CHINA

The EU H2020 INVITE project "Innovations in plant Variety Testing in Europe"

Francois Laurens, INRAE France / coordinator of the INVITE project
Joseph Peller, Wageningen, Netherlands / WP2 co-leader
Karl Schmid, University of Hohenheim, Germany / WP3 leader
Aurélia Gouleau, GEVES, France / WP5 leader

Plant Variety Rights online technical training for Chinese experts; 28 Nov 2023

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Directed by the European Commission, IP Key is implemented by the European Union Intellectual Property Office (EUIPO). IP Key由欧盟委员会指导，欧盟知识产权局（EUIPO）实施。

CHINA

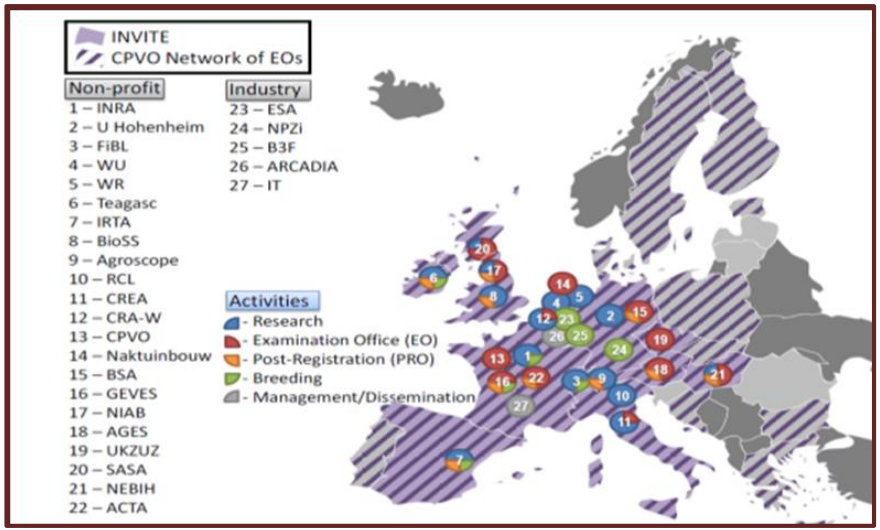
Agenda:

- General introduction on the INVITE project (F. Laurens)
- Designing new tools for phenotyping using Human Centered Design (J. Peller)
- Designing genotyping tools to better characterize varieties and their performance (K. Schmid)
- Integration of new tools in advanced variety testing (A. Gouleau)
- Concluding remarks (F. Laurens)

The INVITE project as a glance

Funded by the European Union

Duration: 5 years : 1/01/2020 – 31/12/2024



29 partners
Consortium ≈ 180 members
From 13 countries
- Academic research
- Breeders
- Technical institutes
- Examination Offices (Eos)
- Post-registration Offices (Pros)
- Management
- Dissemination

General Objective and frame of the INVITE project

- The general objective of the INVITE project is to **improve both efficiency of variety testing** and the **information available to stakeholders** on variety performance under a **range of production conditions and biotic and abiotic stresses.**

- The INVITE project is working on a small but representative set of selected crops:

Maize 

Wheat 

Sunflower 

Perennial Ray Grass 

Apple 

Tomato 

Potato 

+ Soybean 

Lucerne 

oilseed rape 

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Specific objectives

- Identify **bioindicators** associated with **plant resource use efficiency, sustainability and resilience**
- Develop **new phenotyping** and **genotyping tools**
- Implement **models and statistical** tools allowing **to predict variety performance** under a **range of environments and crop management practices**, while considering the economic return for farmers
- **Improve existing variety testing protocols for variety characterisation (DUS) and performance** testing to enhance speed, precision and efficiency
- Define new procedures for the **management of reference collections**
- Propose **organisational innovations** to improve the management of variety testing networks
- Propose **guidelines to policy makers** for including new traits and improving harmonisation of DUS and VCU at EU-level, and for the testing of heterogeneous plant reproductive material
- Facilitate data interoperability and exchanges within the consortium and set up a **prototype of common DB** to store phenotypic and genotypic data
- **Disseminate** results and new technologies **to stakeholders**

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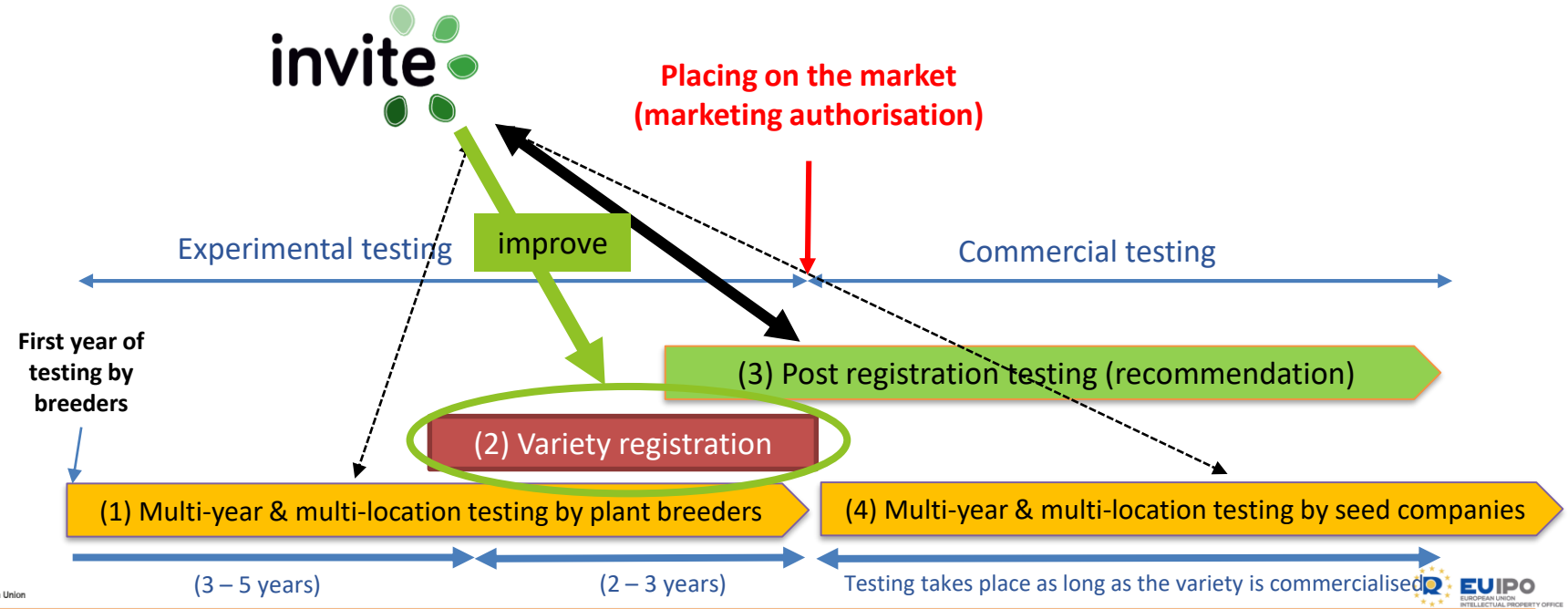
Context of variety testing Europe and positioning of INVITE

The complementary components of plant variety testing (principles)



