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Sant'Anna
School of Advanced Studies – Pisa

The Multi-parent Advanced Generation Inter-Cross (MAGIC) maize population



Why MPPs are better than biparental RILs

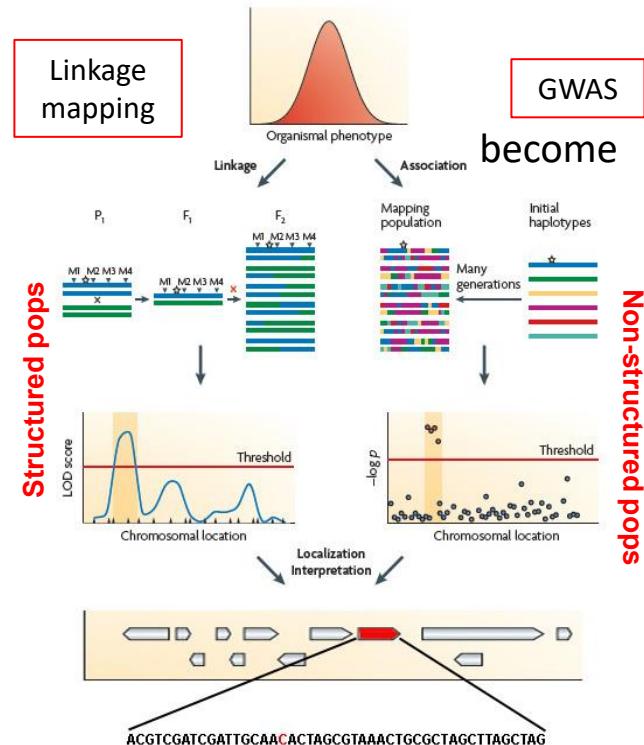
High-throughput DNA sequencing of individuals is becoming routine, but **linking complex phenotypes to their molecular basis remains a challenge**:

- **Marker density** ceases to be a limiting factor
- **Genetic diversity and recombination density** the limiting factor



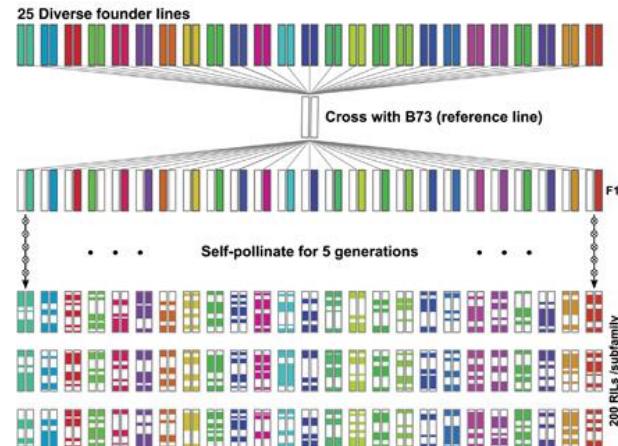
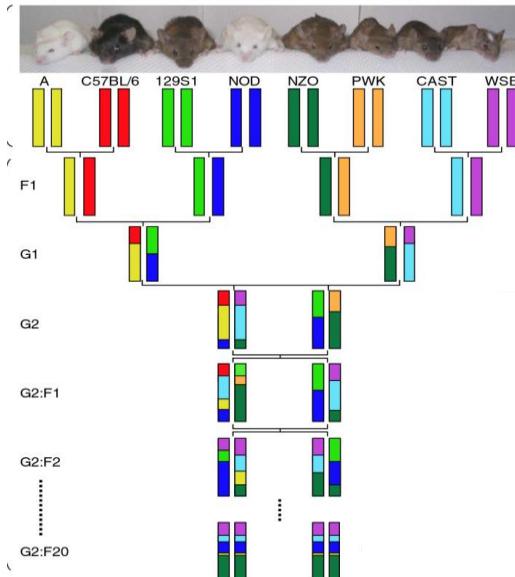
Multi-parent cross designs bridge GWAS and haplotype mapping, and increase QTL detection and characterization by:

- Incorporating **greater genetic diversity**
- Increasing the **number of crossing generations**



Different types of MPPs

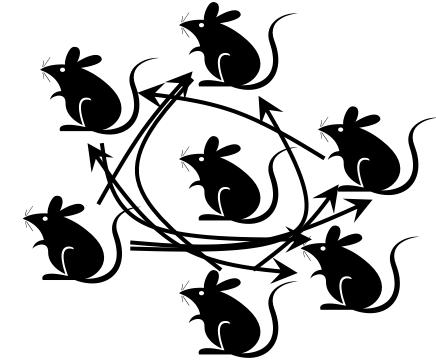
Multi-parental populations achieve **more diversity** and **more recombination events** than biparental populations with different designs



