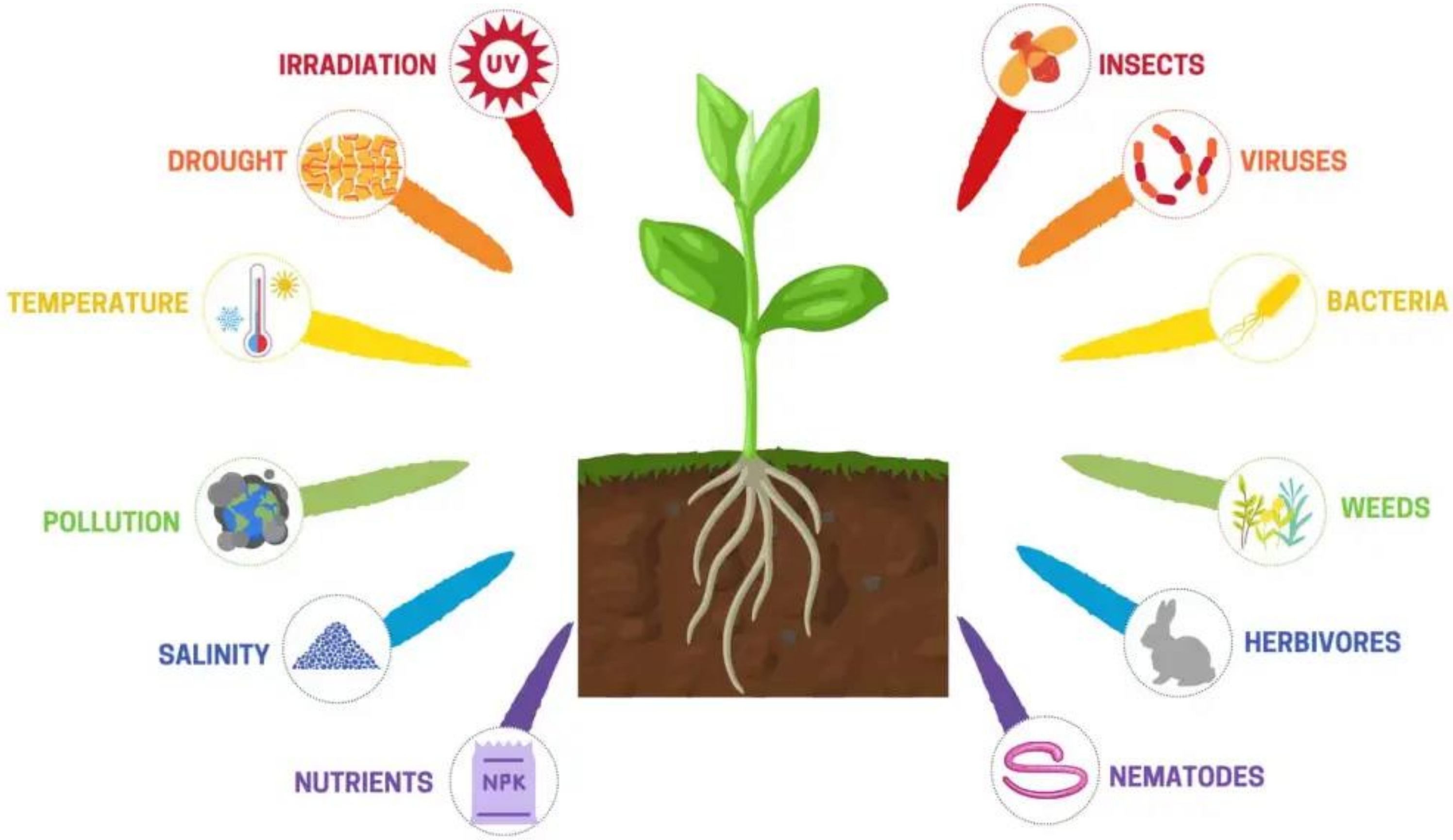
Breeding for Plant Stress Resistance

Dr. Sandra Cvejić

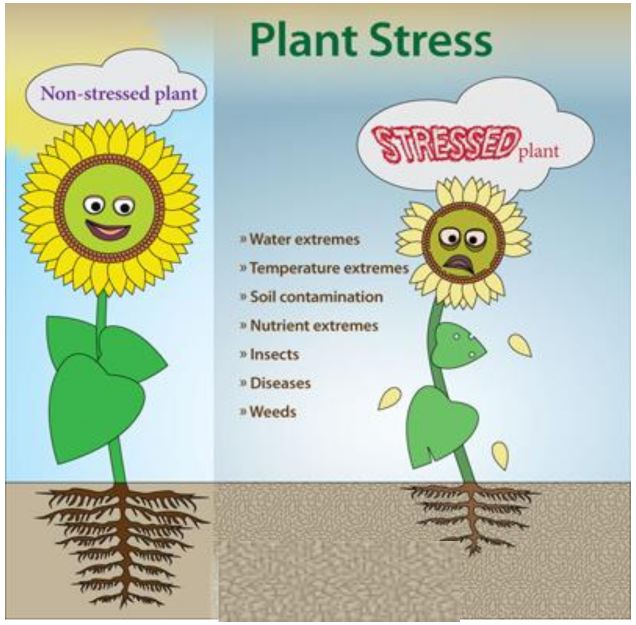


Overview

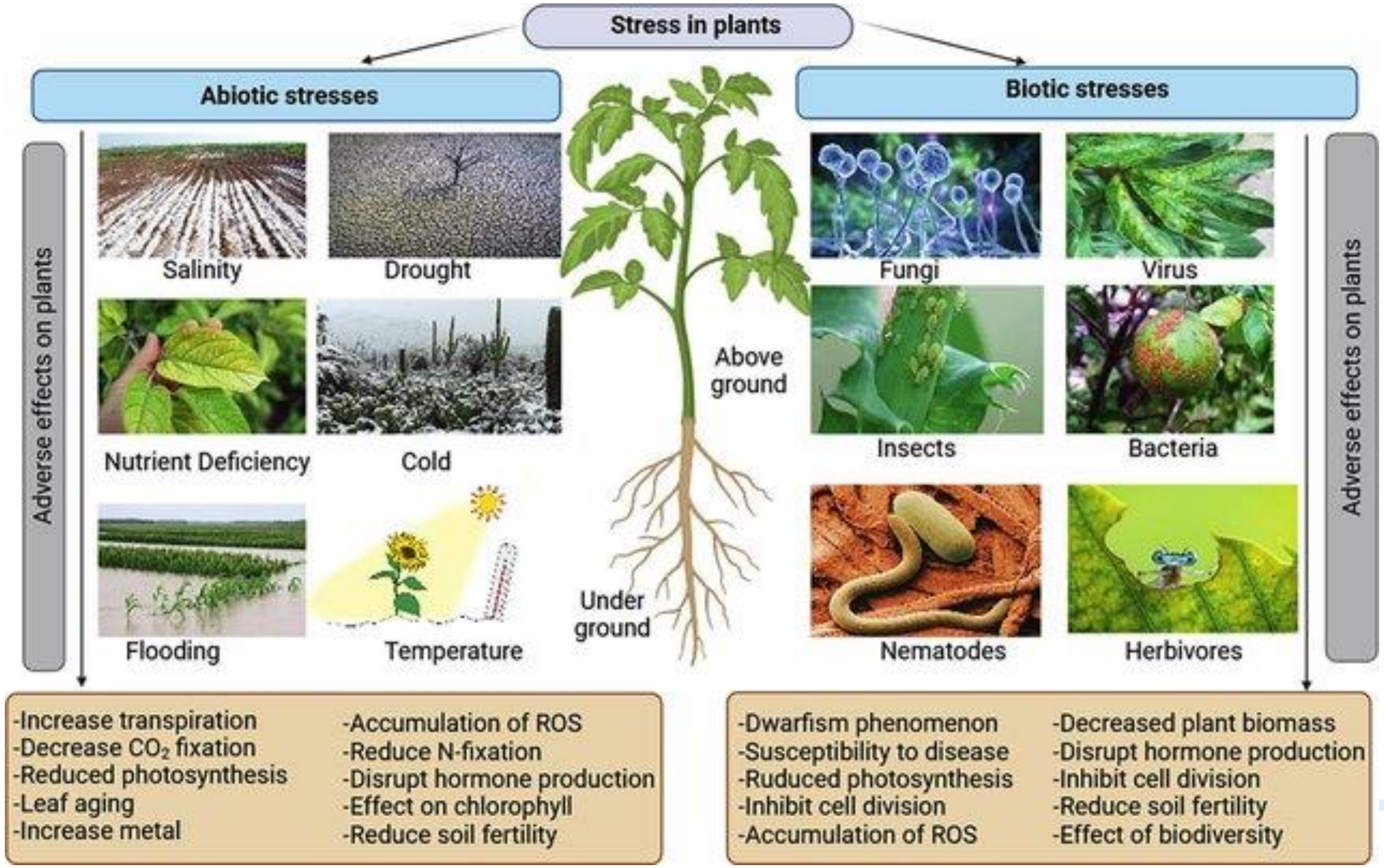
- Introduction
- Abiotic Stress
- Biotic Stress
- Breeding Strategies
- Breeding Methods
- Breeding for Multiple Stress Resistance

Introduction

- An effect that ***negatively impacts*** another entity is termed as stress.
- Stress can be defined as any factor of environment that interferes with the complete expression of the genotypic potential of the plant.
- **Climate change** increases frequency and severity of stress factors.
- Types of environment stress:
  - **Abiotic**
    - Atmospheric (air pollution, light stress, temperature stress) and
    - Edaphic (water stress, salt stress)
  - **Biotic** (human, animals, pests, diseases, weeds).
- **Breeders goal**: Develop cultivars with enhanced resilience without yield penalty.



Types of Stress



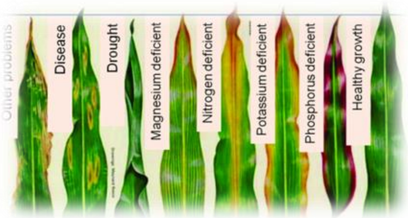
Abiotic Stress

- Refers to the ***negative impacts of non-living environmental factors*** that limit plant growth, development, and productivity.
- Stressors disrupt normal plant metabolism and physiology, forcing the plant to activate defense and adaptation mechanisms to survive and sustain itself against these environmental challenges.

Drought

Heat

Cold/Frost



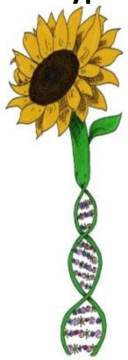
Nutrient deficiency

Salinity

Flood

Main effects

Genotype effect



Environmental effect

G x E interaction

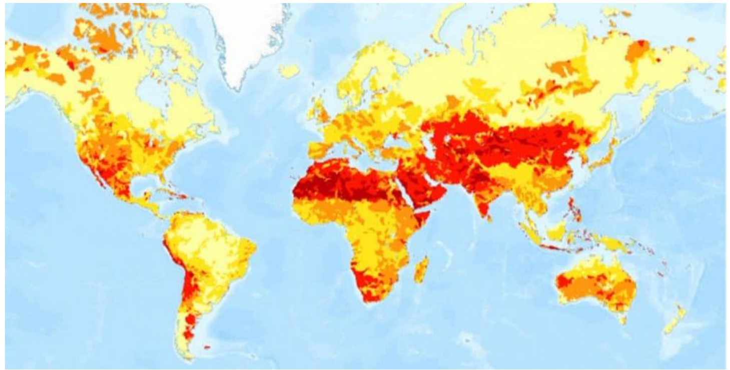
Drought

- Characterized by a ***long period of reduced rainfall***, which leads to insufficient moisture in the soil needed for normal growth and development of crops.
- Significantly affect agricultural production, reducing yields and causing serious economic and social consequences.
- ***Increase of yearly average temperature*** from 0.9 to 2.0°C till 2050.
- It is anticipated that water demand for agriculture could increase two-fold by 2050, with ***freshwater availability decreasing*** by up to 50% due to increasing climatic variations.
- Global economic losses in agriculture stemming from drought totaled approximately ***US $29 billion*** in the last decade.
- Development of high-yielding crops that are climate-resilient and more effective and/or efficient in using water is necessity.

Reference: National Drought Mitigation Center, University of Nebraska-Lincoln

Drought Identification

- **Indicators:**
  - ***Standardized Precipitation Index (SPI)*****:** Measures precipitation over different time scales to quantify meteorological drought.
  - ***Palmer Drought Severity Index (PDSI)*****:** Considers temperature and precipitation data to assess long-term drought severity.
  - ***Crop Moisture Index (CMI)*****:** Evaluates short-term moisture conditions affecting crop yields.
  - ***Normalized Difference Vegetation Index (NDVI)*****:** Uses satellite data to assess vegetation health and density, indicating drought stress.
  - ***Remote Sensing and Geographic Information Systems (GIS):*** Use satellites to monitor vegetation and soil moisture and create maps that visualize drought conditions, helping to identify the most affected areas.



https://klublr.com/ena/drought-in-world

Drought Severity Classification

			Ranges					
Category	Description	Possible Impacts	Palmer Drought Index	CPC Soil Moisture Model (Percentiles)	USGS Weekly Streamflow (Percentiles)	Standardized Precipitation Index (SPI)	Objective Short and Long-term Drought Indicator Blends (Percentiles)
D0	Abnormally Dry	Going into drought: short-term dryness slowing planting, growth of crops or pastures. Coming out of drought: some lingering water deficits; pastures or crops not fully recovered	-1.0 to -1.9	21-30	21-30	-0.5 to -0.7	21-30
D1	Moderate Drought	Some damage to crops, pastures; streams, reservoirs, or wells low, some water shortages developing or imminent; voluntary water-use restrictions requested	-2.0 to -2.9	11-20	11-20	-0.8 to -1.2	11-20
D2	Severe Drought	Crop or pasture losses likely; water shortages common; water restrictions imposed	-3.0 to -3.9	6-10	6-10	-1.3 to -1.5	6-10
D3	Extreme Drought	Major crop/pasture losses; widespread water shortages or restrictions	-4.0 to -4.9	3-5	3-5	-1.6 to -1.9	3-5
D4	Exceptional Drought	Exceptional and widespread crop/pasture losses; shortages of water in reservoirs, streams, and wells creating water emergencies	-5.0 or less	0-2	0-2	-2.0 or less	0-2

Impact on Plants

- Response of a plant to stress is in different ways, some of which include variation in gene expression, cellular metabolism, growth rates, crop yields, and so on.
- Short periods of drought may not greatly reduce seed yield.
- Prolonged drought stress significantly reduces seed yield and quality.
- The effects of drought stress on plant productivity are not the same for all the growth stages.