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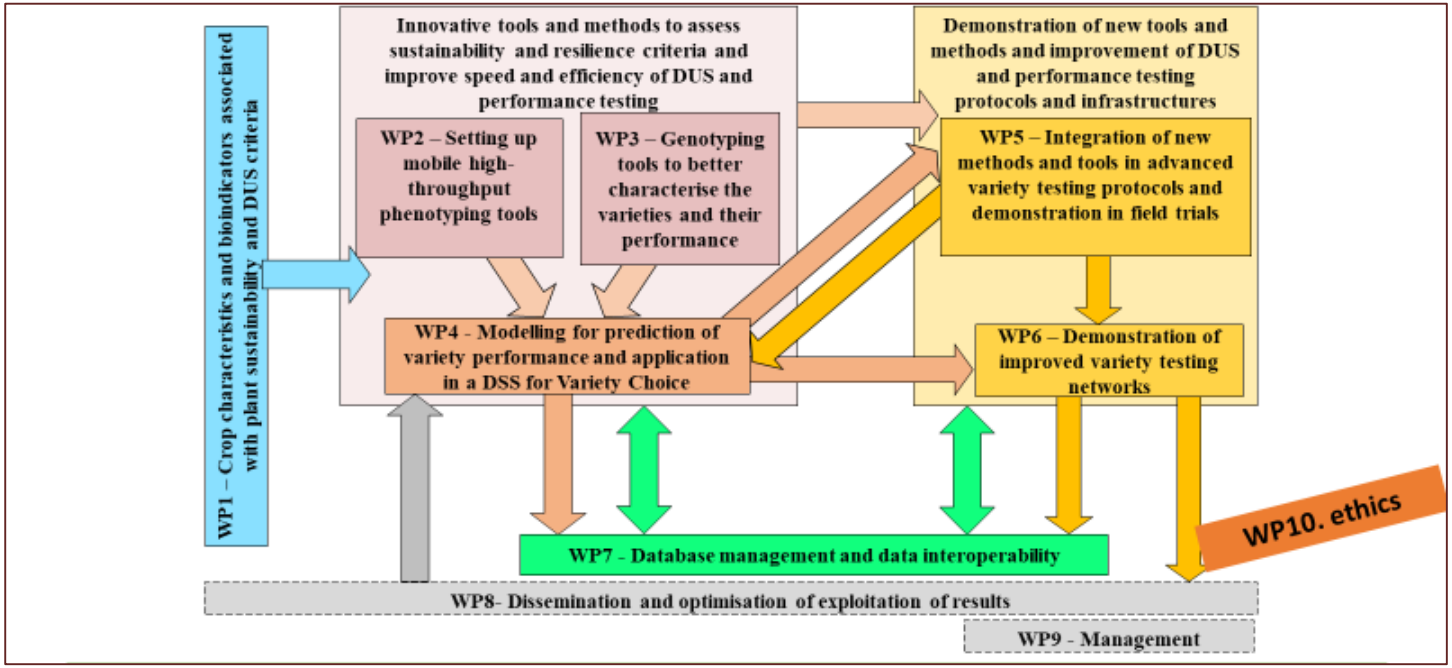
Structure of the INVITE project



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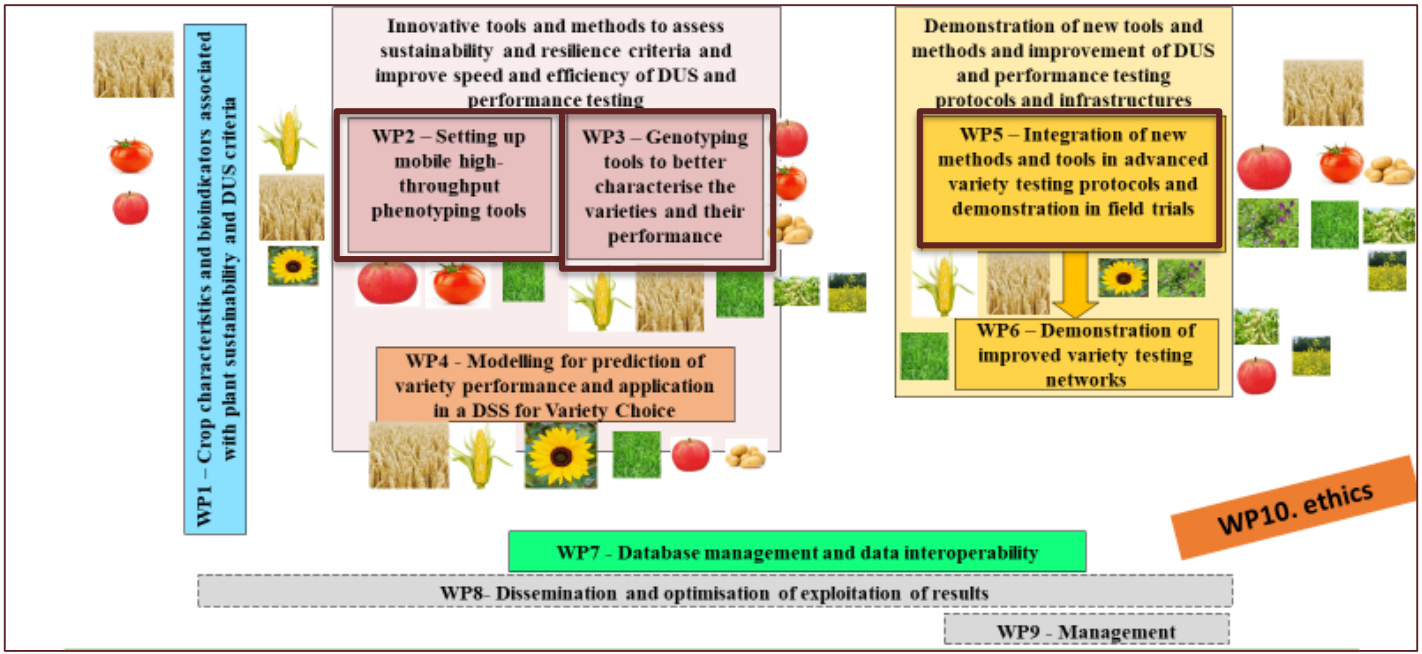
Crops in the project



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CHINA

Designing new tools for phenotyping using Human Centered Design

Joseph Peller | Wageningen, Netherlands | 28-11-2023

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www.ipkey.eu

EUIPO EUROPEAN UNION INTELLECTUAL PROPERTY OFFICE

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Objectives of WP2

To create New Low Cost Phenotyping tools

To make available these tools to Eos and Pros in WP5 (cf A. Gouleau part)

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The Problem with Tool Development

CultivarJ ImageJ Plugin for Plant Variety testing

WAGENINGEN UR
For quality of life

nak tuinbouw

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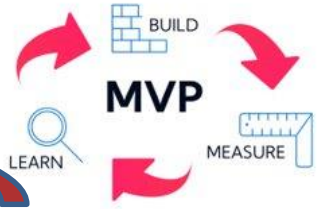
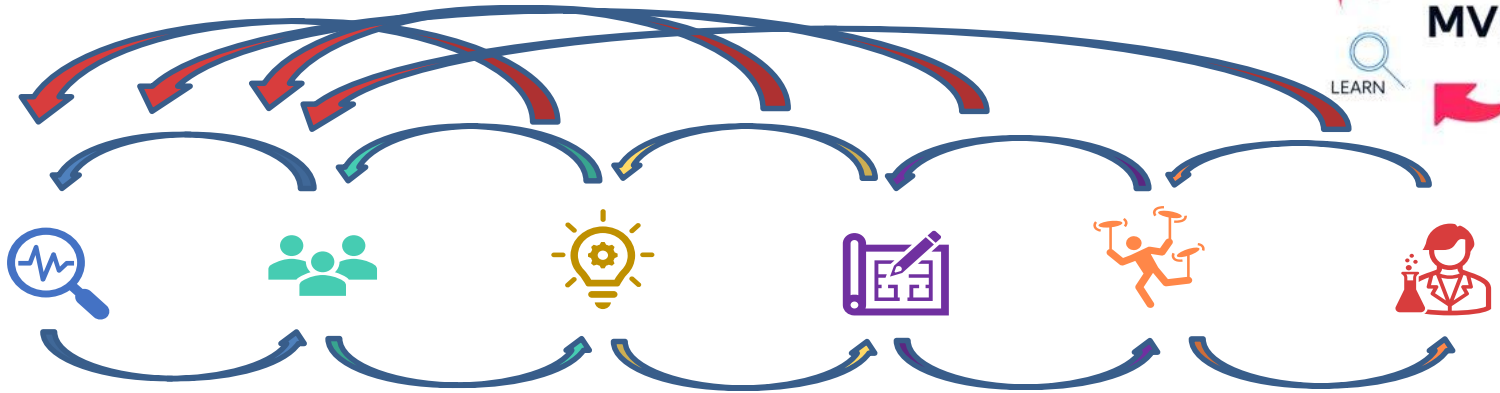
Human Centric Design





User Research

- ✓ Identify users
- ✓ Needs
- ✓ Behaviour
- ✓ Challenges
- ✓ Pain points
- ✓ Desires
- ✓ Values and norms

Create Personas

- ✓ Fictional representers of users

Concept development

- ✓ Generate ideas
- ✓ Brainstorm ideas
- ✓ Consider needs and technical restrictions
- ✓ Consider business and ELSA aspects
- ✓ Match the values
- ✓ interdisciplinary

Design and demonstrate

- ✓ Prototypes
- ✓ Sketches
- ✓ Visuals

Implement and evaluate

- ✓ Identify evaluation method
- ✓ Newer version
- ✓ Involve multiple stakeholders

Test

- ✓ With end-users (farm workers)
- ✓ Feedback
- ✓ With other stakeholders
- ✓ Observe

