

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

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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

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Breeding for adaptation to climate change



Disease resistance



Cytoplasmic male sterile lines for hybrid breeding

Plant Genetic Resources



Insect-pest resistance

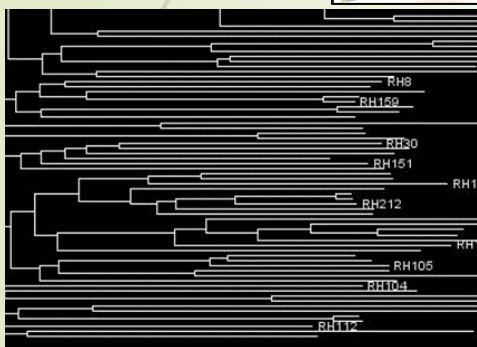


Breeding for non-conventional seasons

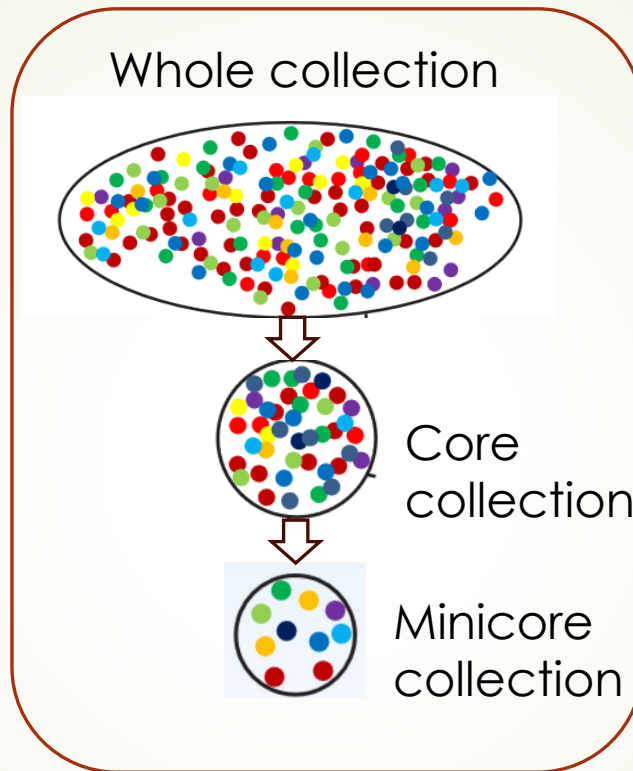
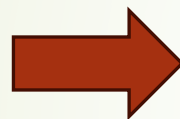


productivity enhancement

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



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

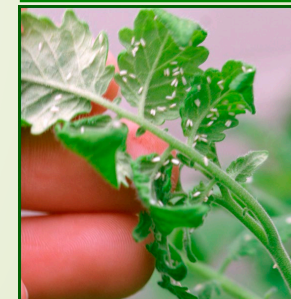


EVALUATION

Agronomic and quality



Resistance to pests and diseases

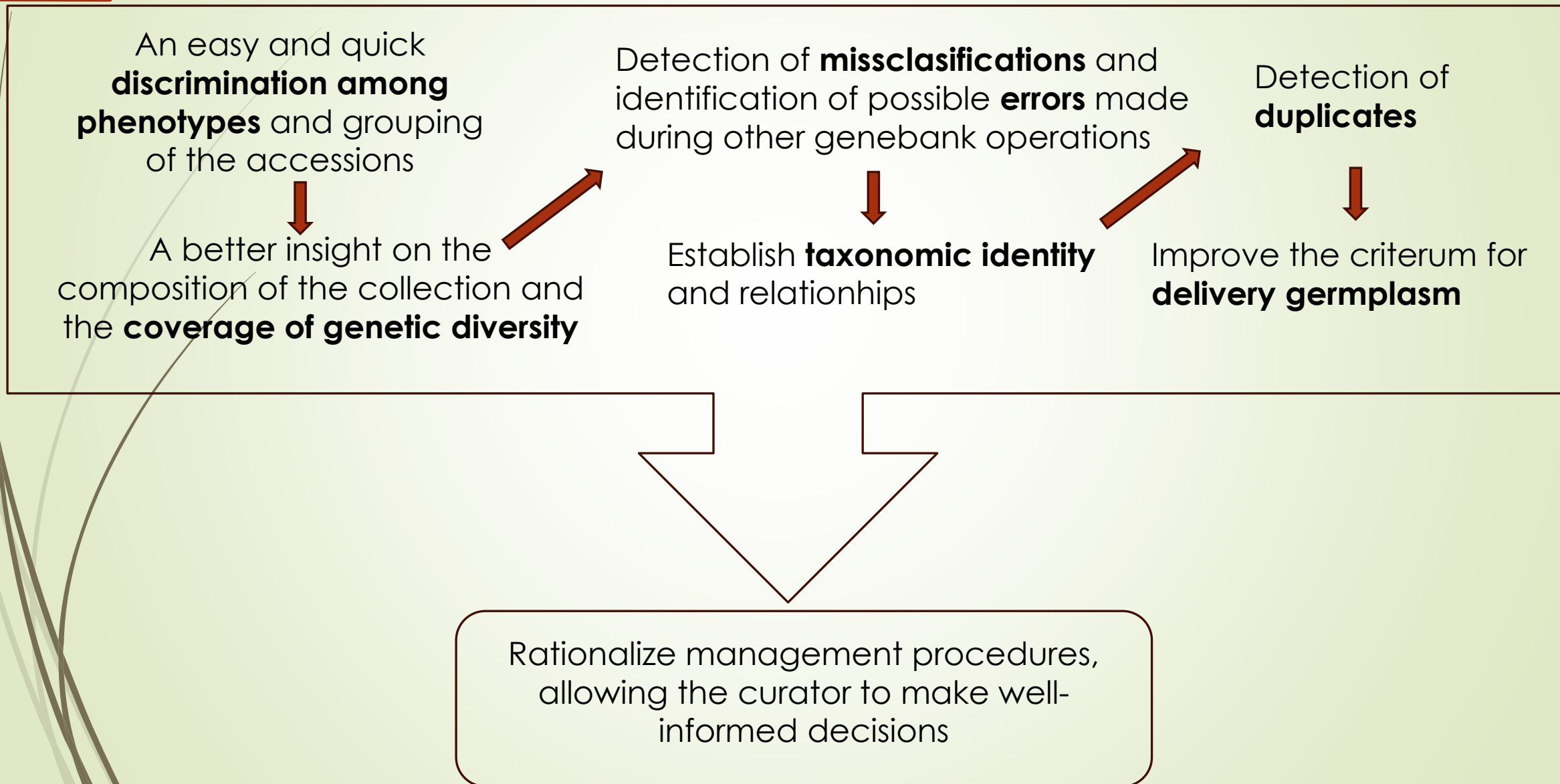


Adaptation to abiotic stresses



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Characterization is necessary to:



Characterization and evaluation

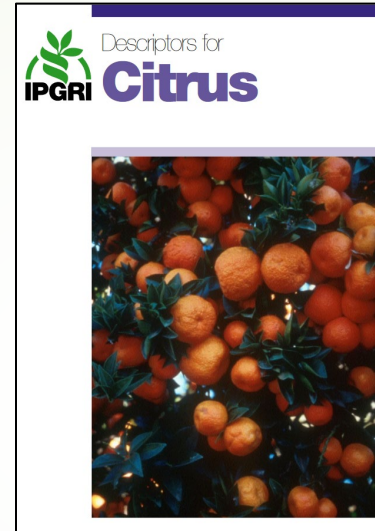
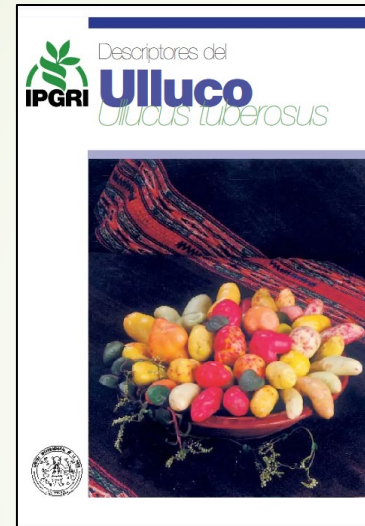
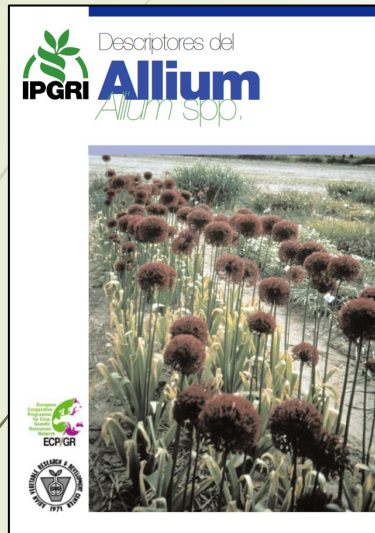
- The **characterization** of germplasm deals with the understanding and recording of **highly heritable traits** which are generally expressed in all the environments. Therefore, it can be performed in a single environment. It ranges from morphological features to seed proteins and molecular and biochemical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



