TRAINING IN ORGANIC BREEDING!

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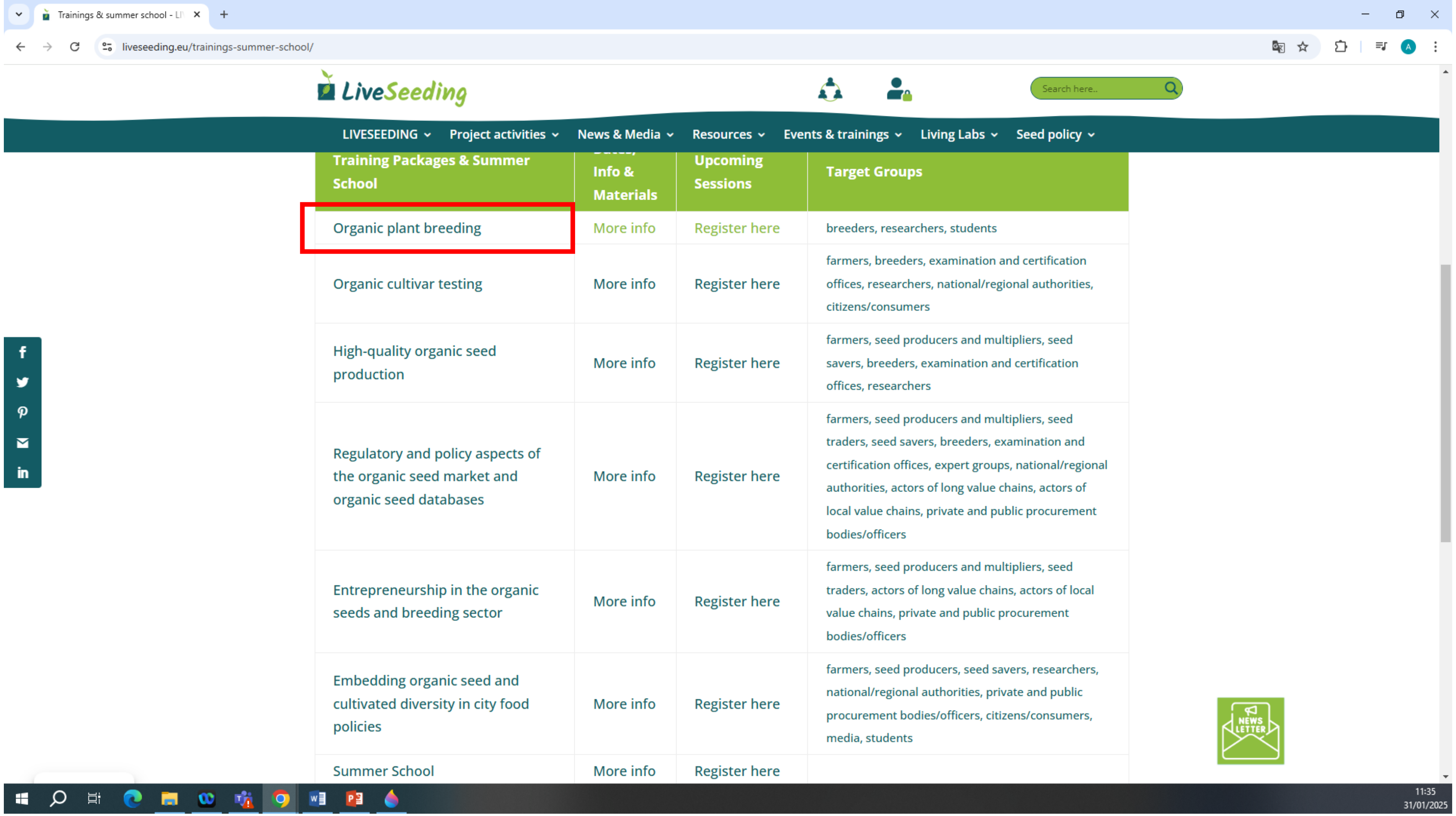
CONTEXT: Training in LIVESEEDING project

https://liveseeding.eu/trainings-summer-school/



Training Packages & Summer School	Info & Materials	Upcoming Sessions	Target Groups
Organic plant breeding	More info	Register here	breeders, researchers, students
Organic cultivar testing	More info	Register here	farmers, breeders, examination and certification offices, researchers, national/regional authorities, citizens/consumers
High-quality organic seed production	More info	Register here	farmers, seed producers and multipliers, seed savers, breeders, examination and certification offices, researchers
Regulatory and policy aspects of the organic seed market and organic seed databases	More info	Register here	farmers, seed producers and multipliers, seed traders, seed savers, breeders, examination and certification offices, expert groups, national/regional authorities, actors of long value chains, actors of local value chains, private and public procurement bodies/officers
Entrepreneurship in the organic seeds and breeding sector	More info	Register here	farmers, seed producers and multipliers, seed traders, actors of long value chains, actors of local value chains, private and public procurement bodies/officers
Embedding organic seed and cultivated diversity in city food policies	More info	Register here	farmers, seed producers, seed savers, researchers, national/regional authorities, private and public procurement bodies/officers, citizens/consumers, media, students
Summer School	More info	Register here	

Training in organic breeding organized in 5 Modules

1. **Module 1** - Plant Genetic Resources (PGRs): collection, conservation and exchange to support the increase of agrobiodiversity in farming systems
2. **Module 2** - Phenomics: approaches and tools for genetic resources and breeding material characterization - FEBRUARY 3rd 2025, 9:00 to 17:30 CET
3. **Module 3** - Breeding methods fundamentals - FEBRUARY 13th 2025, 9:00 to 18:00 CET
4. **Module 4** - Development and application of molecular methods in organic breeding - MARCH 4th 2025, 9:00 to 18:00 CET
5. **Module 5** - Organic heterogeneous material (OHM) design and development - MARCH 7th 2025, 9:00 to 18:00 CET

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Module 4 - Development and application of molecular methods in organic breeding

MARCH 4th 2025 9:00 to 18:00 CET

Unit 4.1: Genome Wide Association and its potential for understanding the genetic basis of complex traits

9:00-11:00 Partner in charge: UPV (Neus Ortega Albero)

11:00-11:30 Break

Unit 4.2: Genomic Selection and its potential in organic breeding

11:30-13:00 Partner in charge: FiBL (Michael Schneider)

13:00-14:30 Lunch Break

Unit 4.3: Application of molecular marker-based selection in practical breeding

14:30-16:00 Partners in charge: KIS (Barbara Pipan) + FiBL (Lupins; Teresa Lazzaro)

16:00-16:30 Break

Unit 4.4: Overview on Experimental populations (e.g. NILs, RILs, introgression families, MAGIC) and their role in molecular breeding approaches

16:30-18:00 Partner in charge: UPV (Neus Ortega-Albero, Adrian Rodríguez-Burruezo) + KIS (Vladimir Meglic)

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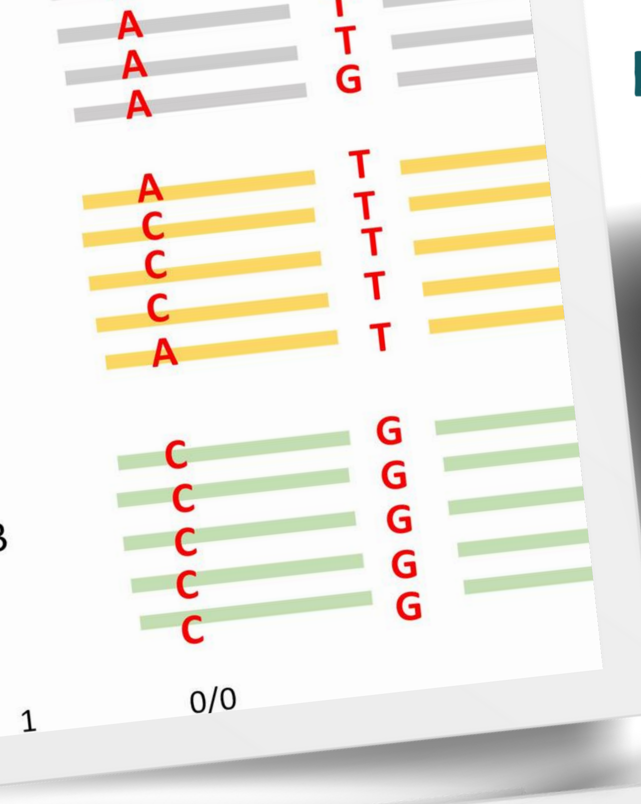

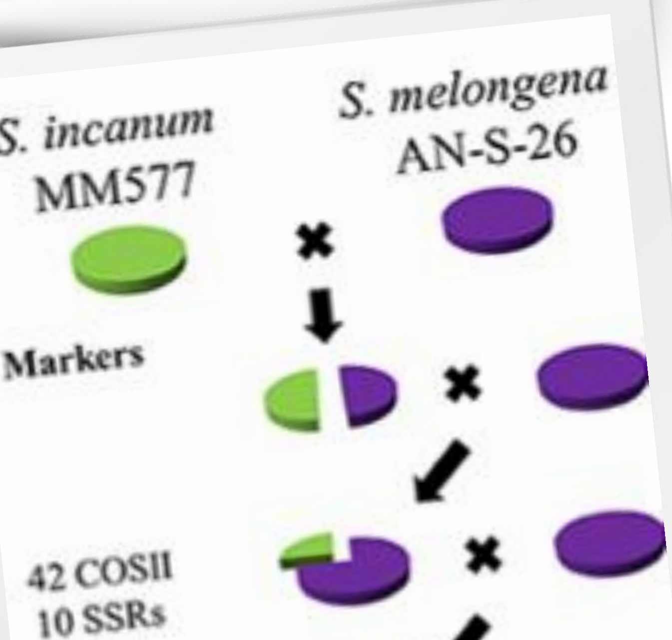

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Training in organic breeding

Module 4: **Development and application of molecular methods in organic breeding**

Unit 4.4: Overview on Experimental populations (e.g. NILs, RILs, introgression families, MAGIC) and their role in molecular breeding approaches

Authors: **Neus Ortega Albero (UPV), Vladimir Meglič (KIS)**

Co-funded by the European Union

Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and UK Research and Innovation (UKRI).

UK Research and Innovation

Traditional breeding populations

- ❖ **Mass selection**
- ❖ **Pureline selection**
- ❖ **Pedigree**
- ❖ **Backcross**

DISADVANTAGES

- × Phenotype based
- × Drag undesirable traits
- × Not precise
- × Slow
- × Low cost effective
- × >10 years for commercializing
- × Narrow genetic diversity
- × Effective for simple traits

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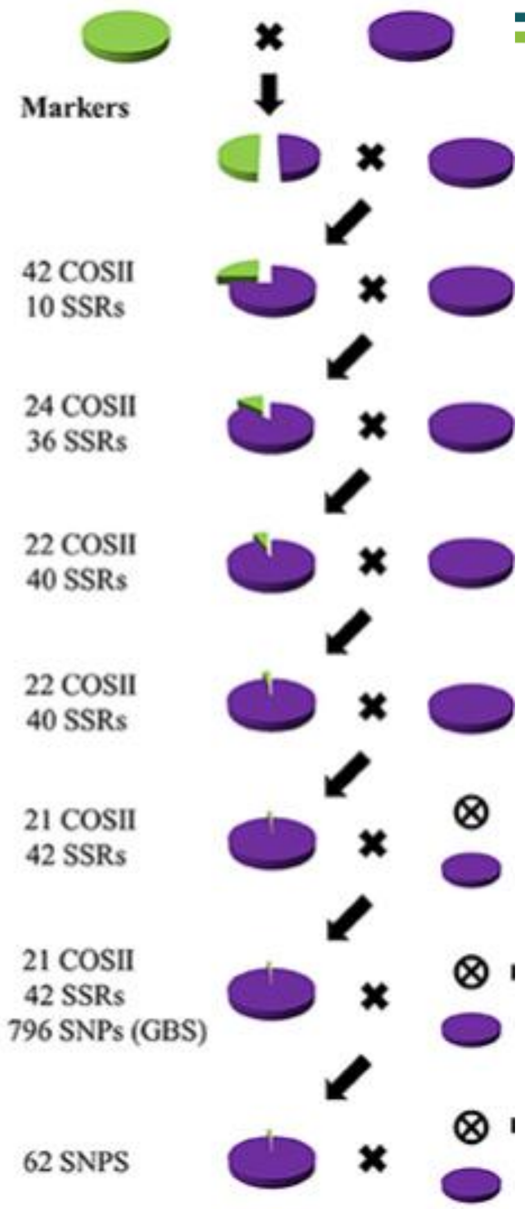
New Breeding Populations

Near Isogenic Lines (NILs)

Contain a single introgression of one variety into a uniform genome.

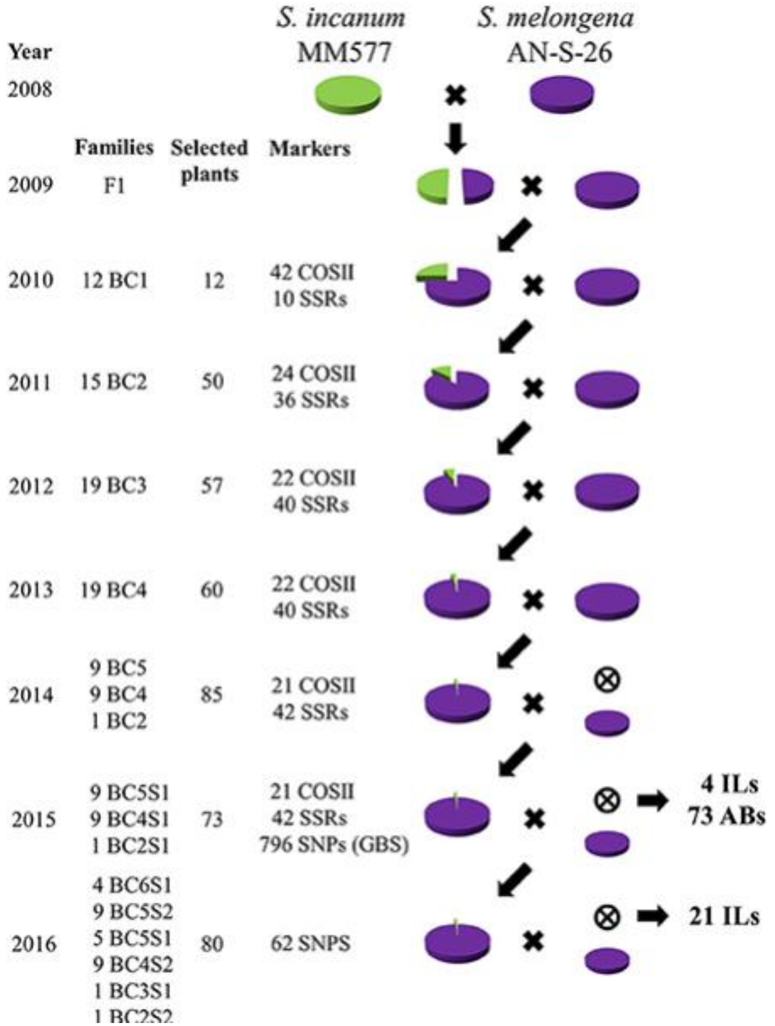
Introgression of one genetic region of one variety containing the genes that control an interesting phenotypic trait that we want into a commercial variety.