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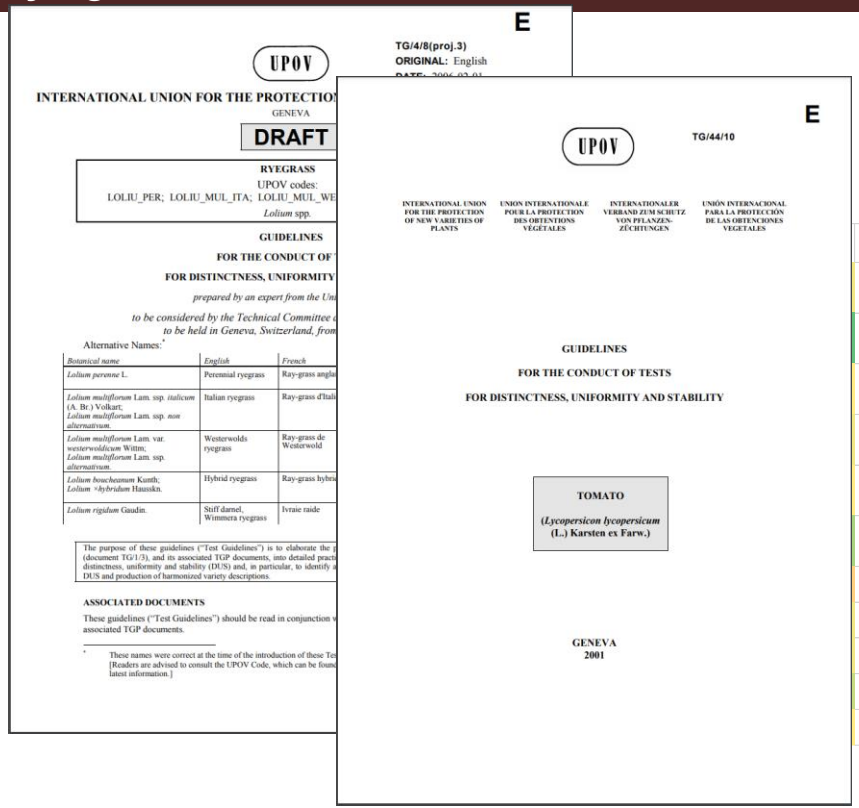
Surveying and Feedback



E

UPOV

TG/4/8(proj.3)
ORIGINAL: English

INTERNATIONAL UNION FOR THE PROTECTIO

GENEVA

DRAFT

RYEGRASS

UPOV codes:
LOLIU_PER; LOLIU_MUL_ITA; LOLIU_MUL_WE

Lolium spp.

GUIDELINES

FOR THE CONDUCT OF

FOR DISTINCTNESS, UNIFORMITY

prepared by an expert from the Uni

to be considered by the Technical Committee
to be held in Geneva, Switzerland, from

Alternative Names:*

Botanical name	*English*	*French*
Lolium perenne L.	Perennial ryegrass	Ray-grass angla
Lolium multiflorum Lam. ssp. *italicum* (A. Br.) Volkart; *Lolium multiflorum* Lam. ssp. *non alternativum*.	Italian ryegrass	Ray-grass d'Ital
Lolium multiflorum Lam. var. *westerwoldicum* Wittm.; *Lolium multiflorum* Lam. ssp. *alternativum*.	Westerwolds ryegrass	Ray-grass de Westerwold
Lolium boucheanum Kunth; *Lolium* ×*hybridum* Hausskn.	Hybrid ryegrass	Ray-grass hybri
Lolium rigidum Gaudin.	Stiff darnel, Wimmera ryegrass	Ivraie raide

The purpose of these guidelines ("Test Guidelines") is to elaborate the p
(document TG/1/3), and its associated TGP documents, into detailed practi
distinctness, uniformity and stability (DUS) and, in particular, to identify
DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These guidelines ("Test Guidelines") should be read in conjunction w
associated TGP documents.

* These names were correct at the time of the introduction of these Te
[Readers are advised to consult the UPOV Code, which can be foun
latest information.]

E

UPOV

TG/44/10

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS | UNION INTERNATIONALE POUR LA PROTECTION DES OBTENTIONS VÉGÉTALES | INTERNATIONALER VERBAND ZUM SCHUTZ VON PFLANZEN-ZÜCHTUNGEN | UNIÓN INTERNACIONAL PARA LA PROTECCIÓN DE LAS OBTENCIONES VEGETALES

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

TOMATO

(*Lycopersicon lycopersicum* (L.) Karsten ex Farw.)

GENEVA
2001

Trait Name	Difficulty Of Measure	Time Needed to	Importance to Discrimin	Ranking
Leaf:Type of blade	9	8	9	26
Leaf: Size of Leaflets	4	7	2	13
Leaf:Intensity of green color	4	7	1	12
Leaf: Glossiness	3	7	7	17
Leaf: Blistering	3	7	7	17
Leaf: Attitude of petiole	8	7	4	19
Inflorescence: type	8	5	5	18
Flower: Colour	7	7	7	21
Flower: Pubescence of style	5	5	9	19
Fruit: Glossiness of Skin	4	7	4	15
Time of Maturity	7	1	2	10

Trait Name	Difficulty Of Measure	Time Needed to	Importance to Discrimin	Ranking
Peduncle: Abscission layer	7	5	1	13
Peduncle: length	7	7	7	21
Fruit: Green Shoulder	4	5	1	10
Fruit: Extent of Green Shoulder	5	5	7	17
Fruit: Intensity of Green color of shoulder	5	5	7	17
Fruit: Intensity of green color excluding	5	5	5	15
Fruit: Green Stripes	9	8	7	24
Fruit: Size	7	3	1	11
Fruit: Ratio length/di	7	7	3	17
Fruit: Color of Flesh	7	7	7	21

Trait Name	Difficulty Of Measure	Time Needed to	Importance to Discrimin	Ranking
Fruit: Shape in longitudinal section	7	7	3	17
Fruit: ribbing at peduncle end	5	7	3	15
Fruit: depression at peduncle	5	7	8	20
Fruit:Size of peduncle scar	5	7	8	20
Fruit: Size of blossom scar	5	7	8	20
Fruit: Shape at blossom end	5	7	8	20
Fruit: diameter of core in	5	7	8	20
Fruit: Thickness of	5	7	8	20
Fruit: Number of	7	3	1	11
Fruit: Color at Maturity	9	9	1	19

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Computer Vision as a Process