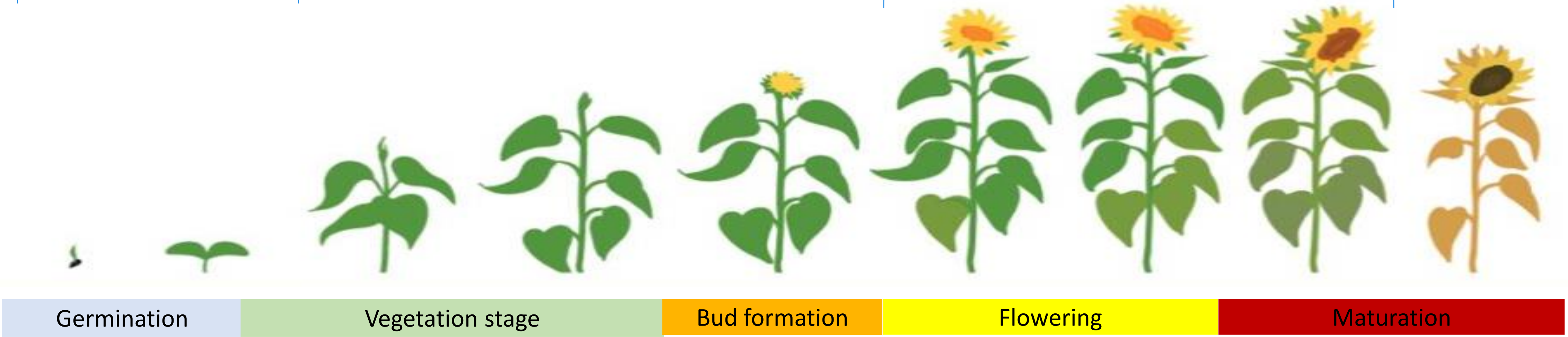

Impact on Plants

Stem elongation
Leaf surface reduction

critical

Pollen infertility
Empty seeds
Reduction of seed yield

critical

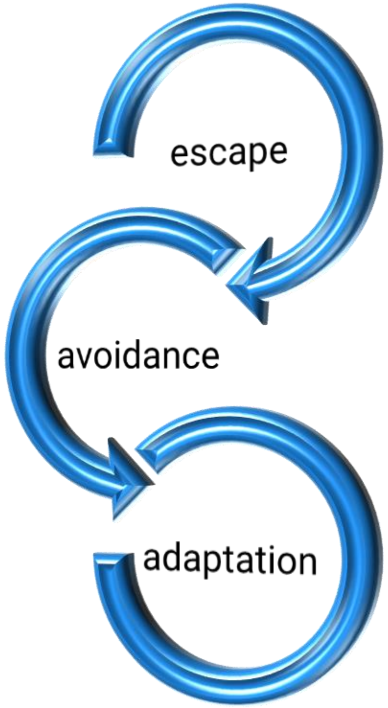


Note: More available water at initial growth stages results in good vegetative growth, but the subsequent low moisture availability at flowering and grain filling stages significantly reduces the yield due to high transpiration demands.

Hussain et al. 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives, Agricultural Water Management, https://doi.org/10.1016/j.agwat.2018.01.028.

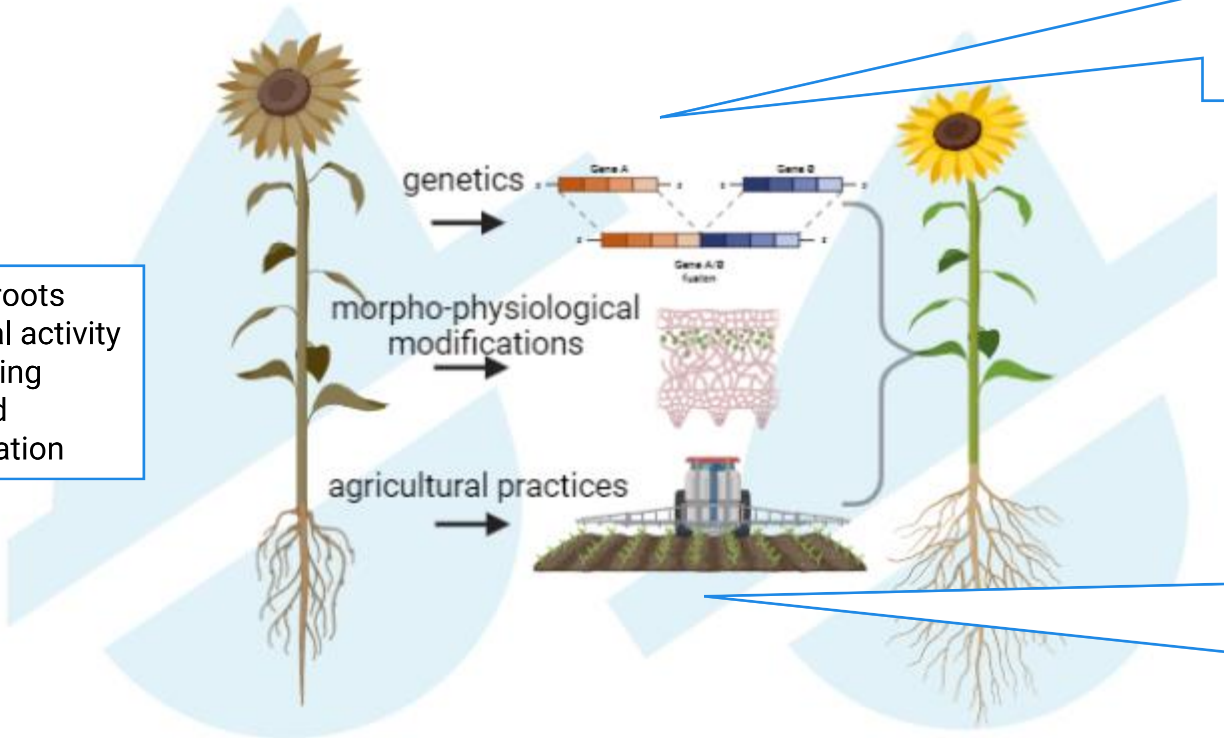
Drought Stress Management

Early flowering
Short life cycle
Early maturity



Hormonal regulation
Antioxidant production
Osmotic adjusment

Longer roots
Stomatal activity
Leaf rolling
Reduced
transpiration

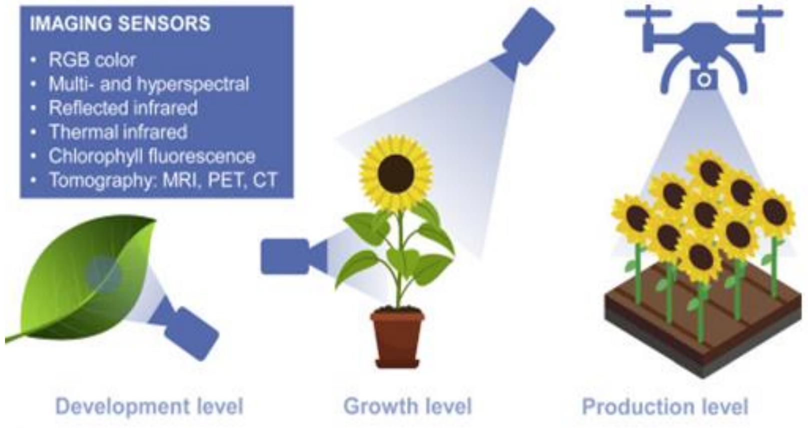


Conventional breeding
Molecular breeding
Epigenetics
Gene editing

Irrigation
Nutrient application
Tillage
Application of
osmoprotectant
Seed priming

Phenotyping

Plant phenotyping is a rapidly emerging research area concerned with ***quantitative measurement*** of the structural and functional traits of plants.

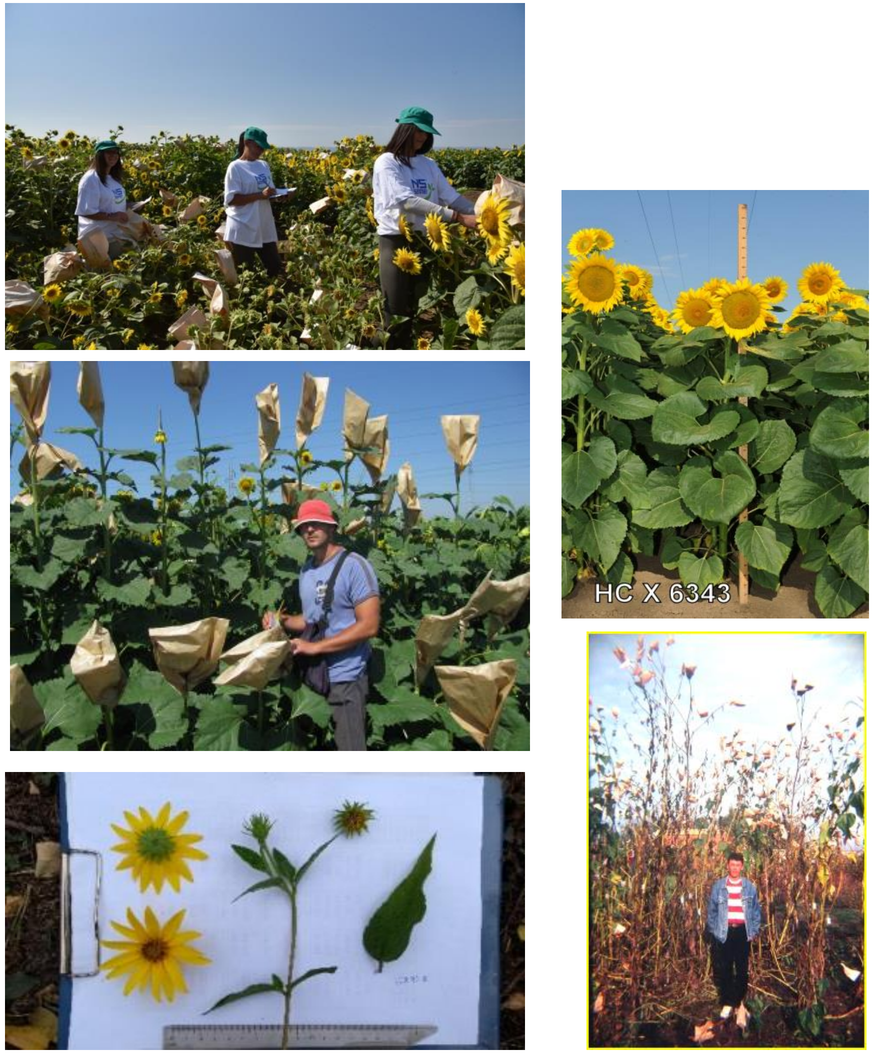


High-Throughput Phenotyping:

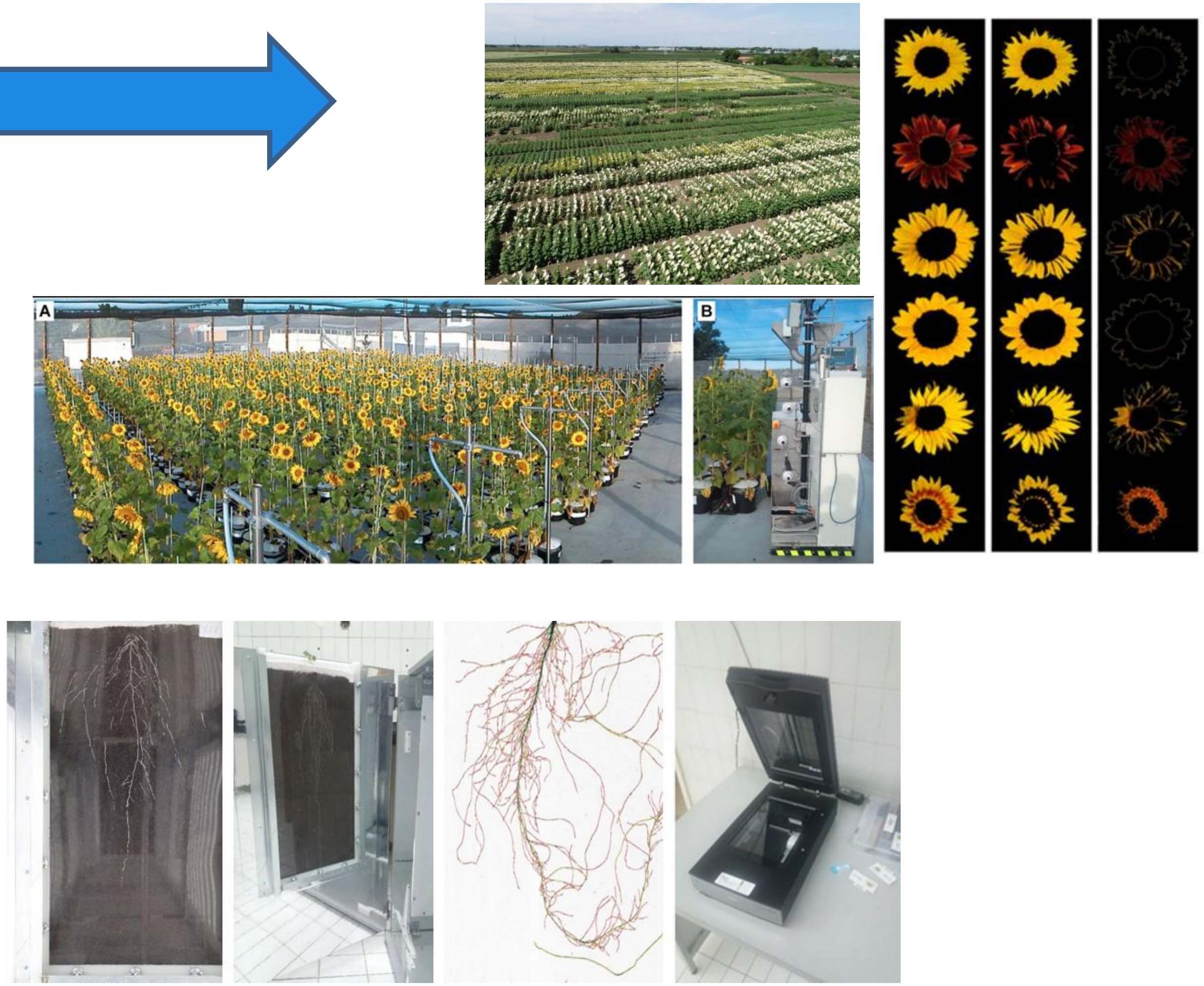
1. **Imaging Techniques:** RGB (color), hyperspectral, thermal, and fluorescence imaging. These images can be analyzed using software to measure parameters like leaf area, chlorophyll content, and water stress.
2. **Automated Systems:** Platforms like drones, and robotic systems. These systems can quickly collect data from large numbers of plants, making it possible to assess vast populations in breeding programs.
3. **Sensors and Remote Sensing:** Sensors placed in fields or on plants measure environmental factors and plant responses, such as soil moisture, temperature, and photosynthetic activity. Remote sensing via satellites or UAVs (drones) can also provide large-scale data on crop performance.

