PRO-GRACE
PROMOTING A PLANT GENETIC RESOURCE COMMUNITY FOR EUROPE

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Tools and methods for the evaluation of Plant Genetic Resources

Entry-level training school on Plant Genetic Resources (PGR)

5-6 October, Mediterranean Agronomic Institute of Chania, Chania, Greece

María José Díez
Polytechnic University of Valencia
SPAIN

Content

- The need for evaluation of plant genetic resources
- Characterization and evaluation
- Agro-morphological characterization: the use of descriptors
- Evaluation for resistance to pests and diseases
- Evaluation for adaptation to abiotic stresses
- Speeding evaluation by high throughput phenotyping techniques

Characterization and evaluation is the key to assess the potential and actual value of germplasm

Breeding for adaptation to climate change

Disease resistance

Cytoplasmic male sterile lines for hybrid breeding

Plant Genetic Resources

Insect-pest resistance

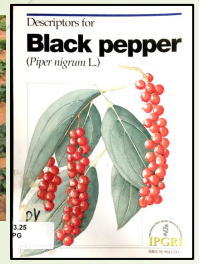
Breeding for non-conventional seasons

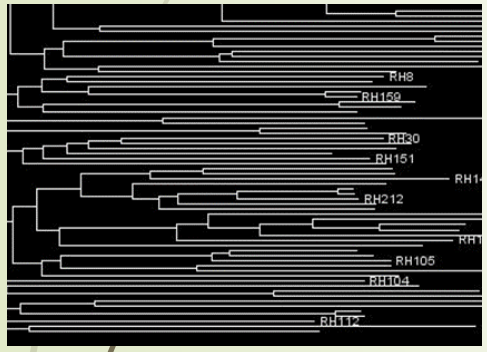
productivity enhancement

PRO-GRACE
PROMOTING A PLANT GENETIC RESOURCE COMMUNITY FOR EUROPE

4

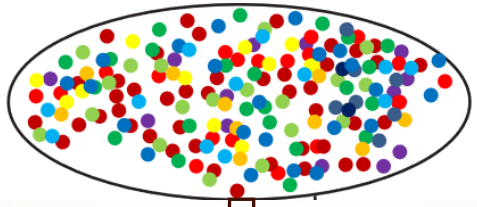
Germplasm characterization and evaluation in the broad sense and in the context of genetic resources is the description of a particular accession

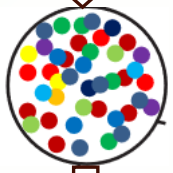




Primary characterization by universally accepted descriptors

Whole collection





Core collection

Minicore collection

Categorization of the collection, core and minicore collections, reference sets of germplasm

EVALUATION

Agronomic and quality

Resistance to pests and diseases

Adaptation to abiotic stresses

Characterization is neccesary to:

An easy and quick **discrimination among phenotypes** and grouping of the accessions

↓

A better insight on the composition of the collection and the **coverage of genetic diversity**

Detection of **missclasifications** and identification of possible **errors** made during other genebank operations

↓

Establish **taxonomic identity** and relationhips

Detection of **duplicates**

↓

Improve the criterum for **delivery germplasm**

↓

Rationalize management procedures, allowing the curator to make well-informed decisions

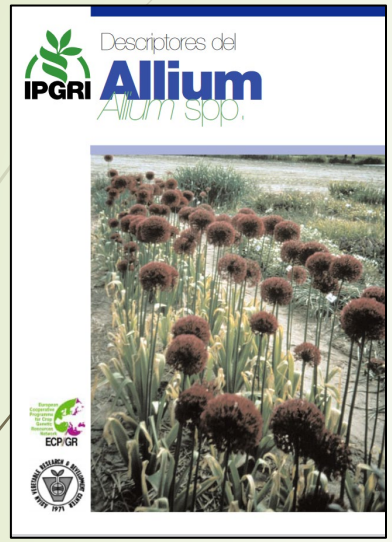
Characterization and evaluation

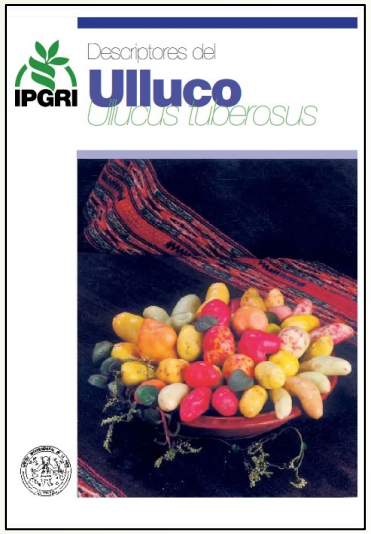
- The **characterization** of germplasm deals with the understanding and recording of **highly heritable traits** which are generally expressed in all the environments. Therefore, it can be performed in a single environment. It ranges from morphological features to seed proteins and molecular and biochemical markers

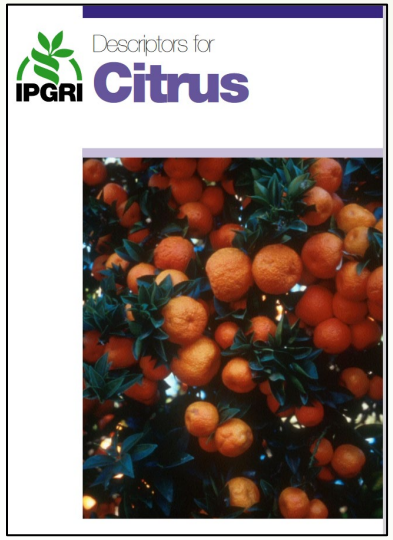
- Germplasm **evaluation** deals with the assessment of the agronomic potential of an accession including quality parameters and response to various abiotic and biotic stresses

Morphological characterization

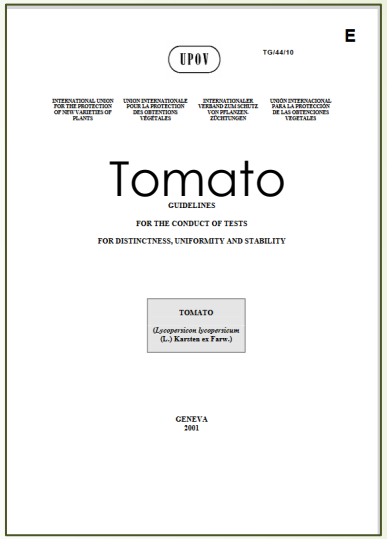
IPGRI Descriptors

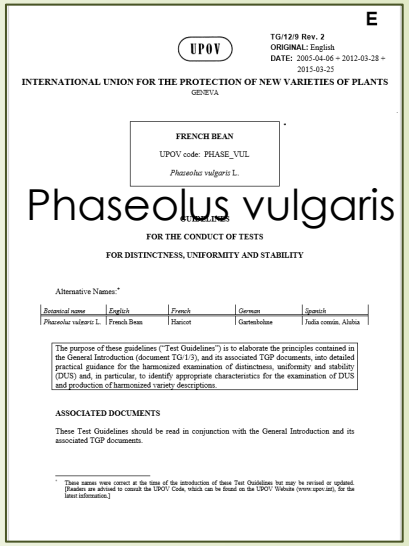






UPOV Guidelines





Passport descriptors
Management descriptors
Environment and site descriptors
Characterization descriptors
Evaluation descriptors

DEFINITIONS AND USE OF THE DESCRIPTORS

- **Passport descriptors**: These provide the basic information used for the general management of the accession and describe parameters that should be observed when the accession is originally collected.

  Accession number
  Donor name
  …

- **Management descriptors**: These provide the basis for the management of accessions in the genebank and assist with their multiplication and regeneration.

  Storage address
  Storage date
  …

- **Environment and site descriptors**: These describe the environmental and site-specific parameters that are important when characterization and evaluation trials are held.

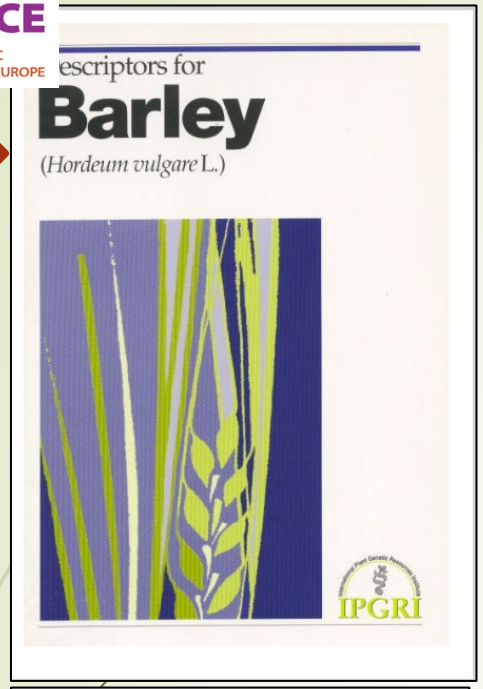
  Latitutude, Longitude
  Elevation
  …

- **Characterization descriptors**: These enable an easy and quick discrimination between phenotypes. They are generally highly heritable, can be easily seen by the eye and are equally expressed in all environments.