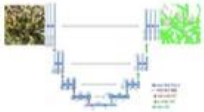
Step 1
image acquisition

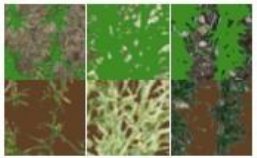
from past and future funded experiments

Step 2
annotation

from collaborative platform

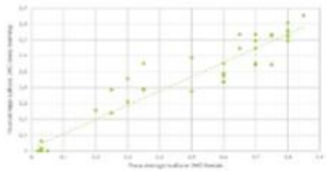
Step 3
Deep learning





from didactic version of U-Net

Step 4
Data analysis



Comparison with ground truth
Automatic cluster of types of errors

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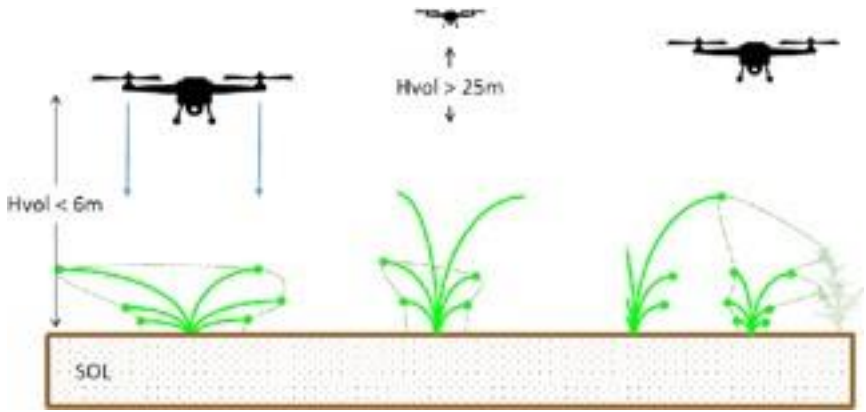
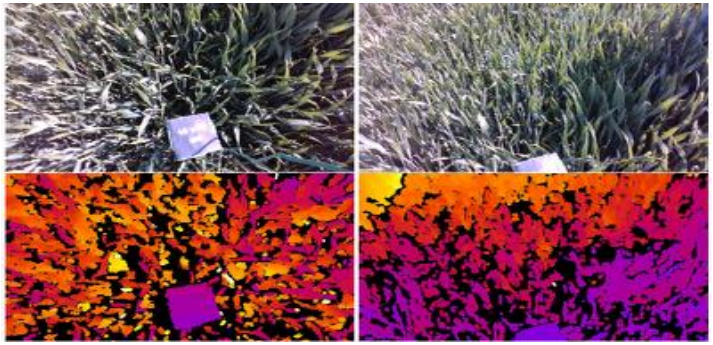
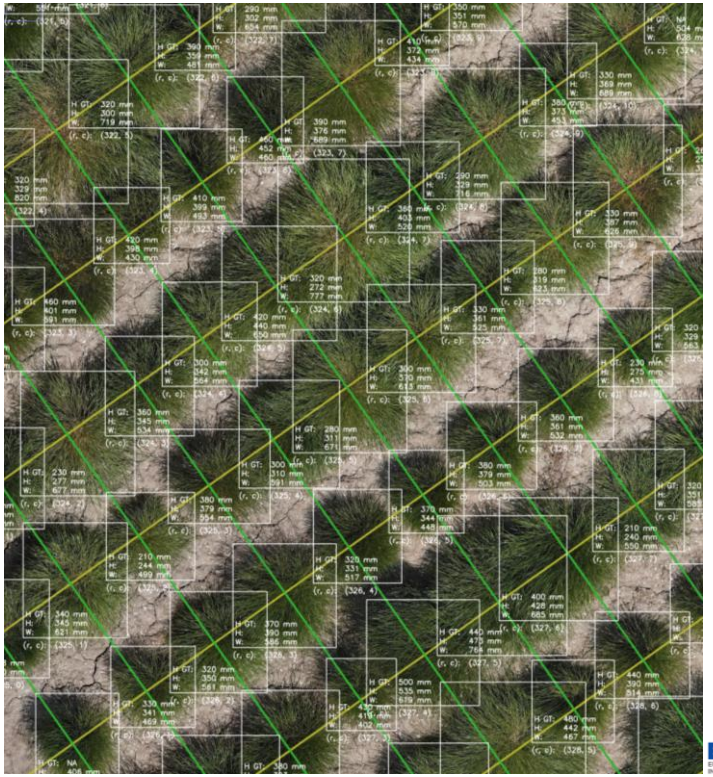
UAV Open Field Applications







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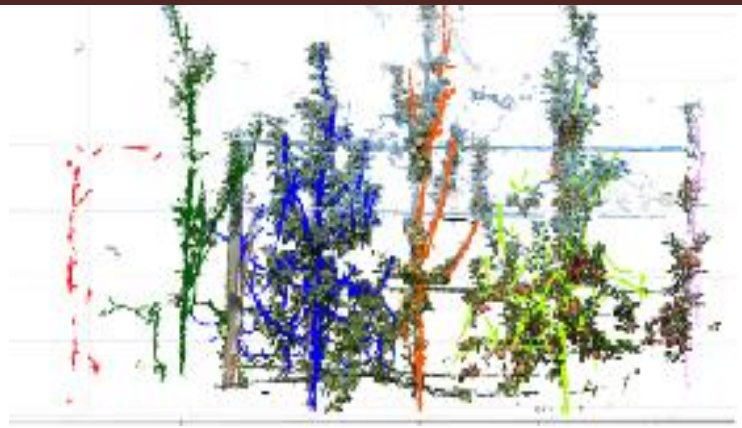
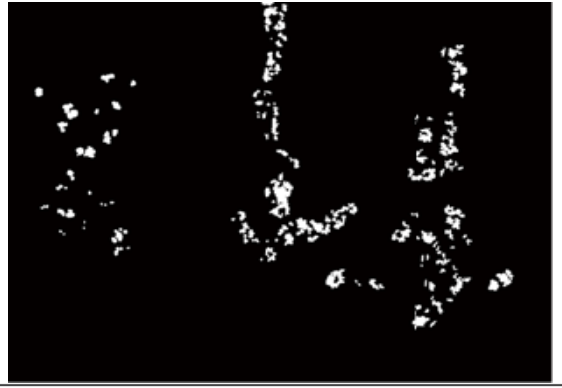
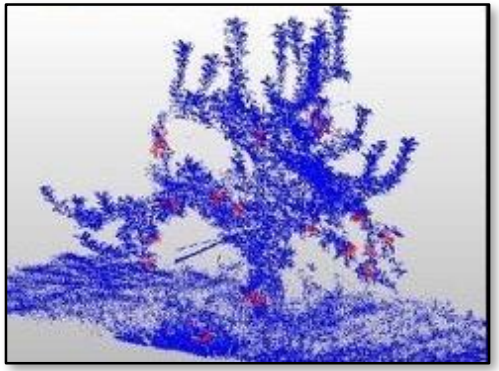
Orchard 3D Reconstructions - Scouting







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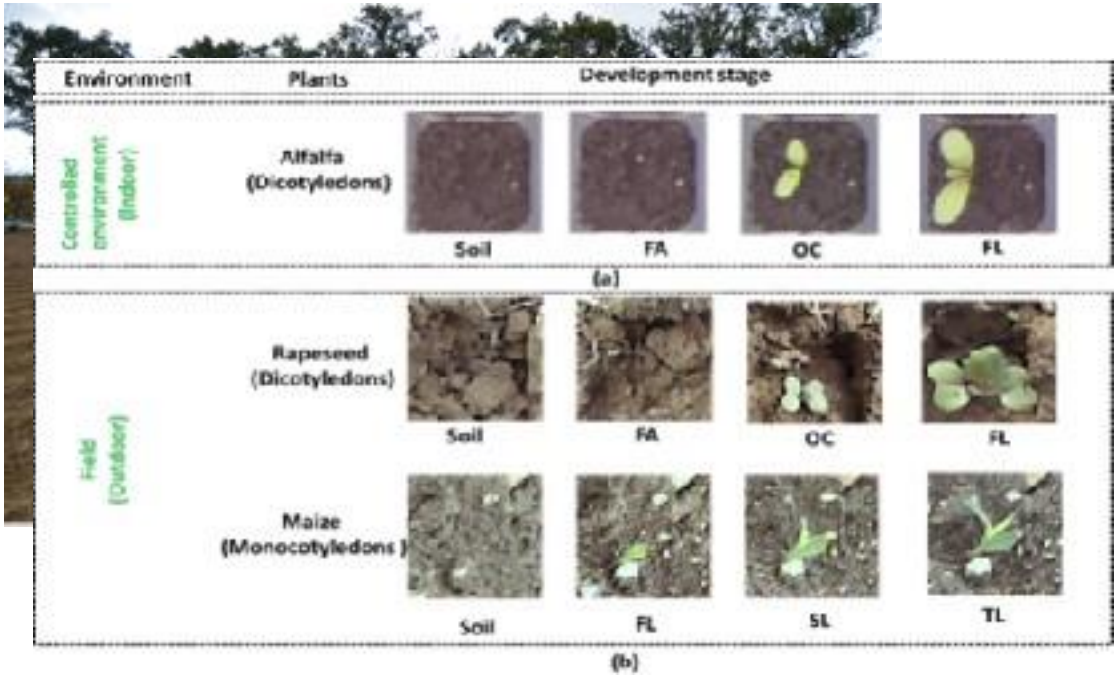
Open field Scouting



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Tomato Phenotyping and Naktuinbouw

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Important Traits

Traits of interest:

1. **Peduncle scar size**
2. **Color**
3. **Shape ratios**
4. **Volume**
5. **Blossom-end scar size**
6. **Ribbing**

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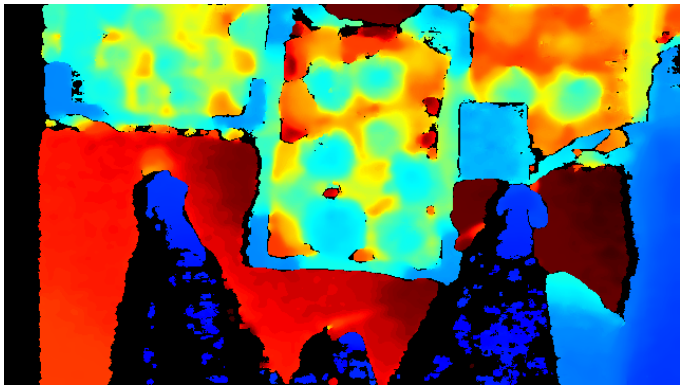
Initial Cycles – 3D Cameras