New Breeding Populations

Near Isogenic Lines (NILs)

Introgression Lines Population in **Eggplant** (ILs)



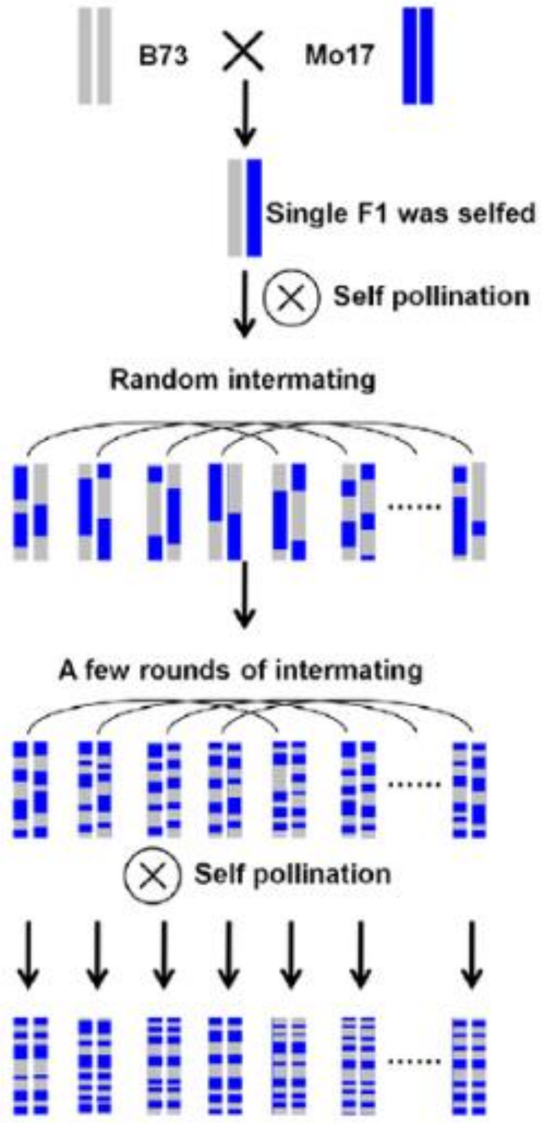
Year	Families	Selected plants	Markers	
2008				
2009	F1			
2010	12 BC1	12	42 COSII 10 SSRs	
2011	15 BC2	50	24 COSII 36 SSRs	
2012	19 BC3	57	22 COSII 40 SSRs	
2013	19 BC4	60	22 COSII 40 SSRs	
2014	9 BC5 9 BC4 1 BC2	85	21 COSII 42 SSRs	
2015	9 BC5S1 9 BC4S1 1 BC2S1	73	21 COSII 42 SSRs 796 SNPs (GBS)	4 ILs 73 ABs
2016	4 BC6S1 9 BC5S2 5 BC5S1 9 BC4S2 1 BC3S1 1 BC2S2	80	62 SNPS	21 ILs

New Breeding Populations

Recombinant Inbred Lines (RILs)

- ❖ Exploit maximum recombination frequency
- ❖ Mosaic of haplotypes
- ❖ Finding associated genetic regions by comparing

  RILs phenotypes
- ❖ Single or multiple parental crosses (MPPs)



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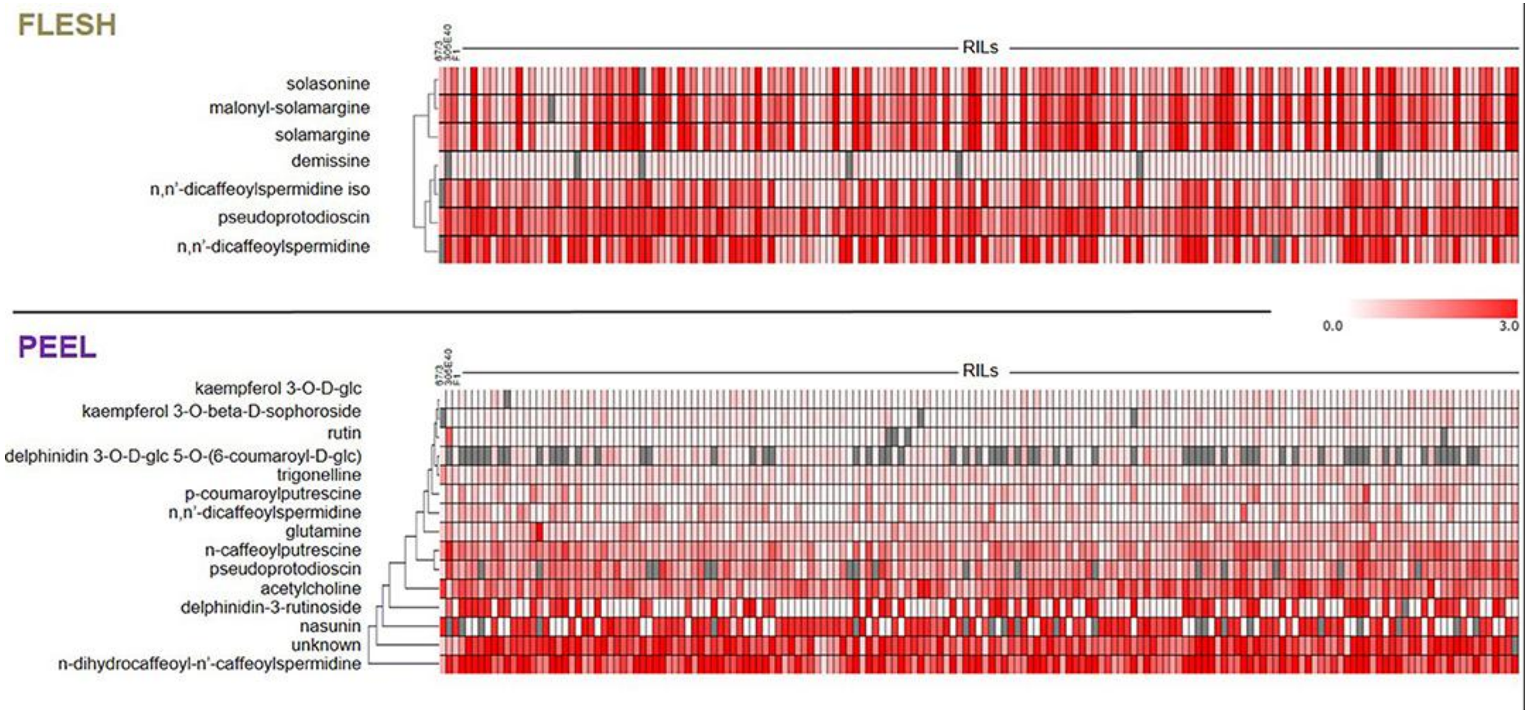
New Breeding Populations

Recombinant Inbred Lines (RILs)

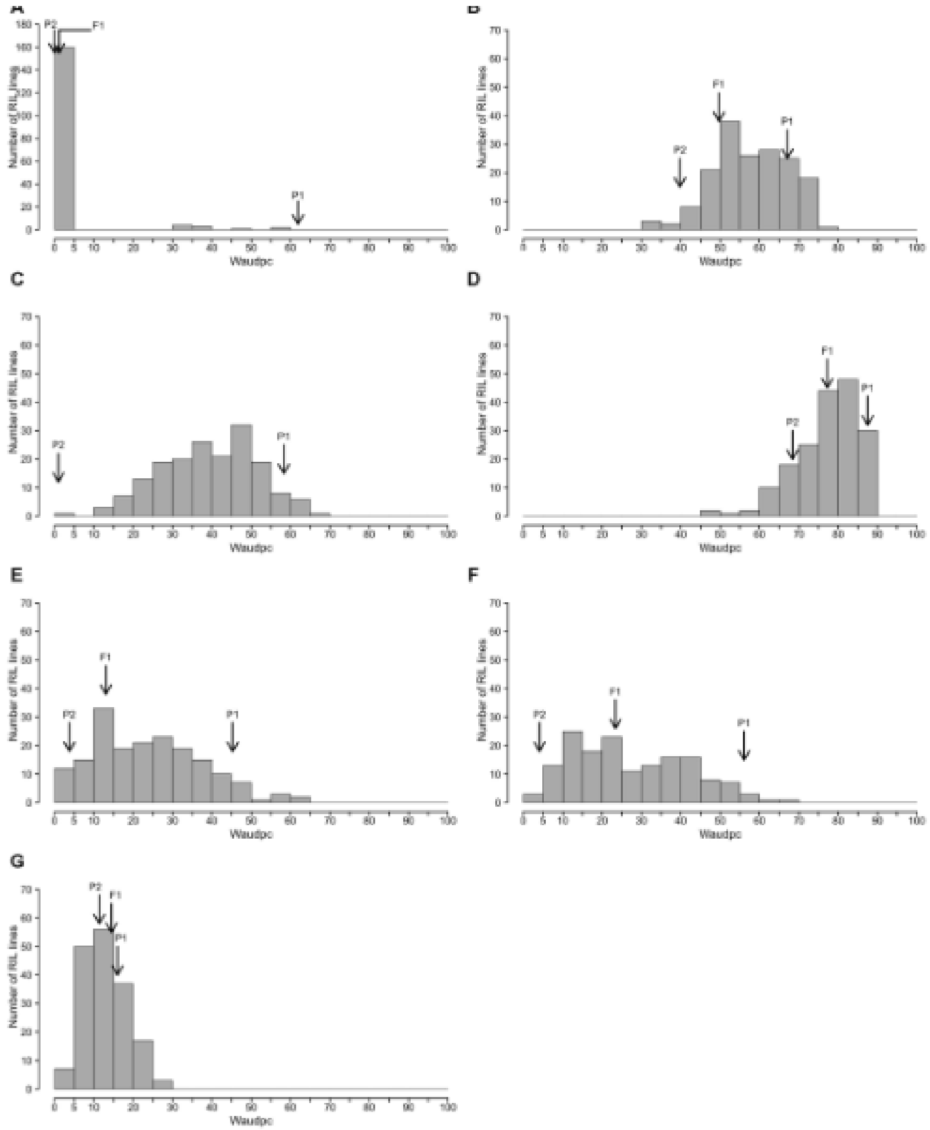
RIL population in **Eggplant** and selection for improved fruit nutritional quality



New Breeding Populations

Recombinant Inbred Lines (RILs)

RIL population in **Eggplant** and screening for resistance to *Ralstonia solanacearum* complex strains

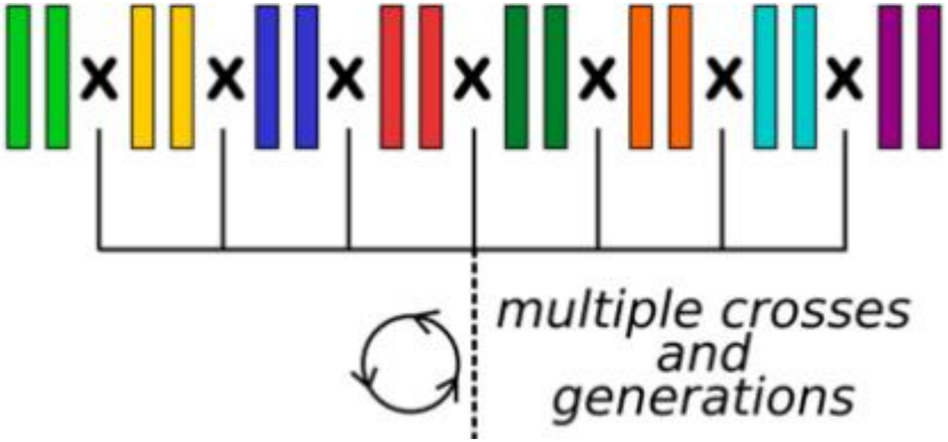


Multiparent Populations (MPPs)

✓ Including more than two parental lines

✓ Aggregate wide variability

✓ Capture additional recombination



OBJECTIVES

1. Introgress more than one interesting traits avoiding genetic loss
2. Obtain genetic information of the traits, relating genes to complex agronomic traits

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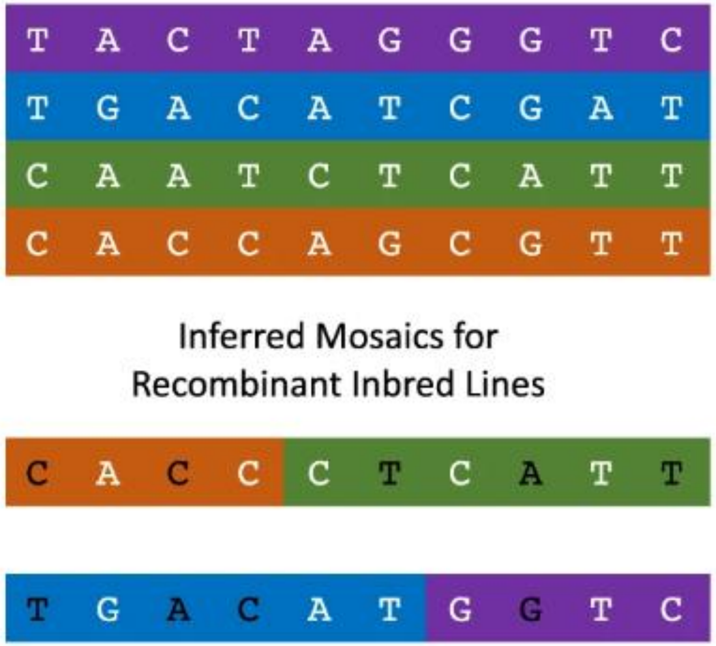
Multiparent Populations (MPPs)