  Plant growth type
  Foliage density
  …

- **Evaluation descriptors**: Many of the descriptors in this category are susceptible to environmental differences but are generally useful in crop improvement and others may involve complex biochemical or molecular characterization. They include yield, agronomic performance, stress susceptibilities and biochemical and cytological traits

  Yield
  Agronomic performance
  Biotic stresses
  Abiotic stresses
  …



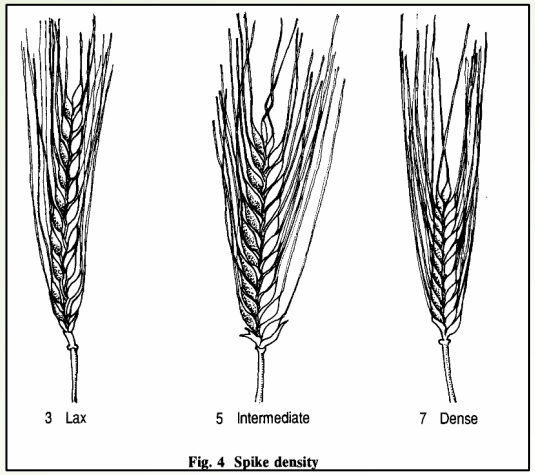


Fig. 4 Spike density

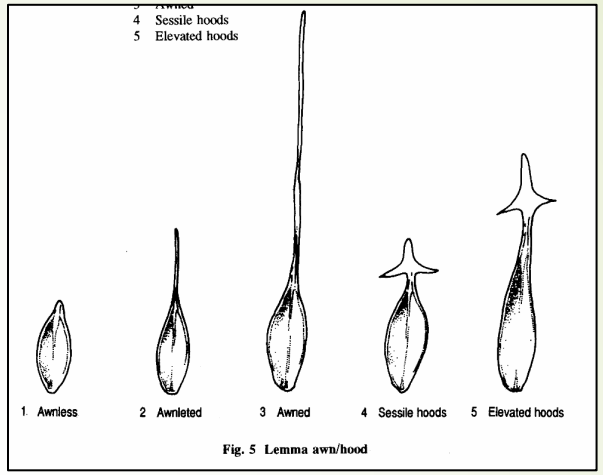


Fig. 5 Lemma awn/hood

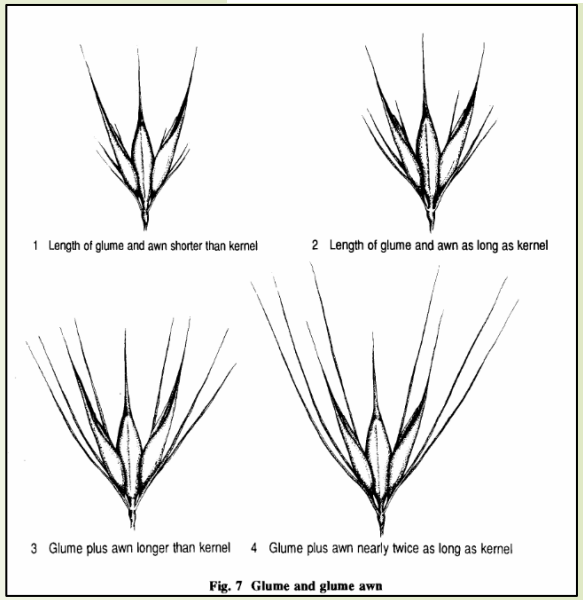


Fig. 7 Glume and glume awn

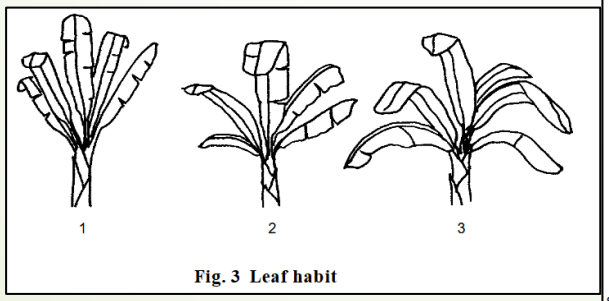


Fig. 3 Leaf habit

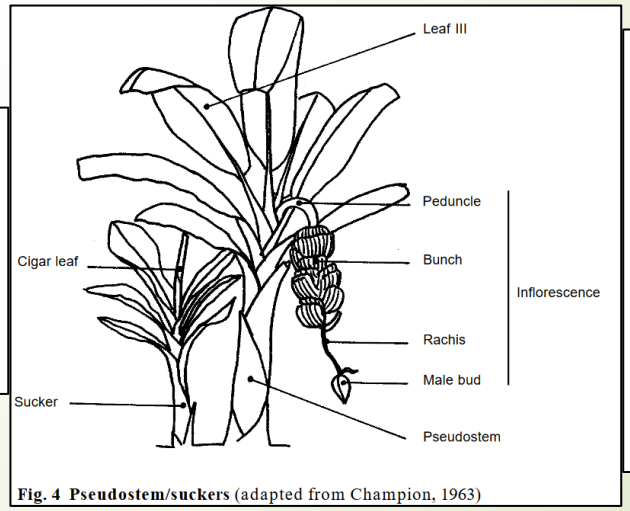


Fig. 4 Pseudostem/suckers (adapted from Champion, 1963)

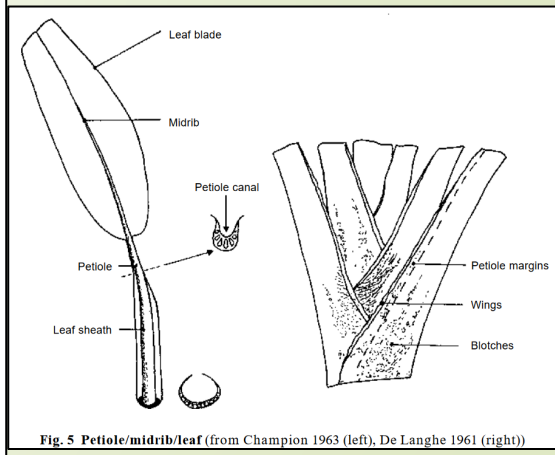
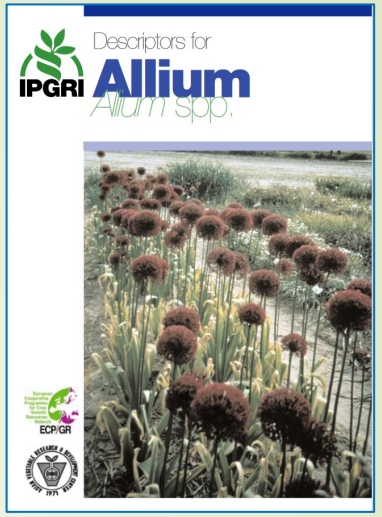


Fig. 5 Petiole/midrib/leaf (from Champion 1963 (left), De Langhe 1961 (right))

Descriptors for *Allium* spp.			
Passport			Number descriptors
	Accessions		12
	Collecting		17
Management			10
Multiplication/regeneration			11
Environment and site			11
Characterization			
	Plant descriptors		
		Vegetative	28
		Inflorescence and fruit	10
	Seeds		2
Evaluation			
	Plant descriptors		
		Vegetative	9
		Inflorescence and fruit	3
	Abiotic stresses		5
	Biotic stresses		6
	Biochemical markers		Isozymes
	Molecular markers		5



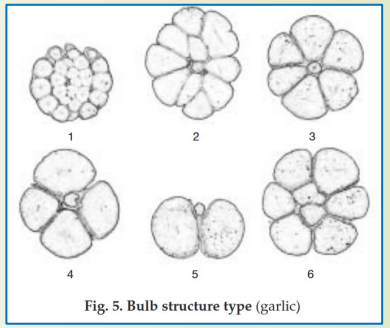


Fig. 5. Bulb structure type (garlic)

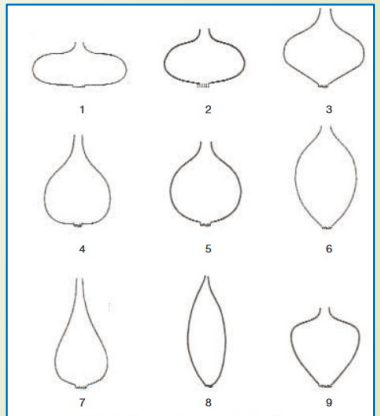
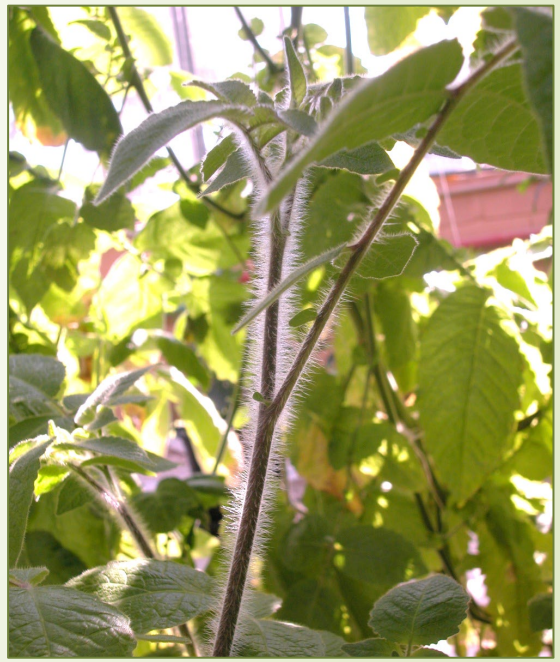


Fig. 3. Shape of mature dry bulbs

Biological status of the germplasm should be known in advance to determine the characterisation strategy

Heterogeneous traditional varieties maintained by farmers

Uniform breeding lines and improved materials



Specific traits: wild species

The basic morphological characterization: plant traits

Indeterminate

Semideterminate

Determinate

Present

Absent

Seedling: anthocyanin coloration of Hypocotyl
Plant: growth type
Plant: number of inflorescences on main stem
Inflorescence: number of flowers per inflorescence
Stem: anthocyanin coloration
Stem: length of internode
Plant: height

Images from Training course on tomato phenotyping (HARNESSTOM)

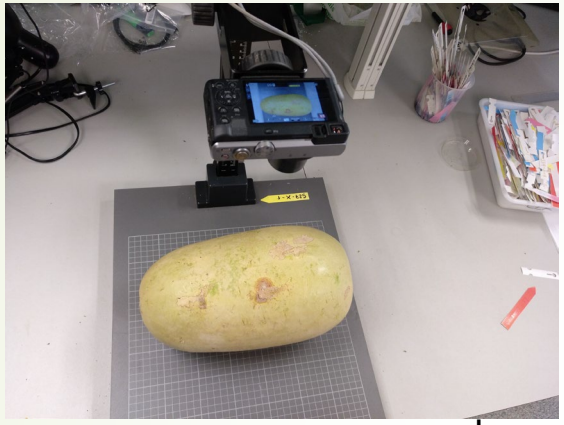
13 The basic morphological characterization: leaf traits