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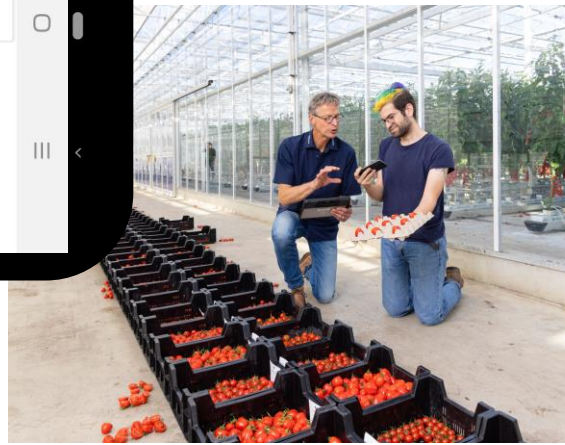
Current Cycles – Phone Applications



Expected applications from INVITE WP2

A general pipeline for any crop to implement low cost Phenotyping

An example App (moRPH) for tomato phenotyping as a baseline for other apps

Feedback and creation of a community of phenotypers in Europe

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CHINA

Genotyping tools to better characterize varieties and their performance

Karl Schmid | University of Hohenheim, Stuttgart, Germany | 28 November 2022

Plant Variety Rights online technical training for Chinese experts; 28 Nov 2023

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Overview

Objectives

Achievements with some examples

Challenges

Expected innovations

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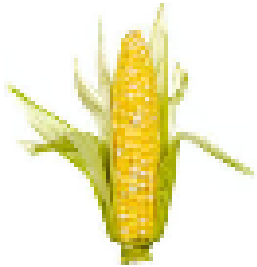
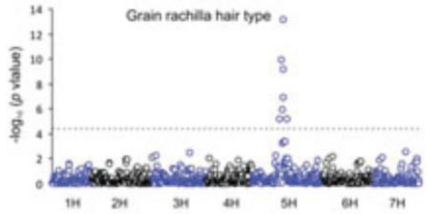
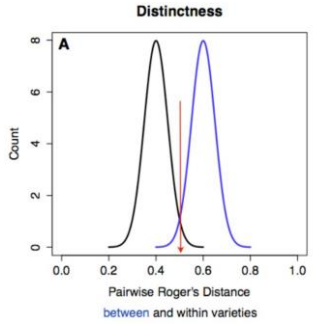
Genotyping tools to better characterize varieties and their performance







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Objectives

- Identify **genome-wide marker sets** in different types of varieties and different crops
- Develop **low-cost genotyping arrays** from genome-wide markers to support evaluation of DUS and VCU criteria
- Identify **novel markers** associated with accepted and novel DUS traits
- Develop **models** for marker-based evalution of DUS and VCU criteria and reference collection management
- **Improved models** for marker-based evaluation of distinctness and uniformity in wheat, maize, PRG, and soybean

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Key achievements

DUS Marker trait associations (GWAS) -> Marker development

Genome-wide markers -> Genomic prediction of DUS traits

New computational/statistical methods for DUS criteria and management of reference collections

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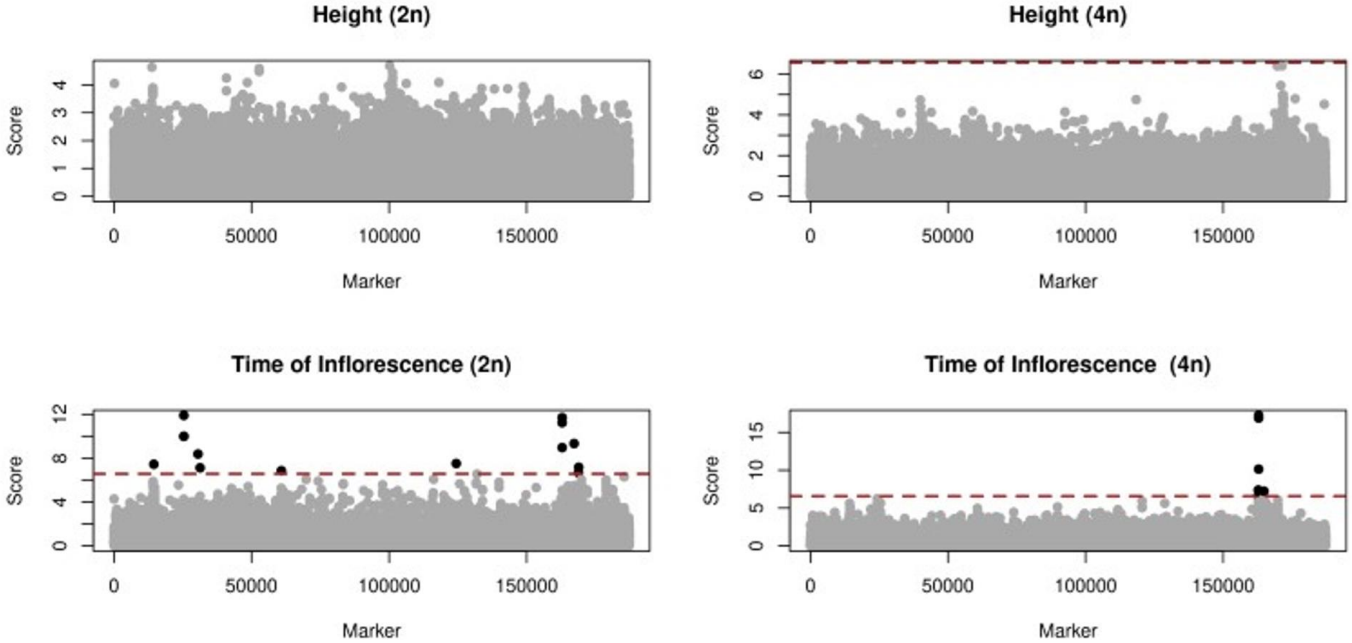
Some selected examples: Genome-wide association studies for DUS traits

Perennial ryegrass (PRG)



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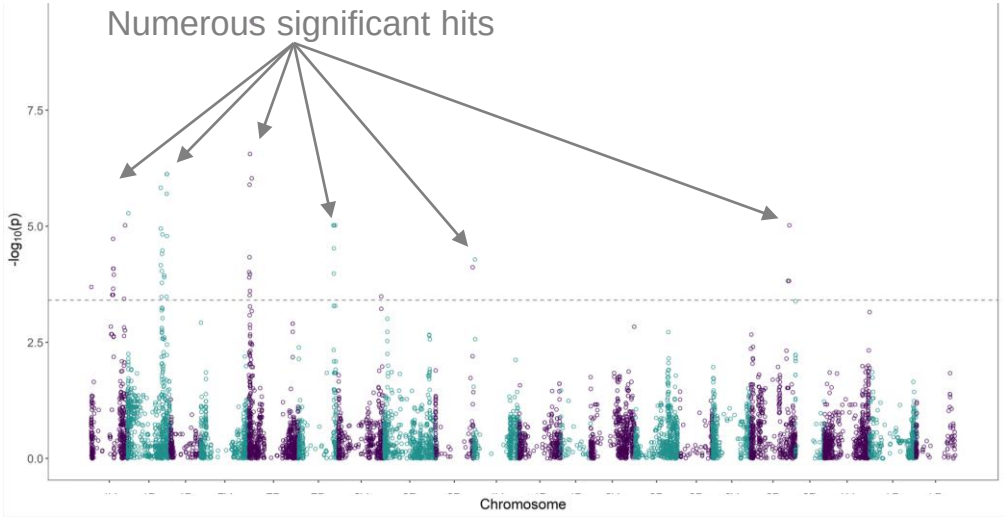
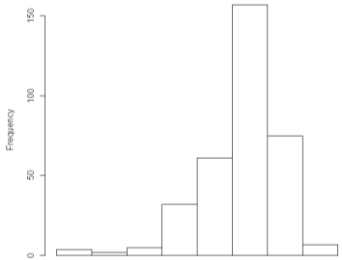
Dan Milbourne TEAGASC, Adrian Roberts BioSS

Some selected examples: Genome-wide association studies for DUS traits

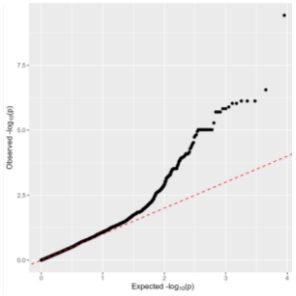
Flag leaf in wheat



Histogram of trait scores



Q-Q plot



Trait scored on a 1-9 (quantitative) scale. Multiple GWAS hits identified.

