UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party on Testing Methods and Techniques **TWM/4/26 Rev.**

Fourth Session
Cambridge, United Kingdom, June 2 to 5, 2026

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CPVO CO-FUNDED PROJECT: INTERNATIONAL VALIDATION OF A SNP MARKER SET FOR TOMATO DUS TESTING

Document prepared by experts from the European Union and the Netherlands (Kingdom of)

Disclaimer: this document does not represent UPOV policies or guidance

The annex to this document contains a presentation “CPVO co-funded project: International validation of a SNP marker set for Tomato DUS testing”, made by experts from the European Union and the Netherlands (Kingdom of), at the fourth session of the TWM.

[Annex follows]

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CPVO R&D Strategy

GUIDING VALUES
Quality, Cost-Effectiveness, Harmonisation, Timeliness and Accountability.

STRATEGIC GOALS

Support R&D projects with the aim :
- to optimize DUS testing within the CPVO network of Examination Offices
- to strengthen EU Plant Variety Rights (PVR) and facilitate enforcement

Support the development of improved varieties in line with the EU Commission policies (Farm to Fork, Green Deal, ..)

Exercise a network function for enhanced cooperation between European MS and with other international partners.

CPVO
Community Plant Variety Office

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IMODDUS working group

(**I**ntegration of **MO**lecular **D**ata into **DUS** testing)

- CPVO BMT working group set up by CPVO Administrative Council in 2016.
- works as a **think-tank** for the development of strategies supporting the integration of BMT into DUS testing.
- IMODDUS assists CPVO in assessing **R&D projects proposals** for species where BMT could improve the **quality** and **cost efficiency of DUS testing**.

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Molecular markers in support of DUS testing for CPVO

- UPOV models for "Combining phenotypic and molecular distance in the management of variety collections" (UPOV TGP/15) :
  applied by each EO individually, possibly harmonized between several EU EOs
  - Selection of varieties to reduce the trial sizes : similar varieties vs super distinct varieties.
  - Acceleration of the procedure : decision on Distinctness in one growing cycle
  - Variety identification (maintenance of reference samples, control of hybrid formulae, …)
- Harmonized characteristics-specific markers included in CPVO TPs / UPOV TGs :
  Detection of specific gene/alleles correlated to characterisitcs used :
  - to replace phenotypic observations → accelerate DUS testing
  - as grouping characteristics (disease resistances, ..) → optimize trial design
- Support to PVR enforcement (legal procedures), to certification and controls

CPVO objectives : ensure quality, optimize cost-efficiency & accelerate DUS testing.

→Need for specific databases to store and use molecular data
(additional cost being balanced by cost gain from the use of markers)

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Overview of validated markers and shared molecular databases set up through IMODDUS projects co-funded by CPVO

+ Lettuce to come...

	Potato	Cannabis	Durum wheat	Tomato	Rapeseed	Hydrangea
Content	adm/morpho (simplified)/molec (SSR)	adm/molec (SNP)	adm/molec (SNP)	adm/molec (SNP)	adm/ molec (SNP)	adm/ molec (SNP)
Molec data production	2 EOs acting as service providers	In house	1 service provider	each EO using his own method	1 service provider	In house
Format	Common	Individual (centralized testing)	Common	Common	Common	Individual
Access/Use	Restricted to contributors / results delivered by labs	Restricted to contributors / % similarity calculated in-house	Restricted to contributors / GD calculated online, adjustable threshold	Restricted to contributors / % similarity in DB	Restricted to contributors / GD calculated in-house	Restricted to contributors / GD calculated in-house
Applications/ Gain	Identification, management of collection (no reduction in trial size, better selection of comparators)	Identification, management of collection (ease material submission)	Management of collection (no reduction in trial size, better selection of comparators)	Identification, management of collection	Management of collection (reduction in trial size)	Identification, management of collection (reduction in trial size)
Maintenance / curating	- Agreement - Rotating coordination - Curating by labs - Cost by each partner	In house	- Agreement - Coordination and curating by AGES - Cost by each partner	- Agreement to come - Coordination and curating by NAKT	- Agreement to come - Coord and curating? - Cost by each partner	- In house by GEVES
Partners	AGES (AT), UKZUZ (CZ), BSA (DE), OEVV (ES), NAKT (NL), COBORU (PL), UKSUP (SK), SASA (UK), Dep Agri Food (IE)	NAKT (NL) NEBIH (HU)?	AGES (AT), INIIA-OEVV (ES), CREA (IT), NEBIH (HU)	NAKT (NL), GEVES (FR), COBORU (PL), NEBIH (HU), INIA-OEVV (ES), DGAV (PT), CREA (IT) + CN, JP, KR	GEVES (FR), BSA (DE), COBORU (PL), UKSUP (SK), , Tystoft (DK)	GEVES (FR) BSA (DE)