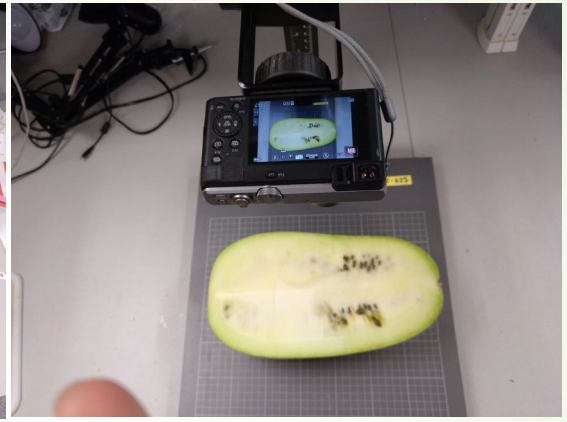
Leaf: attitude
Leaf: length
Leaf: width
Leaf: type of blade
Leaf: size of leaflets
Leaf: intensity of green colour
Leaf: glossiness
Leaf: blistering
Leaf: attitude of petiole of leaflet

Images from Training course on tomato phenotyping (HARNESSTOM)

The basic morphological characterization: fruit

Fruit weight

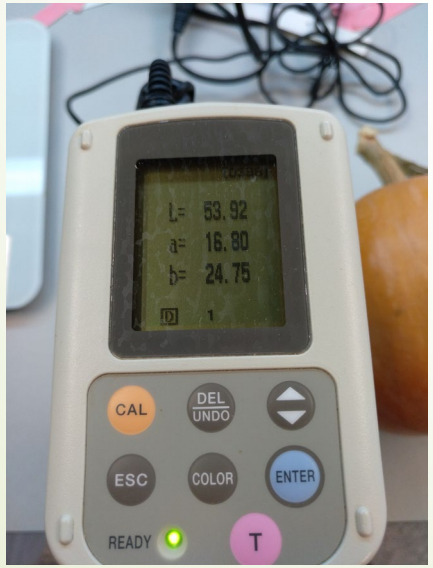




Images

Fruit length and with

ª Brix



Colorimeter

The need for standardization

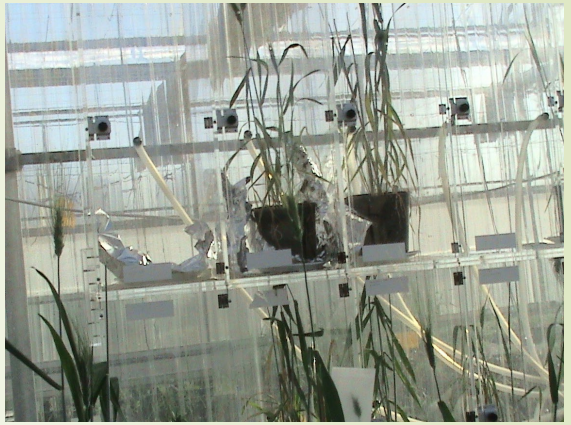
- Standardization for:
  - Traits: IPGRI descriptors, UPOV, etc.
  - MIAPPE (**Minimum Information About Plant Phenotyping Experiments**): MIAPPE is an open, community driven, data standard designed to **harmonize data from plant phenotyping experiments**. MIAPPE provides a specification including a checklist and a data model of metadata required to adequately describe plant phenotyping experiments.
  - Ontologies: The Plant Ontology is a **structured vocabulary and database resource that links plant anatomy, morphology and growth and development to plant genomics data**. The PO is under active development to expand to encompass terms and annotations from all plants.

Evaluation is needed for

- **Identify the appropriate germplasm with a target trait for their further utilization**.
- There is a need for a systematic evaluation in order to know its various morphological, physiological and developmental characters
- Evaluation of germplasm is a **multi-disciplinary approach** and it should be done in collaborative mode involving germplasm **curator, plant breeder, physiologist, pathologist, entomologist, biochemist** etc.
- The germplasm accessions are usually evaluated for **two consecutive years** for an adequate documentation
- For effective evaluation of germplasm, a **close organization and personal contact between curator and breeder** is necessary: It servers **to meet the demands of companies**

Evaluation for resistance to diseases. Previous concepts:

- The response of a plant depends on:
  - The host
  - The pathogen race
  - The environment



Source: F. Martínez

- The identification of a resistance source against a particular race/strain/isolate/biotype within a particular location does not guarantee its resistance response in other locations as **race/strain/isolate/biotype** may vary depending upon the **agro-meteorological conditions** and the presence of **resistance genes** in the commercial varieties

Evaluation for resistance to diseases. Aspects to take into account

- Natural infection conditions vs. controlled conditions: mimic the conditions of development of the disease and the infection mode of the pathogen
- Wild vs. cultivated species: the presence of natural mechanisms of avoidance or antixenosis
- Definition of the inoculation technique
- Standard Evaluation Systems (SES): Development of a reliable diagnostic technique
  - Evaluation of symptoms
  - Quantification of pathogen

Evaluation for resistance to diseases:
The tomato yellow leaf curl virus.

Controlled conditions with the vector (*Bemisia tabaci*)

Agroinoculation

Inoculation techniques

Natural infection conditions

Diagnostic techniques

Standard Evaluation System: severity of symptoms at 15, 25, 35, 45 and 55 after inoculation

0

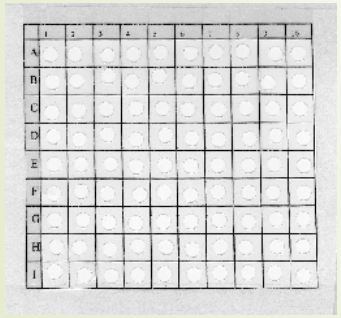
1

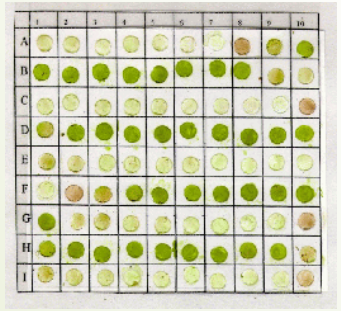
2

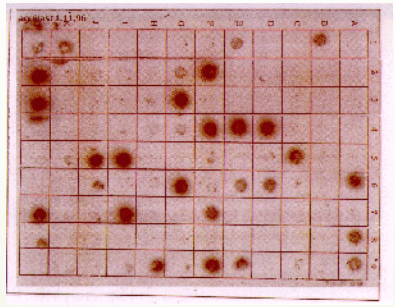
3

4

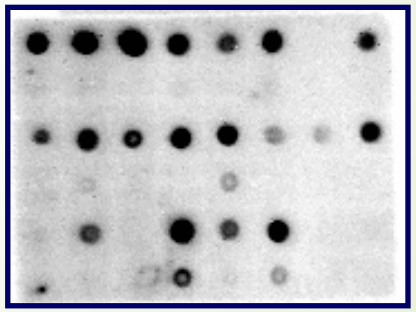
Virus quantification by squash blot or dot blot