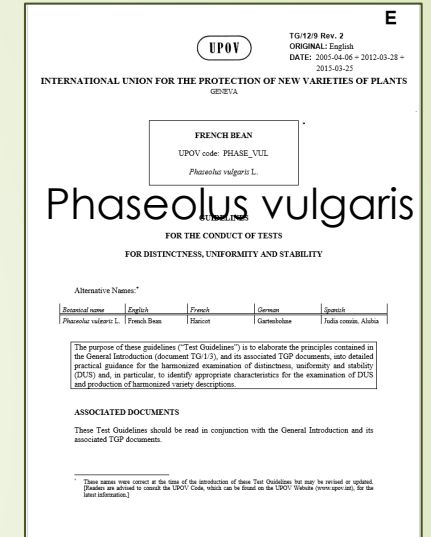
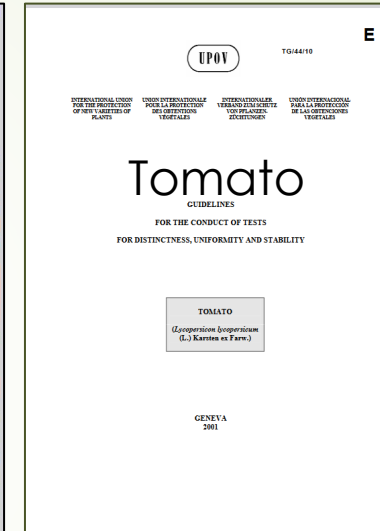
Morphological characterization

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IPGRI Descriptors



UPOV Guidelines

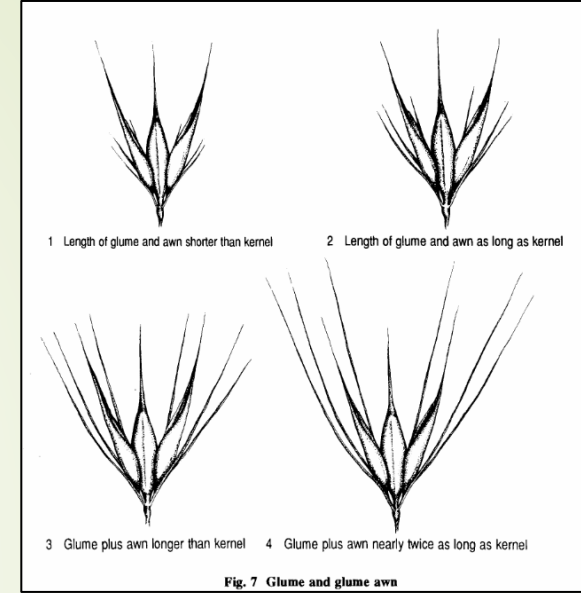
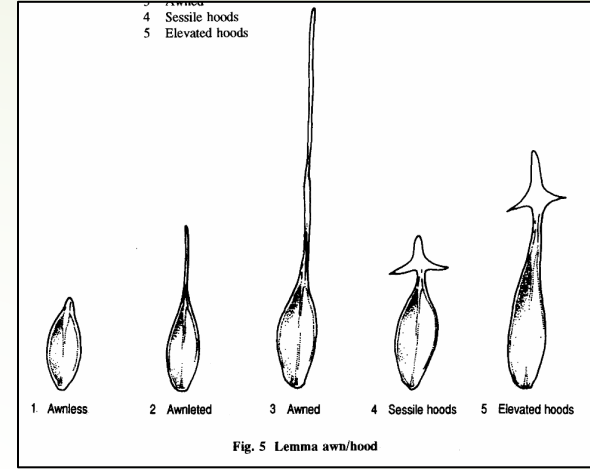
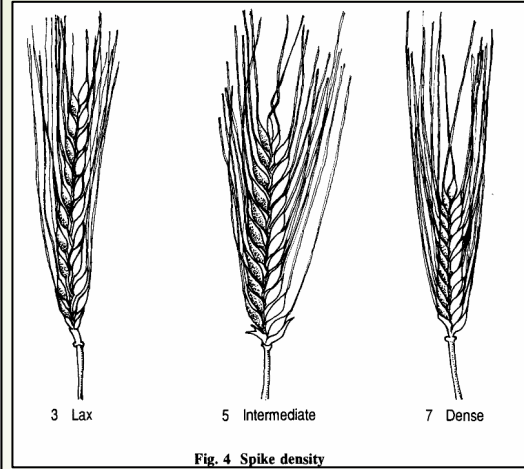


Passport descriptors
Management descriptors
Environment and site descriptors
Characterization descriptors
Evaluation descriptors

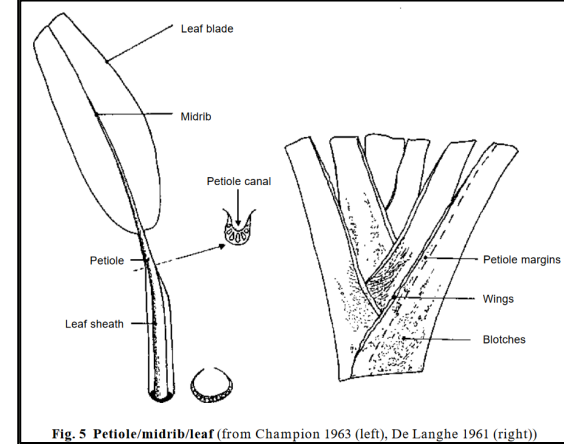
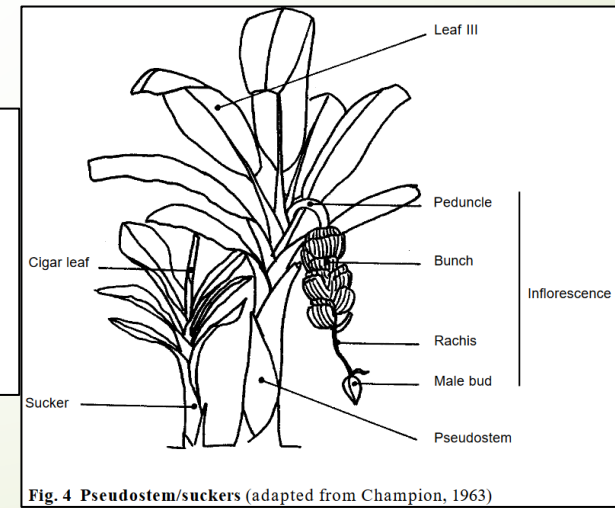
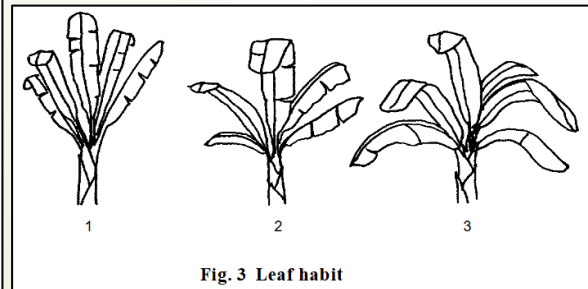
DEFINITIONS AND USE OF THE DESCRIPTORS

- **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.
Latitude, 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
...

Descriptors for **Barley** (*Hordeum vulgare* L.)



Descriptors for **Banana** (*Musa* spp.)