James Cockram, Bethan Love, Tally Wright, NIAB, UK

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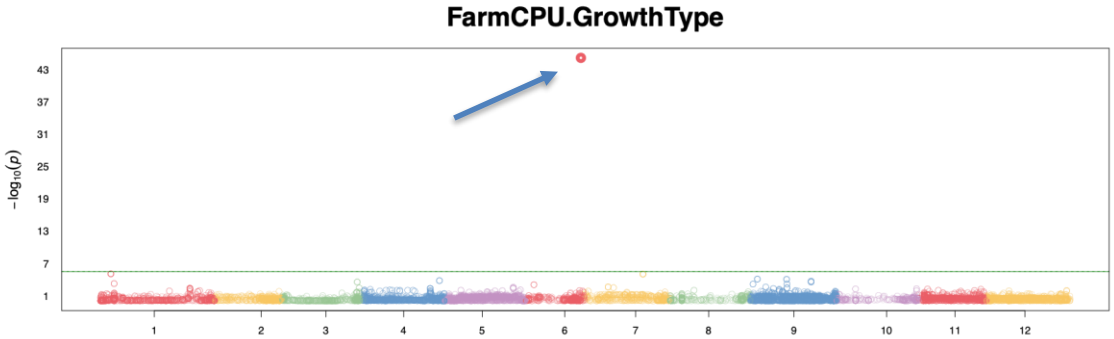
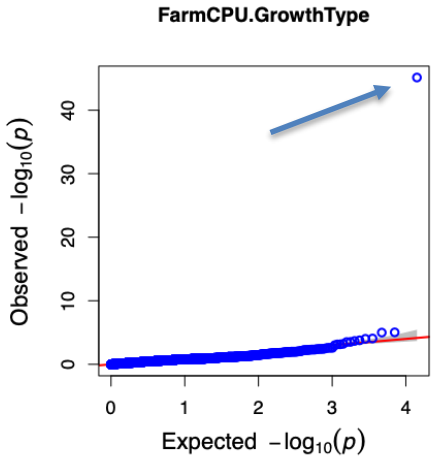
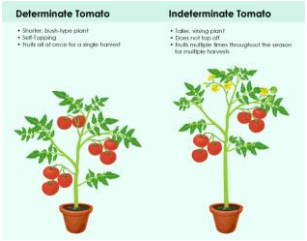
Some selected examples: Genome-wide association studies for DUS traits

Tomato - Trait: Determinate vs. indeterminate growth type







Mathilde Causse INRAE

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Genome-wide markers to differentiate varieties

Select adequate SNPs for verifying uniqueness and identity of any unique apple genotypes

Use SNPs instead of SSRs to facilitate international comparisons

Identify a reference set of 96 SNP with high discrimination power

INRAE

Charles-Eric Durel

Final selection of **96 SNP** allowing at least 6 differences within all pairs of genotypes

currently tested for large set of ~800 individuals

Implementation in cheap and high throughput genotyping array (e.g., KASP, Fluidigm)

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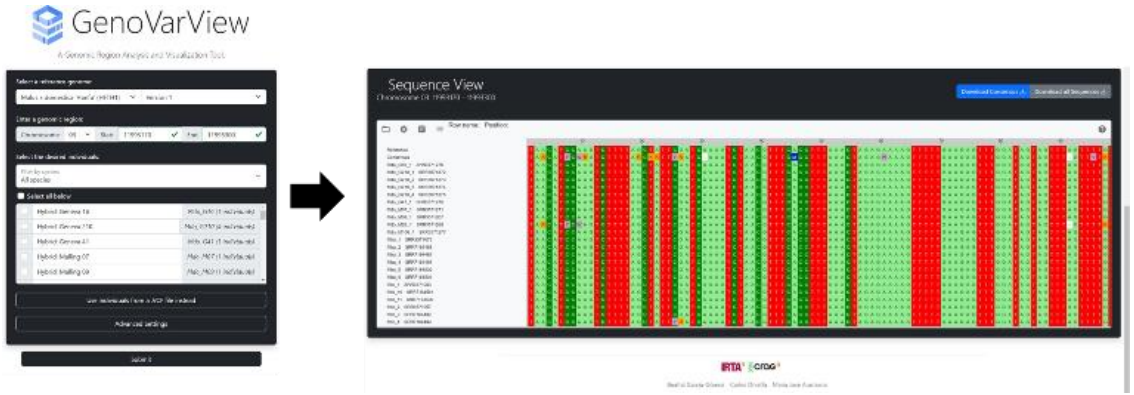
Example: Trait-specific SNP assays for apple

Goal: Gather SNPs known to tag other disease-pest resistances, fruit quality, phenology
-> transfer to KASPas chemistry

Approach: Webtool (GenoVarView) to search for variability in a target genomic region to design primers without mismatches



IRTA

Maria José Aranzana

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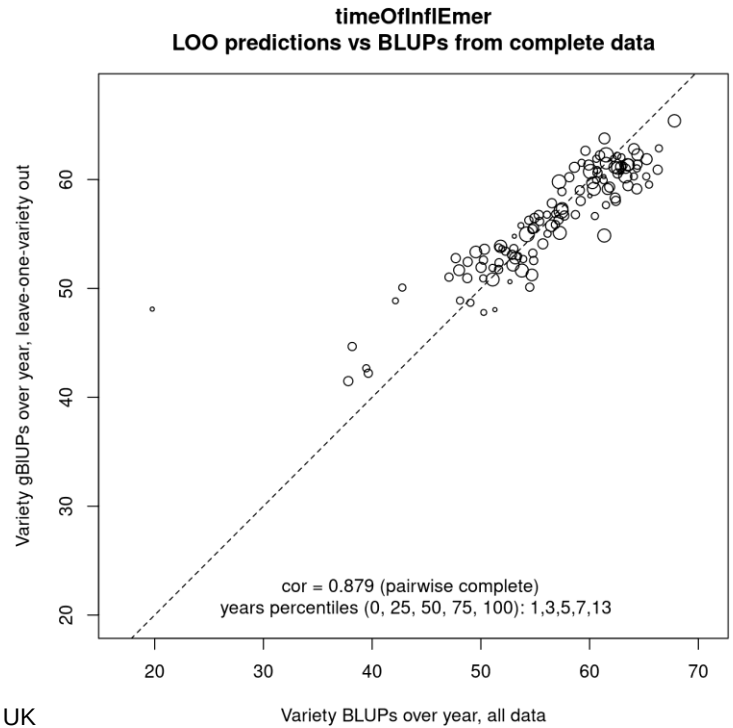
Example: Genomic prediction of DUS traits using genome-wide markers

Genomic BLUP in ryegrass



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Dan Milbourne TEAGASC Ireland, Adrian Roberts BioSS Scotland UK

Example: Improved management of reference collections

Reference collection management using markers

- Use markers to **reduce number of reference varieties** in DUS trials
- **Are markers more efficient** than current UPOV models, whilst staying true to UPOV principles?

Approach to be tested in INVITE:

- Predict distinctness for each character in turn using **genomic prediction**
- Collate information over characters to get overall decision

Potential Advantages:

- **Phenotype driven**
- Easier to **correlate markers with individual characters** than overall distances
- Uses concept that distinctness only needed in one character

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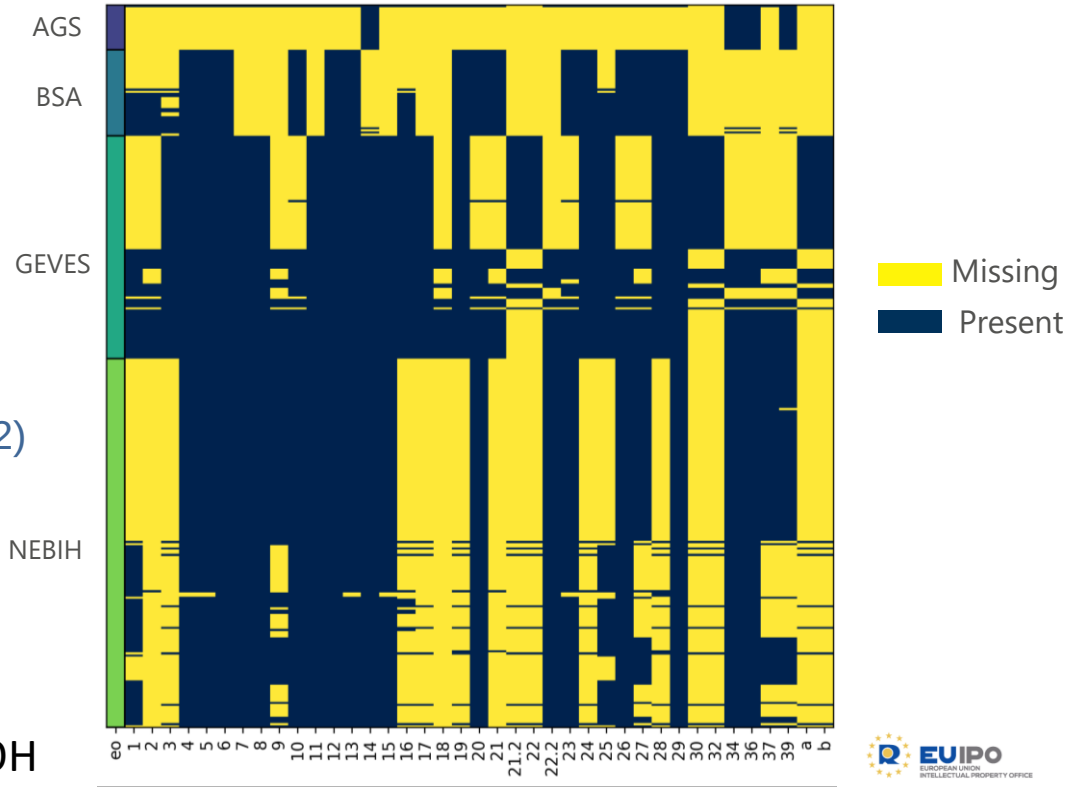
Challenge: Data type, quantity and quality