Intercrossing → Recombination → Breaking genomes into shorter pieces → Study proximal genes independently

- ✓ Reduce linkage disequilibrium
- ✓ Reduce population structure
- ✓ Increase mapping resolution
- ✓ Increase QTL identification



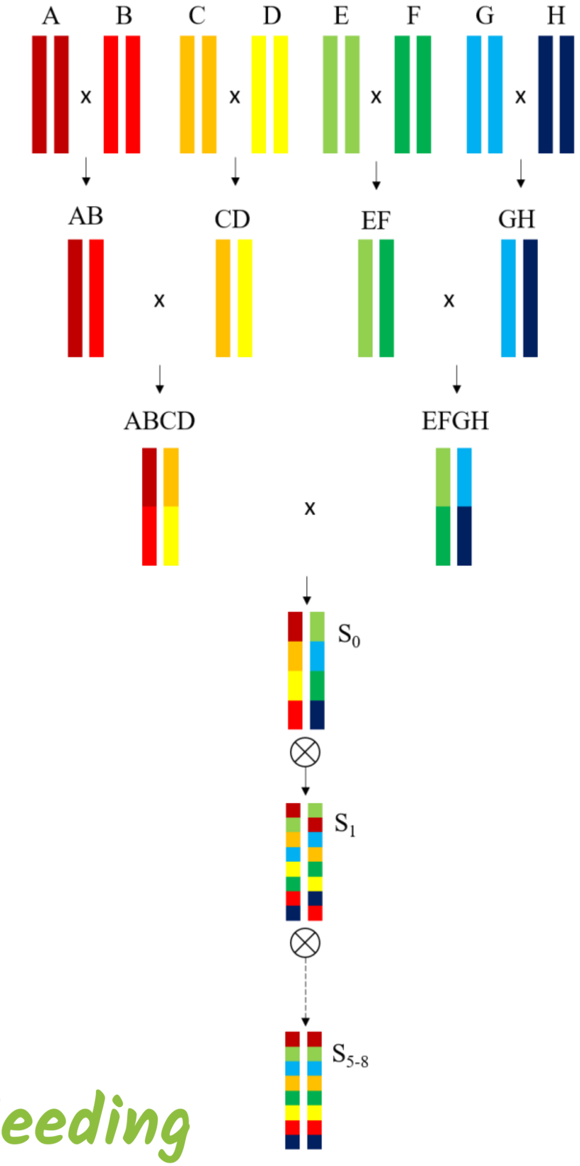
T A C T A G G G T C
T G A C A T C G A T
C A A T C T C A T T
C A C C A G C G T T

Inferred Mosaics for
Recombinant Inbred Lines

C A C C C T C A T T

T G A C A T G G T C

MAGIC populations



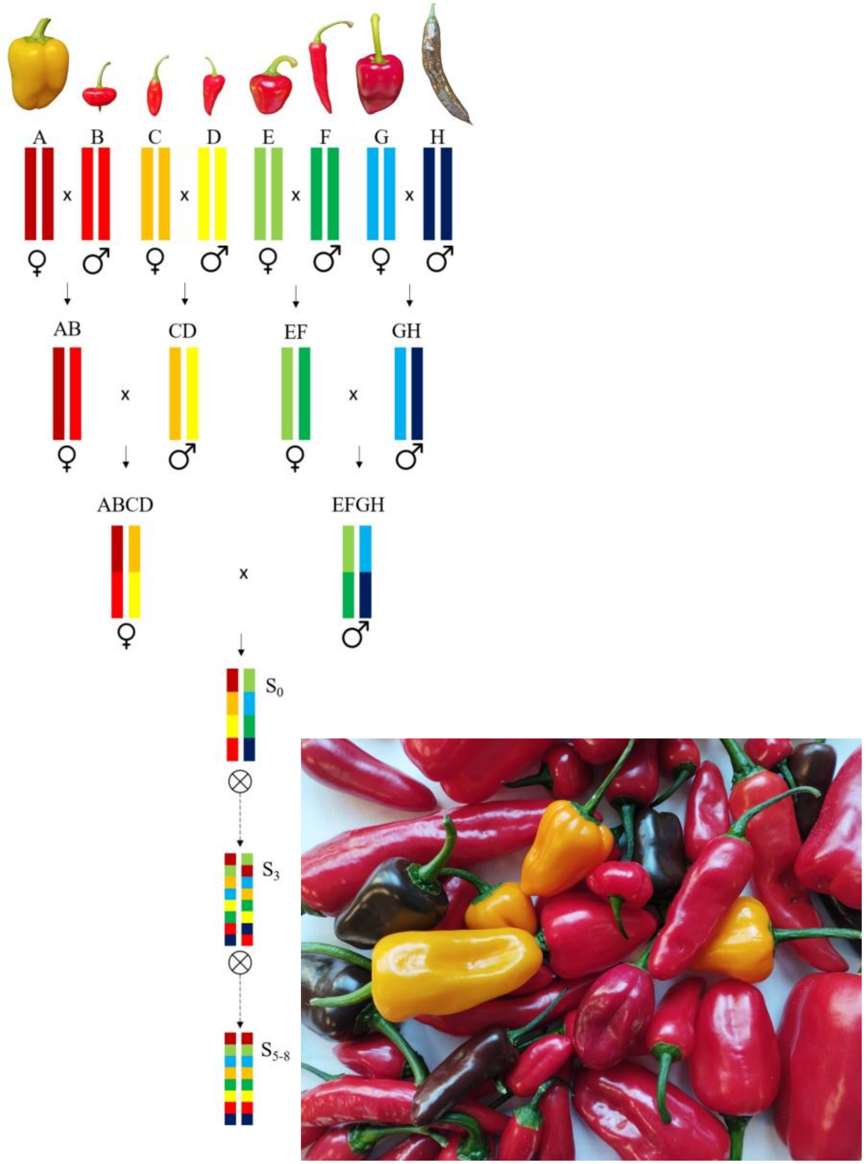
Multi-parent Advanced Generation Intercross

(MAGIC) population

MAGIC populations

Capsicum MAGIC population

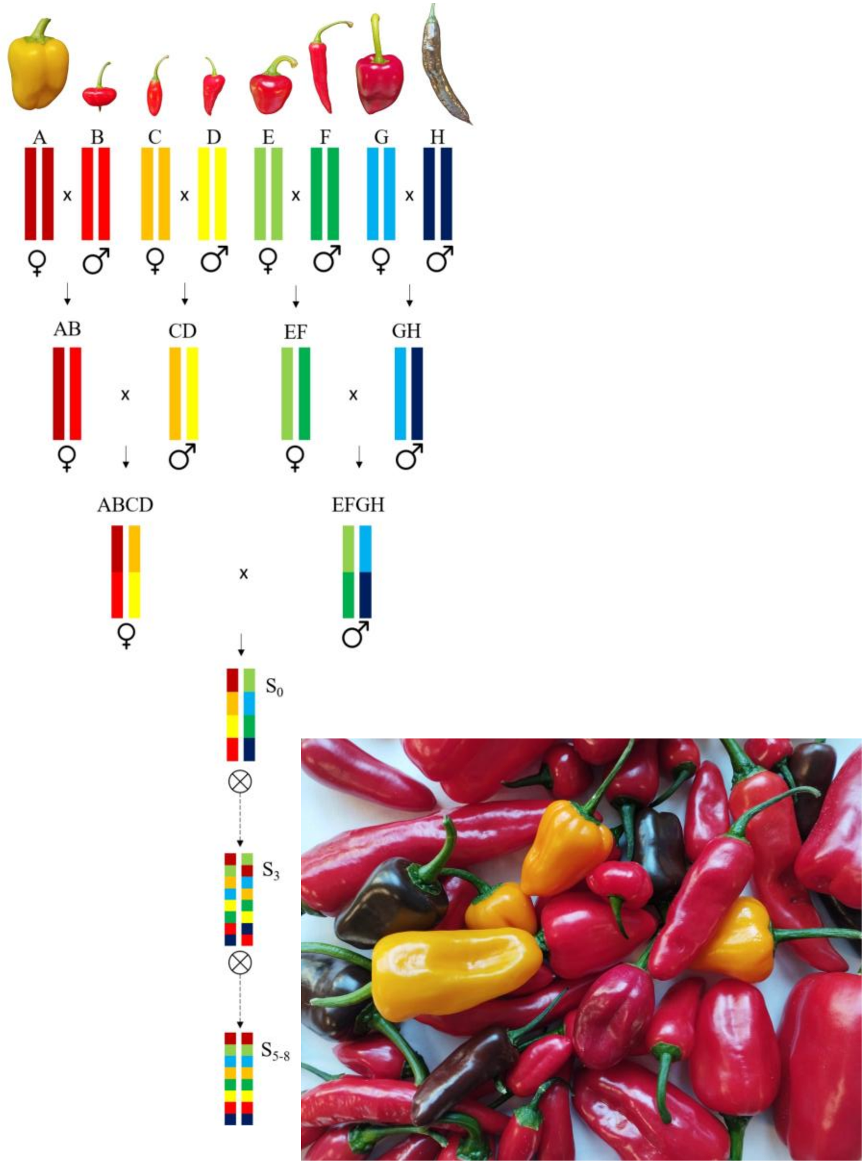
- ❖ Great variability of founder lines
- ❖ Increased recombination rate
- ❖ Reduce population structure
- ❖ High homozygosity
  - ✓ New phenotypes in qualitative traits
  - ✓ Heterosis in quantitative traits



MAGIC populations

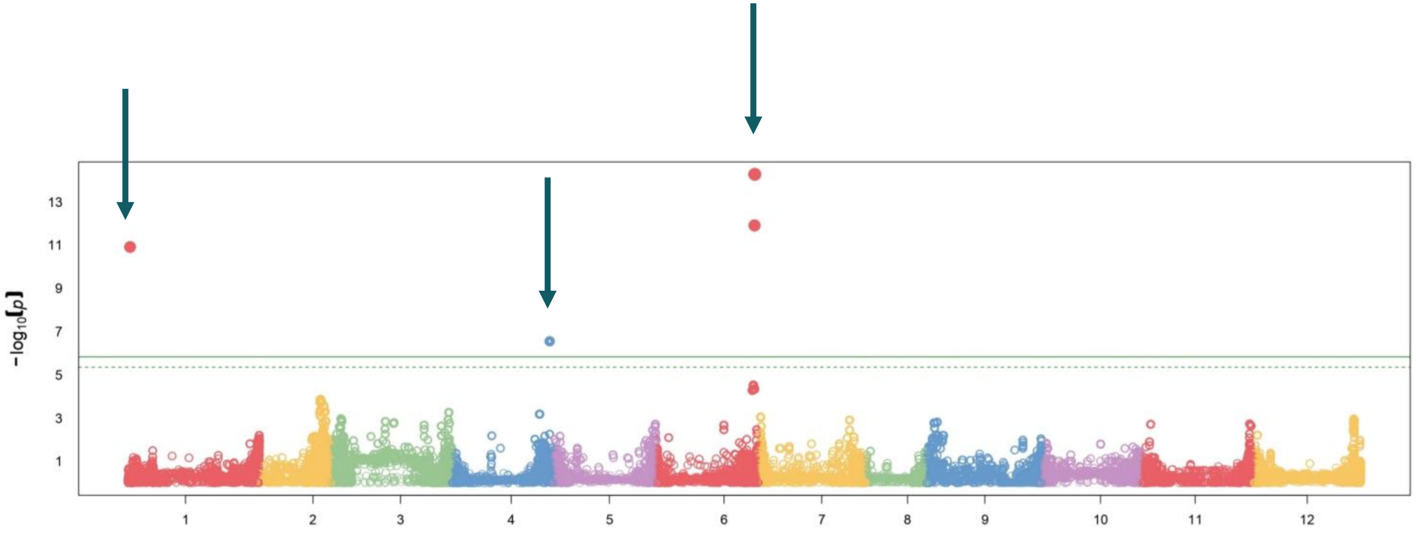
Capsicum MAGIC population

- ✓ New combinations of parental phenotypes
- ✓ New phenotypes in qualitative traits
- ✓ Heterosis in quantitative traits