Descriptors for <i>Allium</i> 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

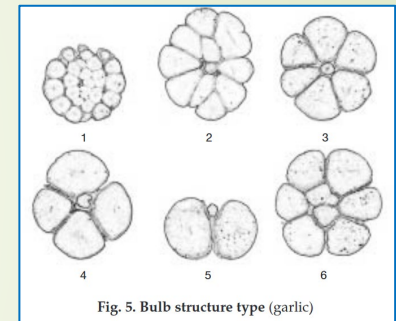
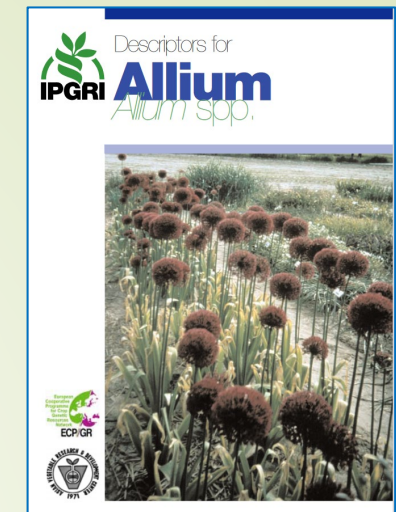


Fig. 5. Bulb structure type (garlic)

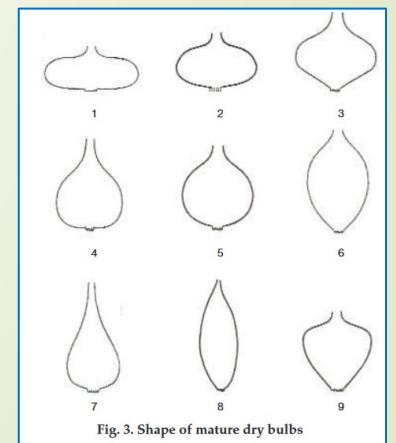


Fig. 3. Shape of mature dry bulbs

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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

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The basic morphological characterization: plant traits



Indeterminate



Semideterminate



Determinate



Present



Absent

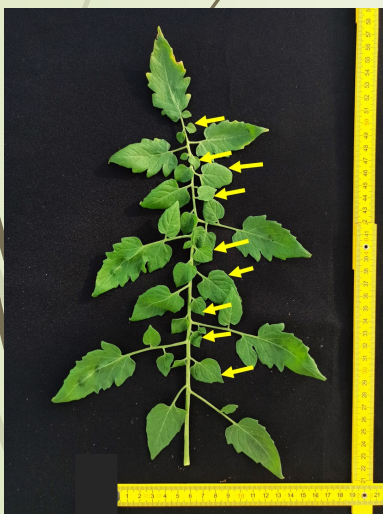
Seedling: anthocyanin coloration of Hypocotyl
Plant: growth type
Plant: number of inflorescences on main stem
Inflorescence: number of flowers per inflorescence
Stem: anthocyanin coloration
Stem: length of internode
Plant: height

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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

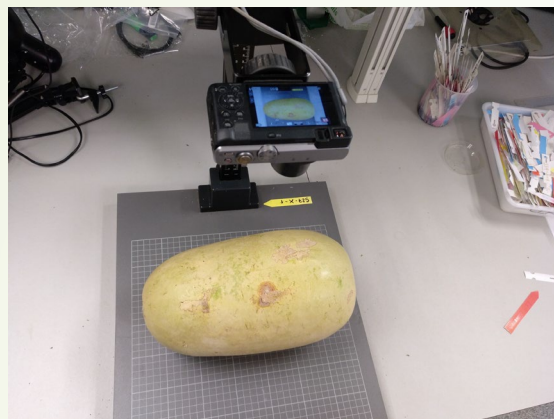


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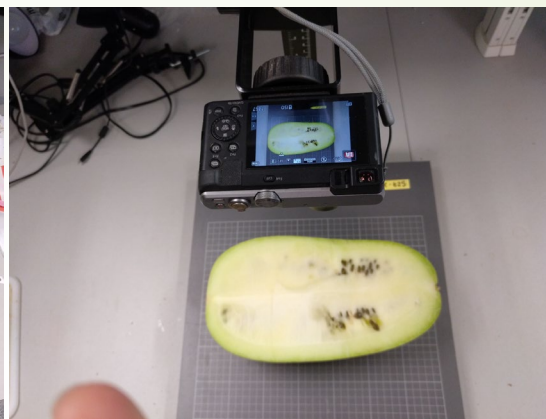
The basic morphological characterization: fruit



Fruit weight



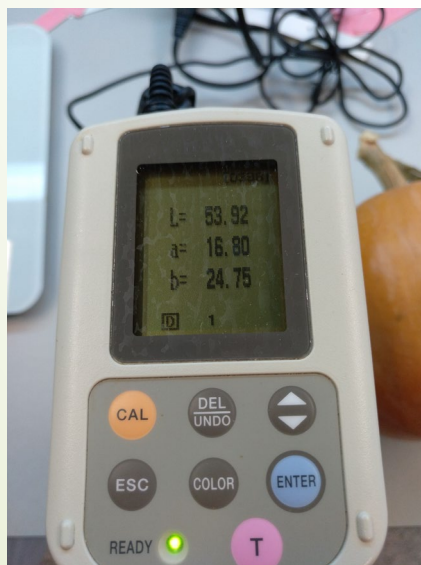
Images



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.

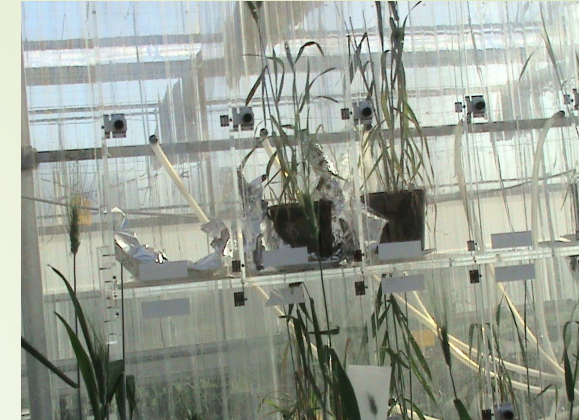
Evaluation is needed for

- **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.

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Controlled conditions with the
vector (*Bemisia tabaci*)



Agroinoculation

Inoculation techniques



Natural infection conditions

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



0



1



2

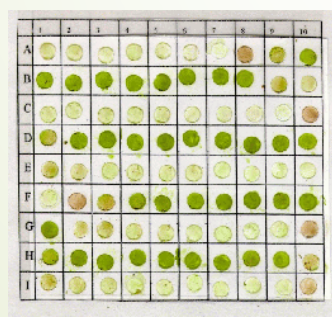
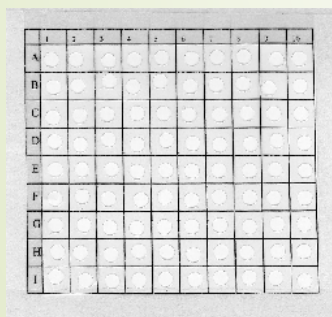


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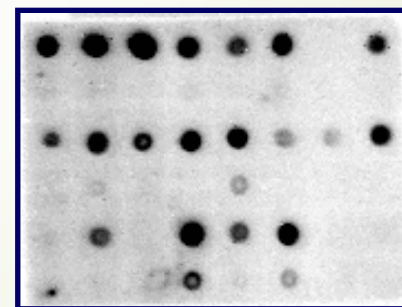
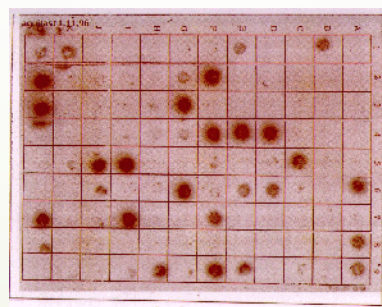


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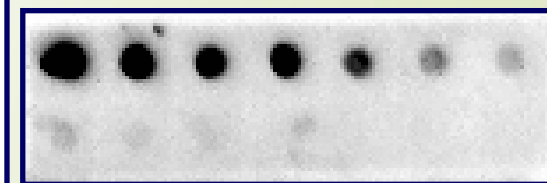
Virus quantification by squash blot or dot blot