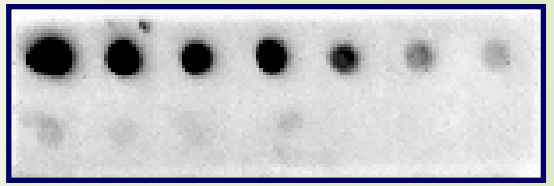






Squash blot





Dot blot

The problem of wild relatives for evaluation of resistance

- Antixenosis or antibiotic mechanisms: presence of dense thrichomes

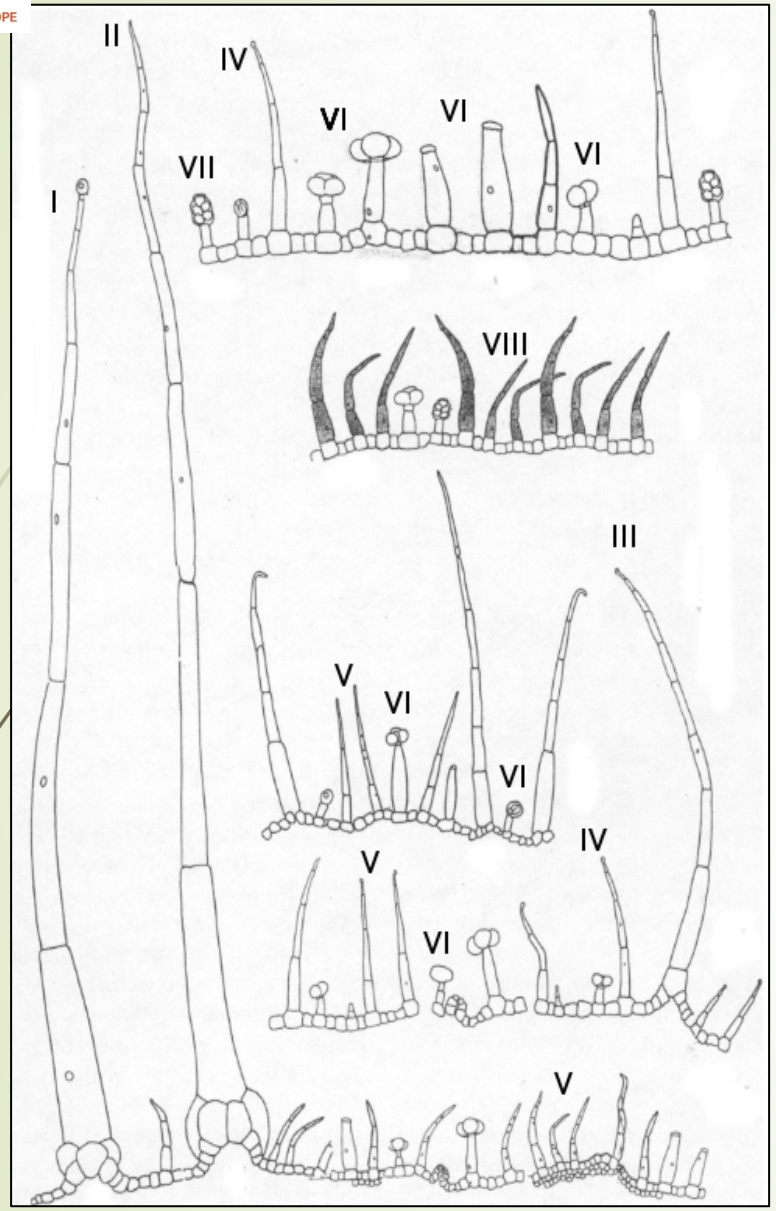
Solanum pennellii

Solanum habrochaites

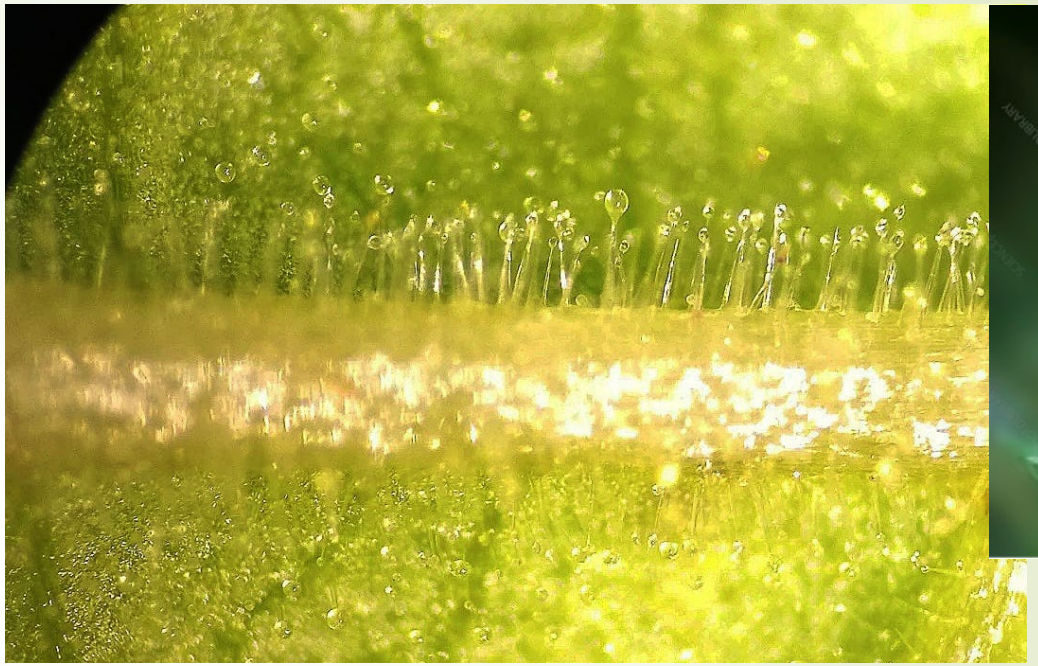
- Symptomatic and asympomatic infected plants

Solanum peruvianum

Evaluation for resistance to arthropods: the glandular trichomes

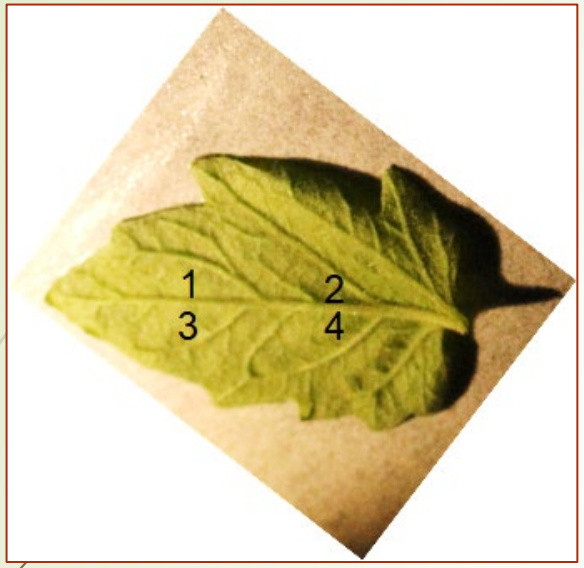


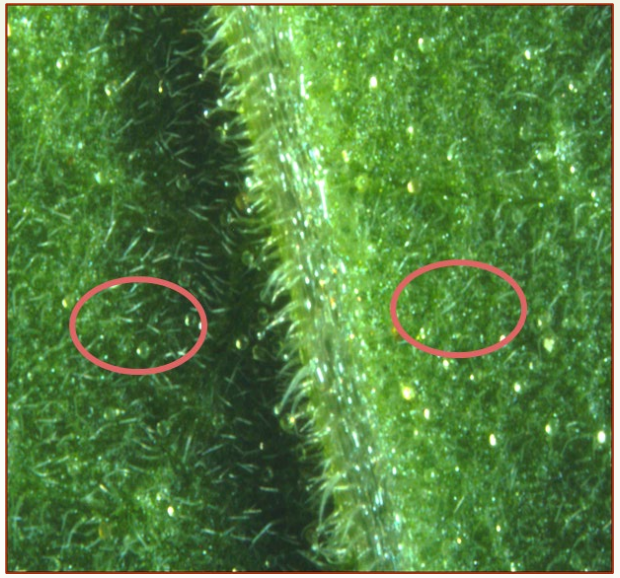
Types of trichomes in tomato and wild relatives (Modified Peralta, Spooner & Knapp, 2008)



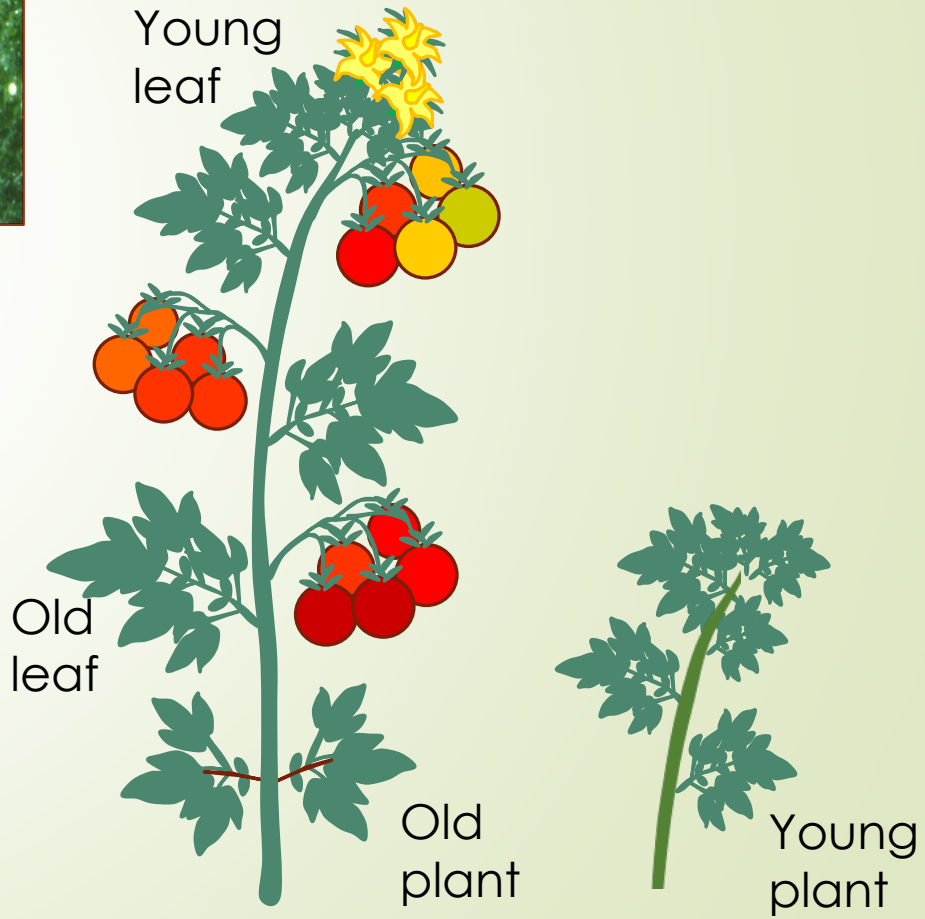
Glandular trichomes

Looking deeper: factors affecting the development of trichomes

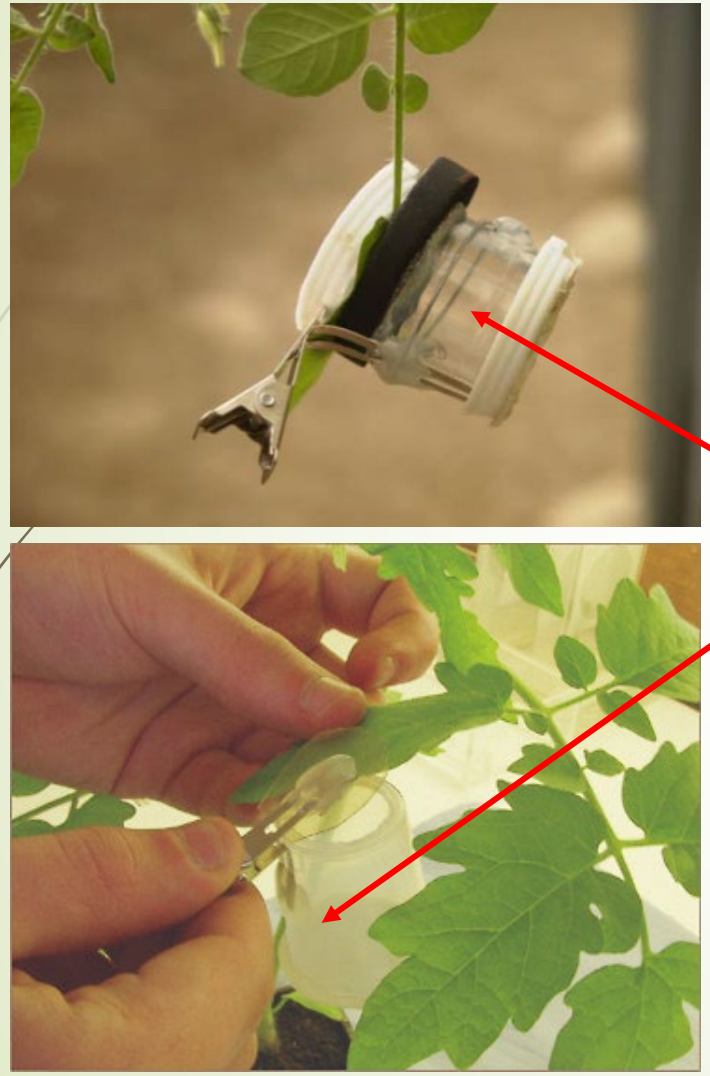




- Factors affecting the development of trichomes:
  - Planta age
  - Leaf age
  - Temperature
  - Humidity