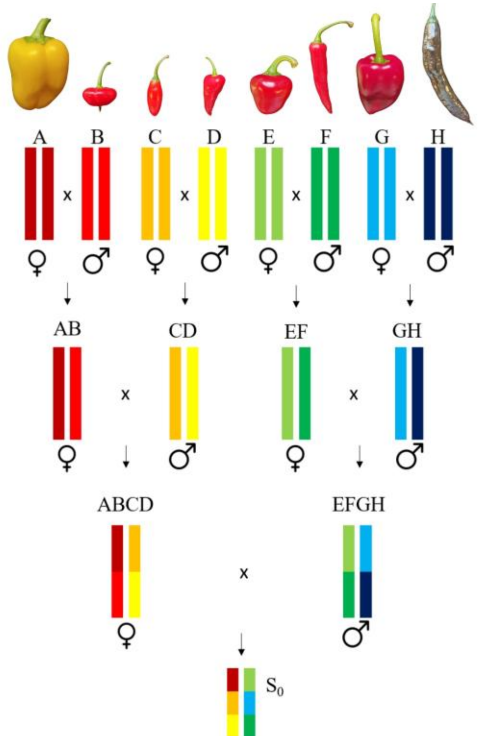


MAGIC populations

Capsicum MAGIC population

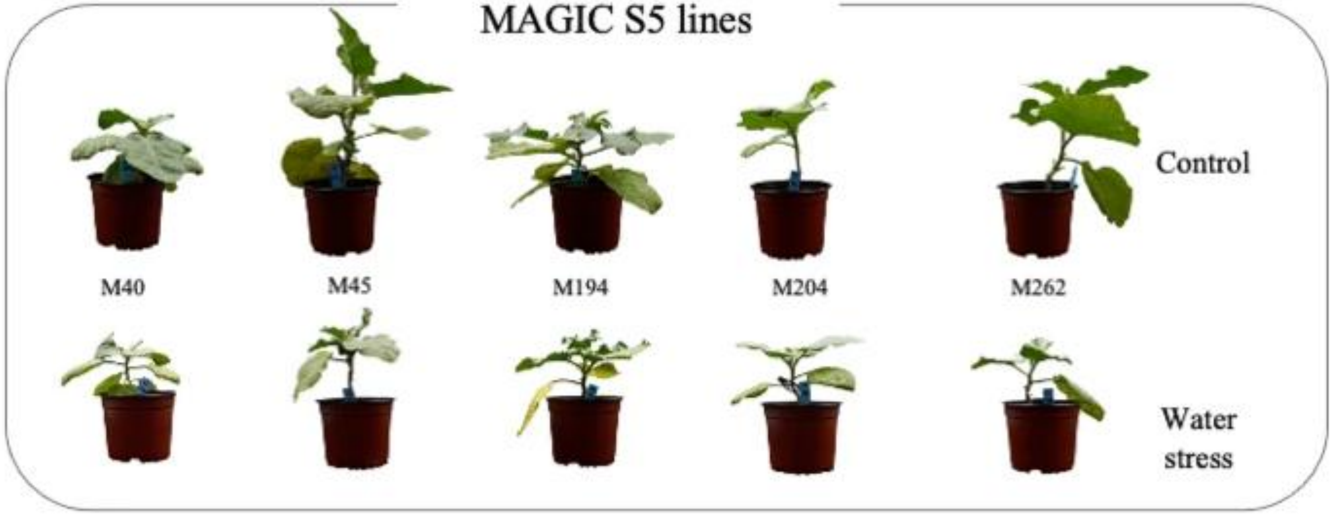


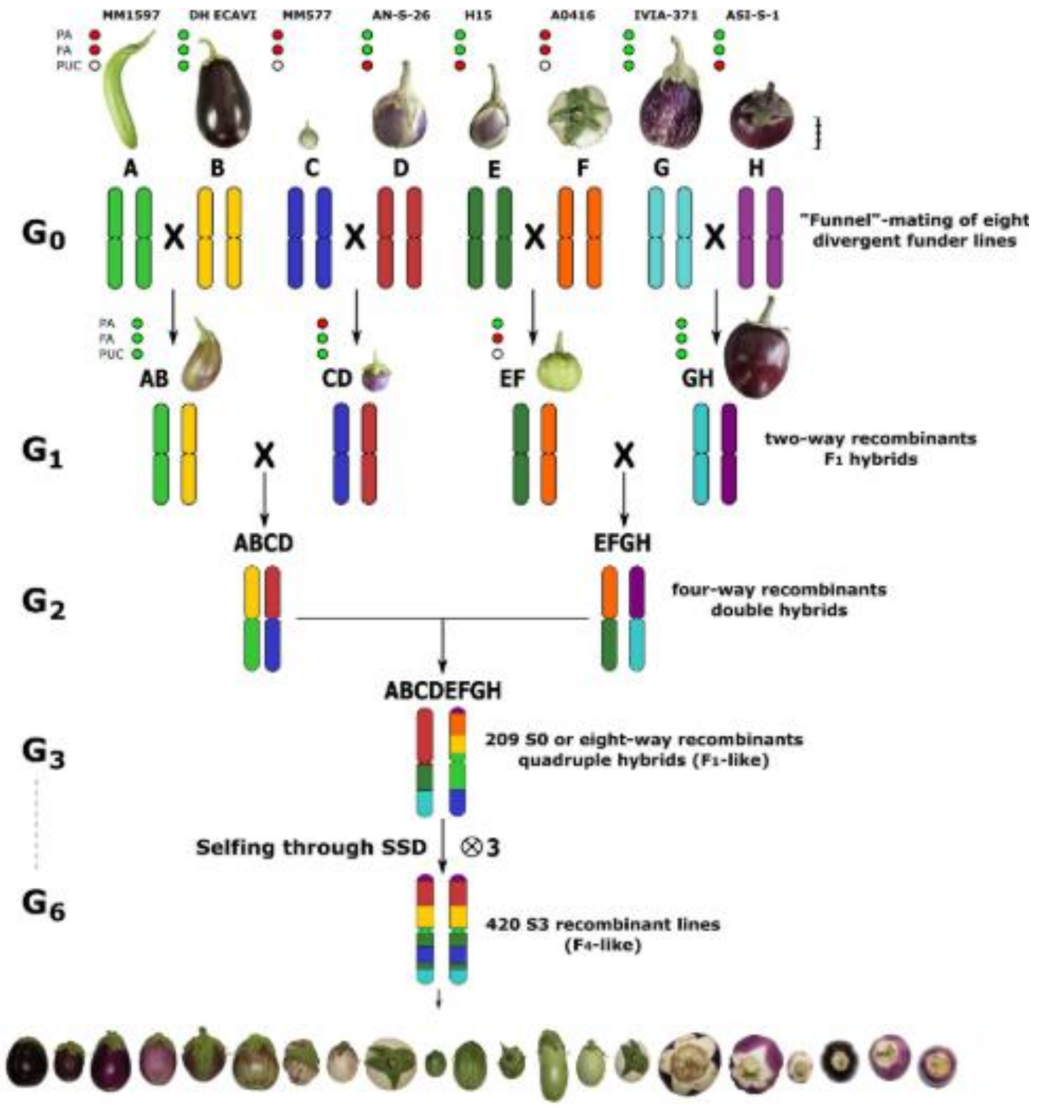
- ✓ Genetic association of QTLs with fruit quality traits