Phenotyping

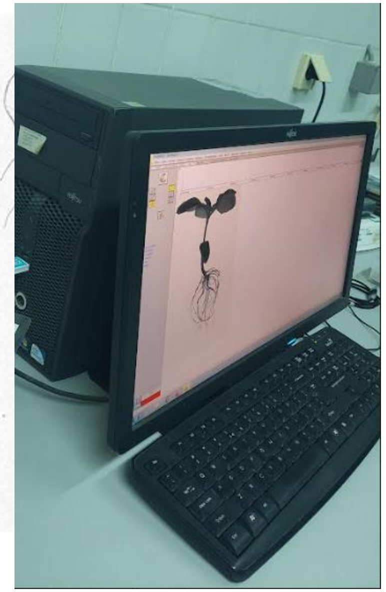
Classical methods

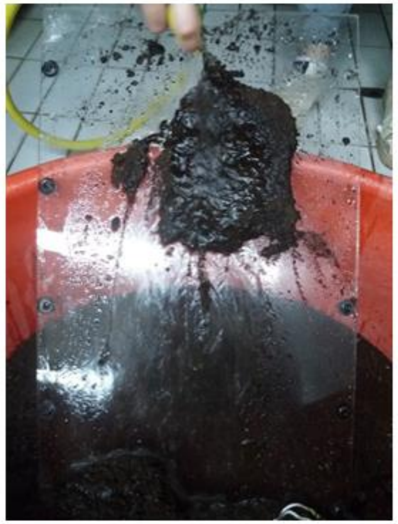


New methods

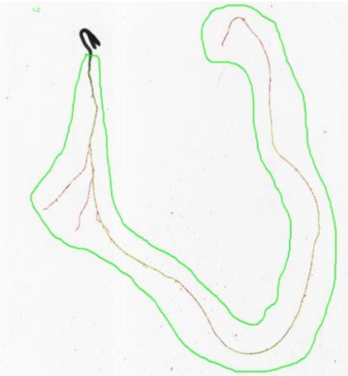


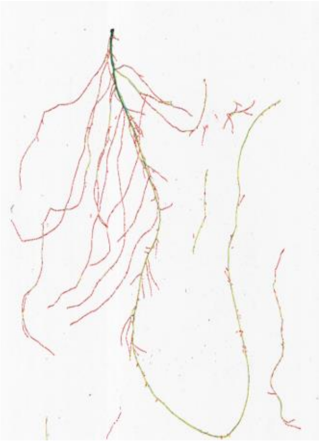
Root Phenotyping

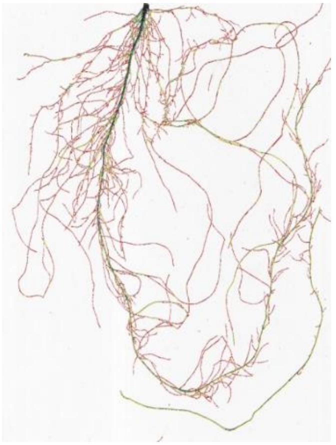




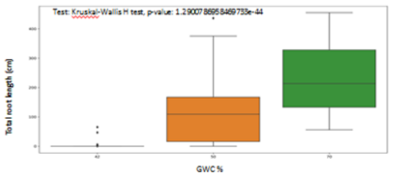
Root Phenotyping for Drought Tolerance



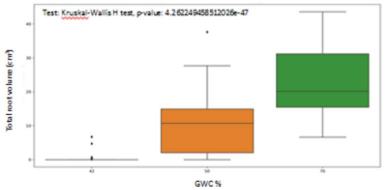




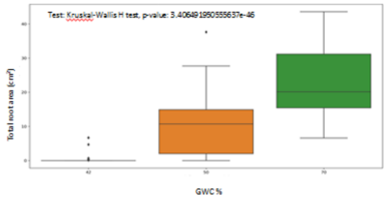
Total root length (cm)



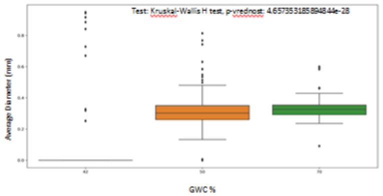
Total root volume (cm^3)



Total root area (cm^2)

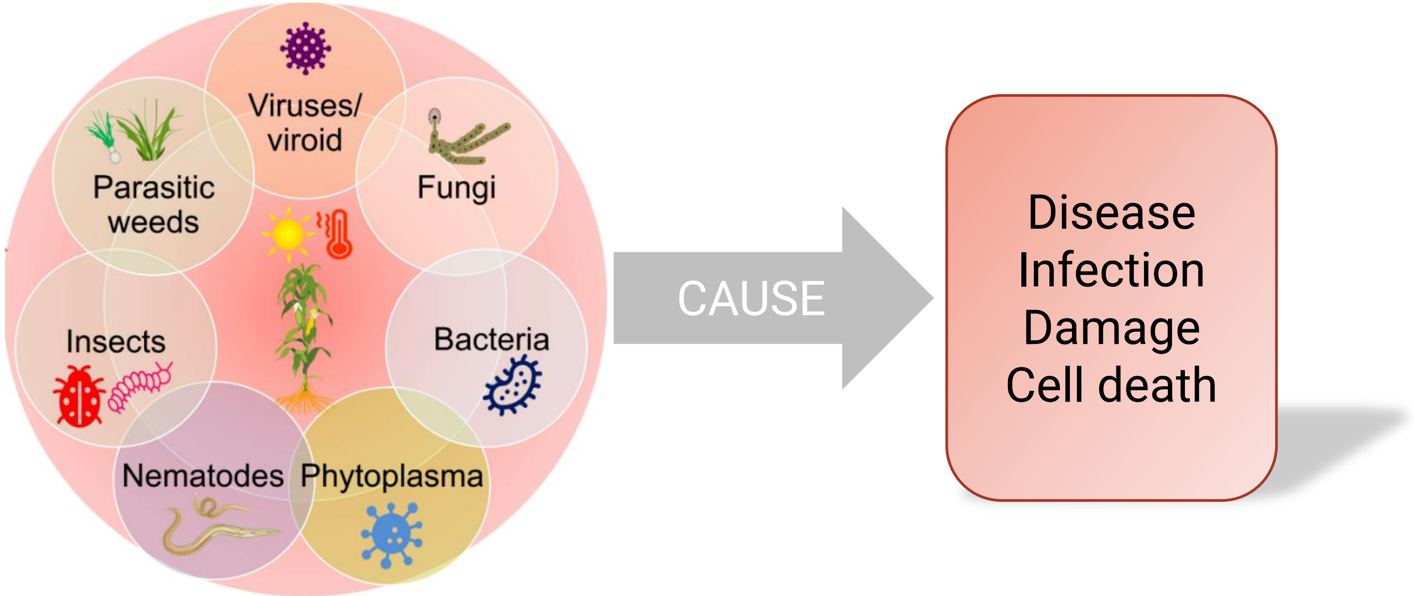


Average diameter (mm)



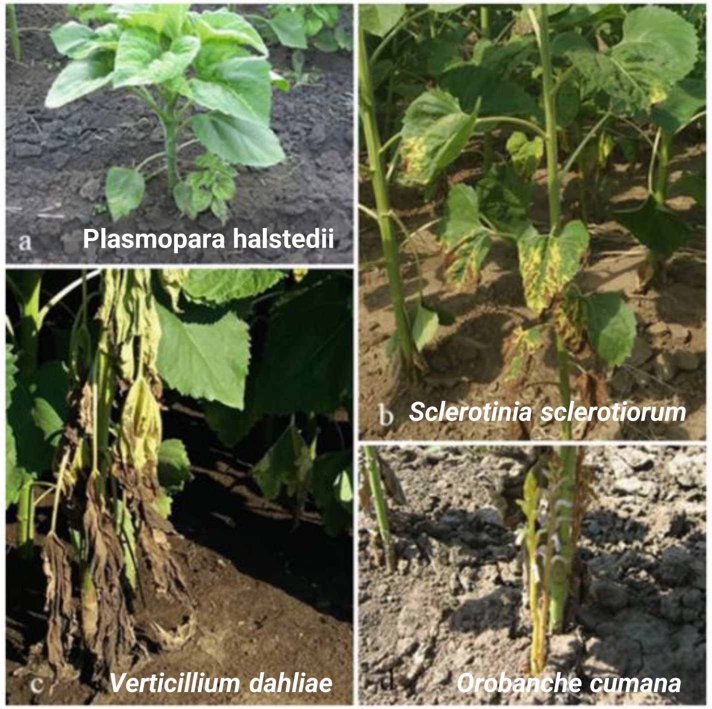
Biotic Stress

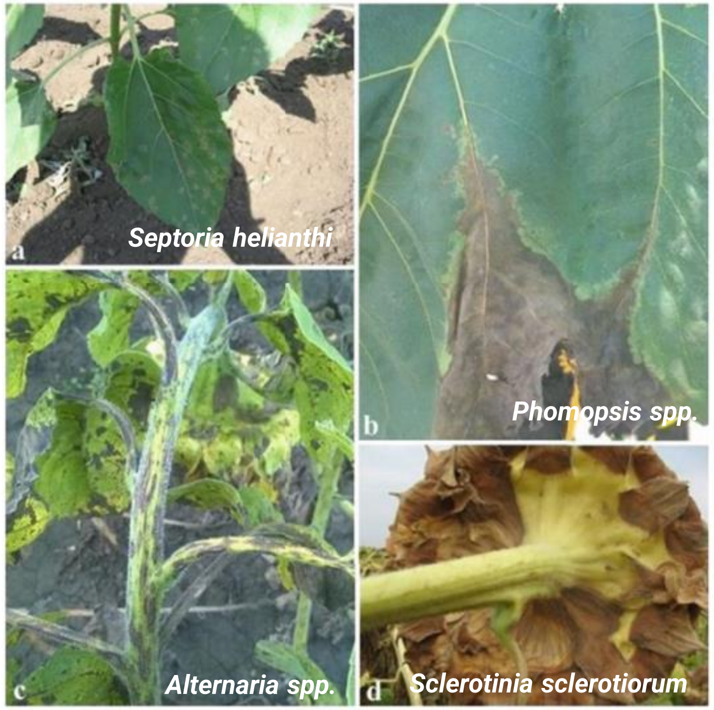
- Caused by other ***living organisms*** like viruses, fungi, bacteria, nematodes, insects, and weeds to plants, resulting variety of diseases, infections, and damage to crop plants, lowering agricultural yields.
- Examples of complete failure of the crops: potato blight in Ireland, coffee rust in Brazil (Rogers, 2004), and maize leaf blight in the USA (Ullstrup, 1972).
- Today, an estimated 20% to 40% of all crop yield losses are caused by *pests and plant pathogens* https://www.cabi.org/projects/global-burden-of-crop-loss/