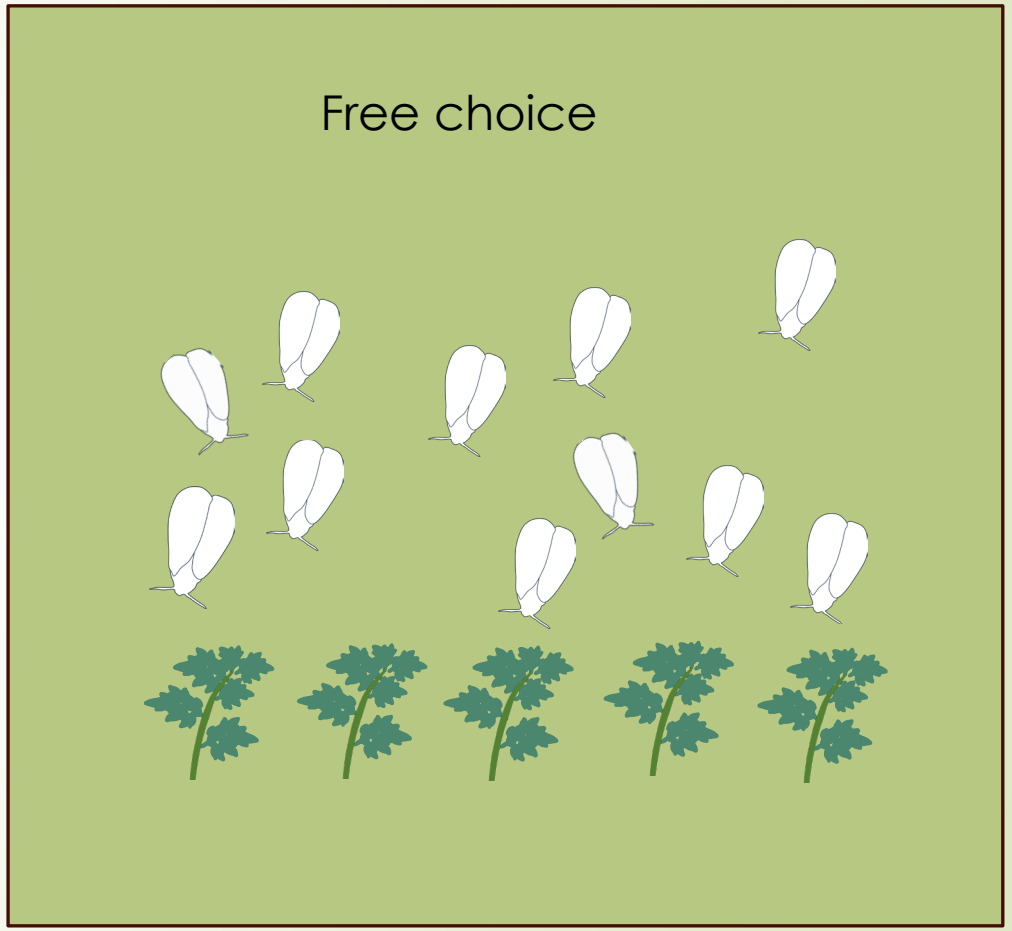


Looking deeper: - the resistance mechanism: antibiosis or antixenosis



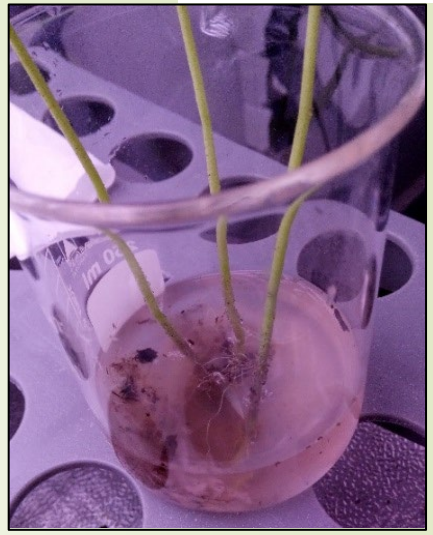
Controlled infestation

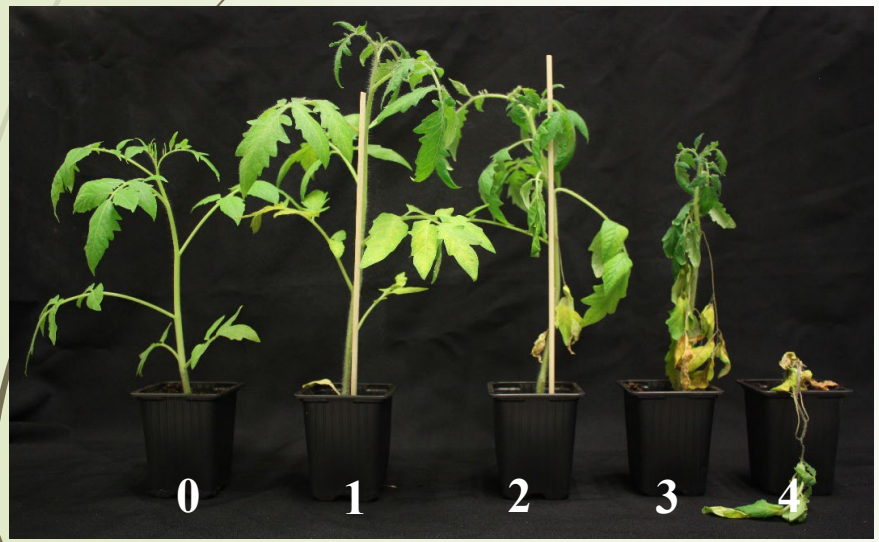
Antibiosis mechanisms: mortality, fecundity



Antixenosis mechanisms: epidemiology

Screening for *Fusarium oxysporum* f. sp. *lycopersici*

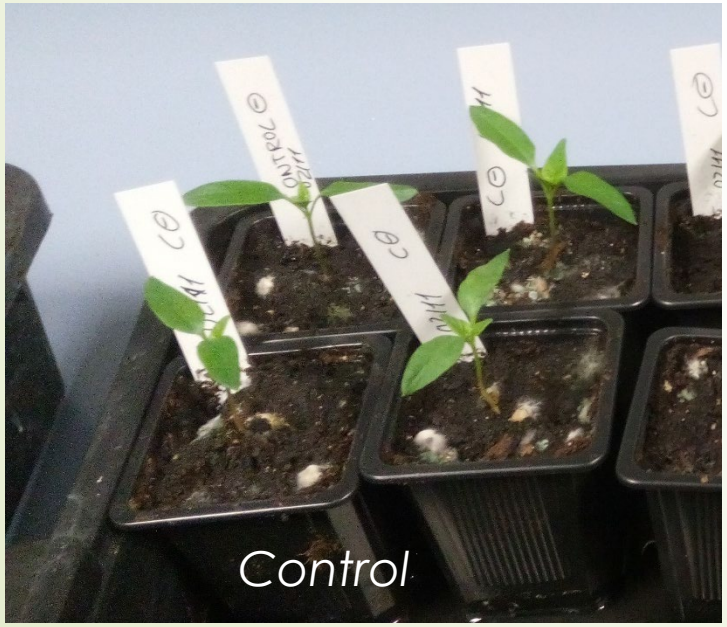


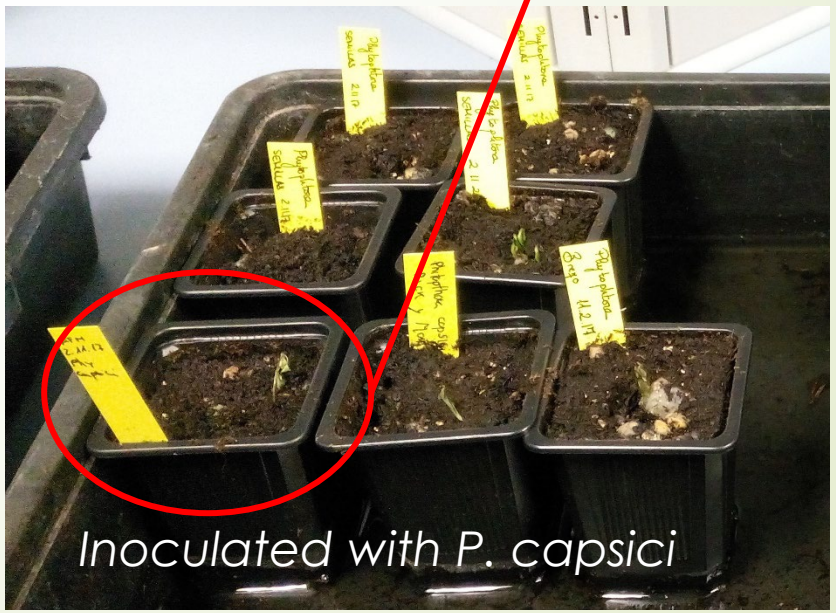


Symptoms scoring

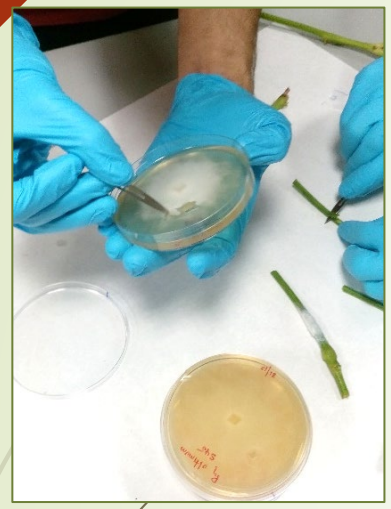
Necrosis in a susceptible plant

Screening for resistance to *Phytophthora capsici* in pepper





Screening for *Phytophthora capsici* resistance in pepper



Control in a wet box

Progress of infection in inoculated stem

Dealing with abiotic stresses
Aspects to take into account

- Preliminary screening/phenotyping should be done with large number of accessions in field conditions specific to the stress