Squash blot



Dot blot



The problem of wild relatives for evaluation of resistance

- Antixenosis or antibiotic mechanisms: presence of dense trichomes



Solanum pennellii

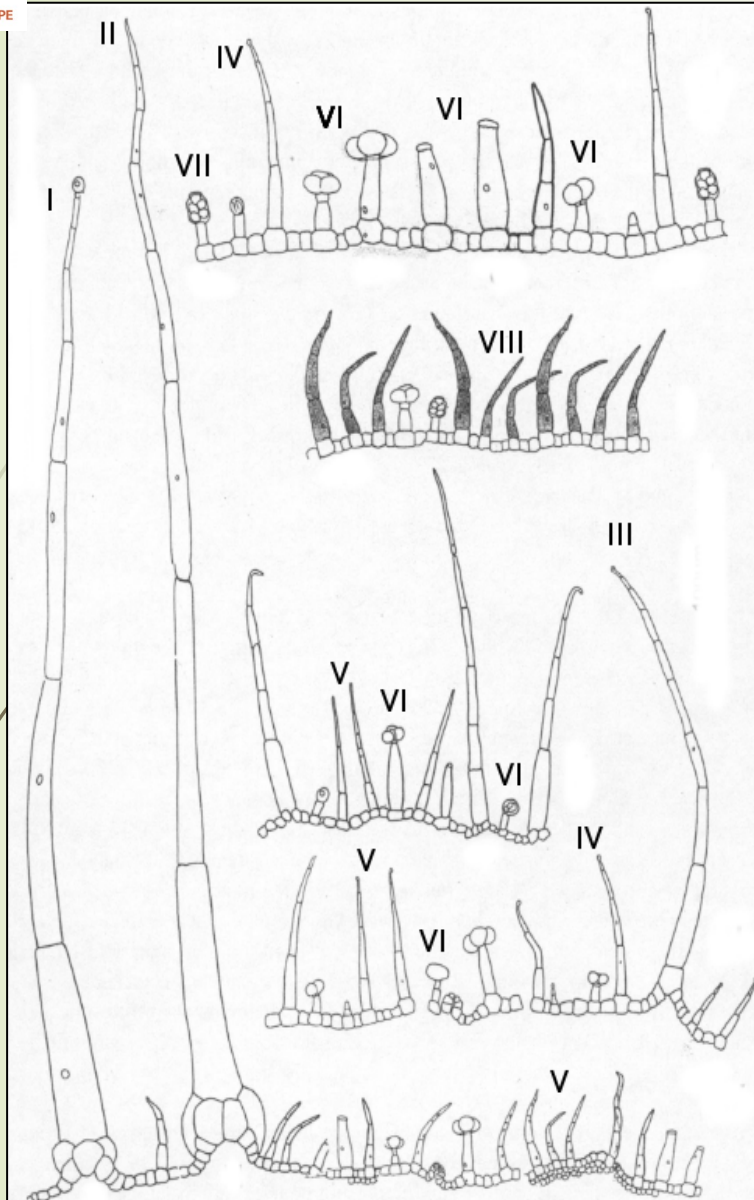


Solanum habrochaites

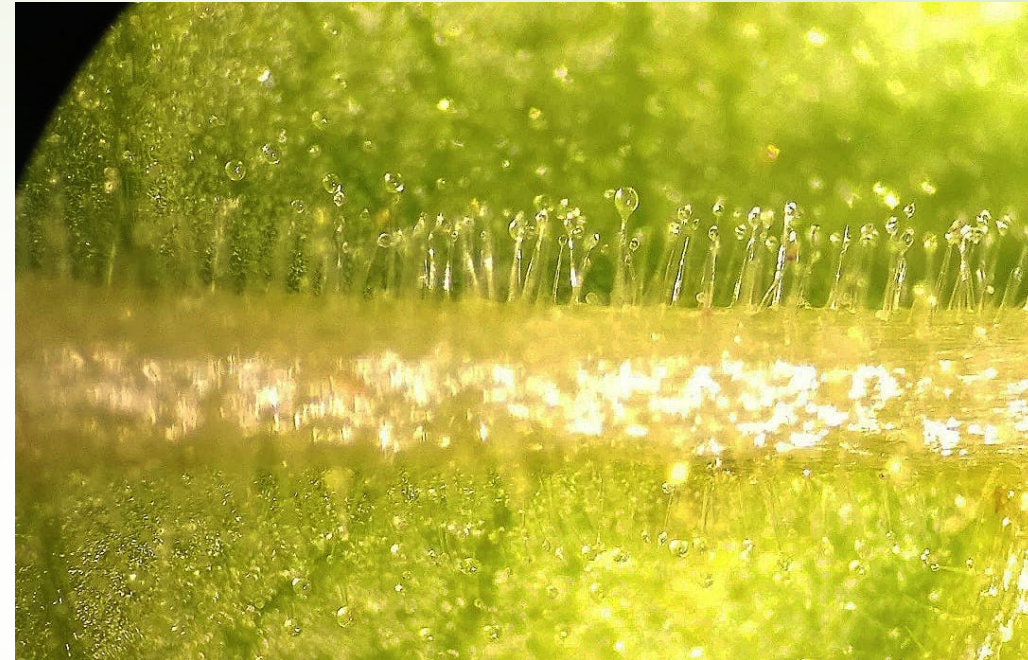
- Symptomatic and asymptomatic infected plants



Solanum peruvianum



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

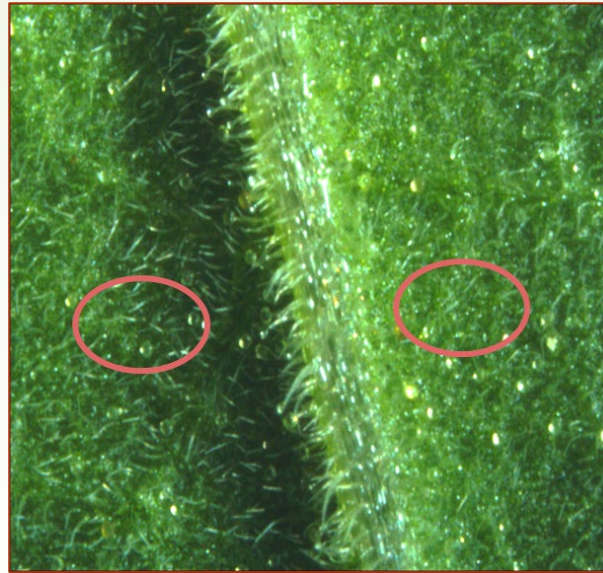
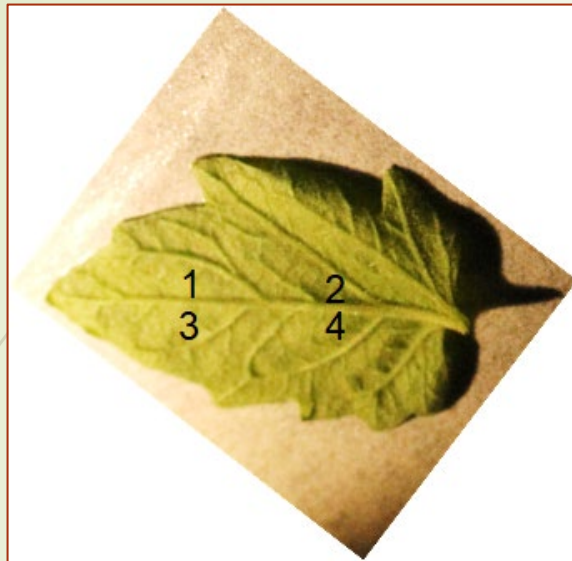


Glandular trichomes



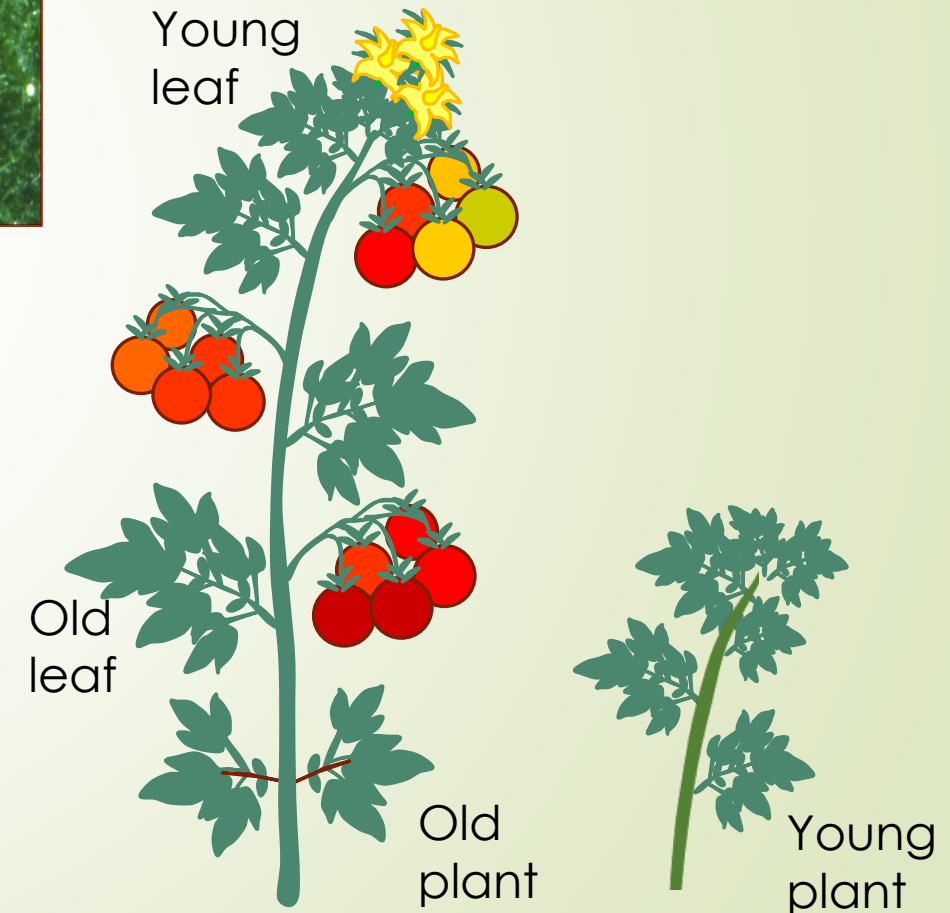
Looking deeper: factors affecting the development of trichomes