- Collaborative Cross (CC)
- Multiparent Advanced Generation InterCross (MAGIC)

Nested Association Mapping (NAM)

- Diversity Outbred (DO)
- Heterogenous Stock (HS)

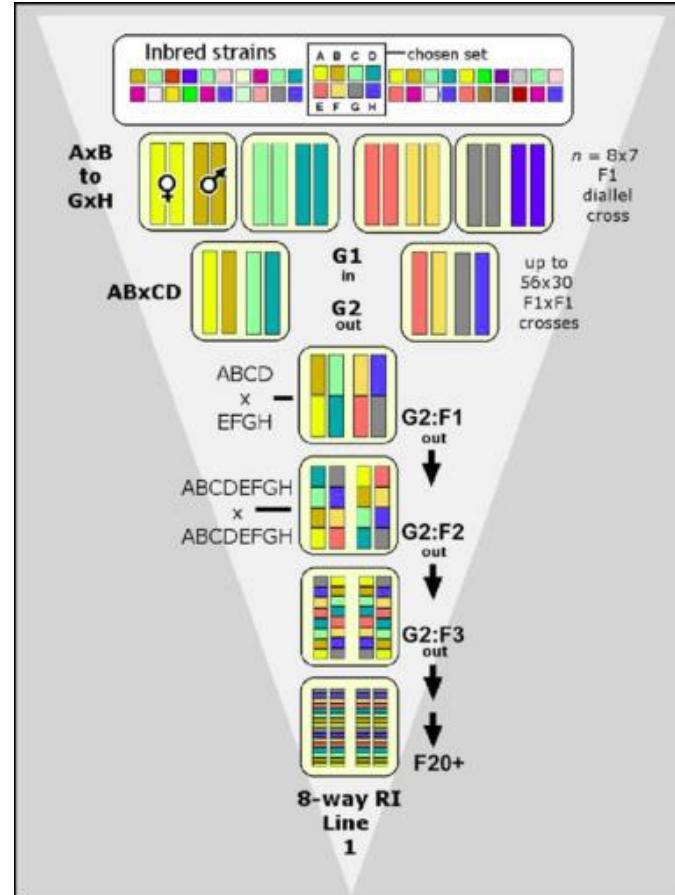


CC like, or MAGIC (Mulitparental Advanced Generation InterCross)

- A set of diverse founders
- Multiple intercrossing generations
- Inbreeding to produce **Recombinant Inbred Lines (RILs)**

Results:

- **Closed**, pooled population of RILs deriving from the founders
- **Small numbers** provide many recombination events and much diversity
- **Eternal**, immutable

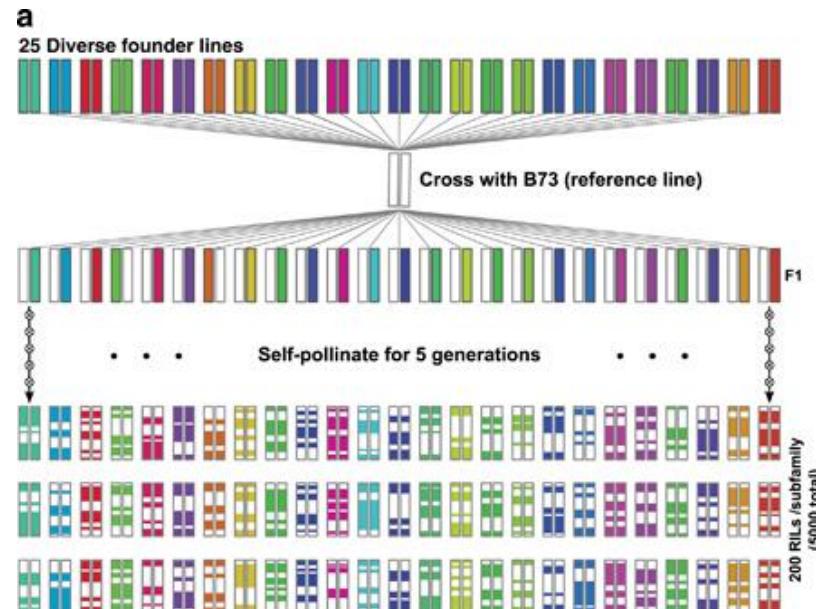


NAM (Nested Association Mapping population)