MAGIC populations

Eggplant MAGIC population

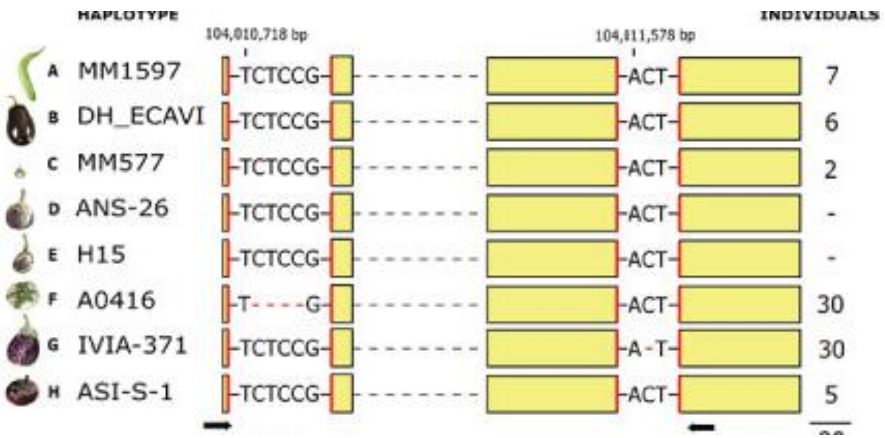


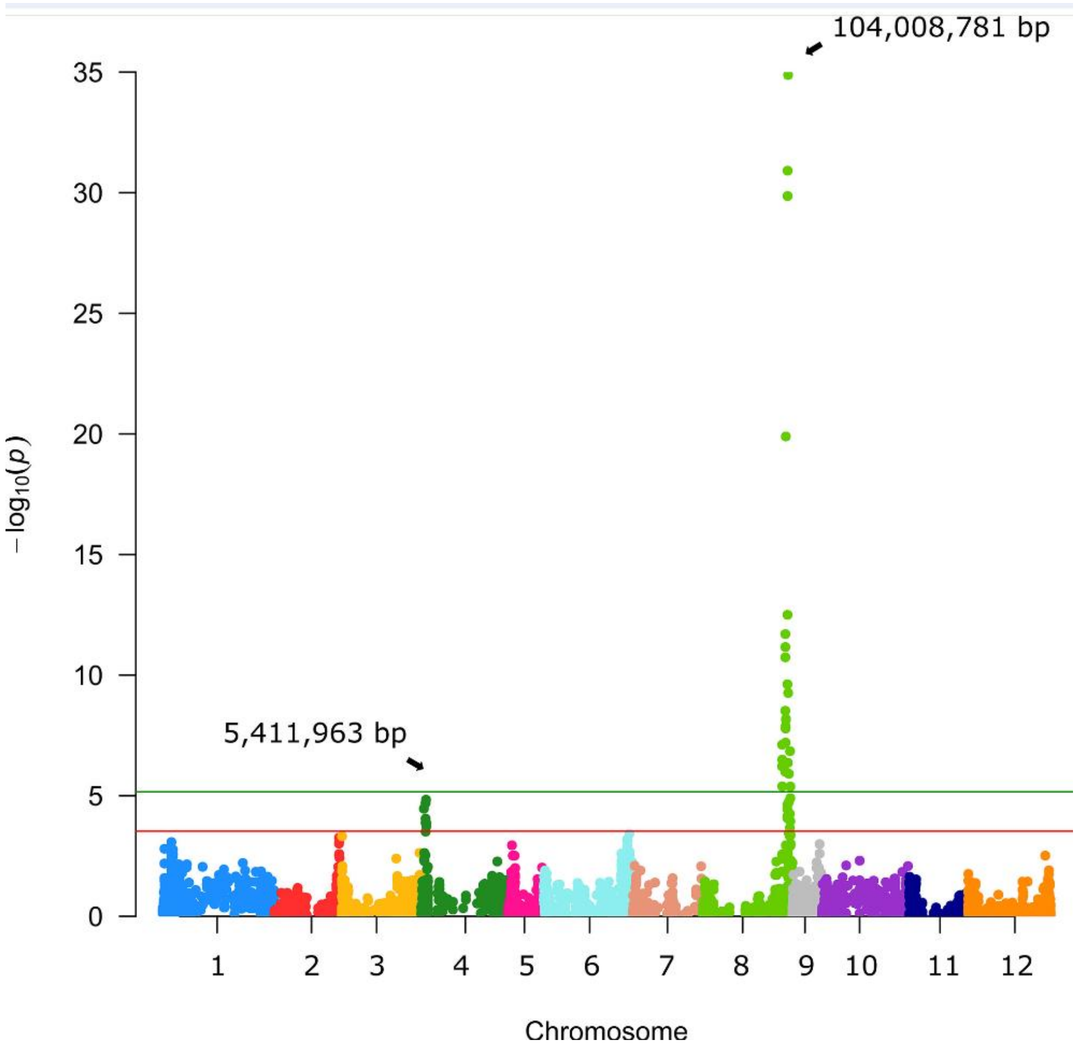


- ✓ RILs tested for water deficit conditions

MAGIC populations

Eggplant MAGIC population

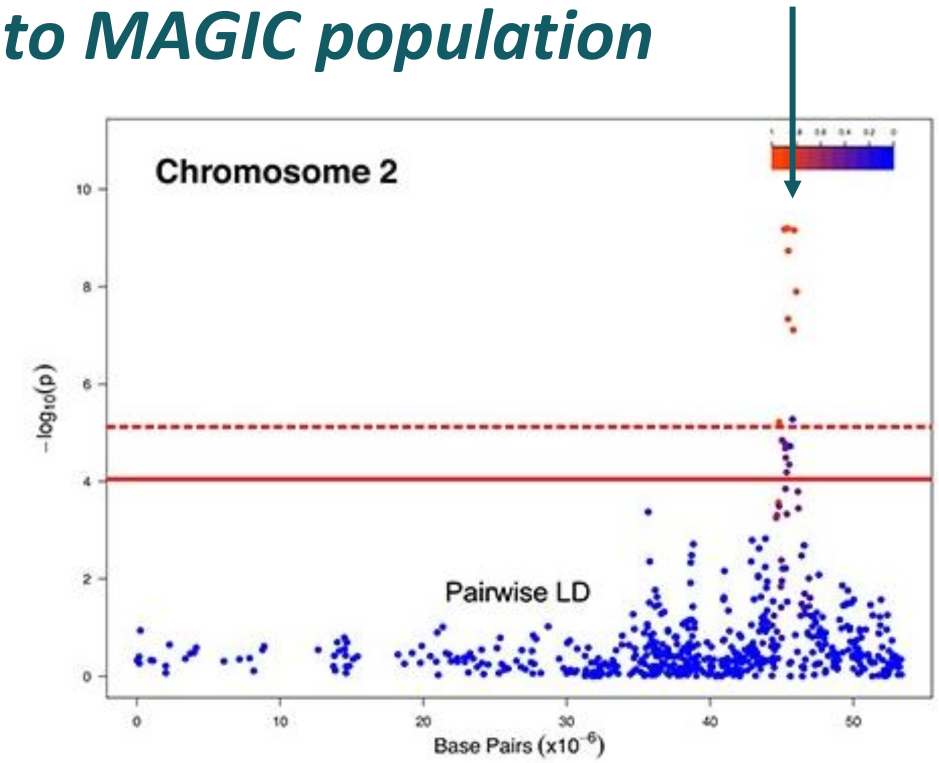


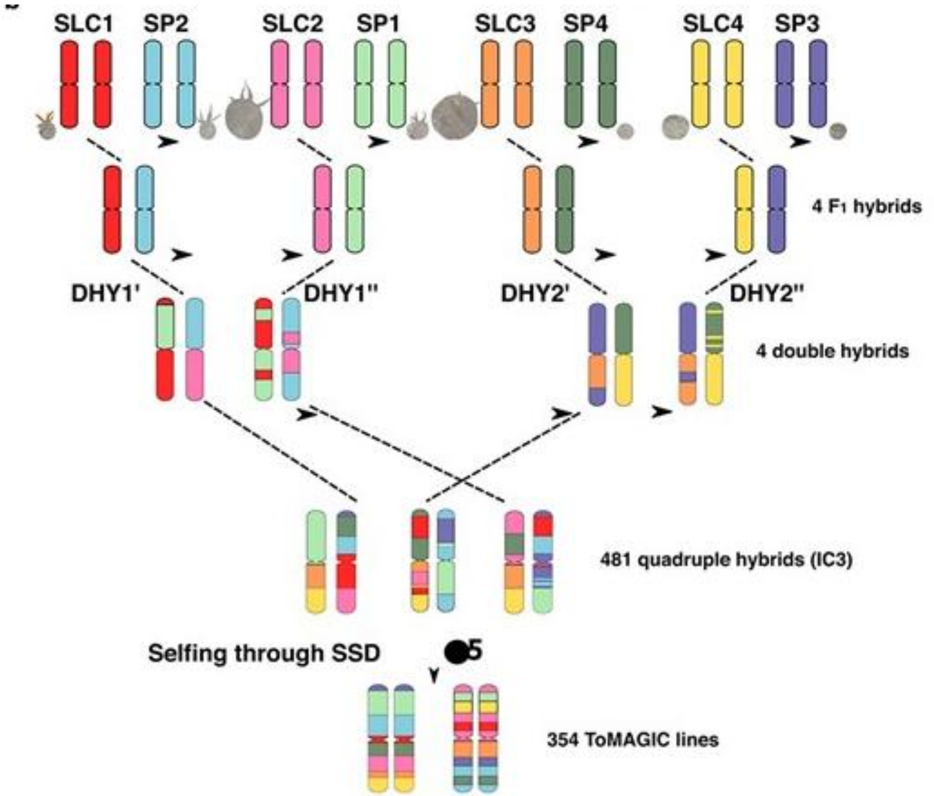


- ✓ Discover the gene and the mutation for peel color

MAGIC populations

Tomato MAGIC population



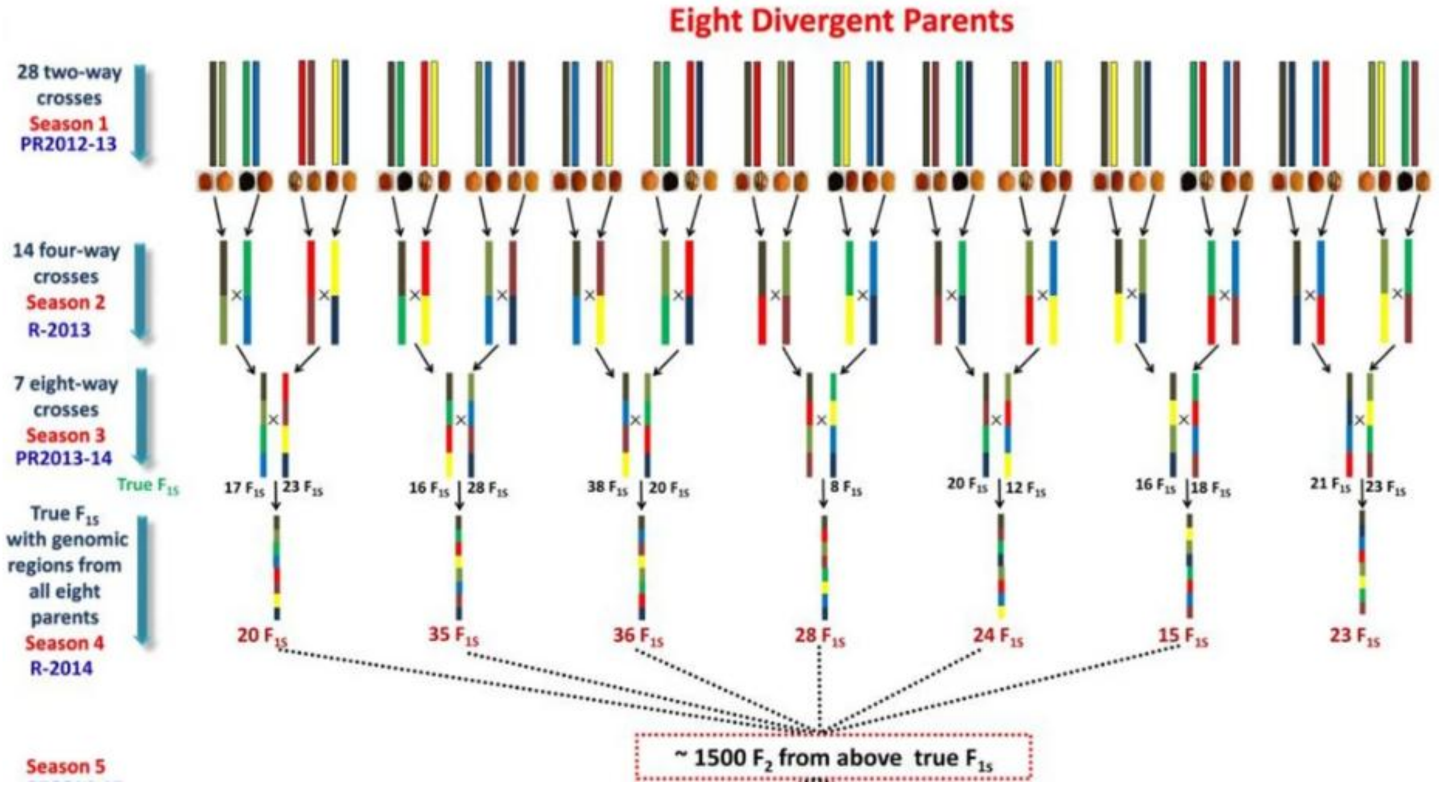


- ✓ Associating fruit quality to QTLs

MAGIC populations

Tomato MAGIC population

- ✓ Accumulation and creation of new diversity
- ✓ Evaluation and screening of outstanding performances under drought conditions



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Why do we use evolutionary populations?

Problem

- There are only a limited number of varieties adapted to the environmental variability of low-input organic farming.
- Genetically homogeneous varieties are more susceptible to spatio-temporal changes in biotic/abiotic stresses and therefore have limited adaptability to local growing conditions.
- Environmental variability often affects crop yields more under low-input and organic conditions than in conventional agriculture.

Solution

- Growing genetically diverse populations could improve tolerance to a wide range of environmental stresses and different growing conditions by allowing them to evolve and adapt to changing conditions.
- Composite cross populations (CCPs) are populations of segregating individuals that are crossed with each other (e.g. by bulking the progenies of these crosses) to produce heterogeneous and diverse material. The development of CCPs is an example of evolutionary breeding.
- Many breeders and researchers have developed and tested heterogeneous populations over the past two decades. In ECOBREED, the Hungarian variety ‚Mv Elit CCP', produced by crossing seven different parents, was tested in organic field trials in 2020 and 2021 and showed stable grain yields and high protein content.
- Compared to the variety 'Capo', which is popular with organic farmers and growers, 'Mv Elit CCP' showed a significantly higher protein content with a similar yield.

Ecobreed example - wheat

Development of new wheat germplasm for organic farming

MAGIC populations are created by several generations of inter-crossing among multiple founder lines. Multiple founders contribute more allelic diversity and multiple cycles of inter-crossing give greater opportunities for recombination. Such multi-parental populations are nowadays widely used for the mapping of genes/QTL, however, may also be used for the creation of heterogeneous material for on-farm selection.

Multi-parent advanced generation inter-cross populations

Two 8-parent MAGIC populations were developed for further selection of either pure line breeding lines or development of organic heterogeneous material (syn. evolutionary bulks or populations) under organic conditions.

The first crosses started in 2019 and the final cross was done in 2021. Afterwards, the two populations were multiplied in 2022 and 2023 as bulks in Hungary, and in Austria as ear-to-row progenies. Material was further distributed in autumn 2023 as bulks to partners in Germany, Czechia, Slovakia and Slovenia.

The first MAGIC population contains mainly varieties from the EARLY panel:
Glosa/NS Ilina//Mv Karej/PS Dobromila/3/Ursita/NS 40S//Ehogold/Nexera 923

The second MAGIC population includes mainly varieties from the LATE panel:
IS Laudis/Mv Kolompos//Arminius/Xt88.5R/3/Viki/Wendelin//Tobias/Spontan

Bulk	Early MAGIC		Late MAGIC	
	BBCH 24/05/2023	Plant height (cm)	BBCH 24/05/2023	Plant height (cm)
I	58-59	80-90	47-49	75-85
II	51-59	95-125	47-49	90-110
III	61-67	65-90	51-53	80-90
IV	61-65	95-130	51-57	95-115

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ecobreed
IMPROVING CROPS

TGRC UCDavis Example: Genome transfer of Solanum lycopersicoides into L. esculentum using introgression

Morphology: perennial – bush with hard fruits
Found in: Peru and Chile; found at 3600m
Self incompatible, cross pollinating sp.
Num. of chromosomes: 24 (2x)
Possibility to cross: F1 hybrid with tomato male sterile and incompatible

- **Resistance to abiotic stress**
  - Low temperature
  - Frost
  - Drought
- **Tolerance to mechanical damage**
  - Fruit firmness

- **Resistance to biotic stress**

- **Viruses**
  - **Tomato Mosaic Virus (ToMV)
  - **Cucumber Mosaic Virus (CMV)
  - Curly Top Virus (CTV)
  - Tomato Yellow Leafcurl Virus (TYLCV)
- **Bacteria**
  - (*Xanthomonas campestris*)
  - (*Clavibacter michiganensis*)
- **Fungi**
  - **Phytophthora (*P. parasitica, P. capsici*)
  - **Siva plesen (*Botrytis cinerea*)
- **Insects**
  - On fruit (*Manduca sexta, Heliothis zea*)
  - Leaves (*Liriomyza trifolii*)

Hybridisation scheme for development of *S. lycopersicoides ILs*

L. esculentum* x *S. lycopersicoides
(cv VF36)

F1

VF36 x (1 male-fertile)

BC1

VF36 x (281 total, 32 fertile)

1. **self-pollinate**
2. **map *S. lycopersicoides* segments**
3. **select homozygotes**

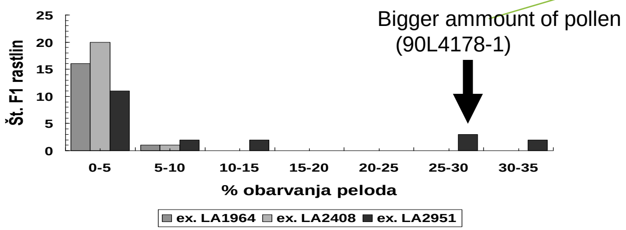
BC2

VF36 x BC2F6

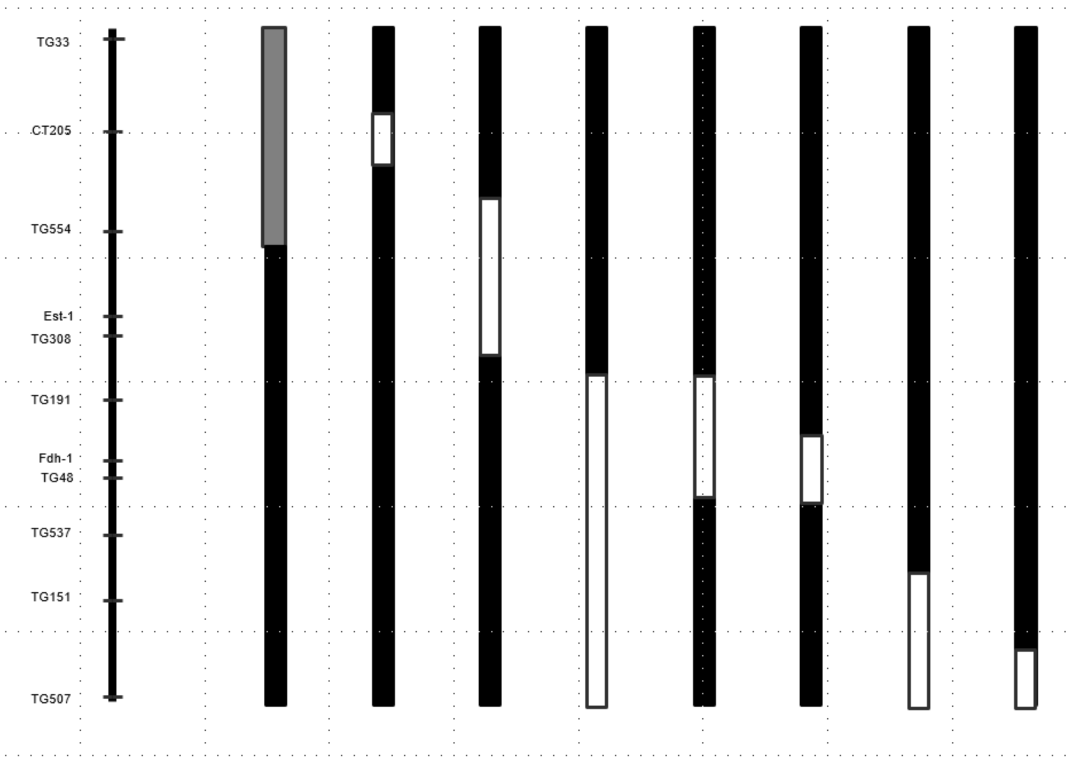
4. **select against extraneous markers**
5. **fix homozygous segments**

<u>**Introgression Lines**</u>

Male fertility of F1 hybrids
(*L. esculentum x S. lycopersicoides*)