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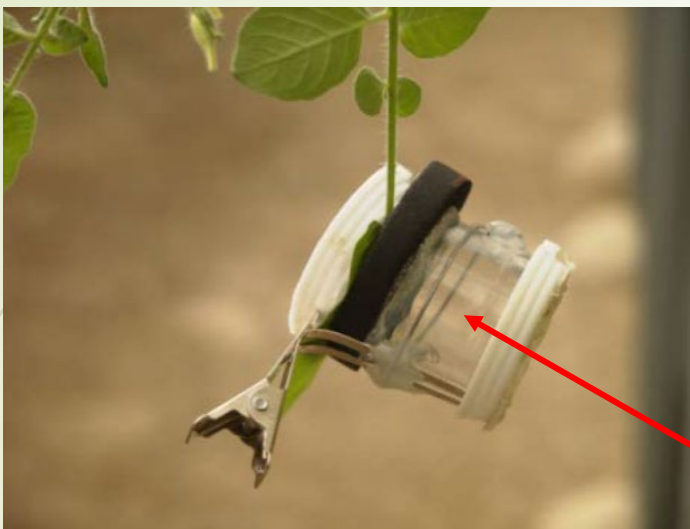


Factors affecting the development of trichomes:

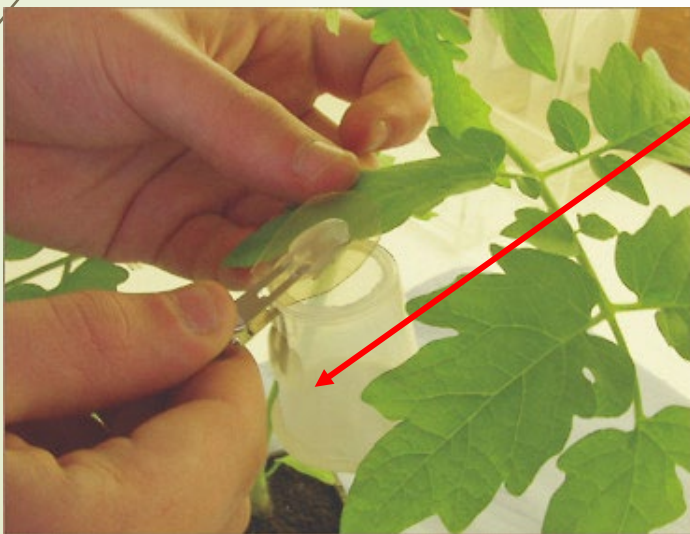
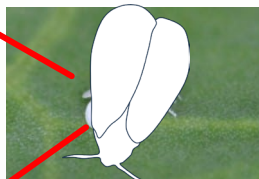
- Planta age
- Leaf age
- Temperature
- Humidity



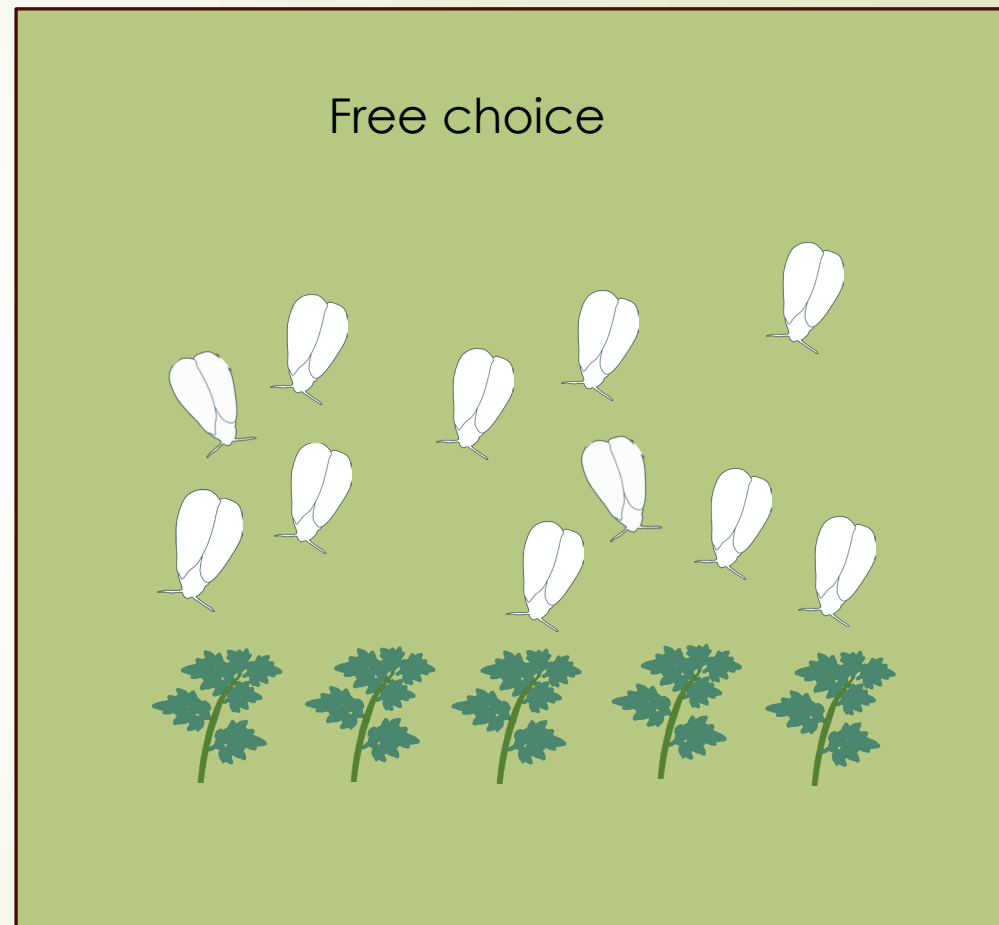
Looking deeper: - the resistance mechanism: antibiosis or antixenosis



Controlled
infestation



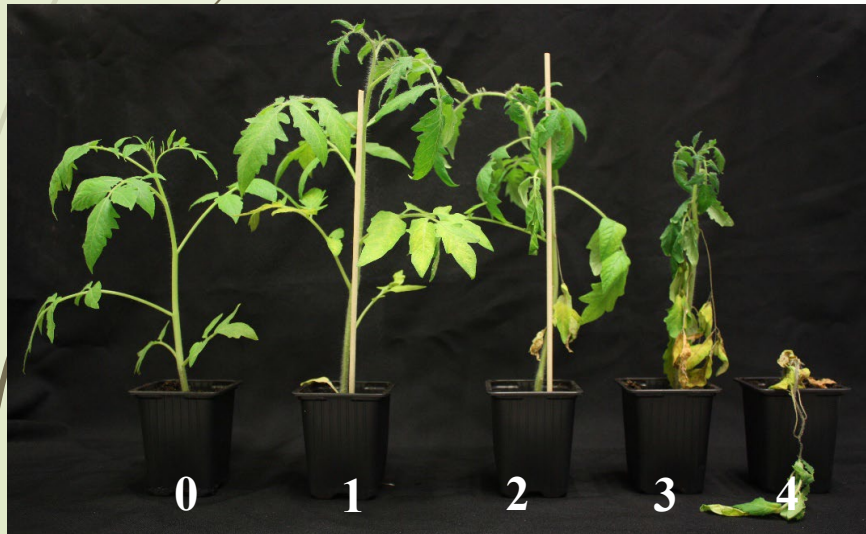
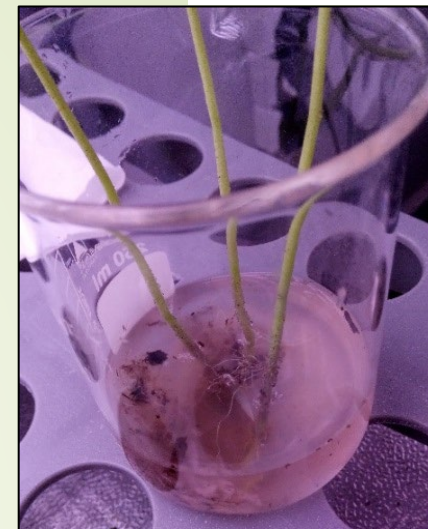
Antibiosis mechanisms: mortality, fecundity