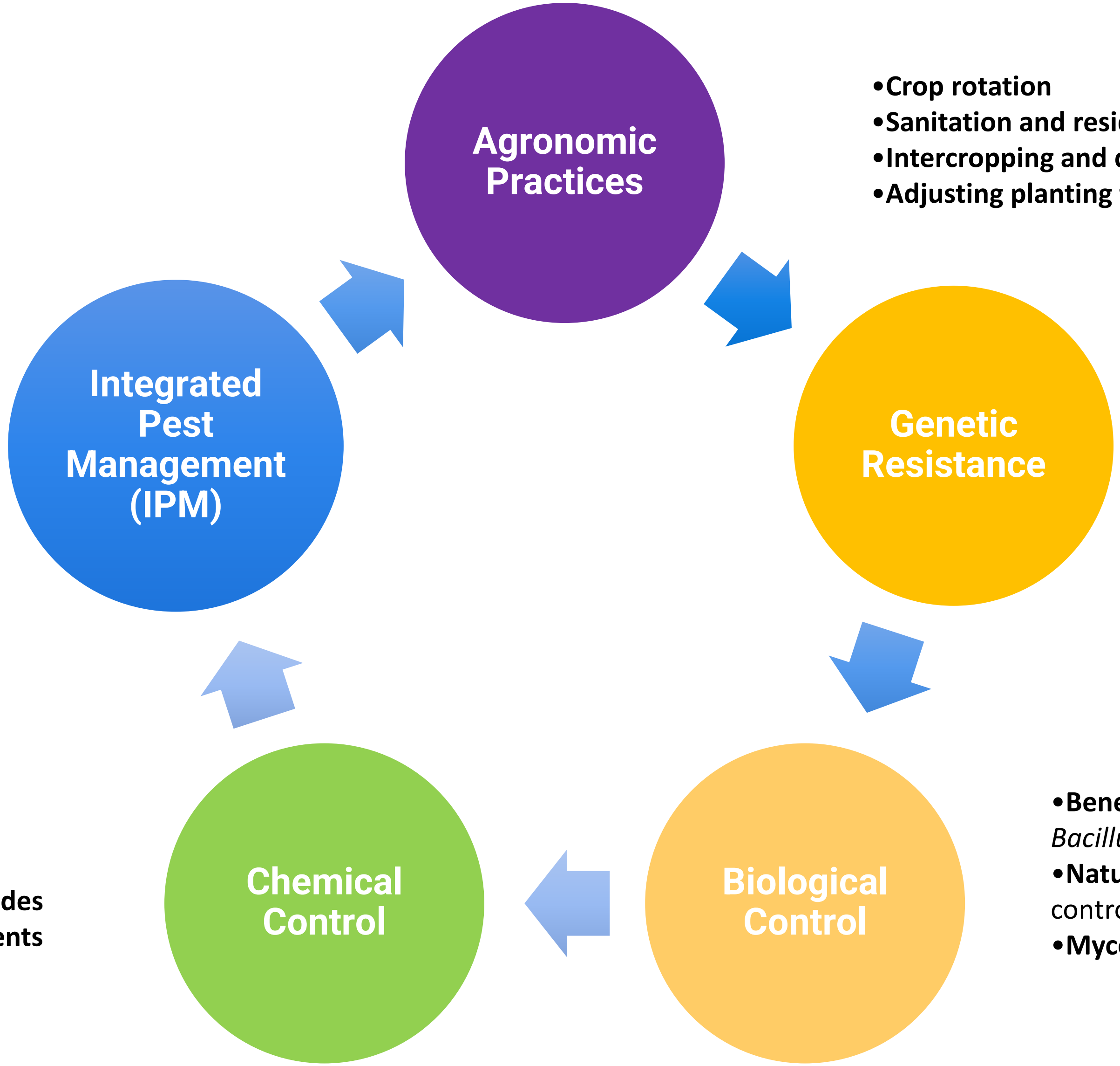
Impact on Plants

- Biotic stress agents induce a variety of ***diseases, infections, and damage to crop plants, lowering agricultural yields.***
- Biotic attacks on crops are determined through manual visual inspections based on physiological signatures such as ***necrosis, chlorosis, lesions, tumors, wilting, stunted growth, discolorations, and cell death***.





Biotic Stress Management



Agronomic Practices

- **Crop rotation**
- **Sanitation and residue management**
- **Intercropping and diversification**
- **Adjusting planting time and density**

Genetic Resistance

- **Breeding for resistance**
- **Omics technologies**
- **Pyramiding of resistance genes**

Biological Control

- **Beneficial microbes:** *Trichoderma*, *Bacillus subtilis*, *Pseudomonas fluorescens*
- **Natural predators and parasitoids:** pest control
- **Mycorrhizal associations**

Chemical Control

- **Pesticides**
- **Seed treatments**

Integrated Pest Management (IPM)

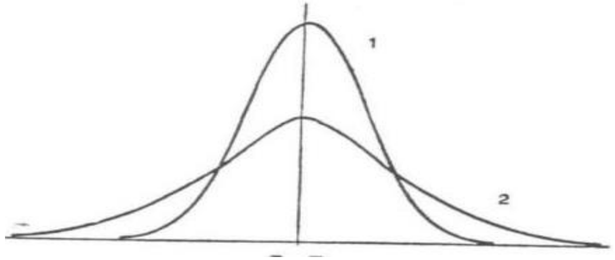
- Combines genetic resistance, cultural practices, biological control, and selective chemical applications.
- Emphasizes monitoring, early detection, and threshold-based interventions.
- Reduces reliance on chemicals, lowering environmental and health risks.

Genetic Resistance

- The most convenient, durable, and eco-friendly approach.
- Plant breeders can exploit the genetic basis to develop resistance in crop plants against diseases and insect pests.

1. GENETIC RESISTANCE:	**2. HIGH LEVEL OF TOLERANCE:**
Plasmopara helianthi	***Phomopsis/Diaporthe helianthi***
Puccinia helianthi	***Macrophomina phaseolina***
Verticillium albo-altrum	***Albugo eragopogonis***
Verticillium dahliae	
Erysiphe cichoracearum	
3. CERTAIN LEVEL OF TOLERANCE:	**4. INSUFFICIENT TOLERANCE:**
Sclerotinia sclerotiorum	***Alternaria ssp.***
Phoma macdonaldii	***Rhizopus ssp.***
	Botrytis cinerea etc.

Types of Genetic Resistance



Vertical

Qualitative – controlled by one or several genes (**MAJOR GENES**)

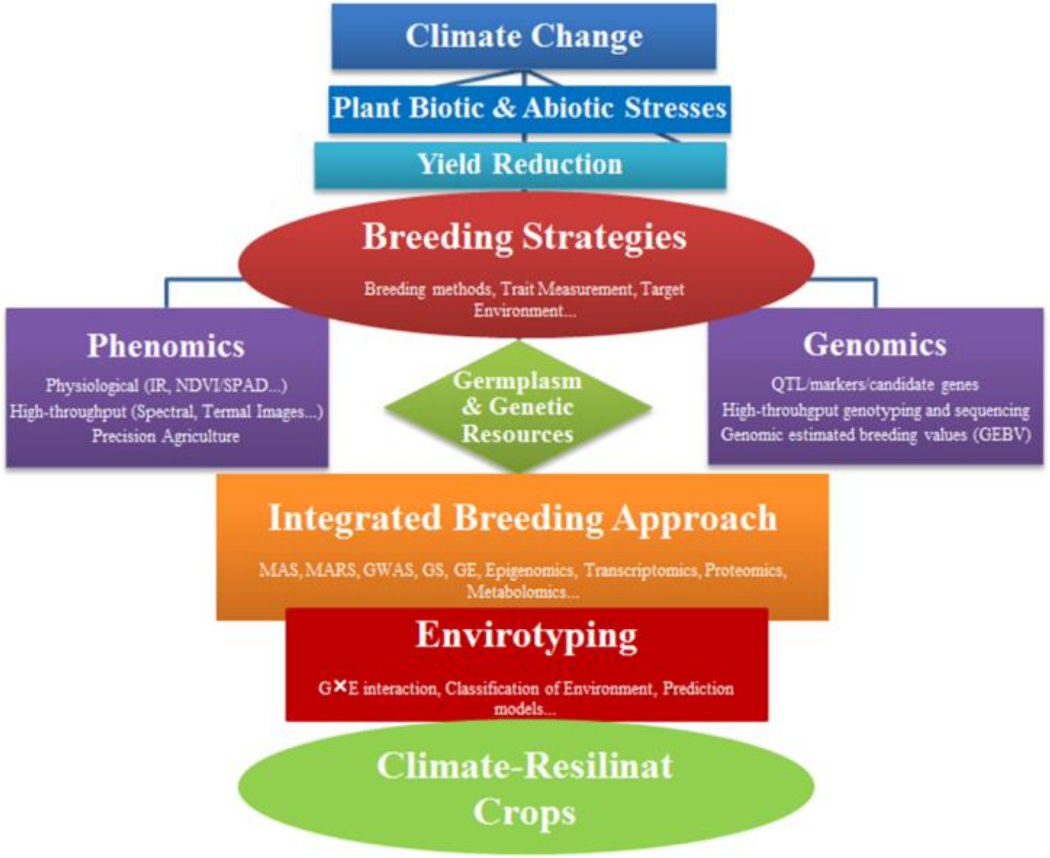
Plants are classified as R, MR, MS, S

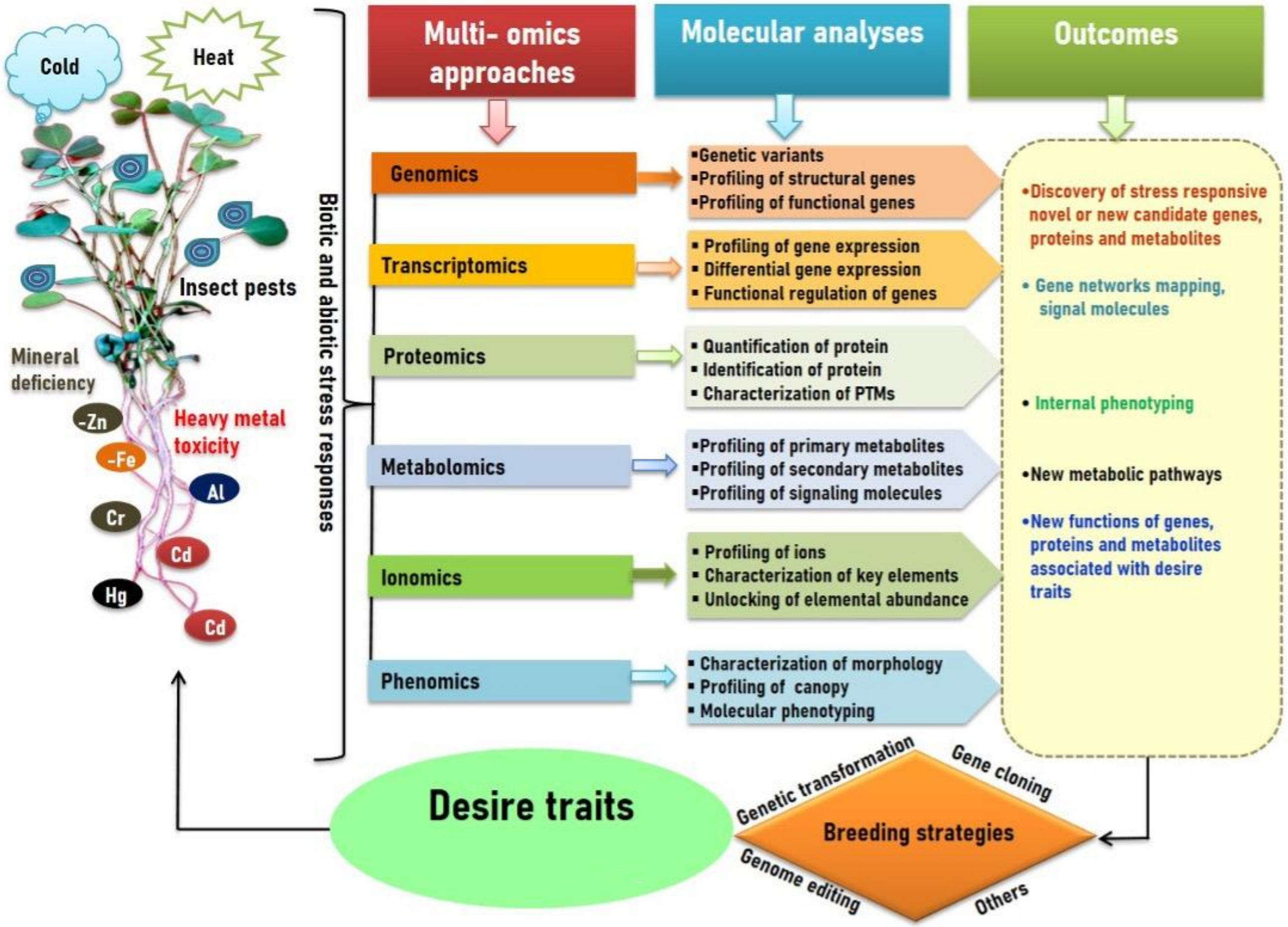
Horizontal

Quantitative – polygenic (**MINOR GENES**)

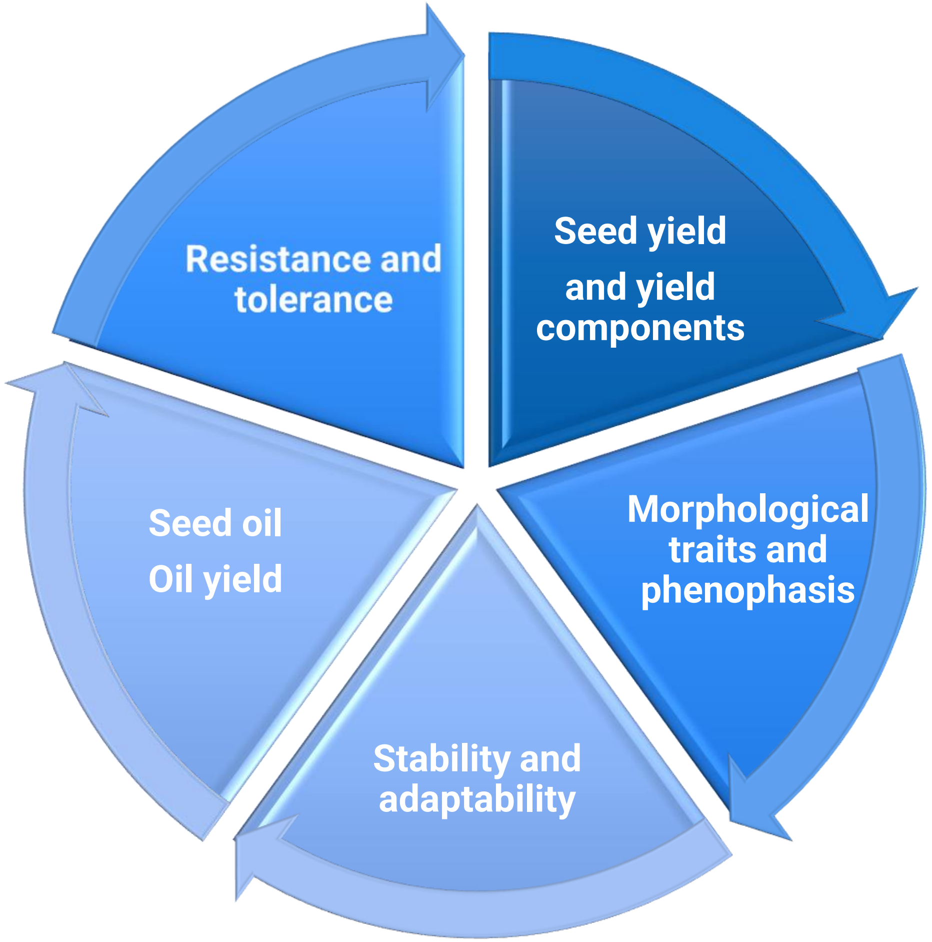
Continuous variability between genotypes – scales 0-100; 1-9

Breeding Strategies





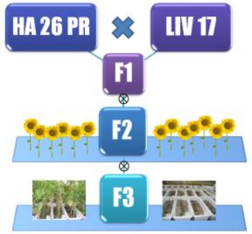
Breeding Objectives for Stress Resistance



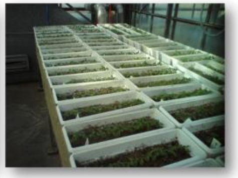
Steps in Breeding for Resistance



Sources of resistance (varieties, inbred lines, wild species, mutations...)