- Alternatively, trials in lab conditions can be carried out if reliable protocols are available

- Assays should be conducted under well-defined controlled conditions: the optimum or different levels of the stress can by applied

Screening for drought tolerance in the lab

Dealing with abiotic stresses

Aspects to take into account

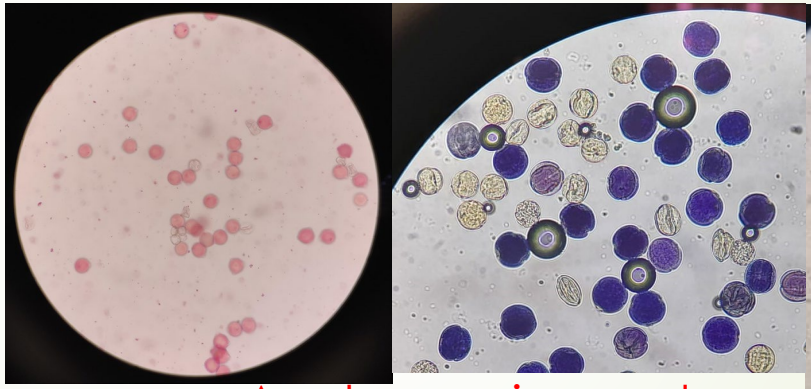
- Evaluation should be conducted with proper **experimental desing** depending on the **number of accessions** to be evaluated.
  - **Augmented block desing** (ABD) is being practiced in large number of accessions
  - For few acessions evaluation should be done in **randomized block design** (RBD) where the checks should be randomized along with the accessions in each replication

- In ABD, the **checks should be replicated in each block after separate randomization of checks within a block**. The number of checks will depend upon the crop and the parameters under study and representative of the type of germplasm. Three or more checks in which one national as well as one locally adapted check used for comparative assessment of germplasm

Dealing with abiotic stresses

Aspects to take into account: traits to be recorded

- Plant growth
  - Plant height, fresh weight, dry weight,
- Flowering
  - Earliness (male and female flowers in monoecious plants)
  - Fruit set
  - Pollen quality
- Fruit traits
  - Fruit weight
  - Fruit size
  - Fruit quality traits
- Fisiological traits
  - Content of proline, glicine betaine, etc.
- Roots



Acetocarmine and tetrazolium staining

Pollinic tube stained with acetocarmine

Testing adaptation to drough: main roots

Angel of the root in the upper part

Length of the main roots

Diameter neck

Testing adaptation to drough: fine roots

S Soler, J Prohens

Dealing with abiotic stresses

Looking for a needle in a haystack

Dealing with abiotic stresses

Genetic diversity and **digitalization** to save water resources in the cultivation of Cucurbitaceae

100% irrigation

50% irrigation

Salinity conditions

Assessing abiotic stresses: water deficit

Platforms and Sensors

DJI Matrice 300 RTK + Zenmuse H20T

- **MTOM:** 9000 g
- **Optic sensor RGB:** CMOS 1/2.3", 12 MP
  - DFOV: 82.9°
  - Focal length: 4.5 mm
  - Aperture: f/2.8
  - Focus: 1 m a ∞
- **Thermal sensor:** Microbolometer Vox
  - DFOV : 40.6°
  - Focal length: 13.5 mm (equivalence a 58 mm)
  - Aperture: f/1.0
  - Focus: 5 m a ∞
- **Spectral band**: 8-14 µm

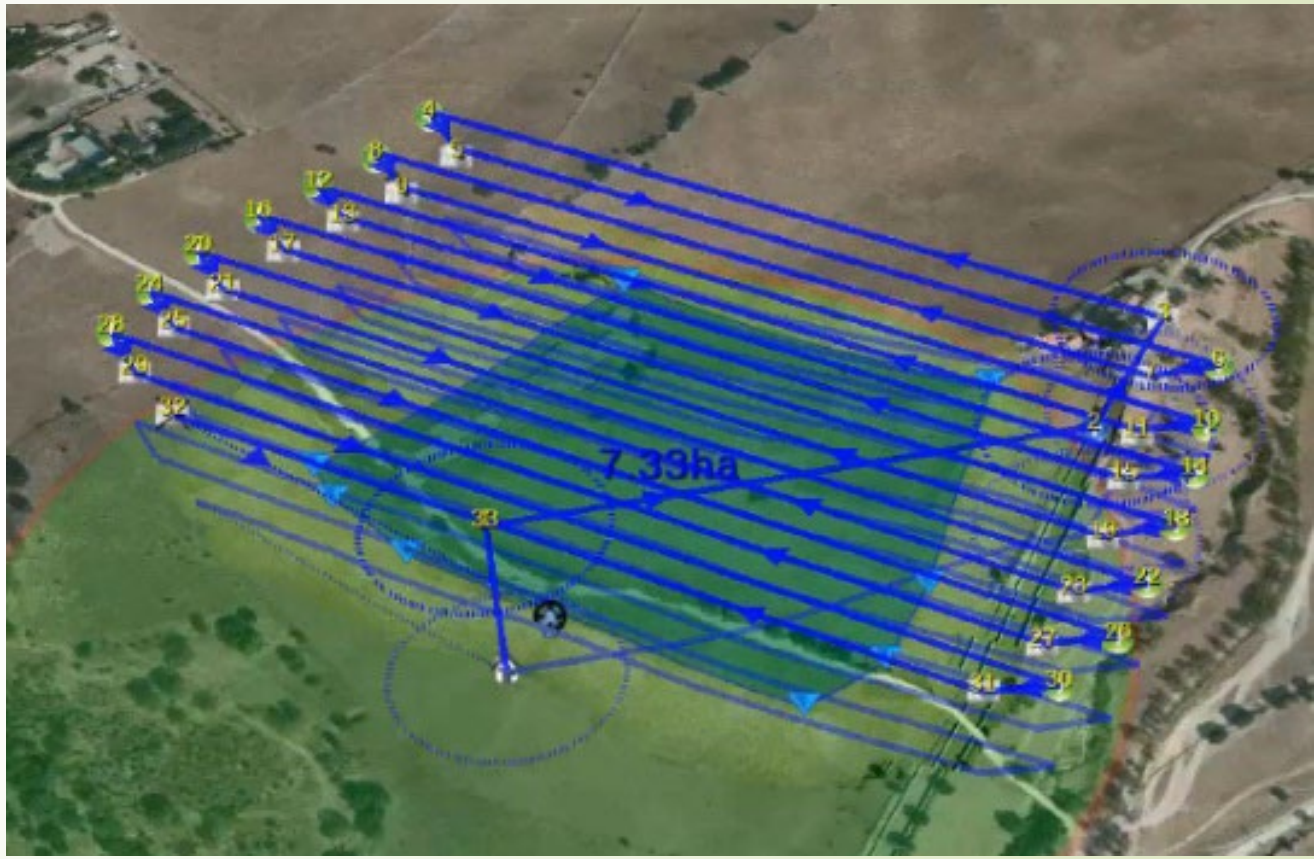
DJI Phantom 4 RTK Multispectral

- **MTOM:** 1487 g
- **Sensor:**
- Six sensors CMOS de 1/2.9", including RGB sensor for the visible spectrum and five monocrom sensors for multiespectral image

- **Bands**
  - Blue (B): 450 nm ± 16 nm;
  - Green (G): 560 nm ± 16 nm;
  - Red (R): 650 nm ± 16 nm;
  - Red border (RE): 730 nm ± 16 nm;
  - Near infrared (NIR): 840 nm ± 26 nm