- Missing data of genotypes (technical causes)
- Missing data of DUS traits
- Numbers of varieties obtained not sufficient for some tasks
- New phenotypes (e.g. arising from WP2)



Anurag Daware, Karl Schmid UHOH

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Challenge diversity of genotyping technologies

Genotyping by sequencing

Low coverage whole genome sequencing

SPETs genotyping

Sequence capture

SNP array genotyping

KASP markers

Take home messages:

- Identification of trait-specific and genome-wide markers easily possible
- Which concepts for using markers given technical possibilities?

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Genotyping tools to better characterize varieties and their performance

Trait-specific markers implemented in **marker assays** (esp. tomato, potato, apple)

Genome wide diversity of European material characterized for wheat, maize, soybean, PRG -> Management of reference collections

Updated **methods and software implementations** using genome-wide markers for distinctness and uniformity testing

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CHINA

Integration of new tools in advanced variety testing

Aurélia Gouleau, GEVES, France | 28th November 2023

Plant Variety Rights online technical training for Chinese experts; 28 Nov 2023

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Objectives of the work

Test and validate phenotyping and genotyping tools and methods for improved speed, precision and efficiency of variety testing

Improve the management of DUS reference collections by the use of molecular tools

Develop new variety testing protocols to integrate sustainability criteria into variety testing

Propose recommendations to policy makers for the evaluation of heterogeneous plant material

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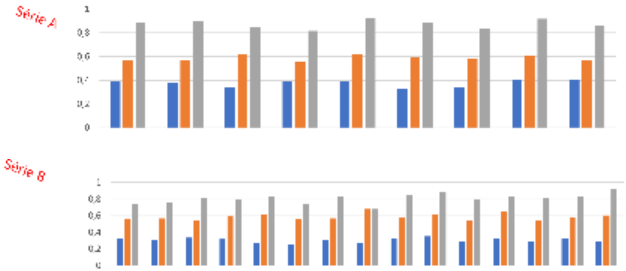
Testing of phenotyping tools - Sunflower

- coverage fraction at early stage

Wallonie recherche CRA-W
GEVES Expertise & Performance
Agroscope
Terres Inovia L'agronomie en mouvement

Drones



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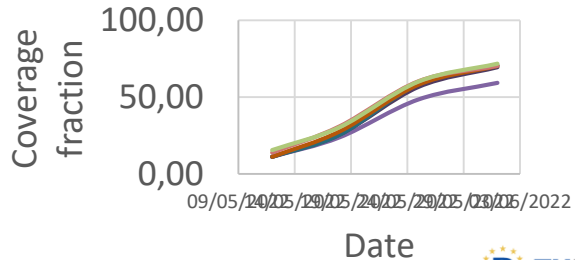
Canopeo software



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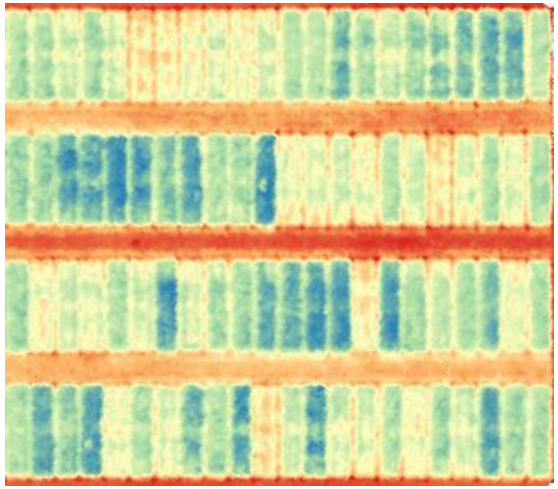
Testing of phenotyping tools - Ryegrass

Forage grasses/*Lolium perenne*: canopy height/persistency

ILVO
Flanders Research Institute for Agriculture, Fisheries and Food



CH model [m]

0

Canopy Height model (CH), variety trial, 20/05/2021

Persistency, RGB orthomosaic, Schelderode, autumn 2021, much variability

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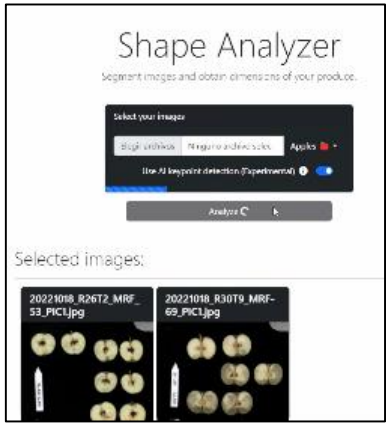
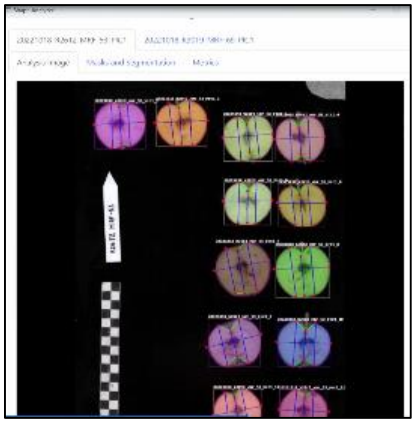
Testing of phenotyping tools - Apple

- Development of a downloadable software for shape analysis previously in INVITE project
- Demonstrated in apple collection of 120 local apple varieties; extended to almond (100 accessions) and pear (130 local varieties). *Publication in preparation*





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Testing of phenotyping tools - Maize

- Early vigor
- Leaf area index
- Plant height
- Senescence

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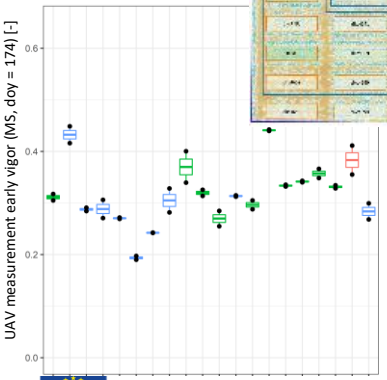

Agroscoe

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ILVO Flanders Research Institute for Agriculture, Fisheries and Food

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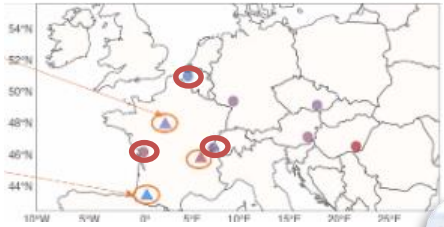
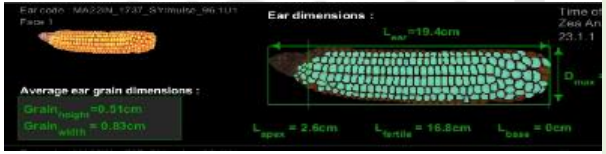


Drones
Literal stick
Phenofield

Km scan
Earbox



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- Ear characteristics

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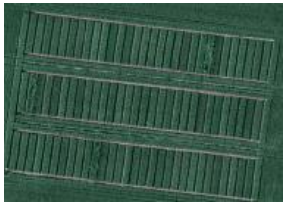
Testing of phenotyping tools - Wheat



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- Heading and flowering time
- Number of spikes
- Height at maturity
- Senescence

- Emergence
- Growth after winter

Drones
Literal stick
Spectrometer
Multispectral and RGB Cameras

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Testing of phenotyping tools – Possible applicability for DUS testing