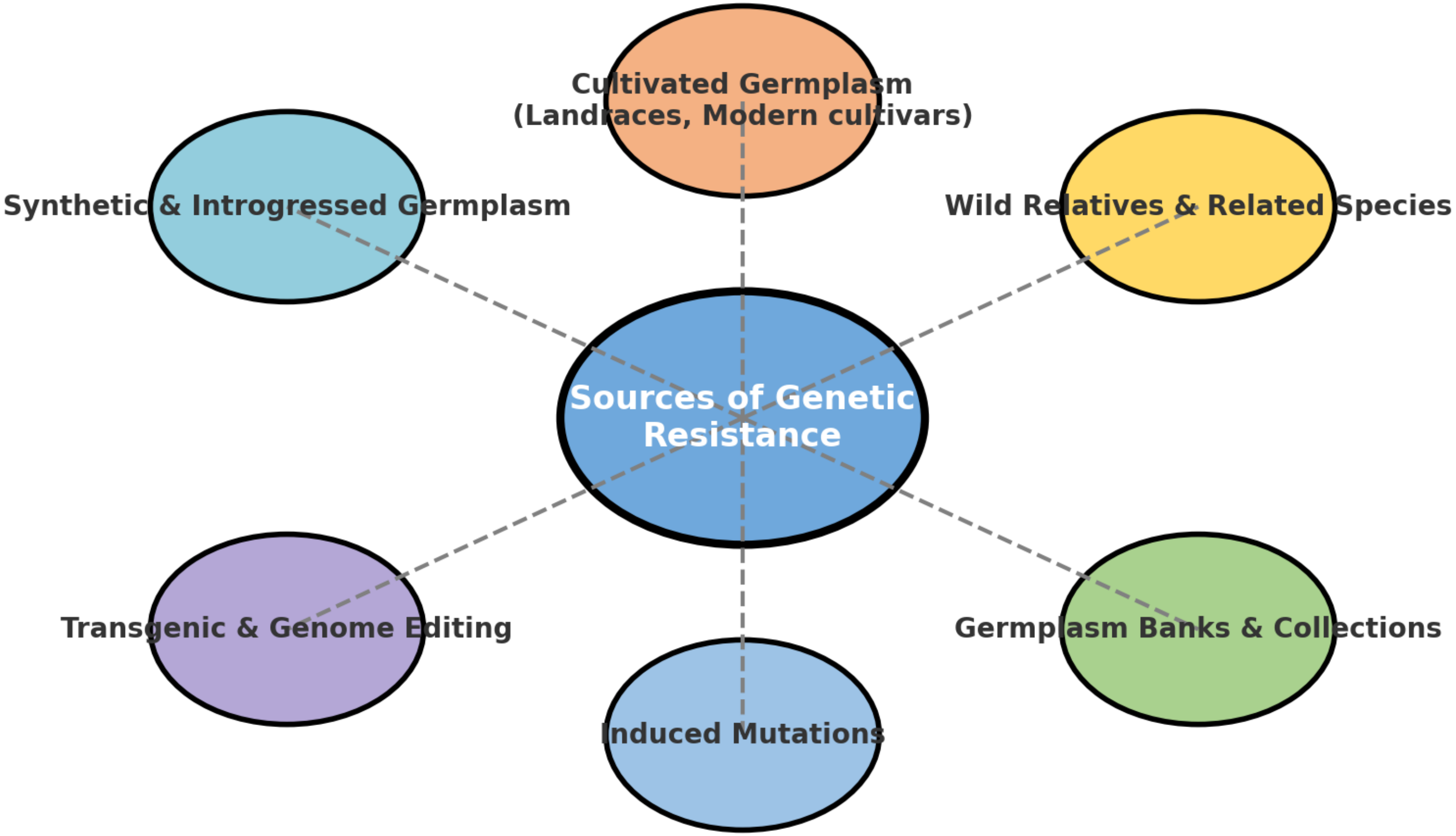
Breeding methods

Methods of analysis (field trials, controled conditions, early-stage...)

Selection of resistance genotypes

Sources of Resistance

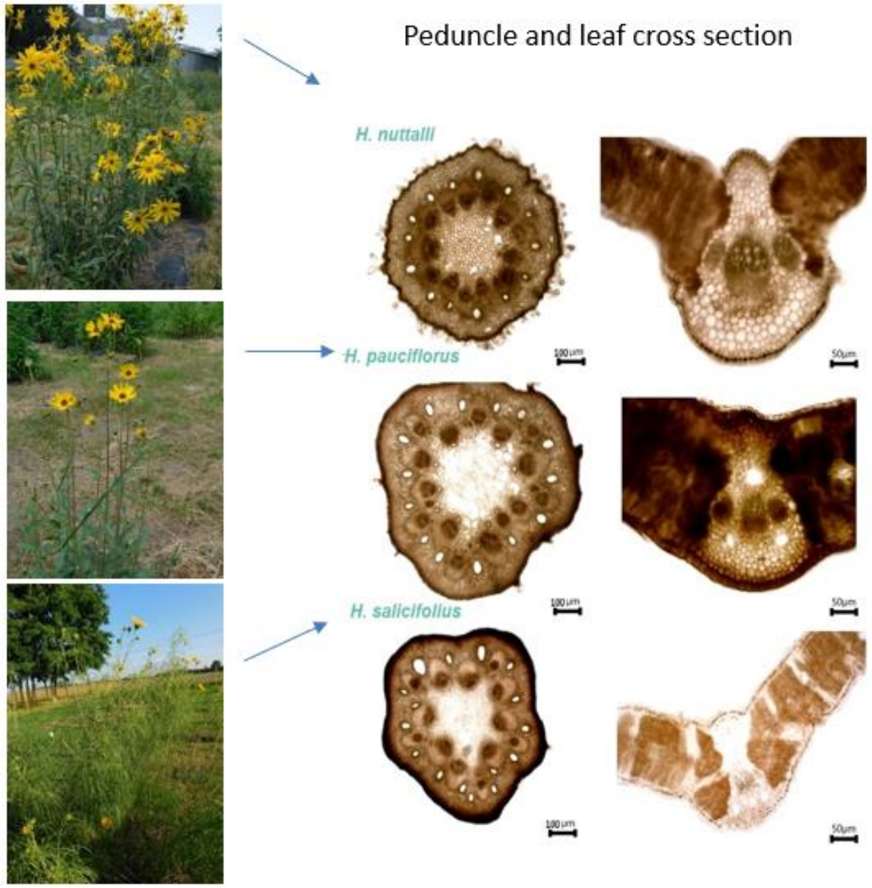


Cultivated Germplasm

- Inbred lines gene-pools, varieties, landraces...
- Breeding efforts traditionally focused on seed yield and yield components, disease resistance, early maturity, oil quality...
- Readily available through national/international gene banks.
- Pre-adapted to agricultural environments, increasing chances of compatibility.

Use of Wild Species

1. **Sources of specific traits**
2. **Advance genetic variability**

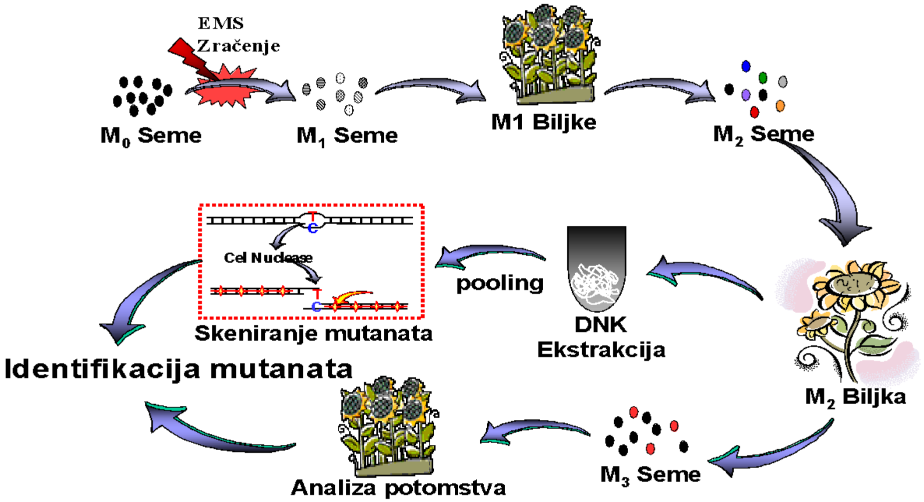


Jocković J. 2023. PhD thesis

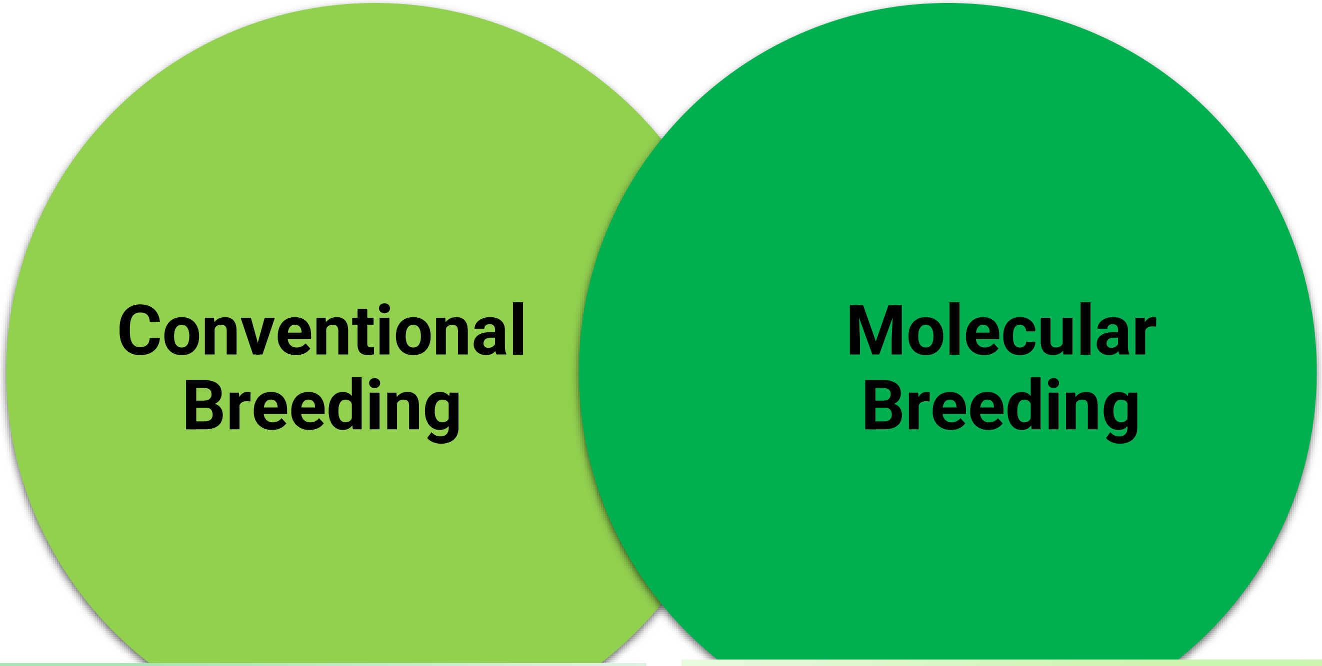
- NEW SOURCES OF CMS AND RESTORER GENES
- INCREASE OF HETEROSIS FOR AGRONOMIC TRAITS
- RESISTANCE TO DISEASES AND INSECTS
- RESISTANCE TO DROUGHT
- RESISTANCE TO HERBICIDES
- ALTERED OIL AND PROTEIN QUALITY
- IMPROVEMENT OF HEAD, STEM AND LEAF COMPOSITION FOR COMPLETE UTILIZATION OF ENTIRE SUNFLOWER PLANT
- ATTRACTIVENESS TO POLLINATORS
- INCREASE OF TOLERANCE TO SALINITY

Mutations

- changes in the genetic material caused by ***different chemical or physical*** means
- mutation breeding
- Mutagens: ***physical*** (X, γ, neutrons)
  chemical (ems, dms, sa)
- Mutational analysis:
  - ***Forward genetics*** (Phenotype to gene): a wide array of characterised morphological mutants
  - ***Reverse genetics*** (Gene to phenotype): tilling...