Assessing abiotic stresses: water deficit

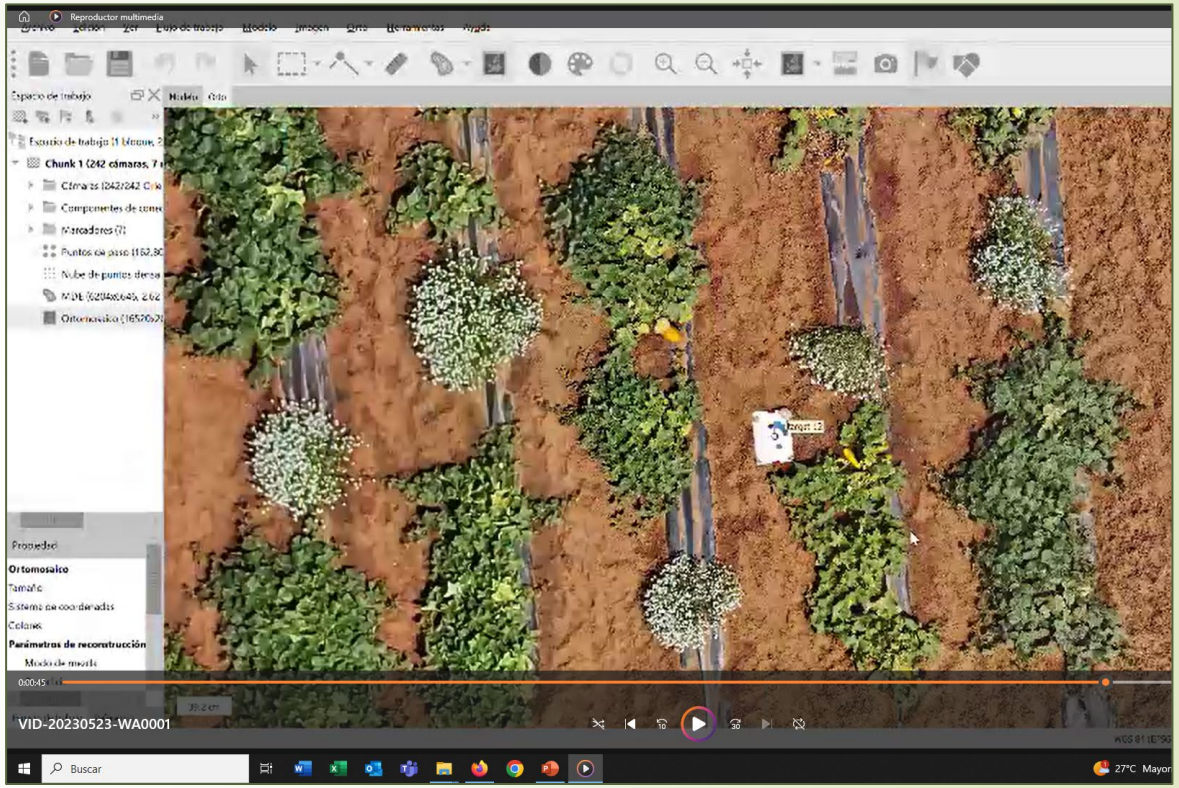
Data collection



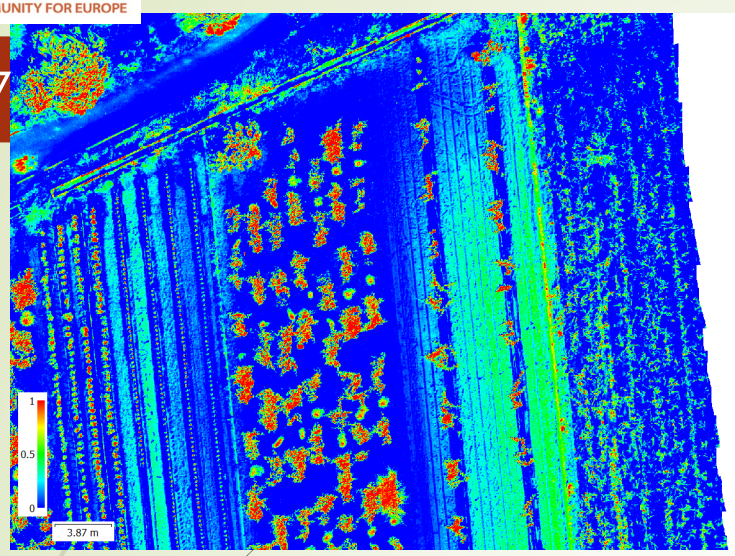
Flight itinerary

Coded targets

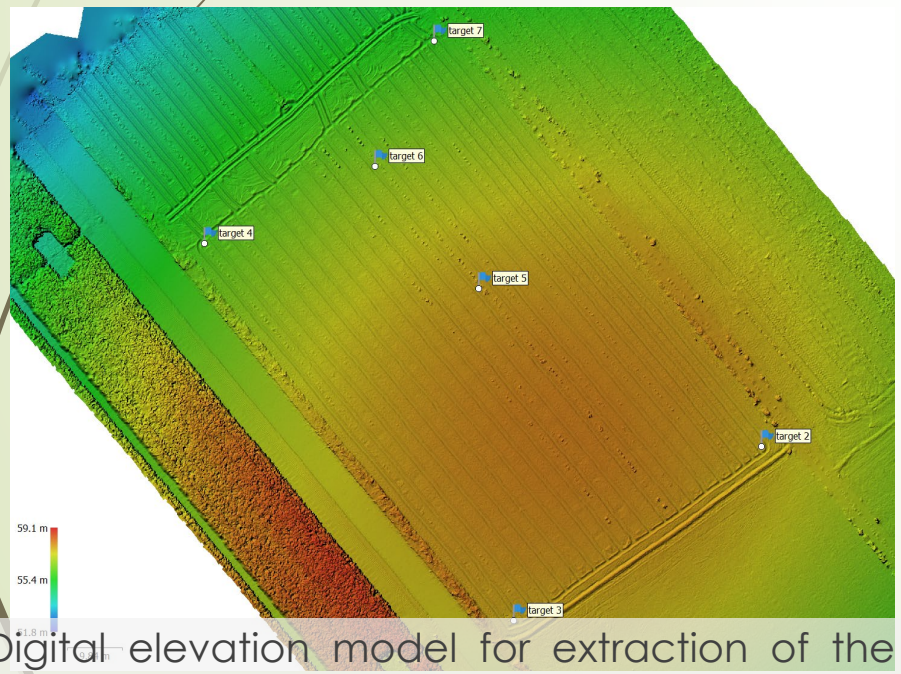
Assessing abiotic stresses



Assessing abiotic stresses: water deficit



Orthophoto in false color for sampling of the Vegetative Index



Digital elevation model for extraction of the digital terrain model and calculation of plant mass volumes

High resolution orthophoto for counting: 8.43 mm/pix

Assessing abiotic stresses

Data processing

Processing is carried out with Agisoft Metashape Professional in order to obtain **high-resolution orthophotos** and **digital elevation models** with which the **volumes of plant mass** will be calculated.

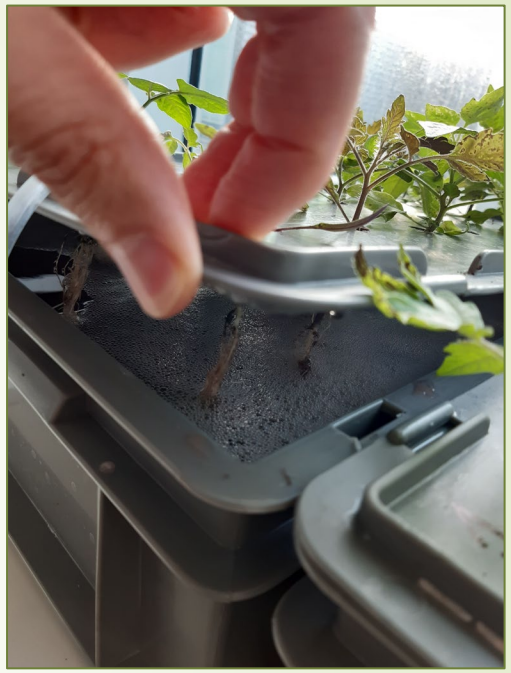
With the **high resolution orthophoto** it can be obtained together with the field data:

- Number of flowers per plant– statistical estimation
- Number of fruits– statistical estimation
- Fruit size– Manually (8mm resolution)
- Number of dead plants- Manually

With **multispectral shots and IR sensors**, information on:

- Vegetative aerial part (vegetative index), surphace, color, and chlorophill content
- Vegetative aerial part (vegetative index), temperature
- Vegetative aerial part (vegetative index), water content

Testing adaptation to drought in the laboratory: the poliethilenglicol



A. Arrones

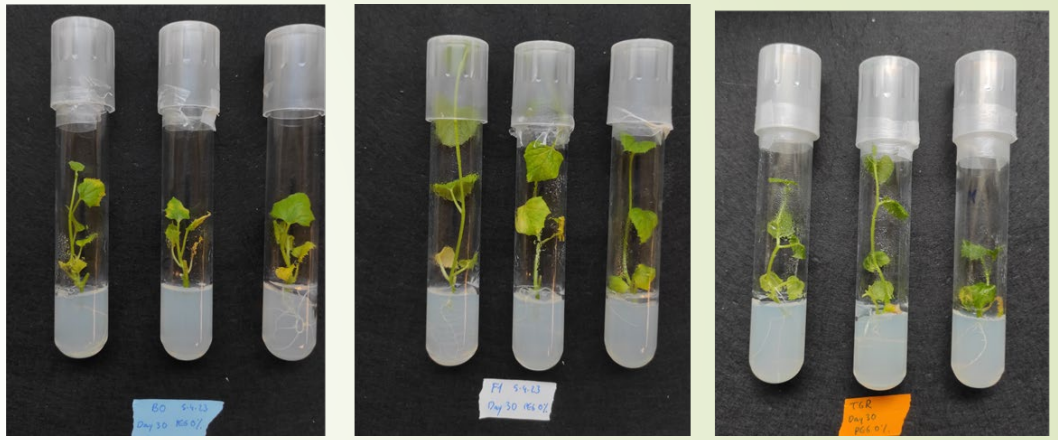
Screening for adaptation to water deficit by *in vitro* culture

- Establishing *in vitro* culture: germination and micropropagation
- Determining PEG and culture media for *in vitro* selection

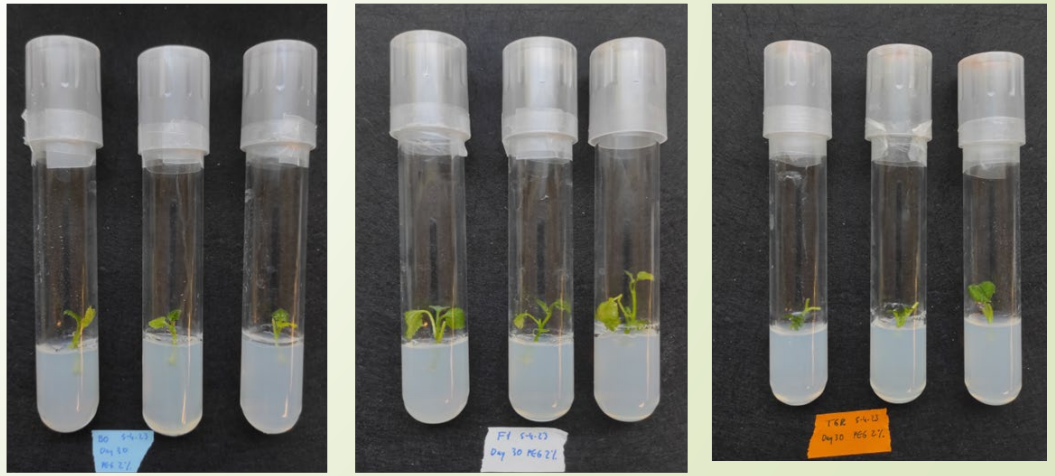
MATERIAL :