Antixenosis mechanisms: epidemiology

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

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Symptoms scoring



Necrosis in a susceptible plant

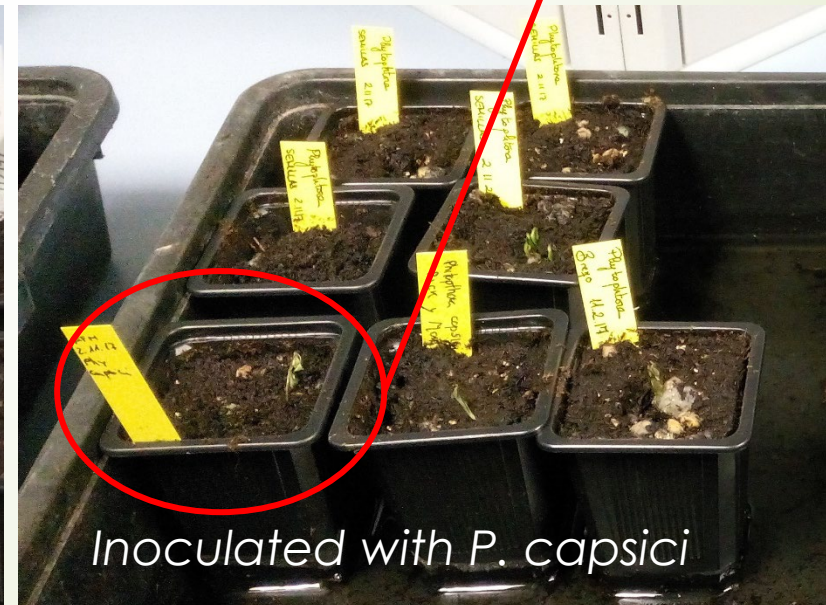


Screening for resistance to *Phytophthora capsici* in pepper

26



Control



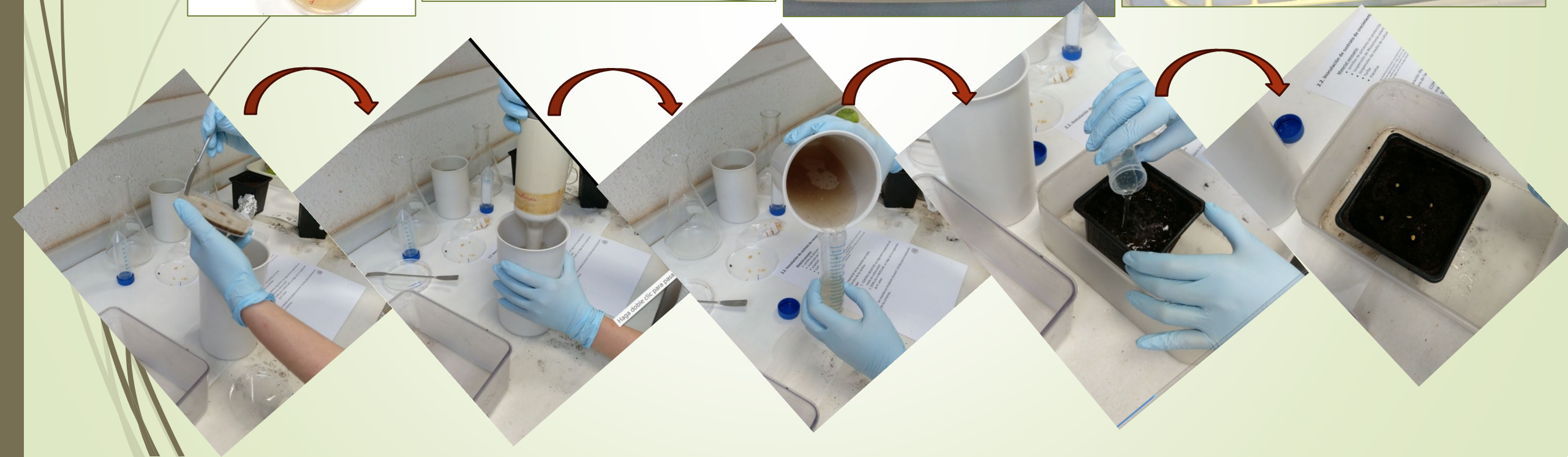
Inoculated with *P. capsici*

Screening for *Phytophthora capsici* resistance in pepper

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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 be applied



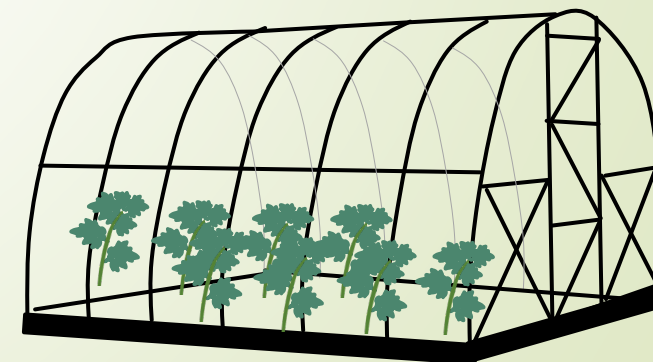
Screening for drought tolerance in the lab



Control



Treatment 1



Treatment 2

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 acesions evaluation should be done in **randomized block design** (RBD) where the checks should be randomized along with the accessions in each replication

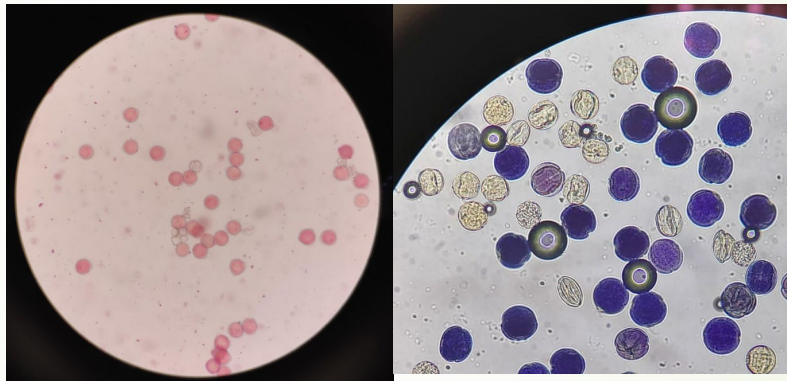


- In ABD, the **checks should be replicated in each block after separate randomization of checks within a block**. The number of checks will depend upon the crop and the parameters under study and representative of the type of germplasm. Three or more checks in which one national as well as one locally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

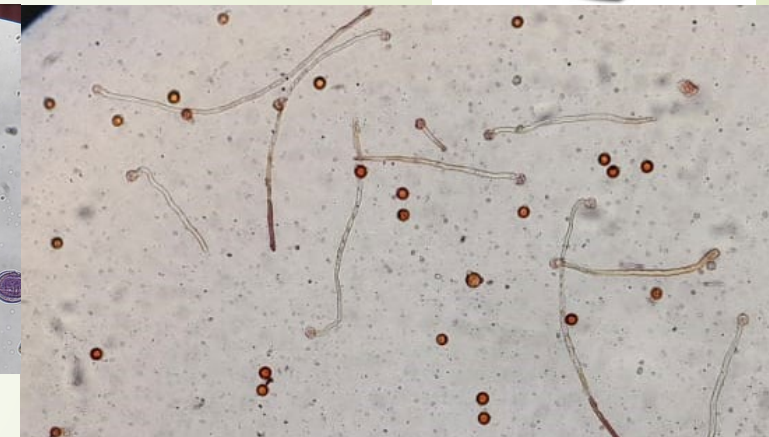
Dealing with abiotic stresses

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 drought: main roots

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Angle of the root in the upper part



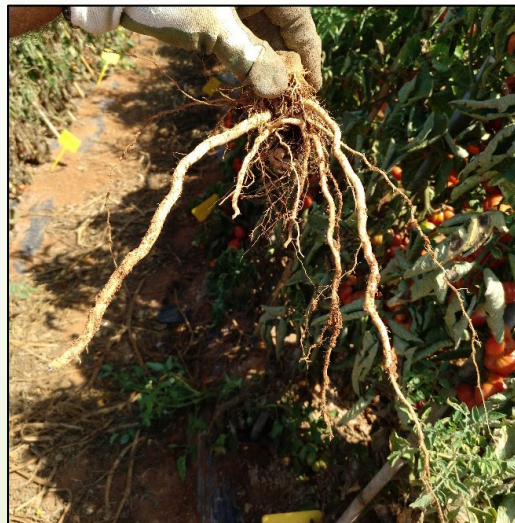
Length of the main roots



Diameter neck



Testing adaptation to drought: fine roots



Dealing with abiotic stresses

Looking for a needle in a haystack

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Dealing with abiotic stresses