Breeding Methods

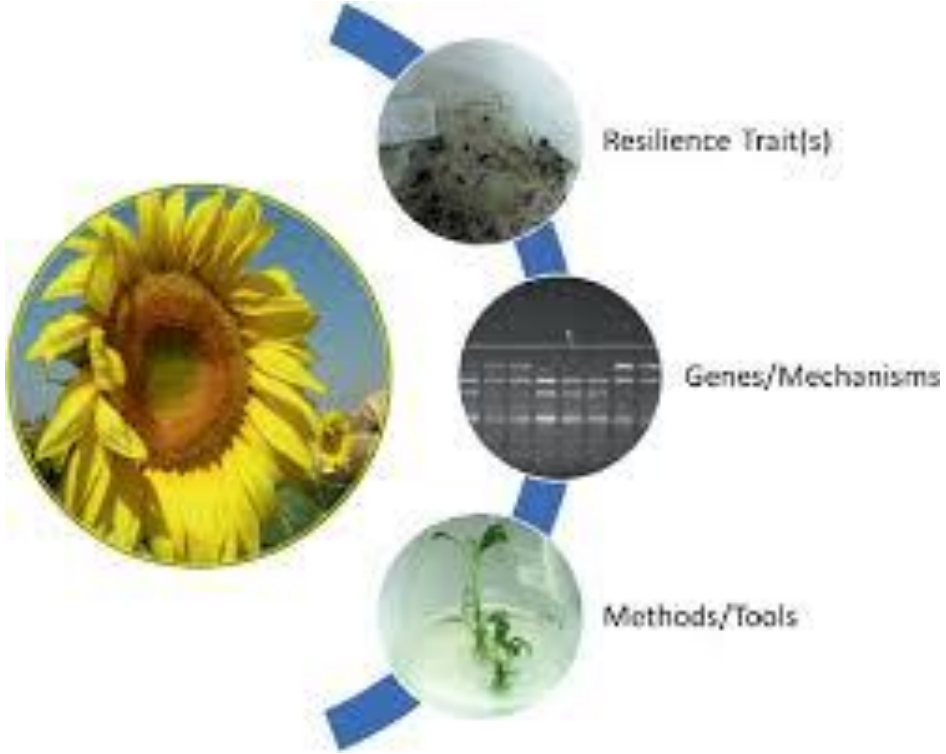


Selection methods (Mass, Individual)
Hybridization methods (Pedigree, Bulk, Backcross, Reccurent, Heterosis breeding)
Mutation breeding (Induced by radiation/chemicals)

Marker-based approaches (MAS, MABC, MARS, QTL mapping)
Genomic approaches (Genomic selection- GWAS)
Biotechnological approaches (Transgenic breeding, Genome editing)

Breeding Methods for Stress Resistance

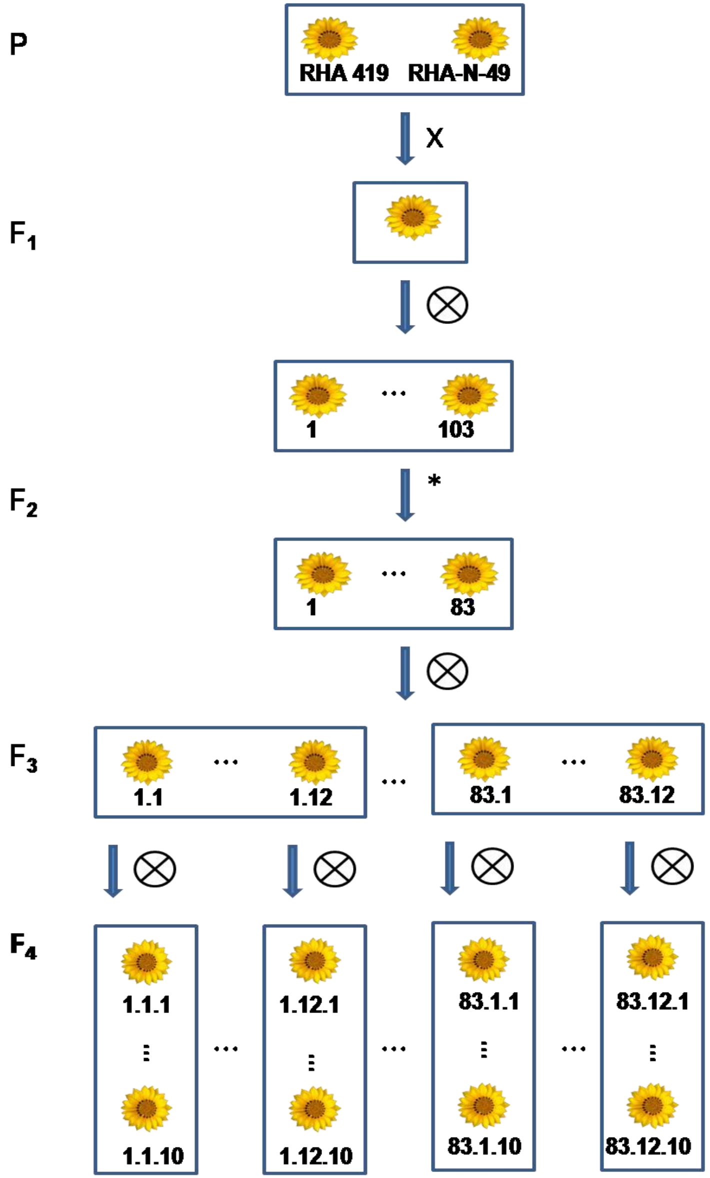
- Conventional breeding with **hybridization and selection** dominates.
- **MAS** & **MABC** are used when known resistance genes are available and reliable markers exist.
- **Genomic approaches** are expanding but are not yet as widespread in routine breeding.



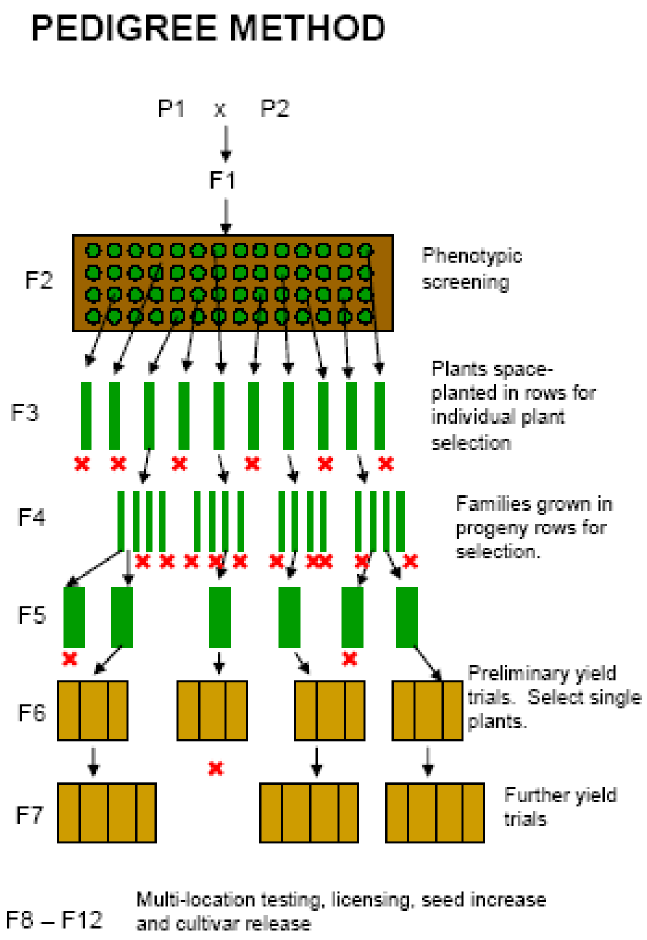
Selection of Resistant Genotype

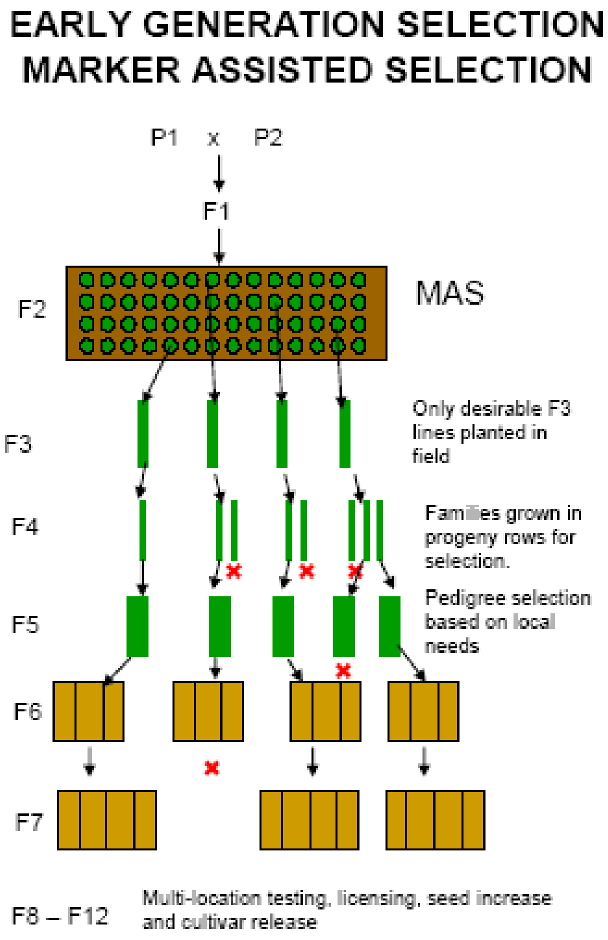
Bulk, Pedigree method, SSD...

- Elite line x elite line
- Elite line x donor



MAS – identification of genotypes with the resistance gene



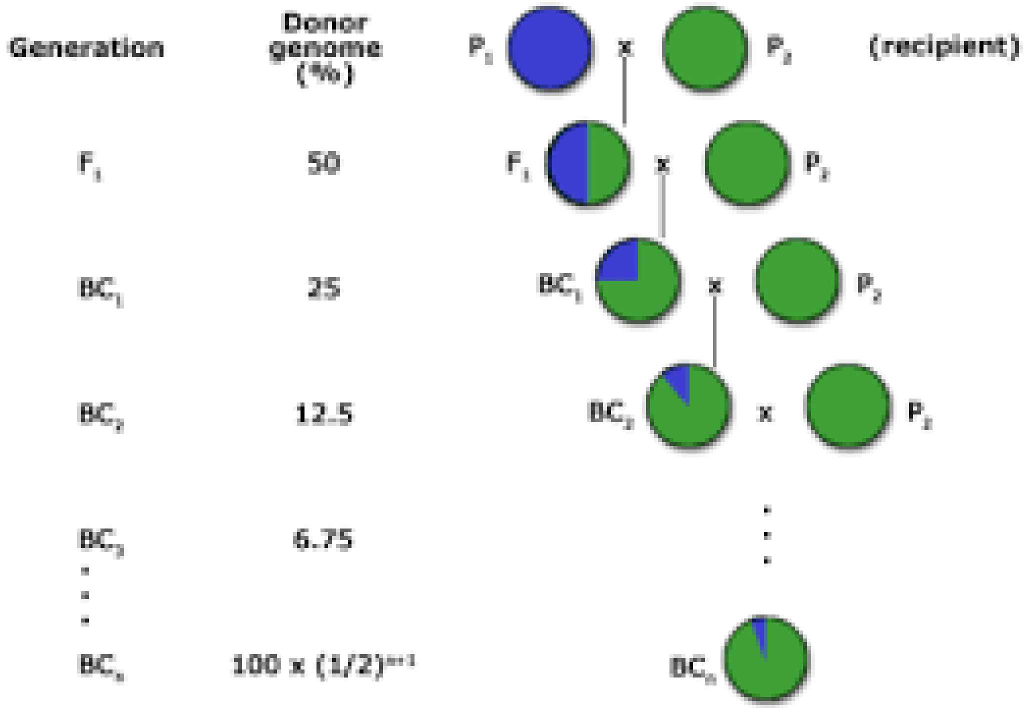


Introduction of Resistant Gene

Donor line X Reccurent line

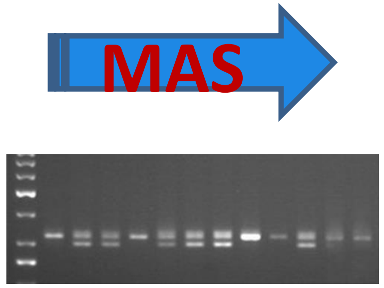
Back cross method

- The stress resistance trait is **controlled by one or a few genes**.
- The goal is to improve an already **high-performing variety** without altering most of its traits.



