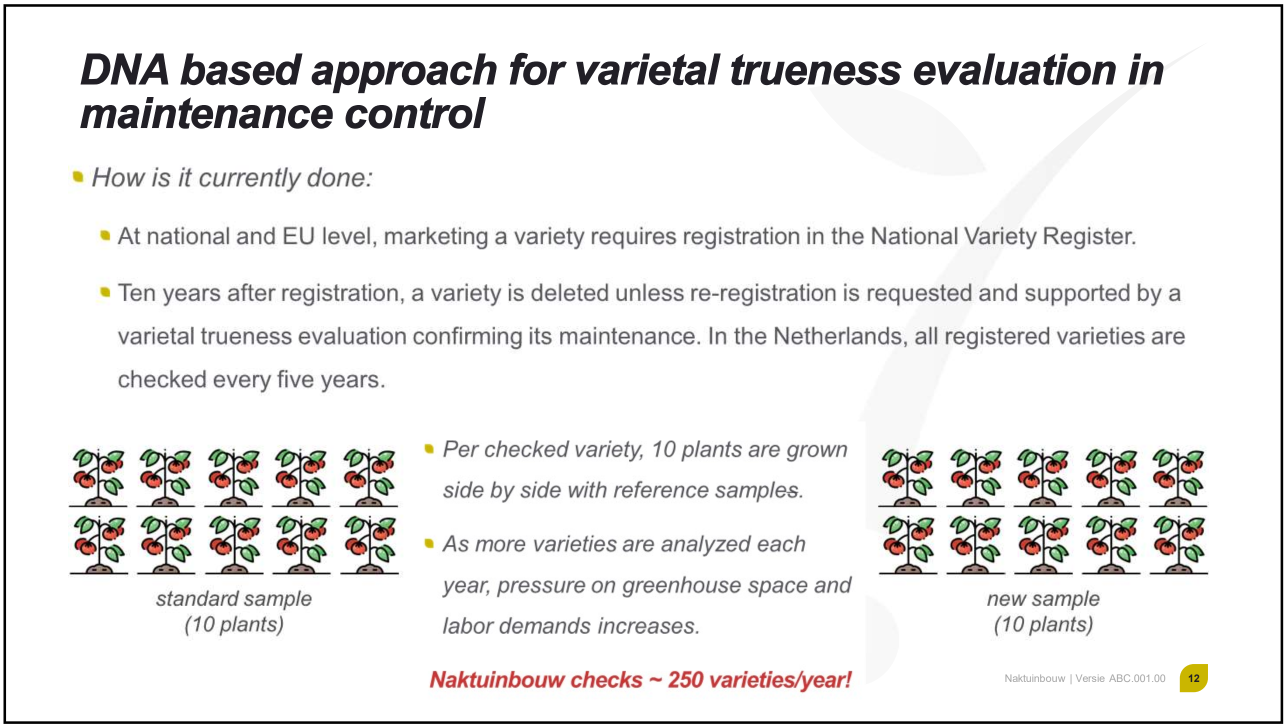

# *DNA based approach for varietal trueness evaluation in maintenance control*

- *How is it currently done:*
  - At national and EU level, marketing a variety requires registration in the National Variety Register.
  - Ten years after registration, a variety is deleted unless re-registration is requested and supported by a varietal trueness evaluation confirming its maintenance. In the Netherlands, all registered varieties are checked every five years.

*standard sample (10 plants)*

- *Per checked variety, 10 plants are grown side by side with reference samples.*
- *As more varieties are analyzed each year, pressure on greenhouse space and labor demands increases.*