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Tomato

FINISHED

CPVO co-funded projects

"*International harmonisation and validation of a SNP for the management of tomato variety collection*"

- Coordinator: **Naktuinbouw**
- Project partners:
  - EU partners: CPVO, Euroseeds, COBORU (PL), NÉBIH (HU), DGAV (PT)
  - EU lab partners: CREA (IT), GEVES (FR), INIA-CSIC (ES), Naktuinbouw (NL)
  - International lab partners (participate on their own funding) :
    - Beijing Sub-Center for DUS testing (IVF-CAAS), China
    - Korean Seed & Variety Service, Rep. of Korea
    - Center for Seeds and Seedlings, NARO (NCSS), Japan
- Granted co-financing in February 2019

Duration : 30 months + extension (breeders' consents) (July 2019 - Dec 2023)

(report available online, https://cpvo.europa.eu/en/about-us/what-we-do/research-and-development)

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«Tomato DB»

STARTED

"Development and implementation of an internationally accepted SNP database to assist the tomato DUS testing"

- Follow-up project for complete characterization of the collection and application following UPOV model "Combining phenotypic and molecular distances in the management of variety collections" (French bean approach).
- Coordinator: **Naktuinbouw**
- Project partners:
  - EU partners: CPVO, Euroseeds, COBORU (PL), NÉBIH (HU)
  - EU lab partners: CREA (IT), GEVES (FR), INIA-CSIC (ES), Naktuinbouw (NL)
- Granted co-financing in September 2025
  Duration : 24 months (01/03/2026 – 28/02/2028)

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Projects aim

- ***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.*
- ***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.

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Objective first project

Development of an international validated and harmonized tomato SNP set, suitable for DUS testing.

Validated: the initial SNP set is tested within all participating laboratories and screened for the SNPs giving the same consistent results.

*Harmonized: SNPs that are consistent regardless of **where** (different laboratories) or **when** (in time) or **how** (different genotyping technologies) they are selected to form the final SNP set.*

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Step I: SNPs detection in a representative set of tomato varieties

- *50K Illumina Axiom array was used to genotype approx. 200 tomato varieties roughly representing the common knowledge:*
  - *Broad genetic diversity (all types and characteristics)*
  - *Morphologically close but distinct varieties (i.e. variety pairs that might have caused some discussion in the DUS test and/or an extra year of testing was required to consider them distinct).*
  - *Varieties originated from different EU countries, different companies (germplasms)*

- *Selection of ~500 most discriminative SNPs, considering:*
  - *No multimapping*
  - *Closely linked (min. 1000bp separation)*

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Step II: Sharing information

- *SNP selection list with positions on reference genome and flanking sequences 100bp upstream and downstream of its position.*
- *Information on used genotyping methods by each partner.*
- *MTA document to all partners.*

At kick-off meeting: *Define variety selections to start seeking breeder's consent requests.*

- *Global Training set*
- *EU Training set*

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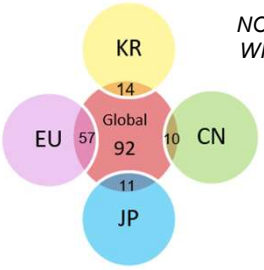
Step II: Global Training set

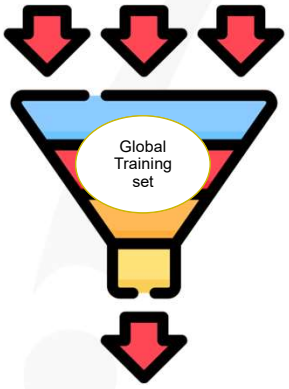
- *Broad genetic diversity*
- *All types, all characteristics*
- *Varieties that are morphological close, but distinct*
- *Different germplasm*
- *Cultivated only, no wild species*



NO OVERLAP WITH EU SET

First SNP selection = 500 SNPs

Partner B *KASP*	***Partner D*** *KASP*	***Partner F*** *Agri-Seq®*
Partner A *KASP*	***Partner C*** *SeqSNP*	***Partner E*** *SeqSNP*	***Partner G*** *GT-Seq*