	Species	Traits	Tools
	Rye-grass	Persistency, plant height and biomass estimation	drones
	Maize	Plant counting, early vigor, canopy height, ear characteristics	drones, connected sticks phenotyping platform, Earbox (Phymea)
	Wheat	Heading and flowering time, number of spikes, height at maturity, senescence, emergence, growth after winter	drones, connected sticks, spectrometer, multispectral and RGB cameras phenotyping platform
	Sunflower	Coverage fraction at early stage	Drones, Canopeo
	Apple	Fruit shape analysis	Shape analysis software

Benefits

- more precise
- adaptation to environmental conditions

Limits

- weather dependent
- training of users

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Testing of molecular markers - Tomato

Test of molecular markers linked to 3 disease resistances

- Tomato Mosaic Virus (ToMV)
- Tomato Spotted Wilt Virus (TSWV)
- *Fusarium oxysporum f. sp. lycopersici* Fol: 1EU/2US

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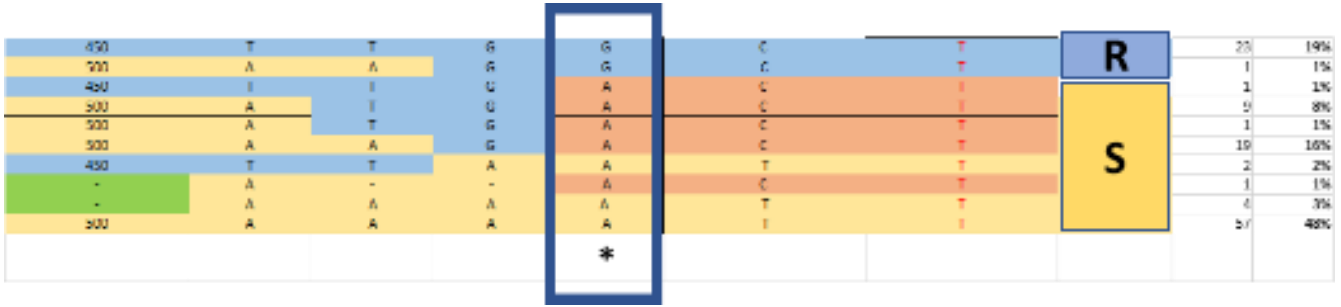
Testing of molecular markers – Apple (1/2)

- Test the SNP marker linked to PI1 resistance gene in progenies.

- The marker predicts well the trait in different genetic backgrounds and is suitable for MAS and could be helpful for DUS



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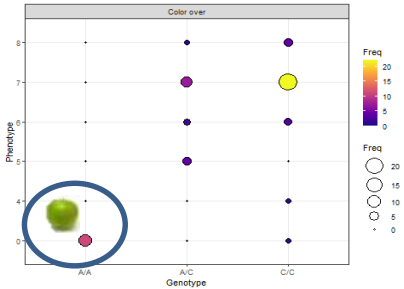
Testing of molecular markers – Apple (2/2)

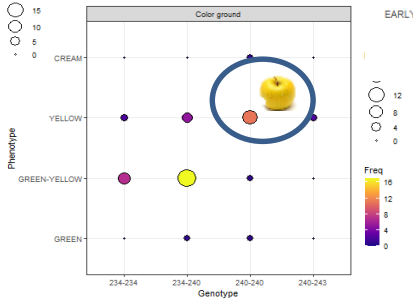
Class	Gene	Type	ID	LG	Referencias
Fruit overcolor	*MYB10*	SNP	MYB10_II	9	Chagne et al. 2016
Fruit background color	*ERF17*	SSR	ERF17	2	Han et al. 2018
Early ripening time	*NAC18.1*	SNP	DY5	3	Migicowsky et al. 2019
Powdery mildew resistance (Pl2)	*Pl2*	SNP	Pl2_PFR	11	Jansch et al. 2015
Scab resistance (Rvi6)	*Rvi6*	SNP	Rvi6_PFR	1	Jansch et al., 2015

Available at: https://bioinformatics.cragenomica.es/projects/genovarview/

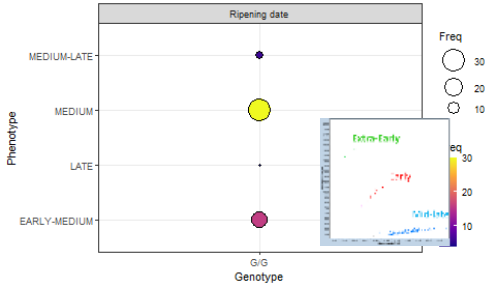
Markers developed from published information and tested in germplasm



Overcolor



Background color



Early maturity

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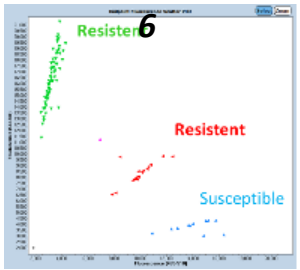
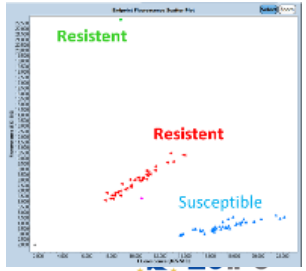
Rvi



Pl2



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Testing of molecular markers - Lucerne

- Test a new technical approach (NGS-like) in order to manage the DUS reference collection
- Choice of markers done: 37 500 genome portions identified; development of a capture tool including 35,500 SNP markers
- Sequencing in progress using the RAD capture tool developed by INRAE instead of genotyping-by-sequencing (GBS)

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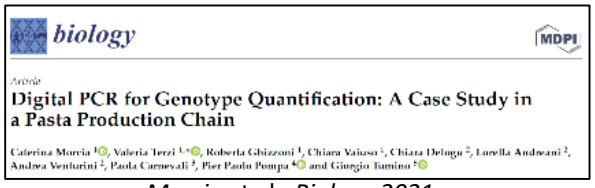
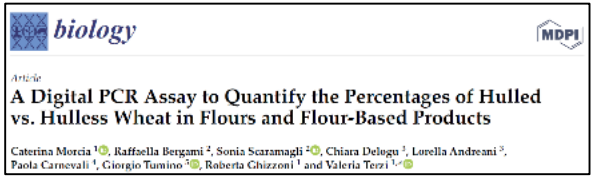
Testing of molecular markers - Wheat

Evaluation of the digital PCR potentialities for a rapid description of genotype content in wheat autogamous, composite crosses and mixture

- Method for the identification and quantification of wheat varieties in grains, flour and derived products
- Chip digital PCR method for the identification and quantification of hulless and hulled Triticum species
- Rapid method for mycotoxin detection for wheat chain
- Study of the adaptive traits and genetic diversity in traditional wheat landraces

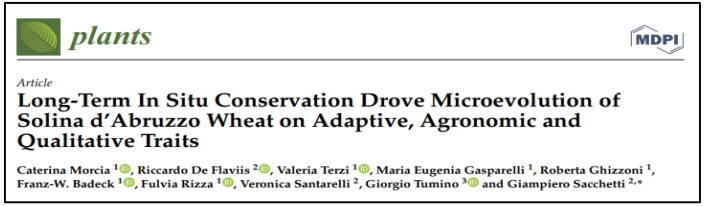


Morcia et al., *Biology 2021a*



Morcia et al., *Biology 2021b*

Salina et al., *Agronomy, 2022*



Morcia et al., *Plants, 2023*

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Expected applications from INVITE

Information about the applicability of phenotyping tools for variety testing

Information about the applicability of molecular markers for distinctness and management of reference collection

Recommendations for the testing of heterogeneous plant material

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IPKey

CHINA

Concluding remarks: general outputs of INVITE

Francois Laurens, INRAE France / coordinator of the INVITE project

Plant Variety Rights online technical training for Chinese experts; 28 Nov 2023

Main general outputs of INVITE (1)

- Setting-up a **research community** dedicated to support the variety testing scheme
- Improve **links between research and users** of research findings:
  - **DUS**: links with the CPVO and several EOs
  - **VCU** and post-registration: Links with VCU Expert network established with agreements that research should be regularly invited to present their activities.
  - **Breeding** testing: EUROSEEDS liaison established
- ↗ strong links outside the consortium with other projects (National, European and international)

Main General outputs of INVITE (2)

- Setting-up a **research community** dedicated to support the variety testing scheme
- Improve **links between research and users** of research findings:
- ↗ strong links outside the consortium with other projects (National, European and international)

- **Collect huge volumes of historical data** that could (should) serve other purposes than just INVITE research.
- **Improve knowledge**
- **Develop tools and methods** to be implemented in the EU variety testing

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THANK YOU

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