*new sample (10 plants)*

***Naktuinbouw checks ~ 250 varieties/year!***

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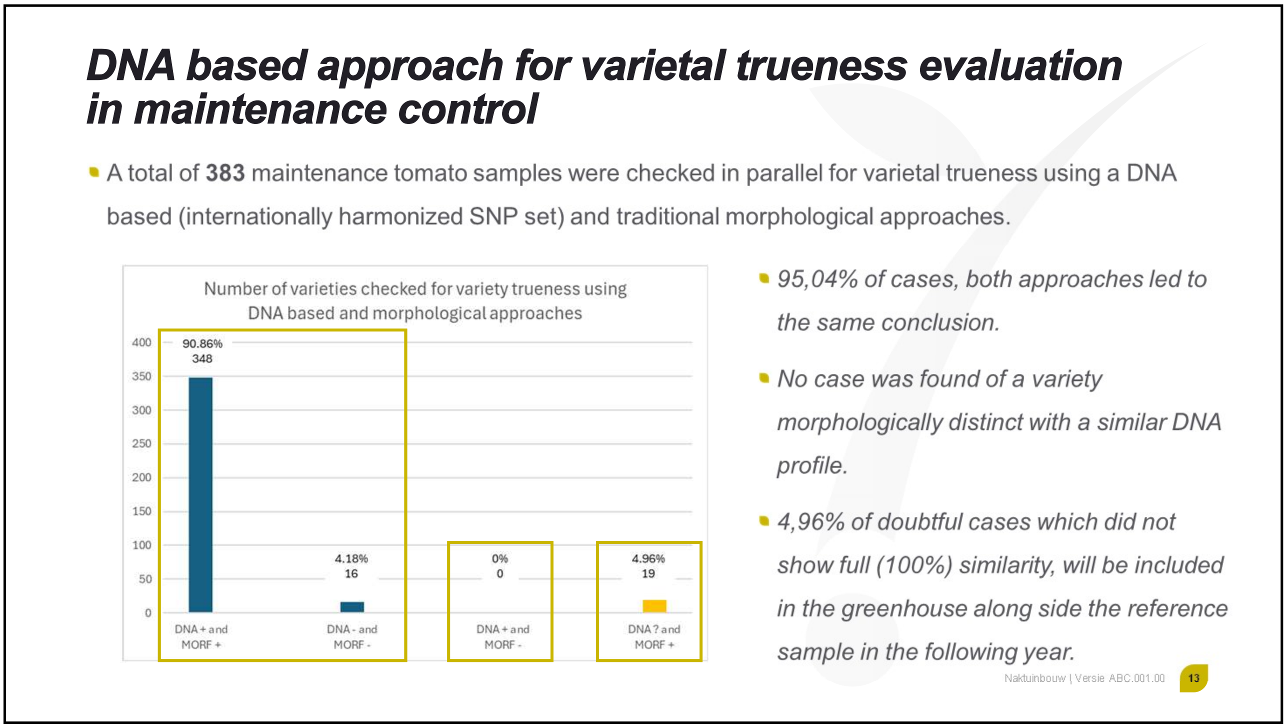
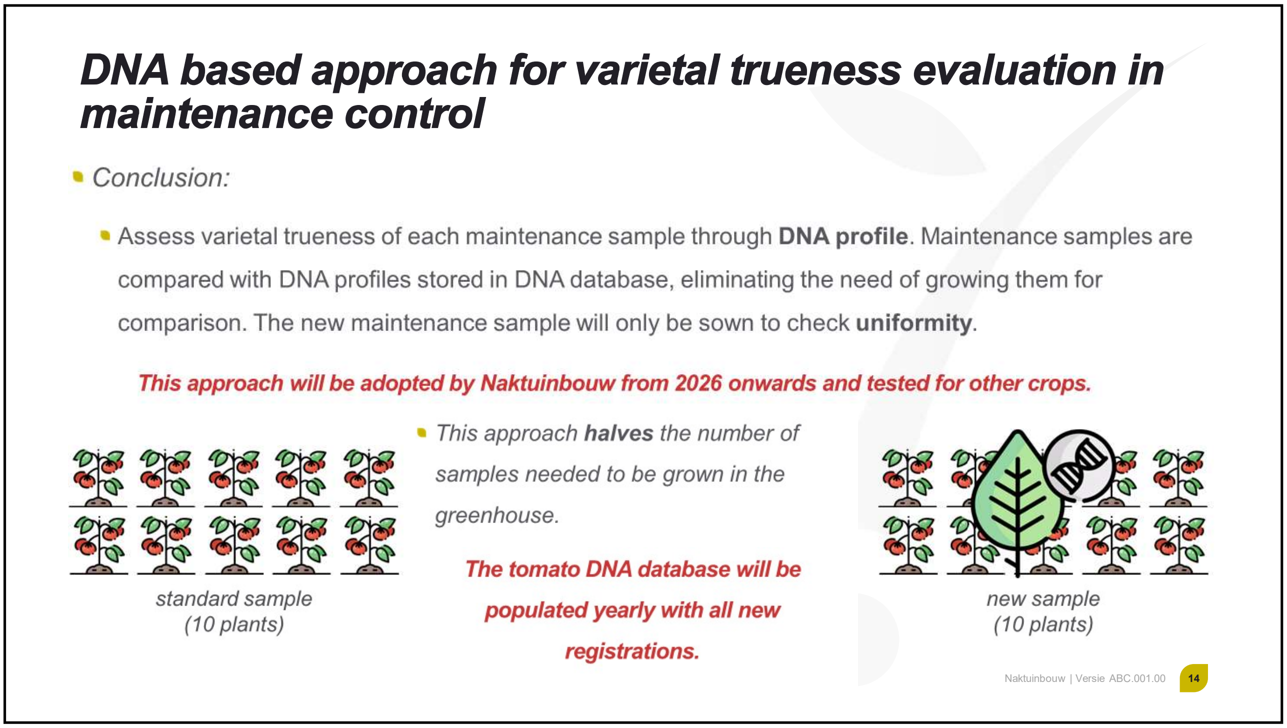


# DNA based approach for varietal trueness evaluation in maintenance control

- A total of **383** maintenance tomato samples were checked in parallel for varietal trueness using a DNA based (internationally harmonized SNP set) and traditional morphological approaches.

Number of varieties checked for variety trueness using DNA based and morphological approaches

- *95,04% of cases, both approaches led to the same conclusion.*
- *No case was found of a variety morphologically distinct with a similar DNA profile.*
- *4,96% of doubtful cases which did not show full (100%) similarity, will be included in the greenhouse along side the reference sample in the following year.*



# DNA based approach for varietal trueness evaluation in maintenance control

- *Conclusion:*
  - Assess varietal trueness of each maintenance sample through **DNA profile**. Maintenance samples are compared with DNA profiles stored in DNA database, eliminating the need of growing them for comparison. The new maintenance sample will only be sown to check **uniformity**.

***This approach will be adopted by Naktuinbouw from 2026 onwards and tested for other crops.***

*standard sample*
*(10 plants)*

- *This approach **halves** the number of samples needed to be grown in the greenhouse.*

***The tomato DNA database will be populated yearly with all new registrations.***

*new sample*
*(10 plants)*

## Thank you for your attention

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[End of Annex and of document]