First round of SNP filtering

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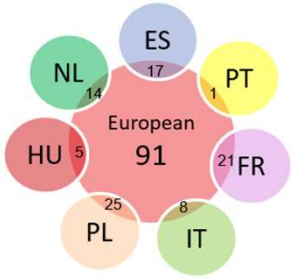
Step II: EU Training set

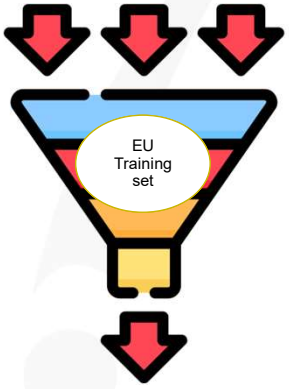
- *Broad genetic diversity*
- *All types, all characteristics*
- *Varieties that are morphological close, but distinct*
- *Different germplasm*
- *Cultivated only, no wild species*



NO OVERLAP WITH GLOBAL SET

First SNP selection = 500 SNPs

	Partner D *KASP*	***Partner F*** *Agri-Seq®*
Partner C *SeqSNP*		***Partner G*** *GT-Seq*



First round of SNP filtering

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Step II: conclusions on the initial 500 SNP set

- *The number of successful SNP assays varied considerably between the 7 partners.*
- *For most partners, when the number of varieties exceeded 80, the number of successful SNPs decreased. As a result, SNP datasets typically contained missing data for approximately 0–20 varieties.*
- *The varieties with missing data differed between partners and appeared to be randomly distribute.*

  These results do not identify a preferred technology or genotyping method.

  Genotyping failure is not driven by DNA quality.
- *When comparing genotyping performance across EU partners, similar results were observed for both the GLB and EU datasets, indicating a high level of consistency between partners.*

  SNP success is independent of the set of varieties used.

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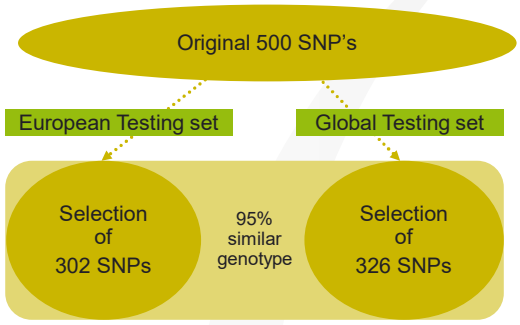
Step II: Proposed SNP selection for validation

- *Based on partner and SNP performance:*
  - *Ability to generate SNP call*
  - *≥ 95% average similarity on scores*



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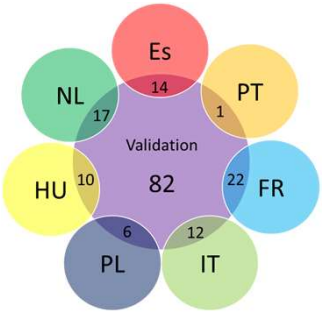
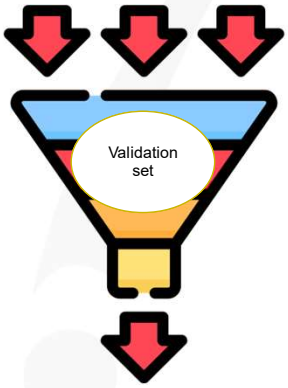
First round of SNP filtering = 326 SNPs from Global set
302 SNPs from EU set

Step II: SNP selection validation

- *Fit for purpose analysis*
- *Biological and technical replicates*
- *Repeatability to define consistency within an experiment*
- *Reproducibility to define consistency between experiments*

Partner B *KASP*	***Partner D*** *KASP*	***Partner F*** *Agri-Seq®*	
Partner A *KASP*	***Partner C*** *SeqSNP*	***Partner E*** *SeqSNP*	***Partner G*** *GT-Seq*