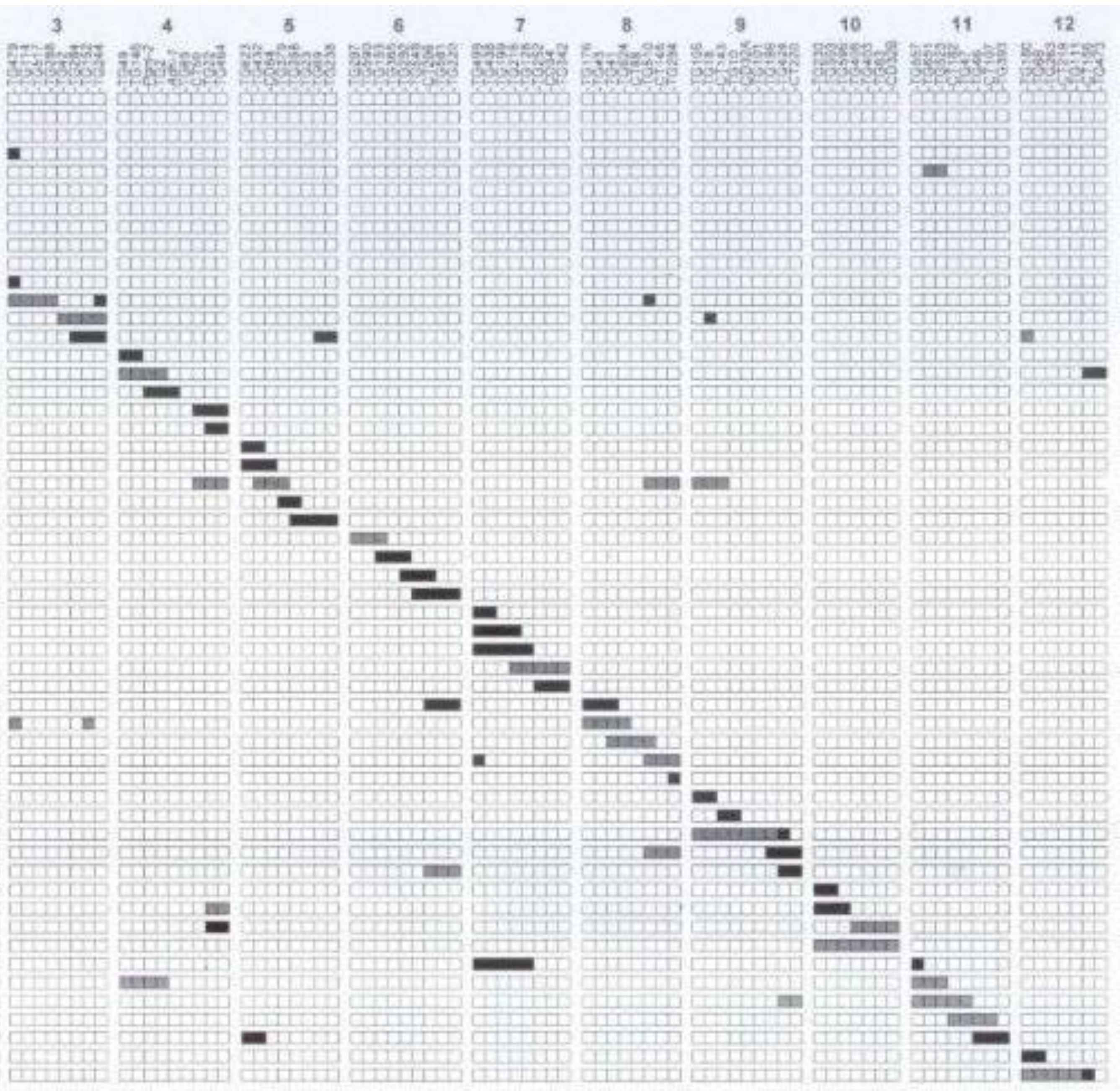


Example of the chromosome no. 2



☐ homozygous

▨ heterozygous



Example: Cucumber QTL mapping

Cucumber Phenotype

Current plant types:

1) Monoecious flowering (*ff*)
2) Unilateral branching (QTL)
3) Crown-set fruit inhibition (?)
4) Indeterminate habit (*De_*)

Future/desired ideotype:

1) Gynoecious flowering (*FF*)
2) Multiple lateral branching (QTL)
3) Sequential fruit setting (QTL)
4) Determinate (*dede*)

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Plant material

H-19

Monoecious
6-20 lateral branches
Poor fruit quality
    3.1 L:D
Sequential fruiting

G-421

Gynoecious
1-2 lateral branches
Good fruit quality
    2.9 L:D
Non-sequential fruiting

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Map Construction and QTL Analysis

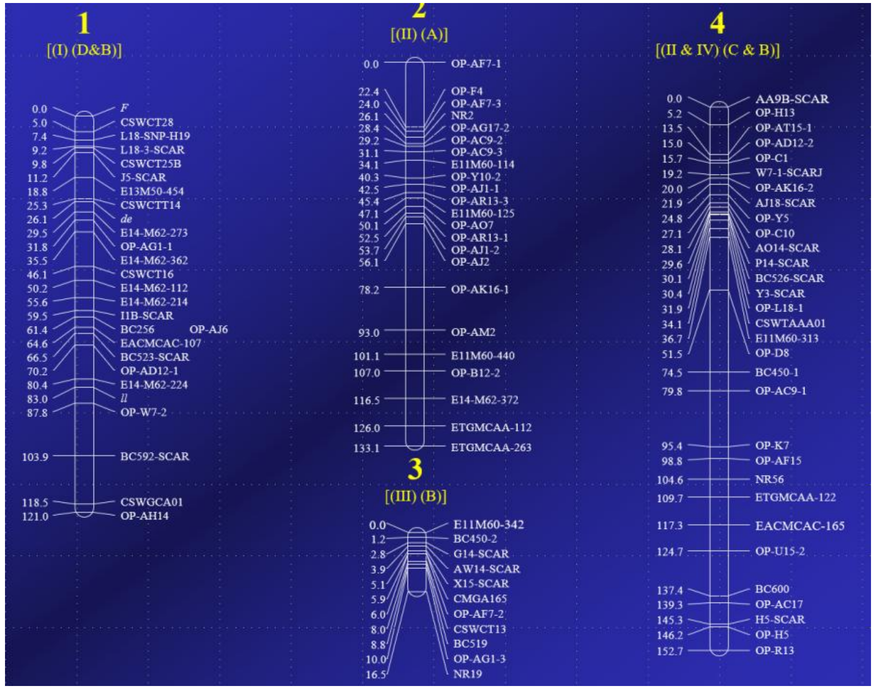
Line G421 and H-19
171 recombinant inbred lines (RIL)
RADP, SCAR, SSR, AFLP & SNP markers
Two locations in two years
RCBD, 3 replications, 12 plants per plot

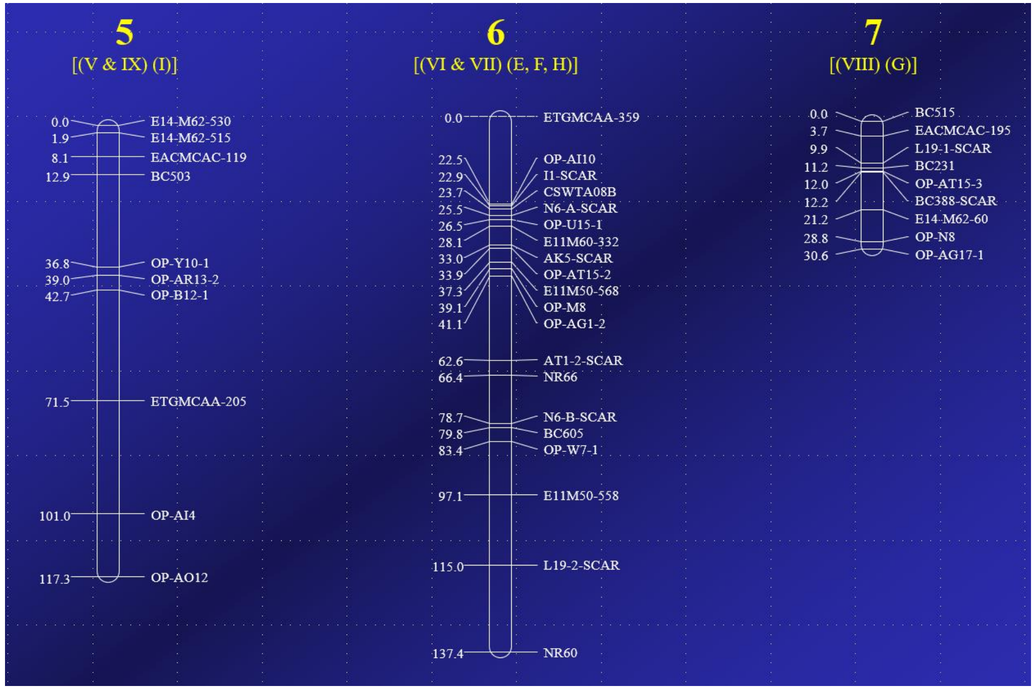
131 point map 14 SSR, 24 SCAR, 27 AFLP, 62 RAPD, 1 SNP

700 cM, mean marker interval=5.6 cM

Largest interval = 29.5 cM

7 linkage groups





Sex expression	6 QTL
Lateral Branches (MLB)	5 QTL
Earliness	2 QTL
Fruit Length	5 QTL

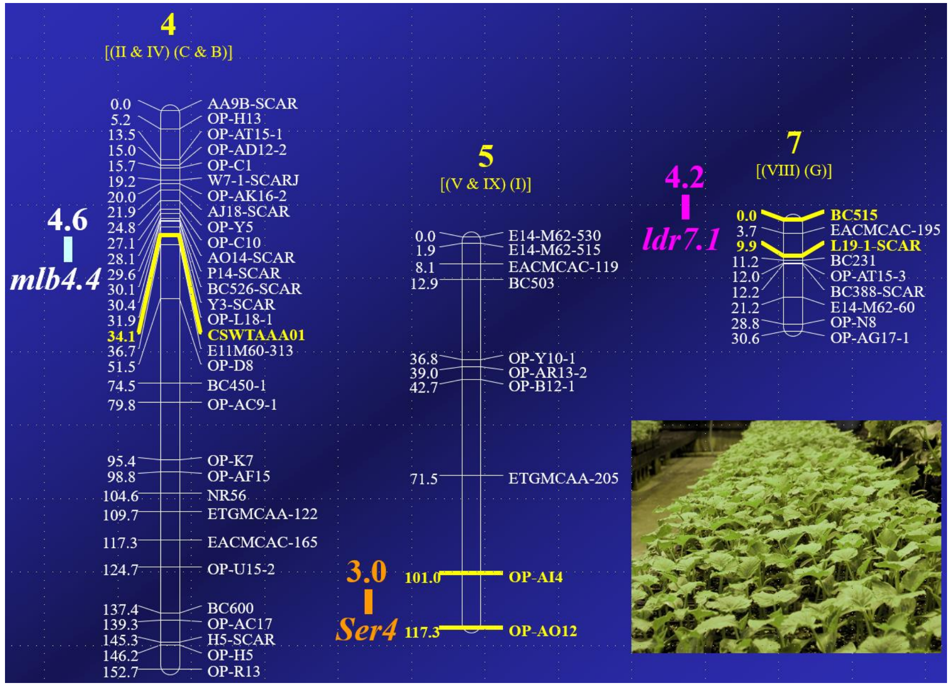
Correlations Among Traits

Trait	**MLB WI**	**Anthesis**	**♀ on mainstem**	**♀on laterals**	**Total fruit**	**Earliness**	**Harvest 1 fruit**	**Mean L:D**	**MLB UT**
MLB WI	1								
Anthesis	-0.47***	1							
♀ on mainstem	-0.29***	0.42***	1						
♀ on laterals	0	0.02	0.57***	1					
Total fruit	0.58***	-0.05	0.05	0.24**	1				
Earliness	0.36***	0.03	-0.63***	-0.55***	0.26**	1			
Harvest 1 fruit	0.30***	-0.01	0.01	0.06	0.50***	0.37***	1		
Mean L:D	-0.20**	-0.09	-0.40***	-0.35***	-0.27**	0.12*	-0.07	1	
MLB UT	0.77***	-0.32***	-0.21**	-0.01	0.42***	0.30***	0.29**	-0.15	1

Multi-trait Selection

Compare phenotypic and marker-assisted selection for multiple traits

- Five traits: Earliness, Multiple lateral branching, Gynoecy, Fruit size (L:D), Normal leaves
- Use of different types of markers for MAS
- Evaluation of cycles (3) selection for gain from selection



Characterize epistatic interactions among QTL conditioning sex expression and MLB

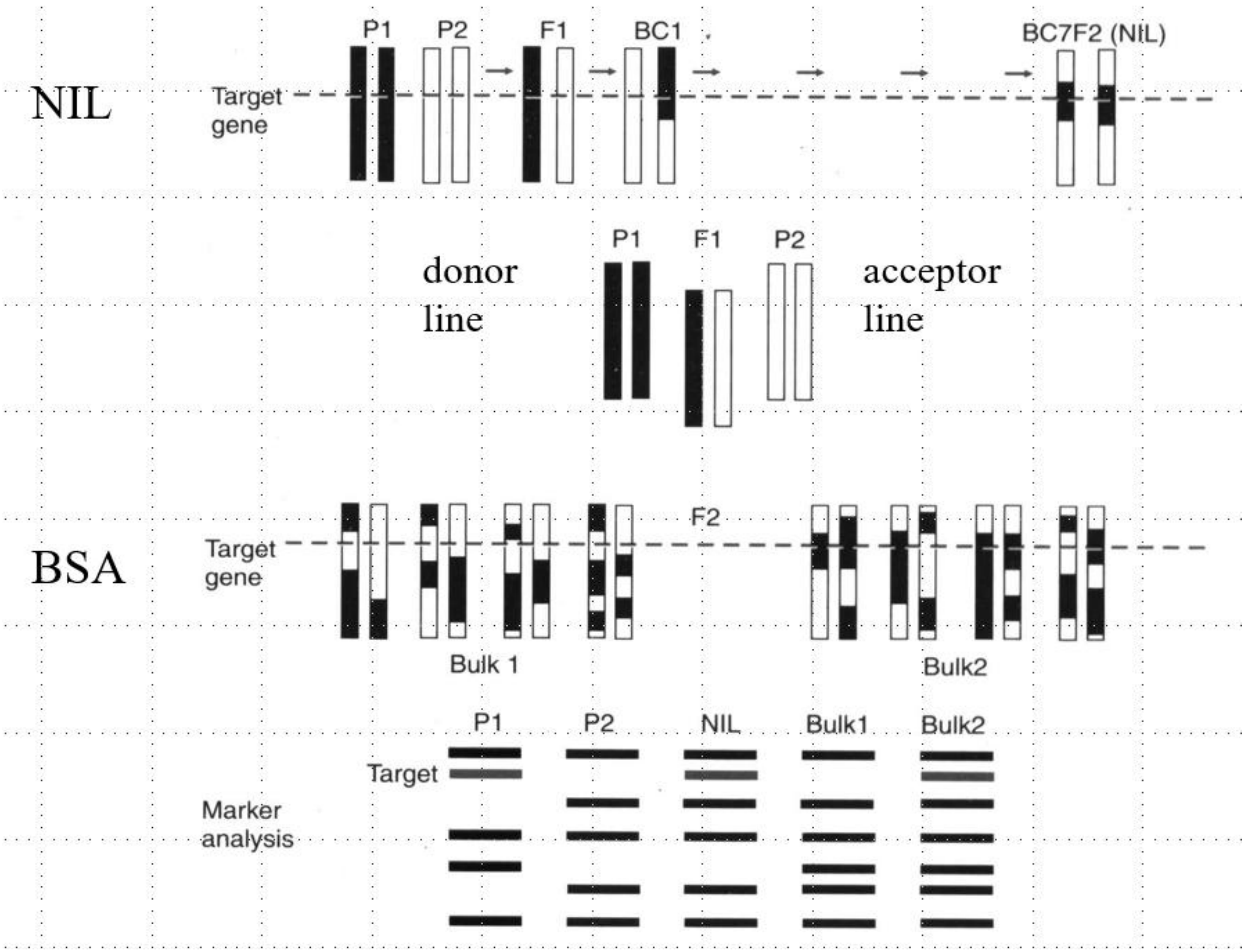
- The creation nearly isogenic BC lines
- Assess these lines under abiotic stress conditions

The steps in marker-assisted selection

Identifying association between molecular marker (RFLP, RAPD, AFLP, SSR) and selected trait.