- Bola de oro (BO; Cucumis melo ssp. melo)
- 'TGR-1551' (TGR; Cucumis melo ssp. agrestis)
- F1 (BO x TGR)

Control: plants at 30 days in vitro culture without PEG



Plants at 30 days in vitro culture with PEG 2%



BO F1 TRG

Study on the adaptation of germplams to water stress in *in vitro* culture

Traits to be recorded

Genotype	% sprouting	% rooting	Index root development	Length aerial part	Number of leaves	Inhibition apical development
BO PEG 0	100	100	4,25	9,68	7,13	0
BO PEG 1	100	100	1,57	2,17	3,42	77,56
BO PEG 1,5	100	98,5	1,13	1,36	3,75	85,92
BO PEG 2	100	62,5	0,87	1,2	3,88	87,6
BO PEG 2,5	87,5	62,5	0,63	1,05	4,57	89,15
F1 PEG 0	100	100	4,63	12,07	9	0
F1 PEG 1	100	100	2,17	4,47	5	62,98
F1 PEG 1,5	100	100	1,8	2,74	5,6	77,29
F1 PEG 2	100	100	2	2,23	4,33	81,49
F1 PEG 2,5	87,5	87,5	1,43	2,24	4,86	81,41
TGR PEG 0	100	100	4,13	8,38	7,75	0
TGR PEG 1	100	100	1,88	2,13	5	74,49
TGR PEG 1,5	100	100	1,83	1,52	4,17	81,9
TGR PEG 2	100	100	1,5	1,18	3,13	85,92
TGR PEG 2,5	100	62,5	0,83	1,35	4,67	83,89

Results at 45 days

The PEG 1 concentration is already selective: it inhibits root formation and plant development.

The F1 shows more vigor and shows less inhibition.

There are no major differences between BO and TRG

Focused Identification of Germplasm Strategy (FIGS)

- It is an efficient technique that combines passport and agroclimatic data for a large number of accessions.

The breeder looks for specific characters in uncharacterized material in germplasm banks

Hipothesis:

If we can identify **geographic regions** in which **limiting environmental conditions** (biotic or abiotic stresses) have occurred during the evolutionary process, we can identify those accessions in a germplasm bank **originating from such regions** or from others subject to the same selection pressures.

Study carried out with the accessions of local bread wheat varieties from VIR, ICARDA and Australia by the Grains Research and Development Corporation

Total number of accessions #17778

Total Number of Accession: 17778

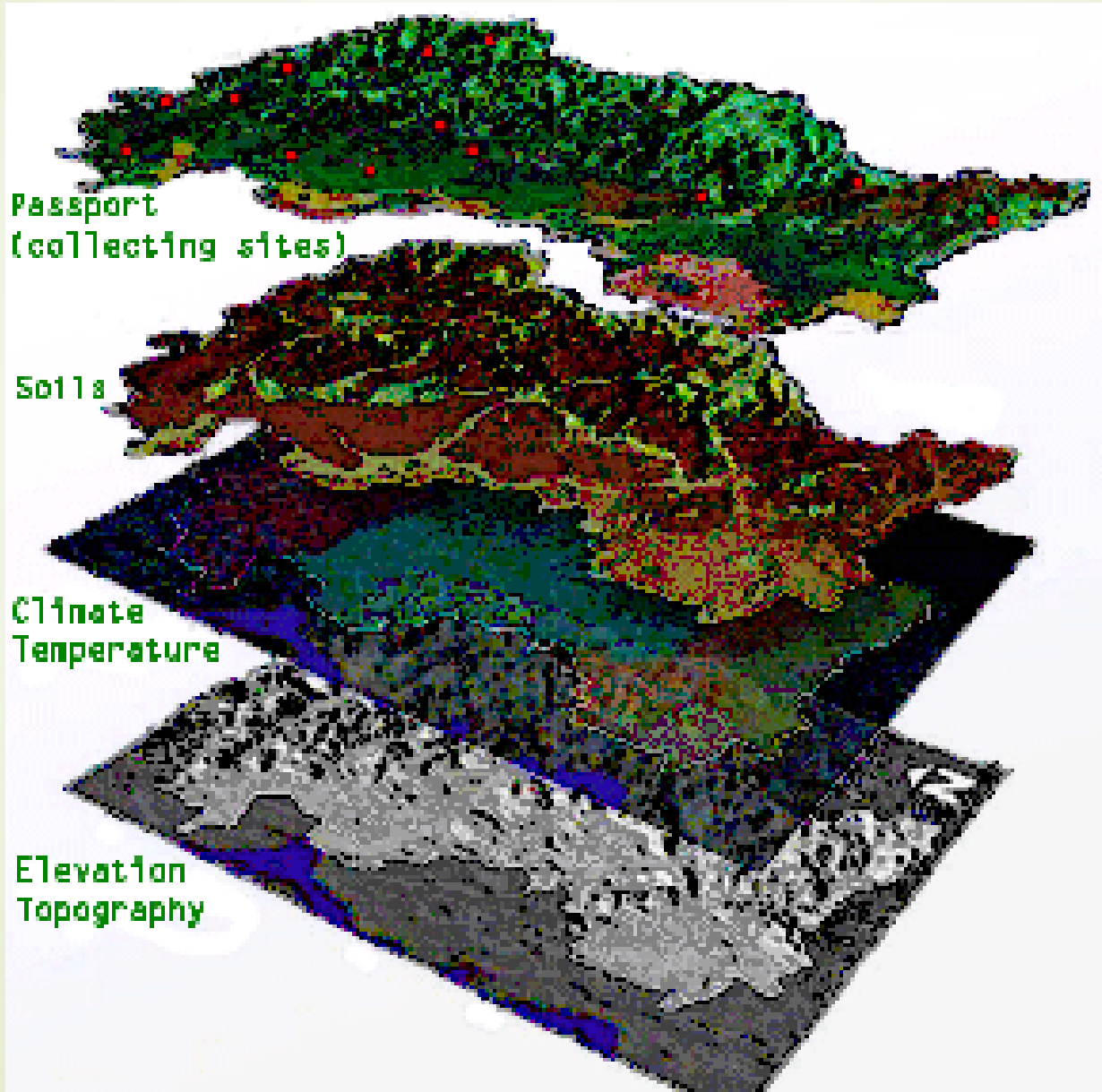
VIR	6983
ICARDA	7175
AWCC	3620

Countries Accessions

Total number of countries #85

ETH	2095	IRN	1780	AFG	1721	RUS	1647	PAK	1218	TUR	1191
TJK	877	IND	670	KAZ	601	UKR	463	DZA	462	TUN	427
CHN	416	AZE	389	UZB	337	YUG	309	MAR	300	ARM	260
PRT	198	ESP	193	TKM	184	GRC	153	IRQ	136	SYR	132
BGR	131	GEO	122	KGZ	119	EGY	110	NPL	110	MDA	99
MNG	96	BLR	89	ITA	72	FRA	59	OMN	55	POL	50
CZE	40	IDN	39	LBN	36	AUS	33	YEM	32	JOR	29
ROM	24	JPN	18	FIN	17	ERI	17	PAL	16	AUT	14
SCG	13	LVA	12	LTU	12	UNK	12	CYP	10	MEX	10
SAU	9	DEU	9	SWE	8	BOL	8	LBY	8	ISR	7
SDN	7	USA	6	EQU	6	HUN	6	MMR	5	SUN	4
COL	4	ALB	4	BTN	3	GBR	3	NOR	3	GTM	3
CSK	2	EST	2	MRT	2	DNK	2	CHL	2	MKD	2
CHE	2	BEL	1	BIH	1	BRA	1	PER	1	KOR	1
SVK	1										

Provision of passport, soil, climate and topography data



Methodology (I)

- 1. Collect all passport data in a single database, verifying geographical coordinates
- 2. Obtaining environmental data from each collection location through geographic information systems (GIS)
- 3. Environmental information from collection sites can be used to create trait-specific subsets of materials for evaluation.

Methodology (II)

Take the 400 entries from the bank with known resistance to downy mildew.

→ Use climatological and ecological data where they evolved to **determine the environmental profile associated with resistance**

↘ Search the full database (16089) for **entries collected in places with similar environmental profiles**

↙ **Identification of 1,320** entries from Iran, Türkiye and Afghanistan

← Screening: 16% of samples showed resistance (211 of 1320)

Methodology (III)

- Molecular characterization to determine allelic variants of the *Pm3* gene

  - More than half (111 of 211) had the resistance gene, some with unknown variants: 7 different alleles

Thanks to:

HARNESSTOM

- HARNESSTOM (grant agreement No. 101000716)
  - Tomato phenotyping training course, MVCRI, Bulgaria, 3rd July 2023
- AGROALNEXT 2022/025, Polytechnic University of Valencia
  - Dept. of Cartographic Engineering, Geodesy and Photogrammetry(I Quintanilla, J Vidal)
  - COMAV (C Gisbert, B Picó, A Pérez, M Izquerdo)

AGROALNEXT

Financiado por la Unión Europea NextGenerationEU

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

Plan de Recuperación, Transformación y Resiliencia

GENERALITAT VALENCIANA
Conselleria d'Educació, Universitats i Ocupació

BRESOV

- BRESOV (Grant agreement ID: 774244) (S Soler, J Prohens)

NATIONAL SCIENCE FOUNDATION

- VARITOME (National Science Foundation, USA) (E van der Knaap)

- MAGIC_Pimpi_Ceras (PID2020-118627RB-I00) (S Vilanova, S Soler)