Harmonized and validated SNP set

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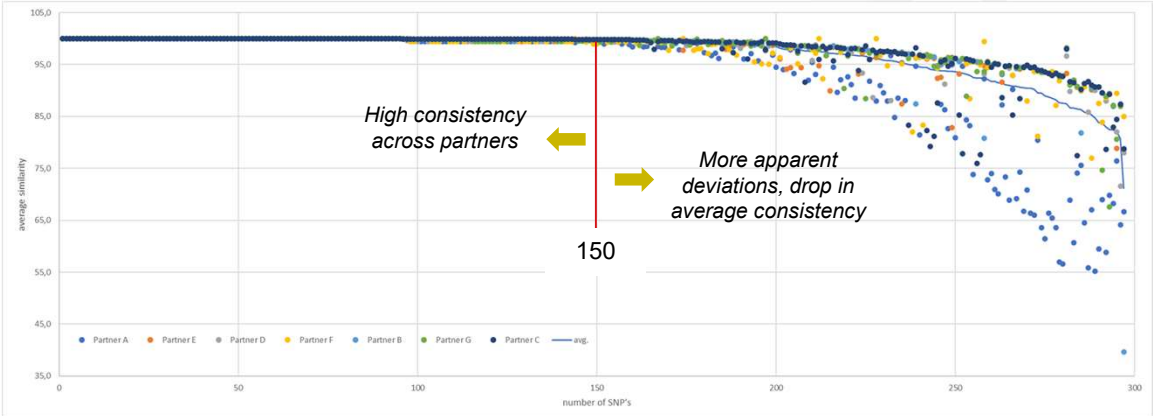
Consistency of SNPs



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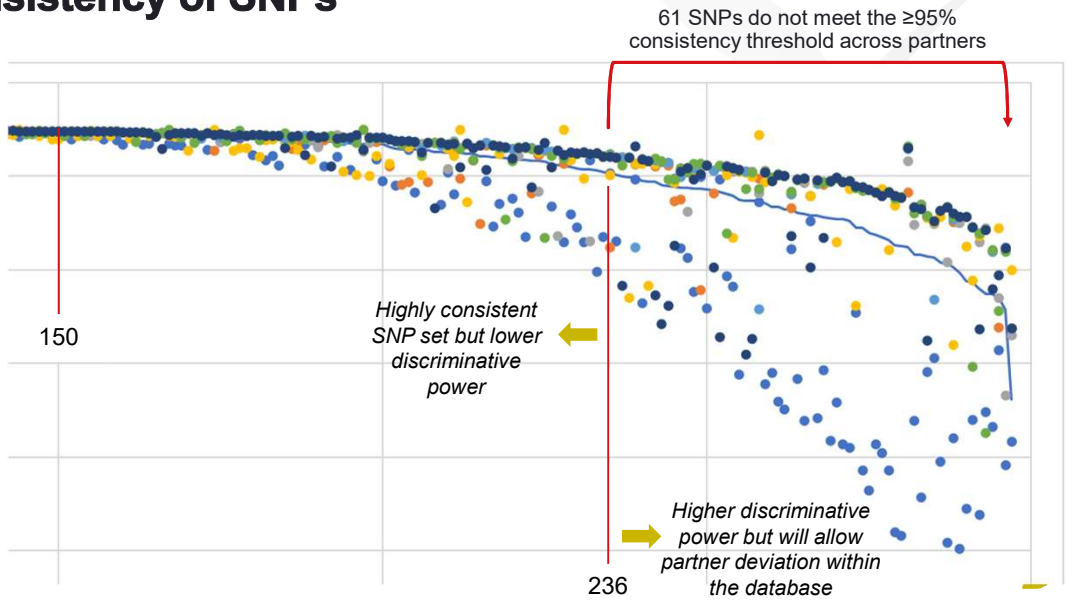
Consistency of SNPs