MABC – introduction of the resistance gene

Elite IFVCNS lines susceptible to downy mildew

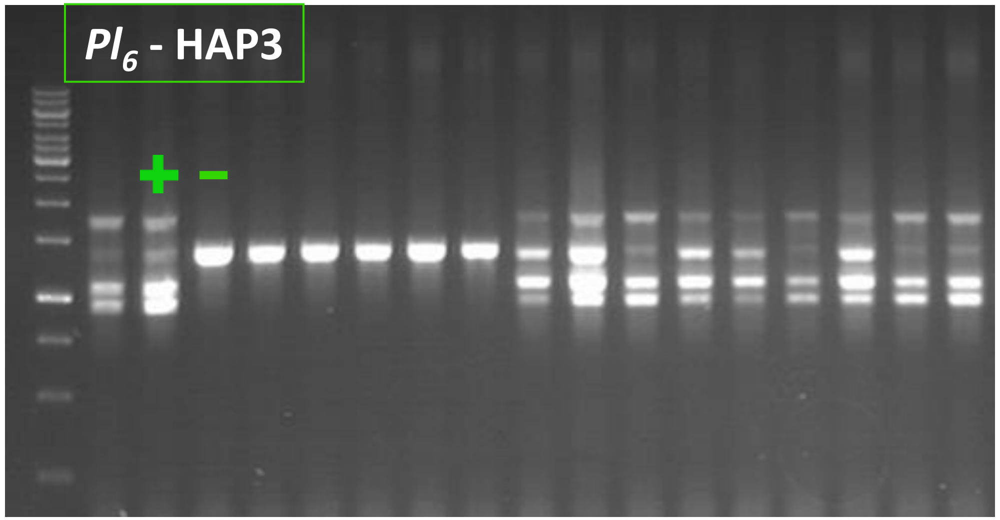


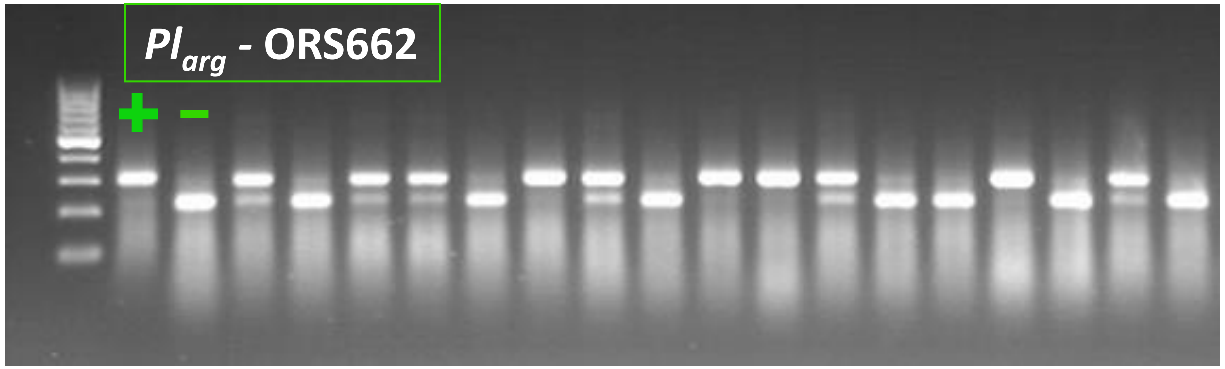
Homozygote resistant lines

Donor x Linija
↓
F1 x Linija
↓ MAS
BC1 x Linija
↓ MAS
BC2 x Linija
…
BC6 ⊗ → BC6F2 → (MAS) Homozygot resistant line

- About 5,000 samples are tested annually.
- Pl gene can be introduced in 3 years.
- Genetic pyramiding helps improve durability and degree of resistance.

MABC

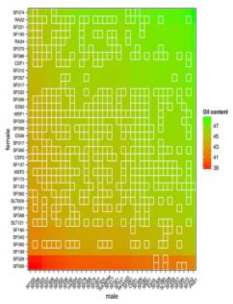


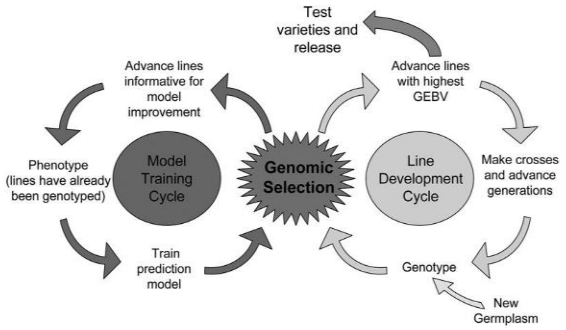


Imerovski et al. (2014) Mol Breeding 34(3):779-788

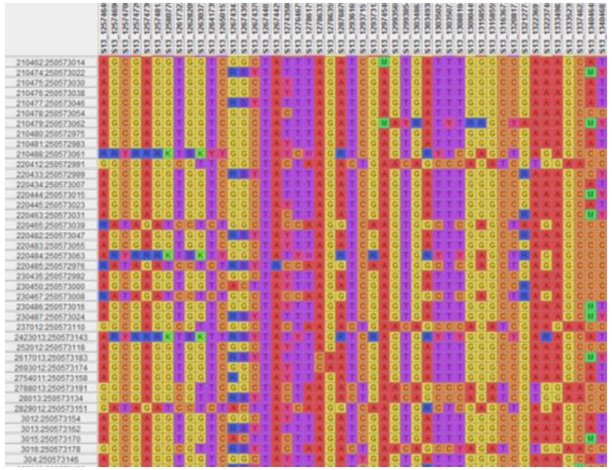
Genomic Selection

- GS = Genome-wide prediction
- Method for improvement efficacy of selection of plant quantitative traits
- GS uses genotypic and phenotypic data from the population to calculate quantitative value of each individual as a parent for future breeding cycles – it is called genome-estimated ***breeding value*.**
- ***Suitable for polygenic traits*** (Quantitative Trait Loci -QTL markers).
- Sunflower genome sequenced (used to predict hybrid performances (Reif et al. 2013), oil content in hybrids (Mangin et al. 2017) and *Sclerotinia* tolerance (Livaja et al. 2016).

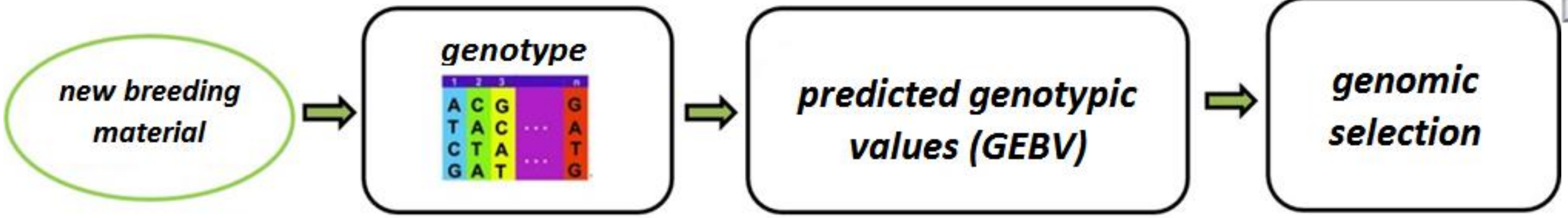


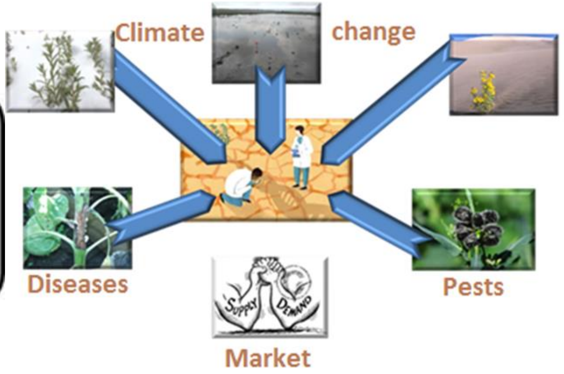


Genomic Selection



Genomic prediction is an approach that uses markers to predict the genetic value of complex traits in progeny for selection and breeding (Meuwissen et al. 2001)

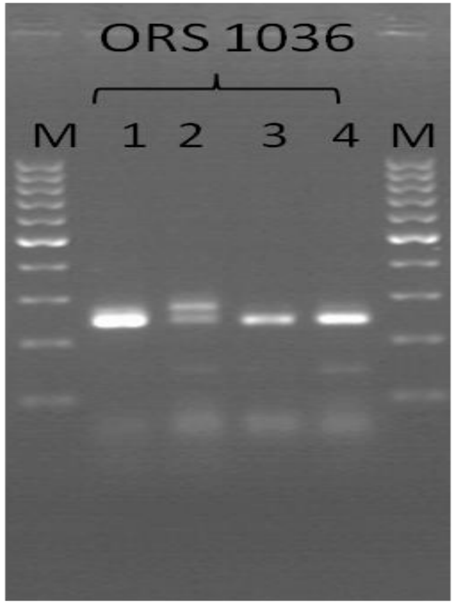


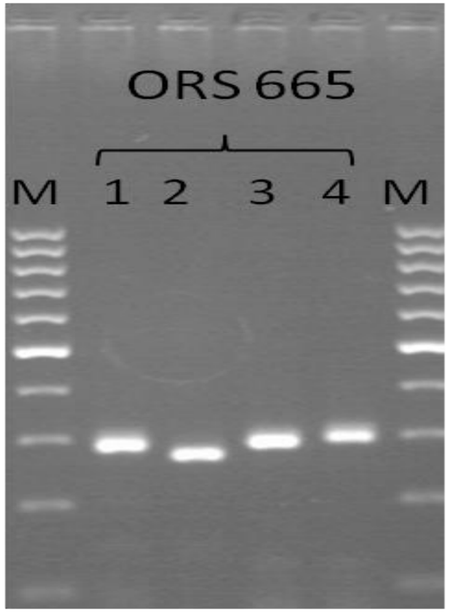


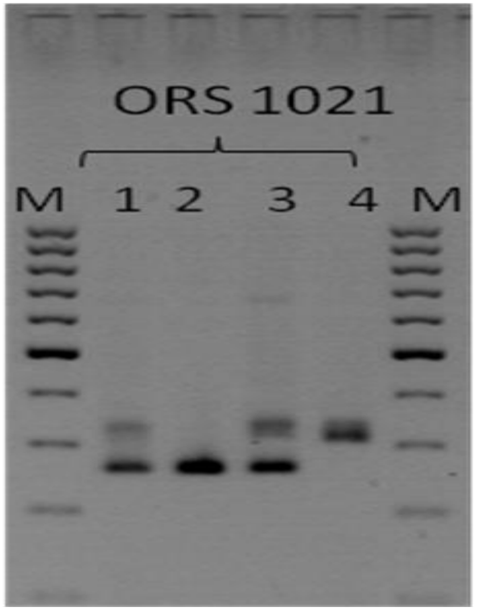
Resistance to Broomrape

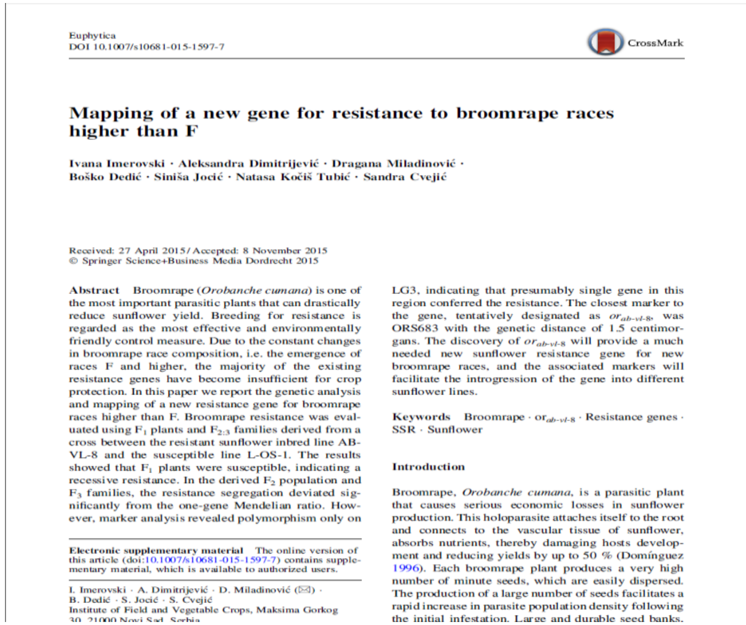
Mapping of new gene controlling resistance to broomrape races higher than F

LG3 – new resistance gene









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CrossMark

Mapping of a new gene for resistance to broomrape races higher than F

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Abstract Broomrape (*Orobanche cumana*) is one of the most important parasitic plants that can drastically reduce sunflower yield. Breeding for resistance is regarded as the most effective and environmentally friendly control measure. Due to the constant changes in broomrape race composition, i.e. the emergence of races F and higher, the majority of the existing resistance genes have become insufficient for crop protection. In this paper we report the genetic analysis and mapping of a new resistance gene for broomrape races higher than F. Broomrape resistance was evaluated using F_1 plants and $F_{2:3}$ families derived from a cross between the resistant sunflower inbred line AB-VL-8 and the susceptible line L-OS-1. The results showed that F_1 plants were susceptible, indicating a recessive resistance. In the derived F_2 population and F_3 families, the resistance segregation deviated significantly from the one-gene Mendelian ratio. However, marker analysis revealed polymorphism only on LG3, indicating that presumably single gene in this region conferred the resistance. The closest marker to the gene, tentatively designated as $or_{ab\text{-}vl\text{-}8}$, was ORS683 with the genetic distance of 1.5 centimorgans. The discovery of $or_{ab\text{-}vl\text{-}8}$ will provide a much needed new sunflower resistance gene for new broomrape races, and the associated markers will facilitate the introgression of the gene into different sunflower lines.

Keywords Broomrape · $or_{ab\text{-}vl\text{-}8}$ · Resistance genes · SSR · Sunflower

Electronic supplementary material The online version of this article (doi:10.1007/s10681-015-1597-7) contains supplementary material, which is available to authorized users.