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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

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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

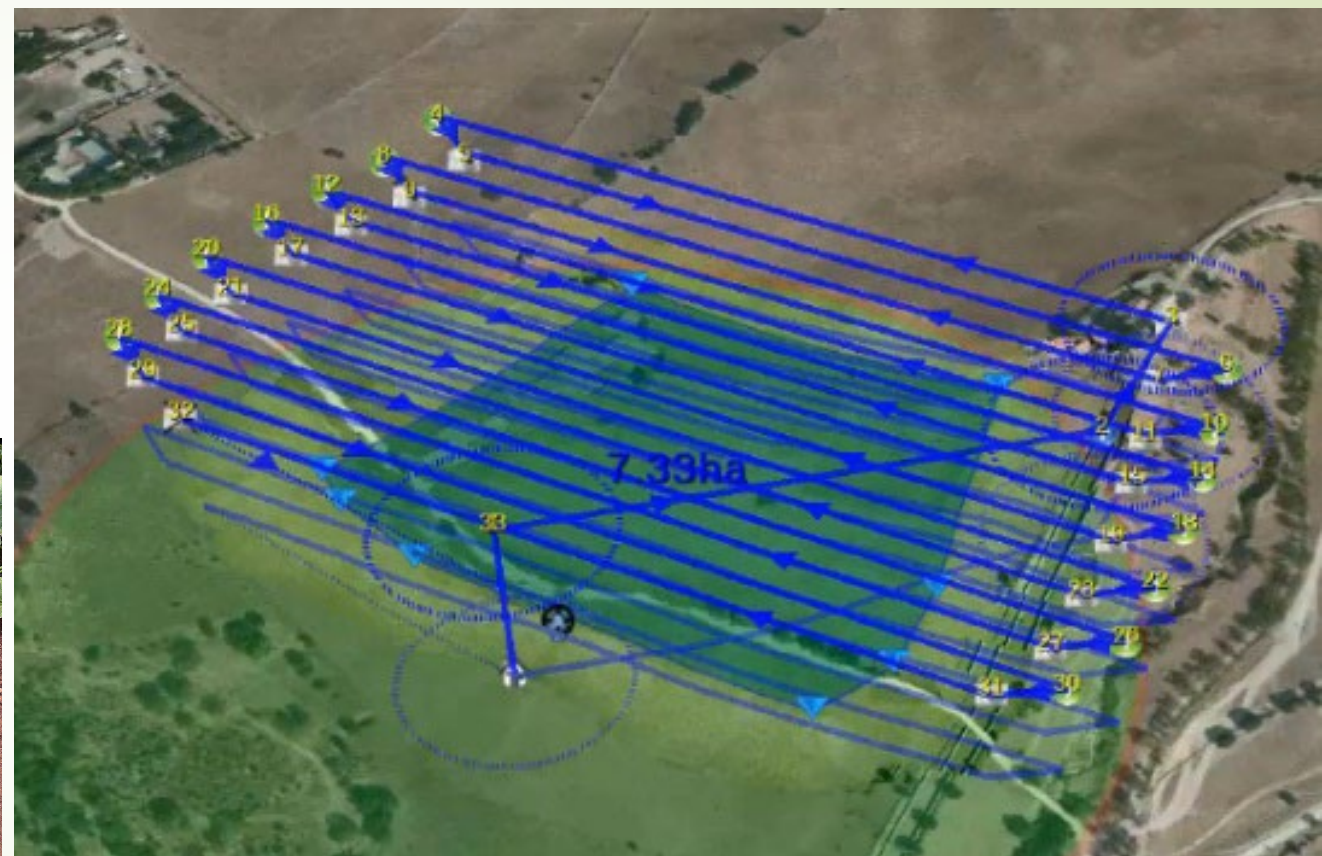
- **MTOM:** 1487 g
- **Sensor:**
- Six sensors CMOS de 1/2.9", including RGB sensor for the visible spectrum and five monocrom sensors for multispectral image
- **Bands**
 - Blue (B): 450 nm \pm 16 nm;
 - Green (G): 560 nm \pm 16 nm;
 - Red (R): 650 nm \pm 16 nm;
 - Red border (RE): 730 nm \pm 16 nm;
 - Near infrared (NIR): 840 nm \pm 26 nm