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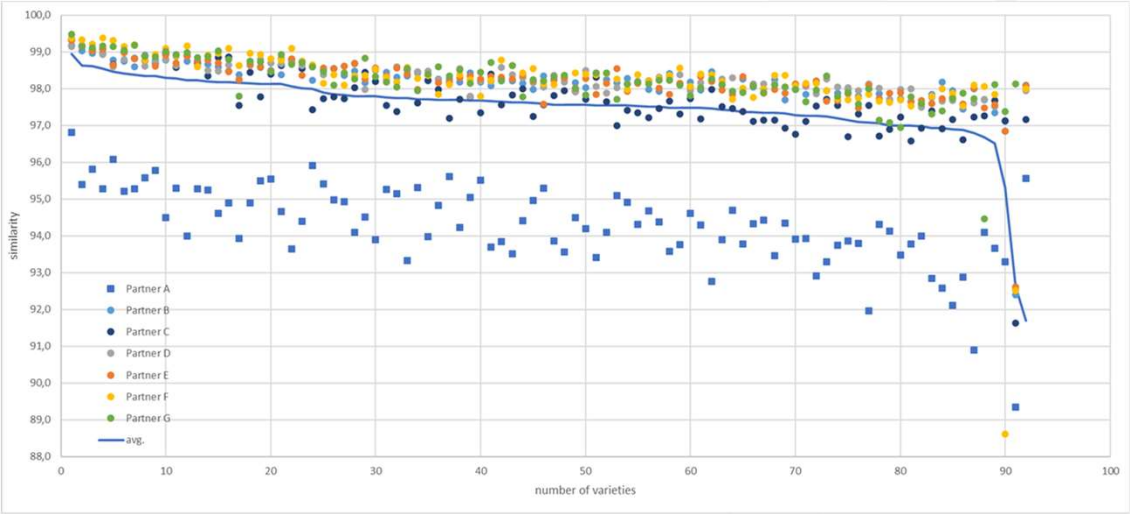
Consistency of genotypes



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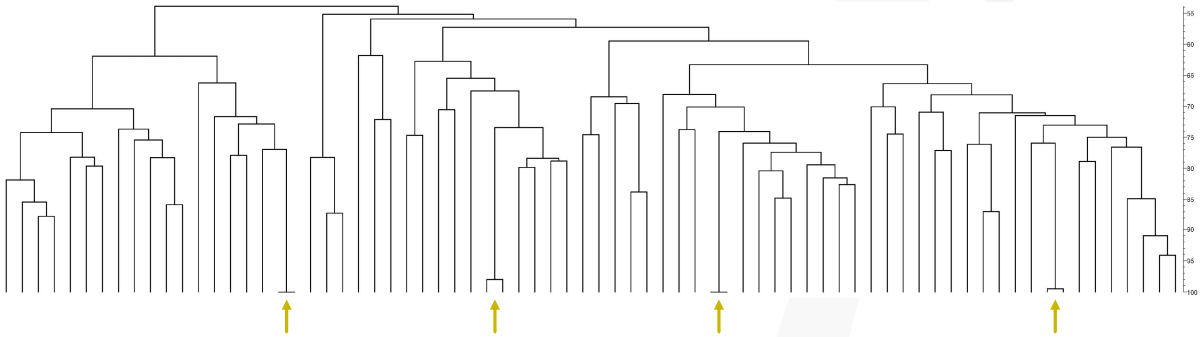
Genetic distances of Validation set



High similar varieties based on genetics (indicated with arrows), explainable by morphology, highly similar or single resistance difference

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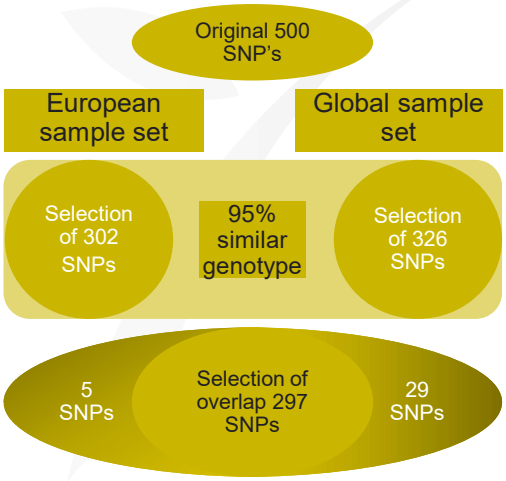
Conclusions

- Final set : 297 SNPs
- Markers publicly available (CPVO website)
- Scientific publication at Prophyta 2024



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Lessons learned early on

Legal framework

Finalizing the legal framework on this scale takes a lot time and should not be underestimated in future projects

Consent

Breeders and companies are willing to work with us and help wherever they can, but are critical and obtaining consent may take some time

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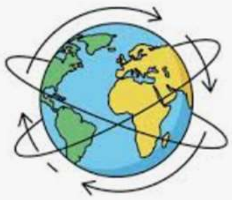
Question now



Markers validated in the EU, China, Rep. of Korea, Japan

→ **Interest of other UPOV Members in the world ?**

(markers freely accessible on CPVO web site)

Make it a **UPOV standard** ?

Recognition by other organizations (**ISTA, OECD**) ?

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[End of Annex and of document]