Methods: near isogenic lines (NIL), bulked segregant analysis (BSA).

Using this association to develop improved lines or populations.

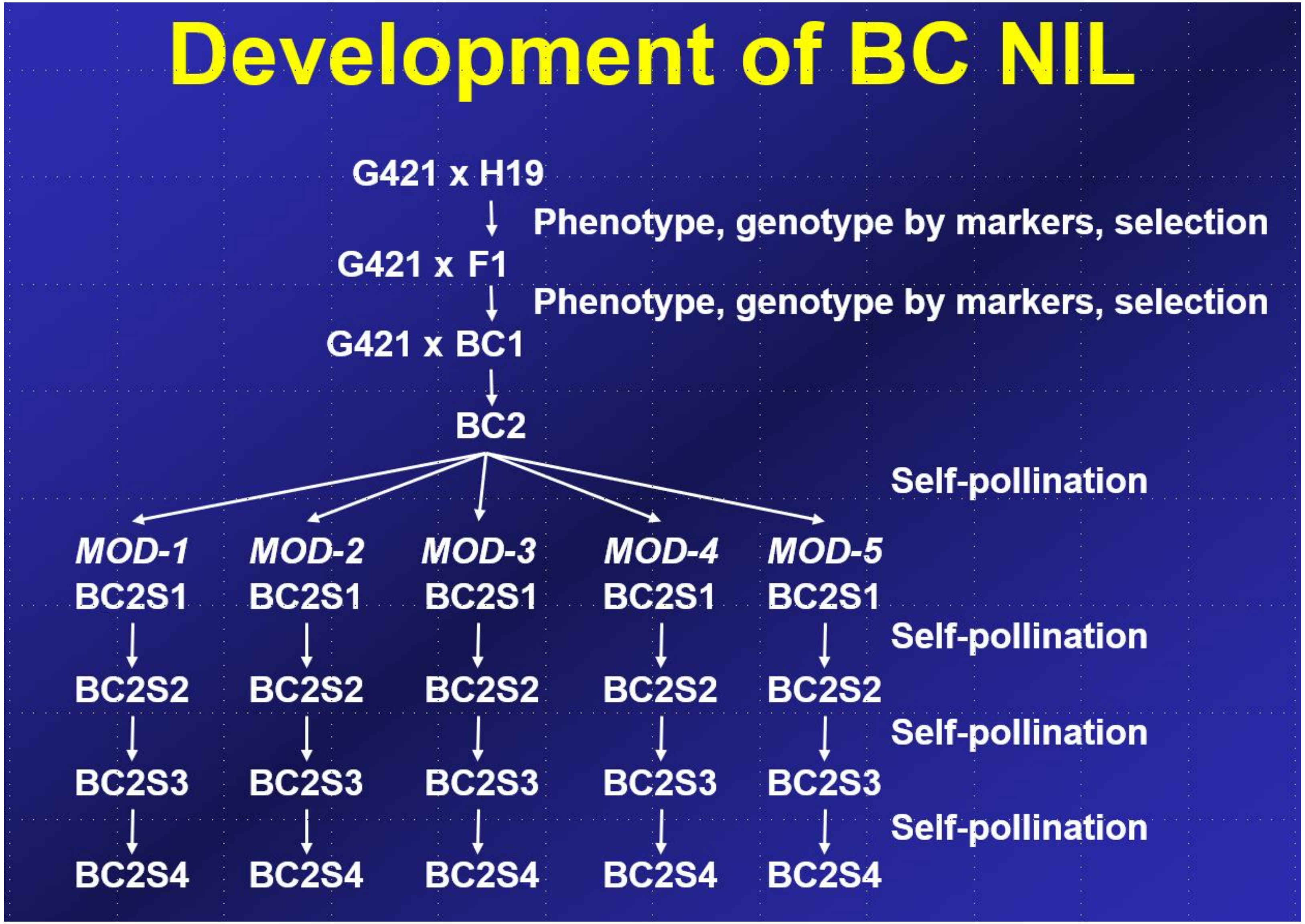


The creation of lines for mapping

Lines that have the same QTL alleles pooled for analysis

Lines compared that carry the opposite QTL allele in a bulk segregant analysis technique

Strategic comparisons involve markers not included in previous maps



Development of BC NIL

G421 x H19

Phenotype, genotype by markers, selection

G421 x F1

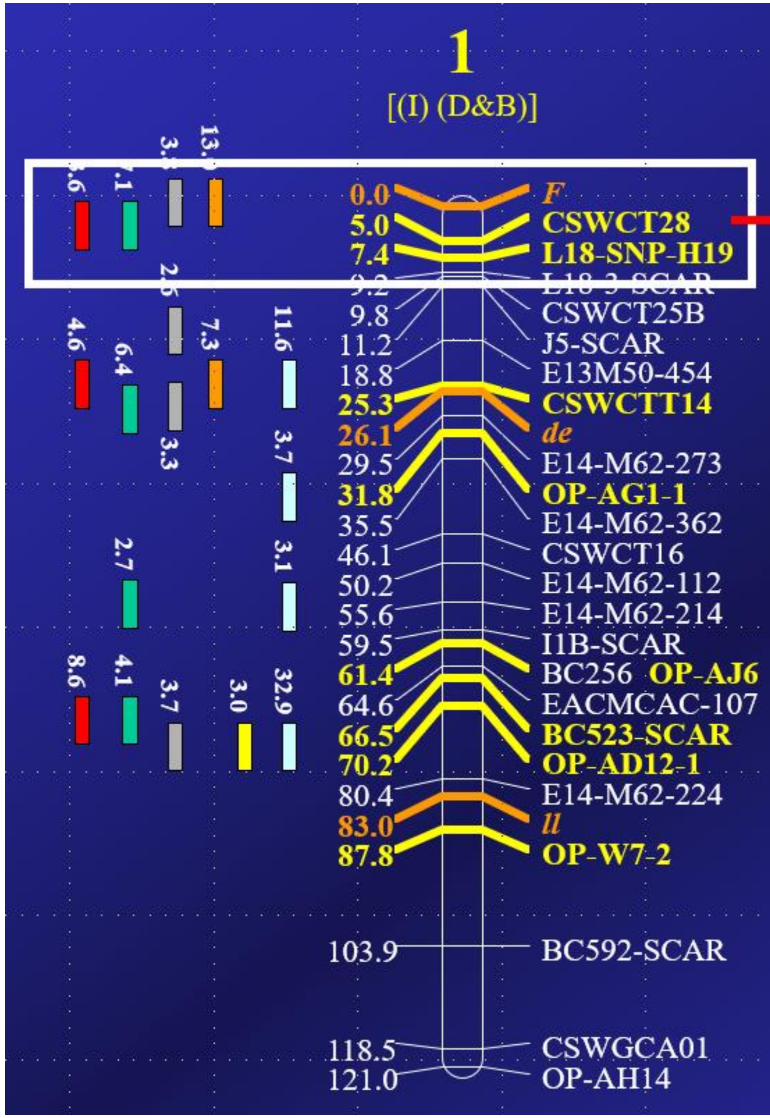
Phenotype, genotype by markers, selection

G421 x BC1

BC2

Self-pollination

MOD-1	*MOD-2*	*MOD-3*	*MOD-4*	*MOD-5*	
BC2S1	BC2S1	BC2S1	BC2S1	BC2S1	
					Self-pollination
BC2S2	BC2S2	BC2S2	BC2S2	BC2S2	
					Self-pollination
BC2S3	BC2S3	BC2S3	BC2S3	BC2S3	
					Self-pollination
BC2S4	BC2S4	BC2S4	BC2S4	BC2S4	

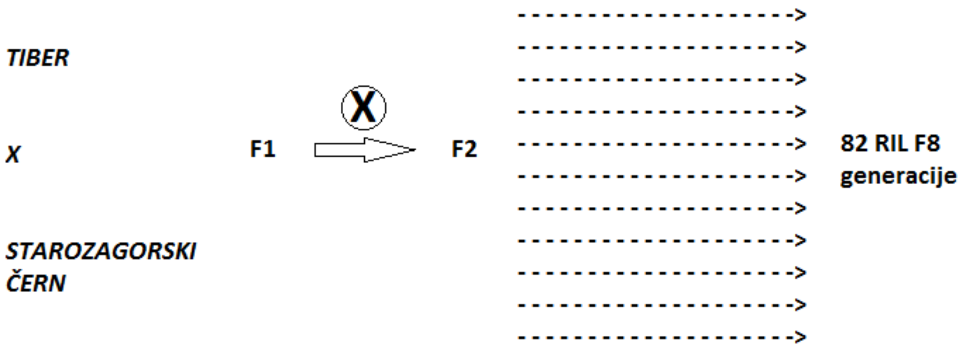


Trait	Type	Name	Location	Ideotype
SE	Gene	F	0	G
SE	QTL	sex1.1	1.5	G
SE	QTL	fnl1.1	1.5	G
LD	QTL	ldr1.1	3.6	H
EAR	QTL	ear1.2	3.6	H
---	Codom SSR	CSWCT28	5.0	Het
---	Dom SNP	L18-SNP-H19	7.4	G

Example: Drought tolerance in common bean (*Phaseolus vulgaris* L.)

The aims of this study were to characterize quantitative traits as a differential response mechanism to water-limitation in two bean cultivars contrasting in their water stress tolerance, and to analyze 82 recombinant inbreed lines (RILs) for polymorphisms with SSR and AFLP markers to construct genetic linkage map of common bean for further QTL mapping.

At the stage of flowering, plants were stressed by withholding irrigation. Morphological and physiological parameters that discern the two parental lines, e.g. seed and flower color, days to flowering, seed yield, one seed mass, water potential and photosynthetic fluorescence were scored at different stage of drought.


TIBER

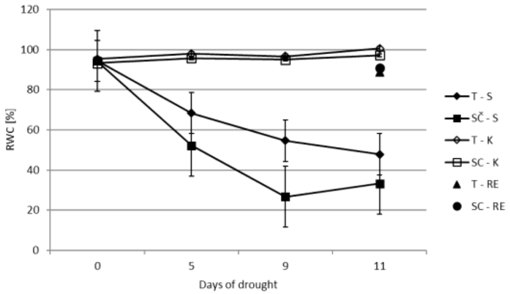
X

STAROZAGORSKI ČERN

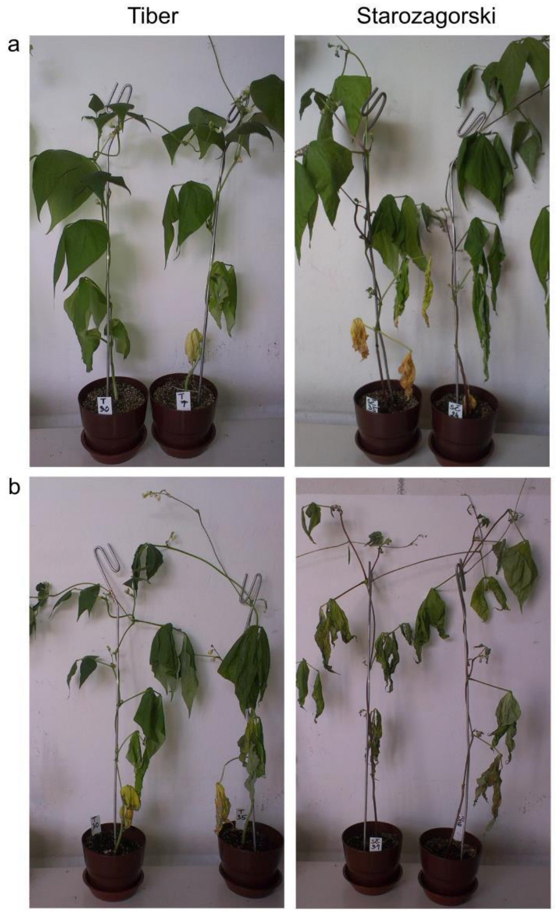
F1 ⓧ **F2**

82 RIL F8 generacije

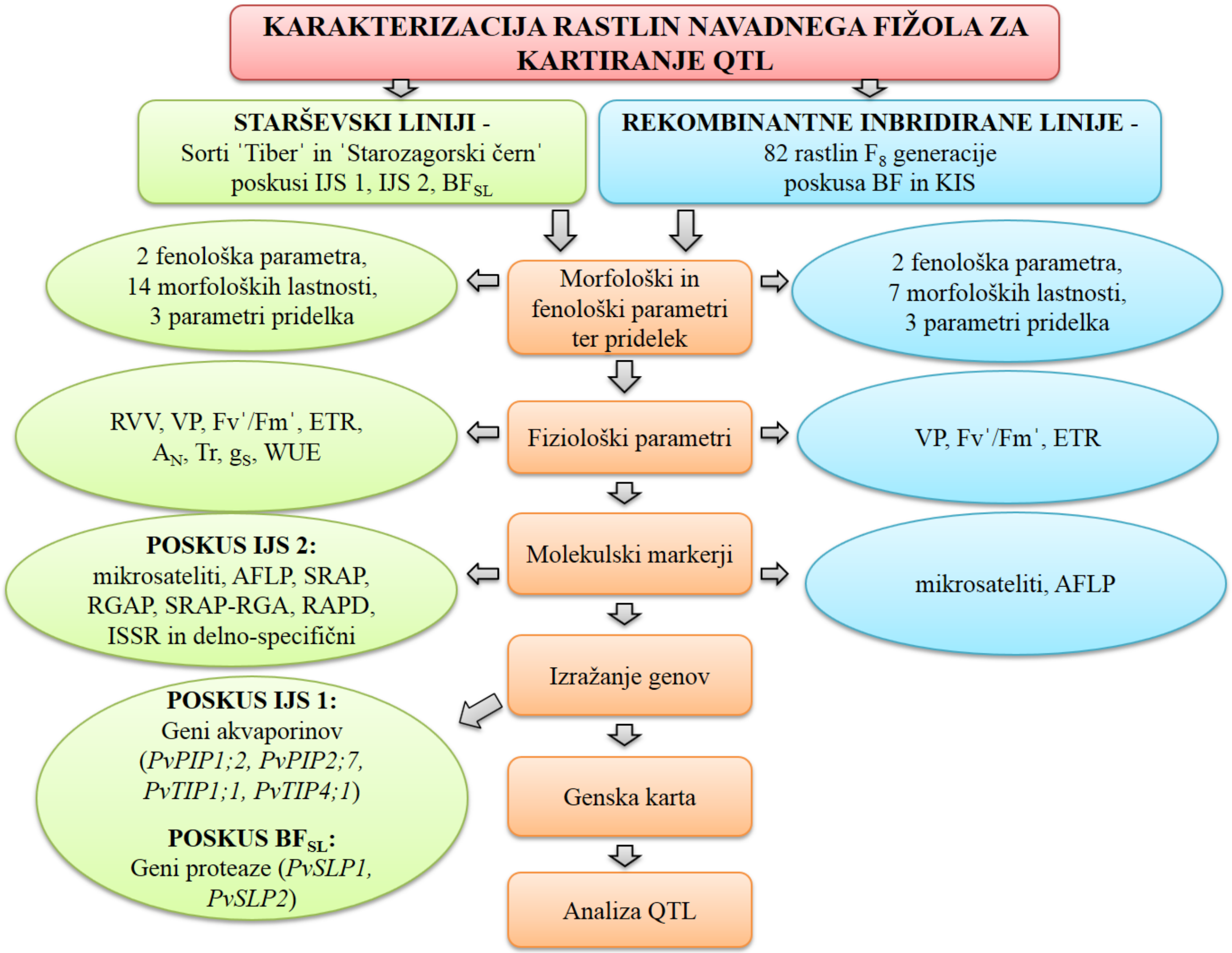
The process of obtaining the population of 82 recombinant inbreed lines of F8-generation for mapping of quantitative trait loci.