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Coded targets

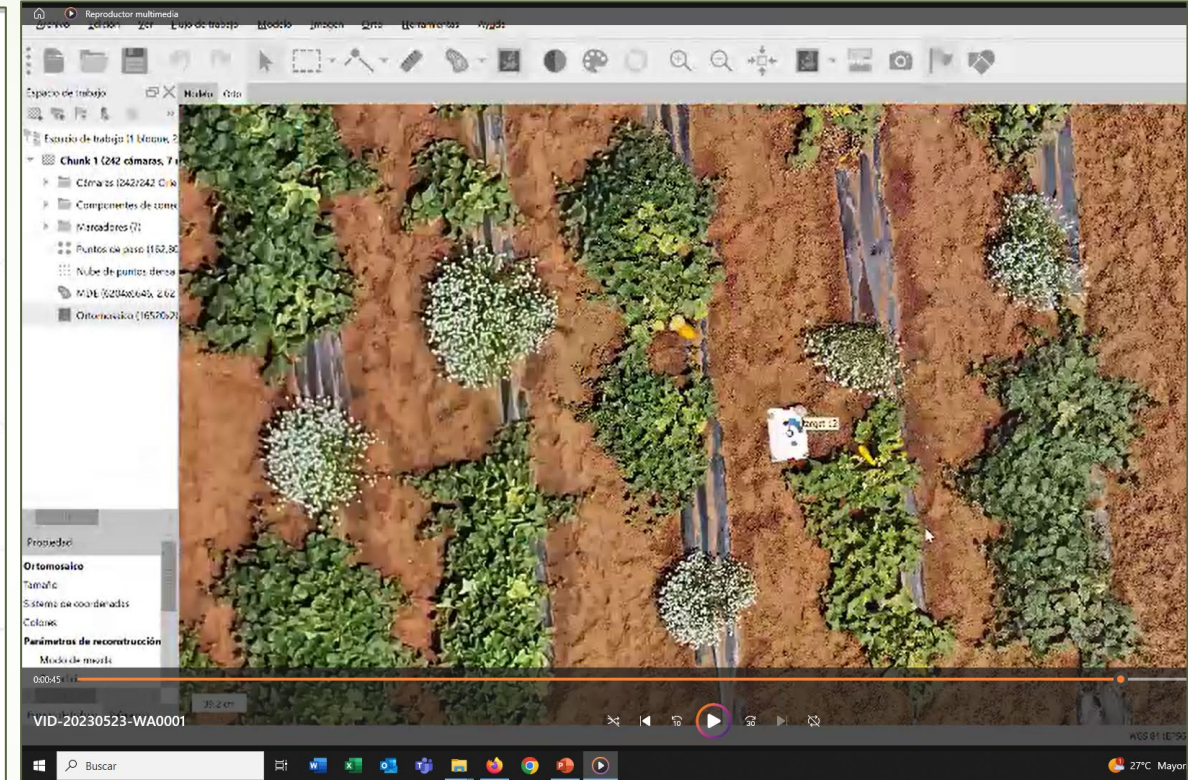
Data collection



Flight
itinerary

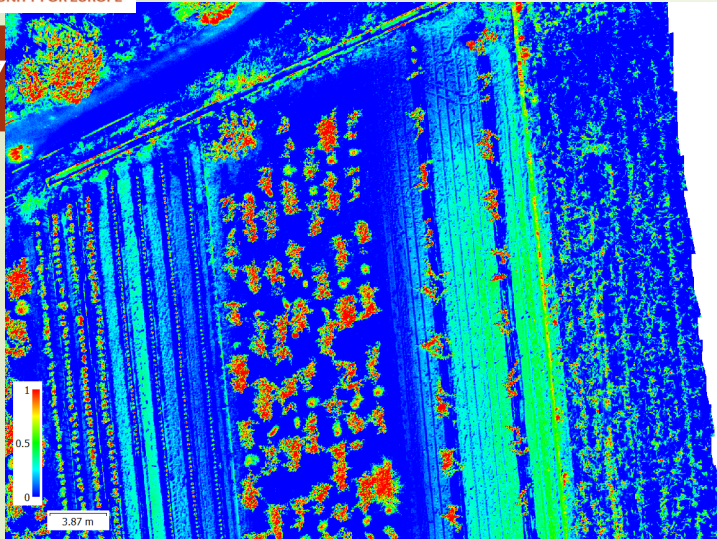
Assessing abiotic stresses

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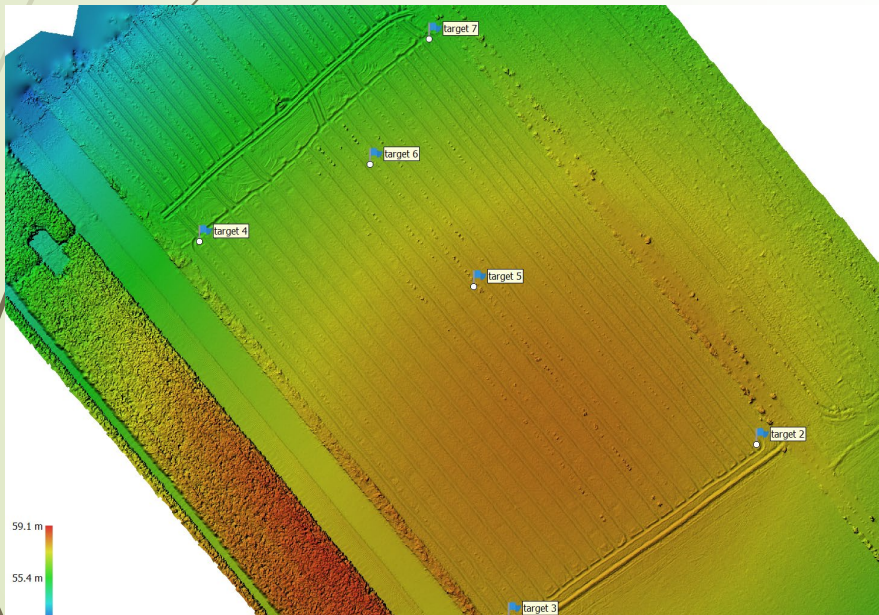


Assessing abiotic stresses: water deficit

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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
AGROALNEXT 2022/025

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), surface, color, and chlorophyll content
- Vegetative aerial part (vegetative index), temperature
- Vegetative aerial part (vegetative index), water content

Testing adaptation to drought in the laboratory: the poliethilenglicol

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A. Arrones

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

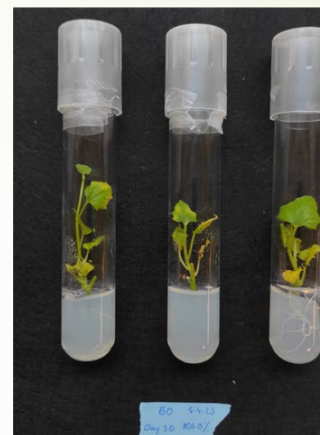
- ❑ Establishing *in vitro* culture: germination and micropropagation
- ❑ Determining PEG and culture media for *in vitro* selection

40

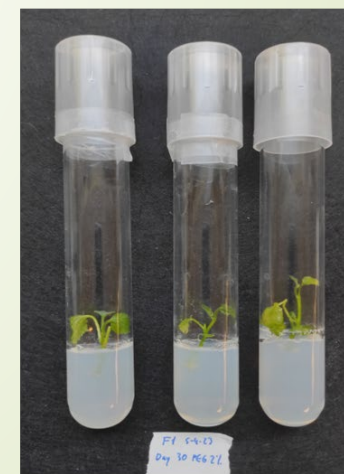
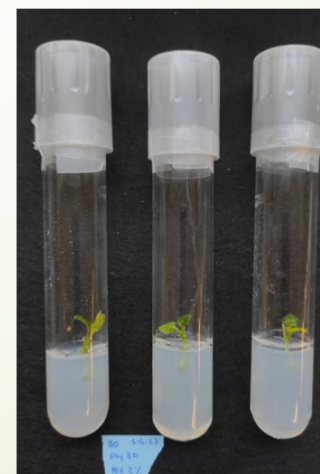
MATERIAL :

- ❑ 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

TGR

Study on the adaptation of germplasm 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

Hypothesis:

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.



Total Number of Accession: 17778

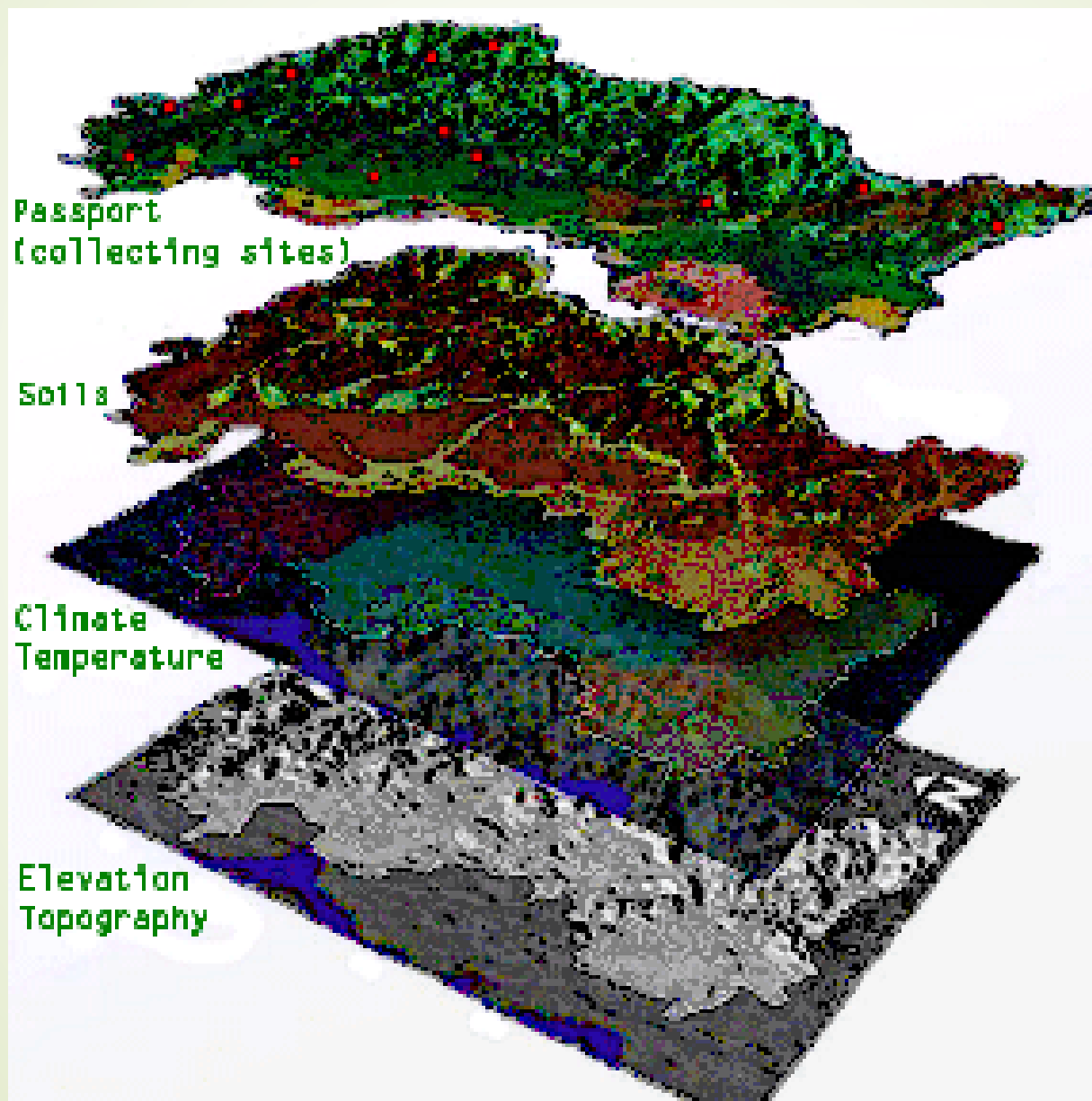
VIR	6983
ICARDA	7175
AWCC	3620

Countries Accessions

[illegible]

Provision of passport, soil, climate and topography data

44

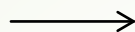


Methodology (I)

- 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)

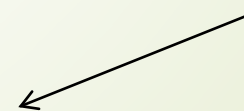
Take the 400 entries from the bank with known resistance to downy mildew.



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

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