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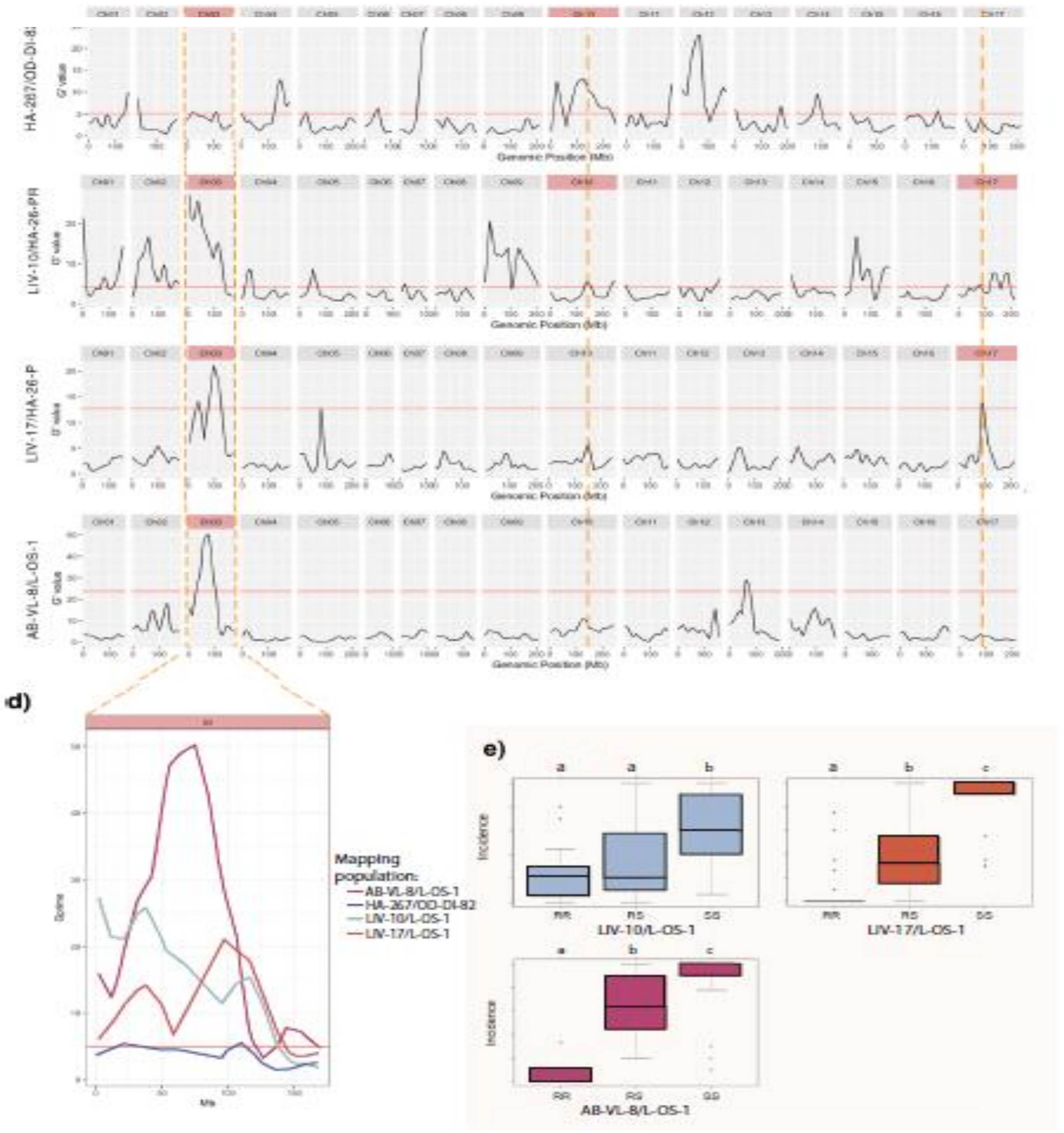
Introduction

Broomrape, *Orobanche cumana*, is a parasitic plant that causes serious economic losses in sunflower production. This holoparasite attaches itself to the root and connects to the vascular tissue of sunflower, absorbs nutrients, thereby damaging hosts development and reducing yields by up to 50 % (Domínguez 1996). Each broomrape plant produces a very high number of minute seeds, which are easily dispersed. The production of a large number of seeds facilitates a rapid increase in parasite population density following the initial infestation. Large and durable seed banks.

- Resistance had a polygenic basis, and numerous QTLs mapping populations.
- Two major QTLs on chromosome 3, which were designated *or3.1* and *or3.2*.

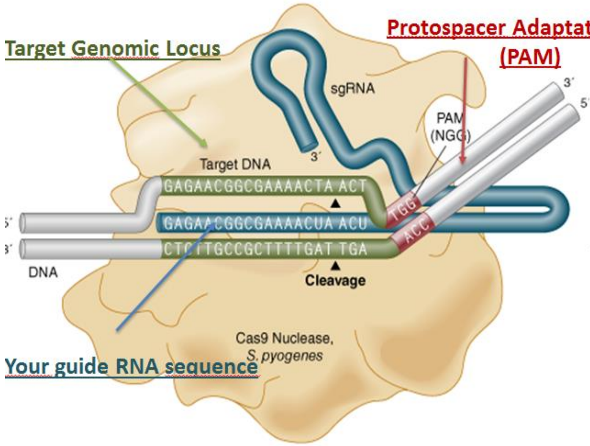
- QTL *or3.1* was positioned in a genomic region where a previous broomrape resistance gene *Or5* has been mapped, which now acts as a „defeated R gene". This gene provides only a moderate level of resistance.

- QTL *or3.2* was identified for the first time. This gene together with QTLs on other chromosomes are required for resistance to races higher than E.



Genome Editing

- In 2016 Nobel Prize in Medicine
- Group of laboratory techniques that change DNA structure (insert gene of interest in DNA structure) at a molecular level
- CRISPR-CAS9 – new genomic tool for locating a region of interest
- In EU the application and even research was arguable



Cas9 nuclease:

- Recruted by sgRNA complementary to specific region of interest

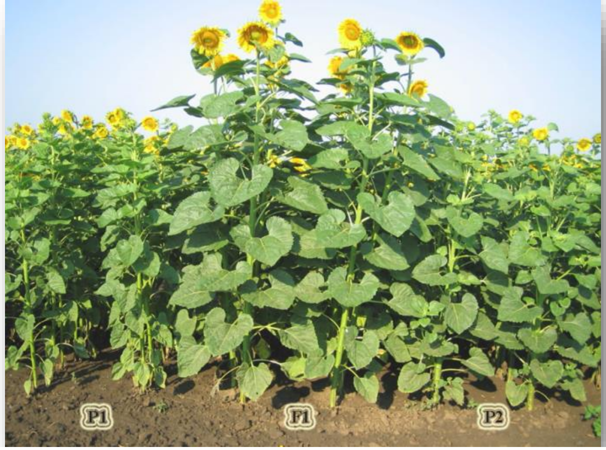
- Generates double strand break DNA

E. Charpentier/J. Doudna

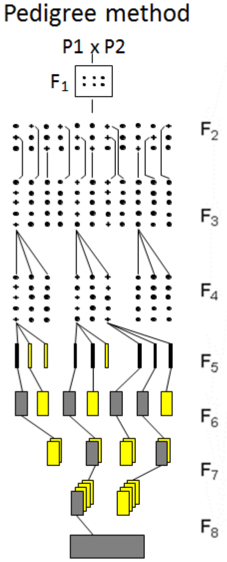
Heterosis (Hybrid) Breeding

- Combining multiple resistance genes, often through techniques like gene pyramiding, to create a stronger, longer-lasting defense against plant diseases.

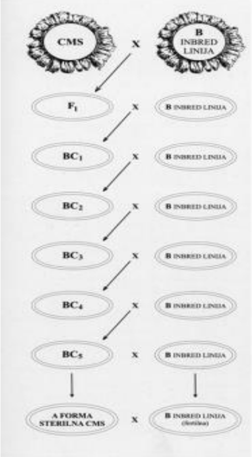
Duration 12-15 years

CMS line → 

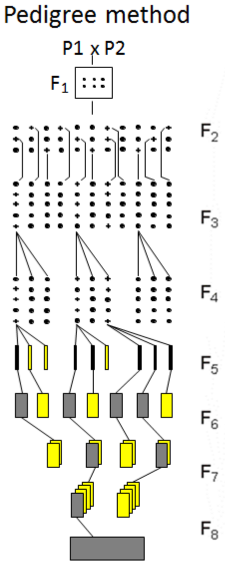
← Rf line

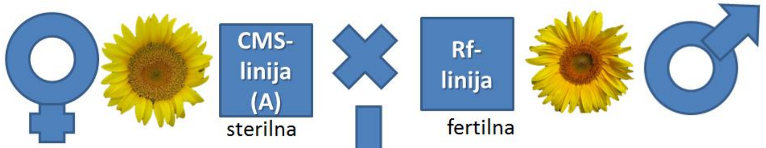


6-8 generation
+
6 generation of CMS introduction



6-8 generation





F1 HIBRID

Multi-environmental testing combining ability 3-4 years

Hybrid registration

Breeding for Multiple Stress Resistance

- Multiple stress resistance = integrated breeding → resilient crops for future climates.

Challenges

- Stress responses are often **complex, polygenic, and environment-dependent**.
- **Trade-offs** may exist: resistance to one stress can increase susceptibility to another.
- Interactions between stresses are not simply additive — combined stresses can trigger **unique physiological responses**.

Key Strategies

- **Pyramiding**: combining multiple resistance genes in a single genotype.
- **Trait introgression**: transferring stress resistance from wild relatives into elite cultivars.
- **Breeding for plasticity**: selecting genotypes with stable performance across environments.
- **Systems approach**: integrating physiology, molecular biology, and breeding.

Breeding for Multiple Stress

Breeding for Multistress Resistance

- *(Drought 🌵 + Heat 💥 + Charcoal Rot 🦠)*

🔴 **Why It Matters**

- Stresses **co-occur** in the field.
- Drought + Heat → weaken plant defense.
- *Macrophomina phaseolina* → charcoal rot, stem breakage, yield loss.

🟢 **Strategies for Resistance**

- **1. Genetic:** resistant germplasm, multi-trait selection.
  2. Physiological: stay-green, deep roots, osmotic adjustment, heat-shock proteins.
  3. Molecular: QTL mapping, genomic selection, omics to identify shared tolerance pathways.

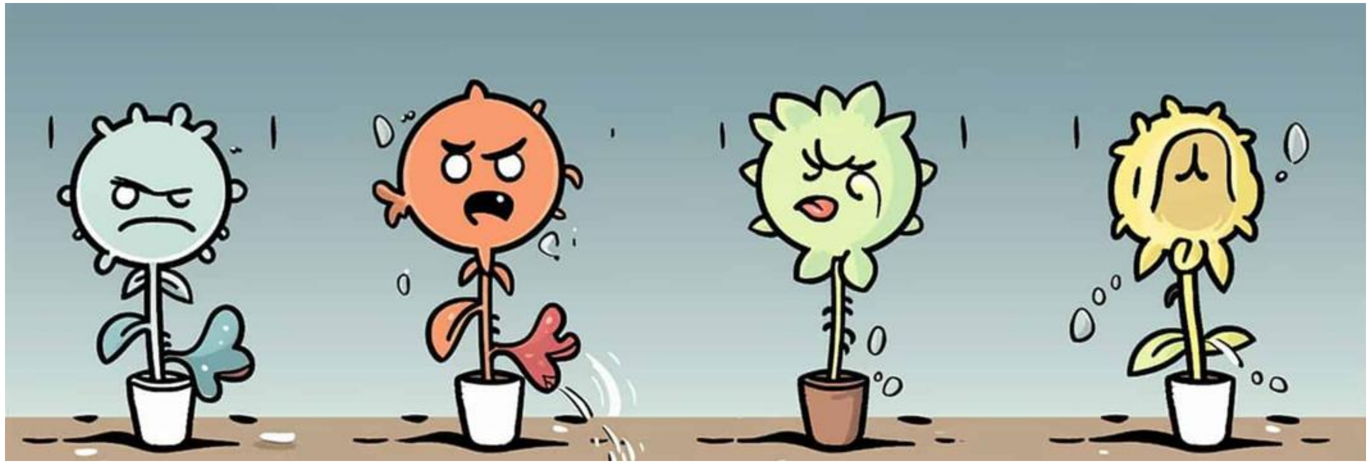
⭐ **Breeding Goal**

- ➡️ **Integrated resilience**: crops that withstand drought + heat **while resisting charcoal rot**.

Methods of Analysis

Stress Type	Key Methods	Parameters Measured
Disease	Field screening, artificial inoculation, markers	Lesion size, disease index, resistance gene presence
Pests	Field and lab assays, antixenosis/antibiosis tests	Damage scores, pest survival, plant defense traits
Drought	Field trials, pot experiments, physiology	Yield, RWC-relative wather content, stomatal conductance, osmotic potential
Salinity	Hydroponics, ion analysis, stress indices	Na^+/K^+ ratio, chlorophyll content, MSI
Heat	Growth chambers, thermal imaging	Membrane stability, chlorophyll fluorescence

QUIZ!!!



Which of the following is not an abiotic stress?

a) Drought

b) Heat

c) Downy mildew

d) Salinity

Which conventional breeding method is most commonly used to combine resistance traits from two parents?

a) Mutation breeding

b) Hybridization

c) Polyploidy induction

d) Tissue culture

Which molecular tool allows breeders to identify DNA regions linked to stress resistance?

a) PCR

b) Marker-assisted selection (MAS)

c) Chromosome doubling

d) Hybrid seed production

A plant's ability to survive with minimal water by altering its physiology (e.g., stomatal closure, deeper roots) is called:

a) Stress tolerance

b) Stress avoidance

c) Stress recovery

d) Stress sensitivity

Which type of genetic resistance is usually controlled by major genes and is race-specific?

a) Quantitative resistance

b) Horizontal resistance

c) Vertical resistance

d) Polygenic resistance

True / False

- Transgenic breeding is the only way to improve stress resistance in crops. (T/F)
- Wide hybridization (crossing distant species) can be used to transfer resistance genes from wild relatives. (T/F)
- Heterosis breeding plays no role in plant stress adaptation. (T/F)
- Drought and heat stress often occur together and require breeding for combined resistance. (T/F)
- Mutagenesis can create new alleles that confer stress resistance. (T/F)

Short Answer

- Name two **sources of genetic resistance** used in plant breeding.
- What is the main advantage of **marker-assisted selection (MAS)** compared to conventional phenotypic selection?
- Give one example of a **biotic stress** and one of an **abiotic stress**.
- Explain briefly why **wild relatives** are valuable in breeding for stress resistance.
- What does **pyramiding resistance genes** mean in plant breeding?

Thank you for your attention!

Any questions?