Relative water content (%) of leaves from Tiber and Starozagorski variety at different stages of drought stress



Response of common bean cultivars ˈTiberˈ and ˈStarozagorski černˈ at drought 1 (a) and drought 2 (b).



KARAKTERIZACIJA RASTLIN NAVADNEGA FIŽOLA ZA KARTIRANJE QTL

STARŠEVSKI LINIJI -
Sorti 'Tiber' in 'Starozagorski čern'
poskusi IJS 1, IJS 2, BF$_{SL}$

REKOMBINANTNE INBRIDIRANE LINIJE -
82 rastlin F_8 generacije
poskusa BF in KIS

2 fenološka parametra,
14 morfoloških lastnosti,
3 parametri pridelka

Morfološki in fenološki parametri ter pridelek

2 fenološka parametra,
7 morfoloških lastnosti,
3 parametri pridelka

RVV, VP, Fv'/Fm', ETR,
A_N, Tr, g_S, WUE

Fiziološki parametri

VP, Fv'/Fm', ETR

POSKUS IJS 2:
mikrosateliti, AFLP, SRAP,
RGAP, SRAP-RGA, RAPD,
ISSR in delno-specifični

Molekulski markerji

mikrosateliti, AFLP

POSKUS IJS 1:
Geni akvaporinov
(*PvPIP1;2, PvPIP2;7,*
PvTIP1;1, PvTIP4;1)

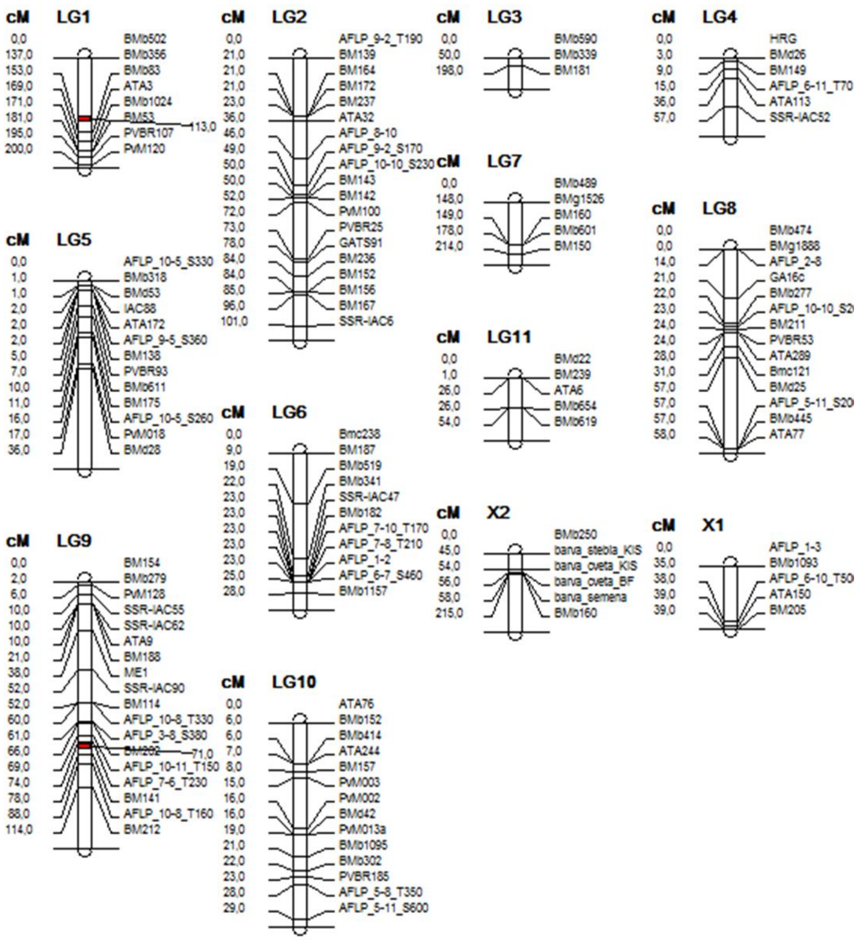
POSKUS BF$_{SL}$:
Geni proteaze (*PvSLP1,*
PvSLP2)

Izražanje genov

Genska karta

Analiza QTL

Scheme of characterization of QTL mapping population



Genetic map of common bean

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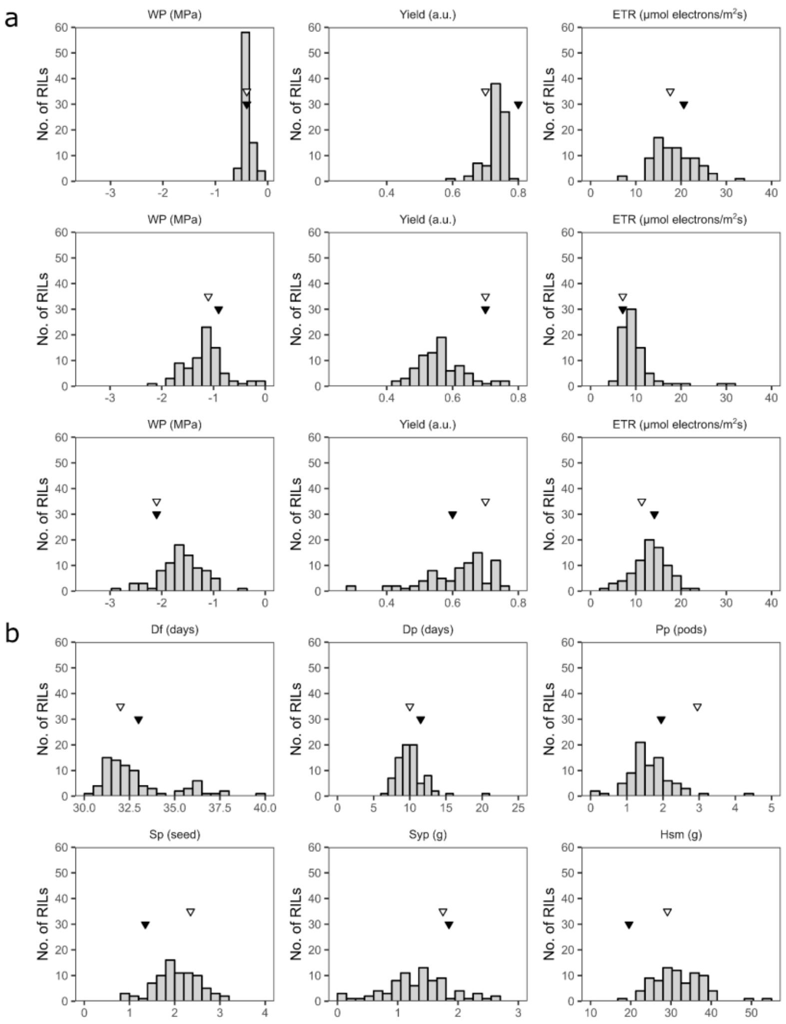

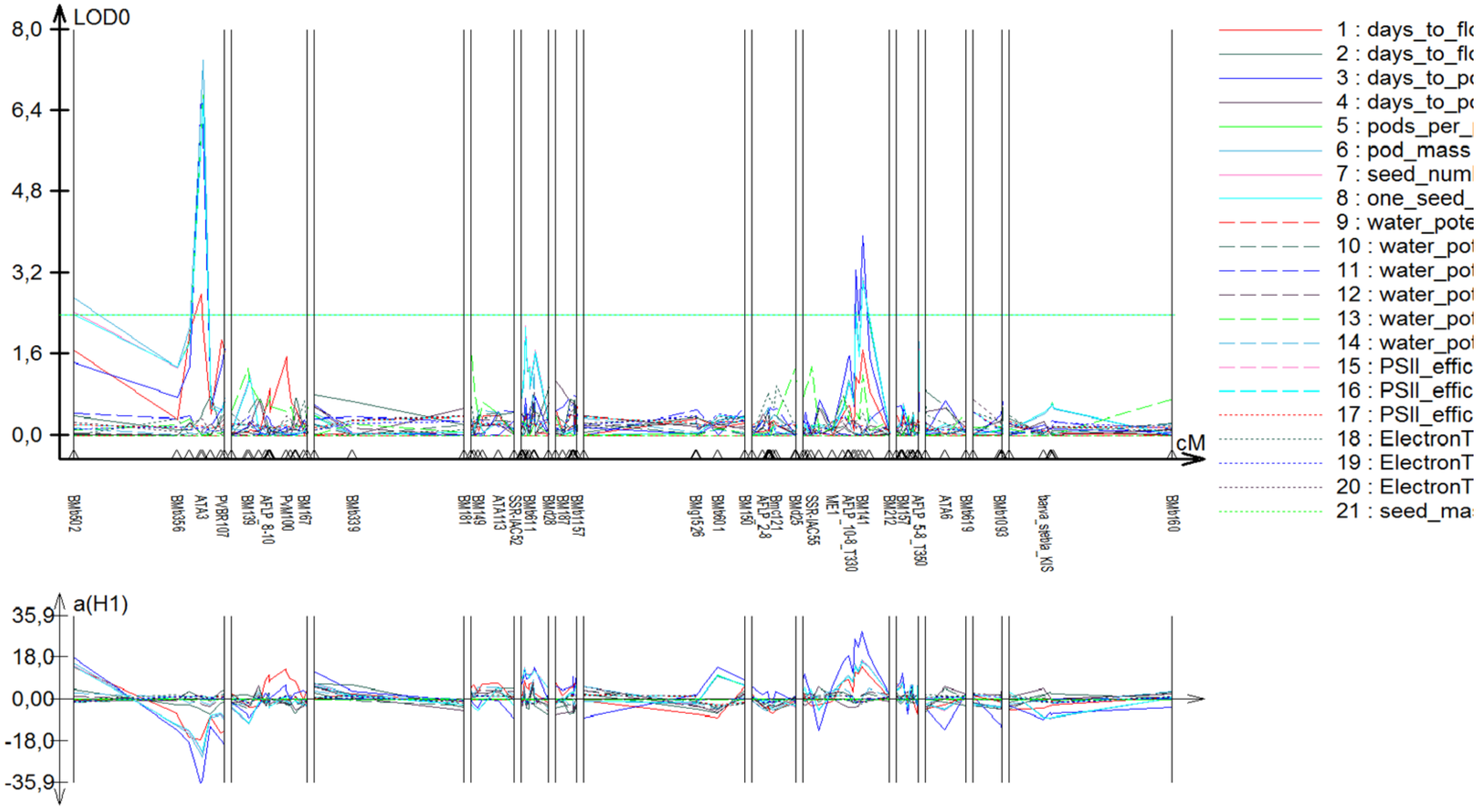

QTL map of 21 quantitative traits linked with genetic markers arranged into 11 LG

Fast Quiz

- ☐ How can new breeding populations be used for organic breeding purposes?
- ☐ Why is it important to maintain genetic diversity?
- ☐ Which precondition is important for QTL mapping?

Send your answers to: anamarija.coric@ips-konzalting.hr

neuoral@doctor.upv.es, adrodbur@upvnet.upv.es

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Take Home Messages

- ✓ Traditional breeding has significant limitations for complex agronomic traits
- ✓ New breeding populations exploit natural diversity in existent crops
- ✓ Multi-parent populations can be used to develop more tolerant varieties for organic and low input agriculture
- ✓ Obtaining new genetic information is key for a more sustainable development of new cultivars

Proposed Homework

☐ Propose in short procedure for QTL mapping of your favorite crop and trait.

Send your answers to: anamarija.coric@ips-konzalting.hr

neuoral@doctor.upv.es, adrodbur@upvnet.upv.es

Additional Resources

Ceccarelli S, Grando S (2020). Evolutionary plant breeding as a response to the complexity of climate change. iScience 23: 101815. doi: 10.1016/j.isci.2020.101815

Döring TF, Annicchiarico P, Clarke S, Haigh Z, Jones HE, Pearce H, Snape J, Zhang J, Wolfe MS (2016). Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. Field Crops Research 183: 235-245. doi: 10.1016/j.fcr.2015.08.009

Suneson CA (1956). An evolutionary plant breeding method. Agronomy Journal 48: 188-191. doi: 10.2134/agronj1956.00021962004800040012x

LiveSeeding

SEMENTES VIVAS

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ARCOIRIS

bingenheimer saatgut

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