- One recurrent founder, n diverse founders
- One intercross generation
- Inbreeding to produce **Recombinant Inbred Lines (RILs)**

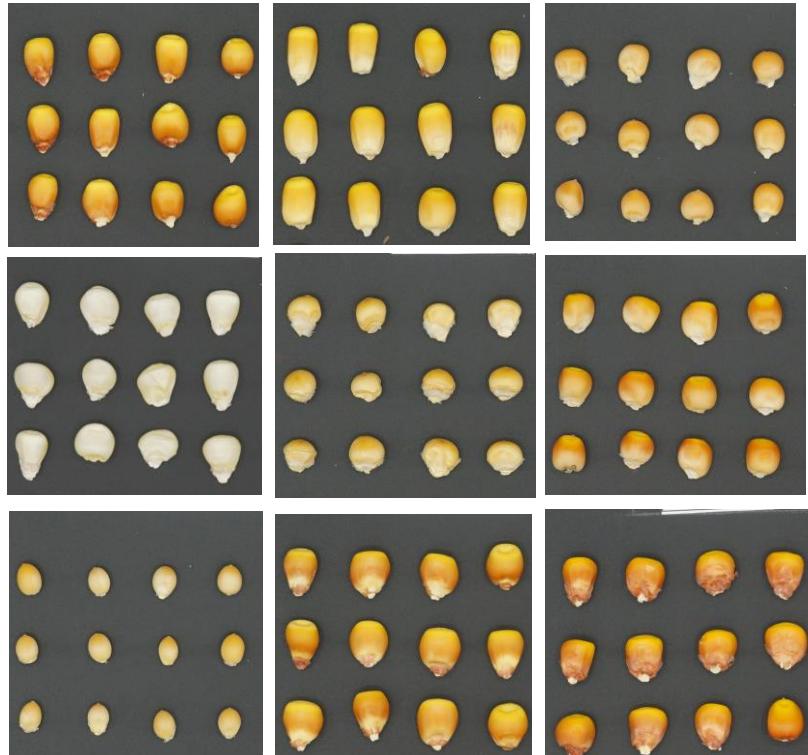
Result:

- **Open** collection of biparental families
- **Higher numbers** provide many recombination events and much diversity
- **Eternal**, immutable

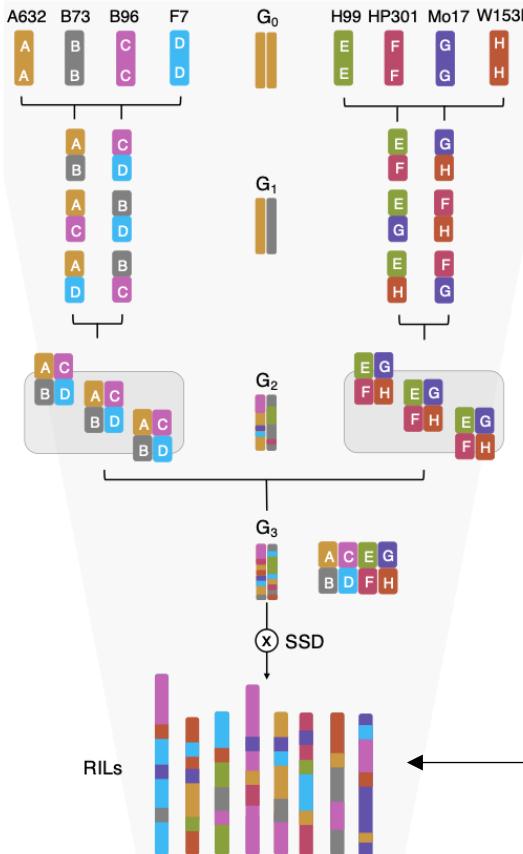


Multi-parent populations in maize

A framework to combine **genomics**,
genetics, and **diversity** in a multi-purpose
tool for mapping and breeding



The Multi-parent Advanced Generation Inter-Cross (MAGIC) maize population



Multi-parent population developed following a MAGIC crossing scheme inter-crossing **8 diverse lines** (founders)



Fig. Founders lines of MAGIC population

After 6 generations: 1,600 Recombinant Inbred Line (RIL)

Each RIL is a **mosaic of the founders' genomes**

→ **High genetic diversity, high recombination density**

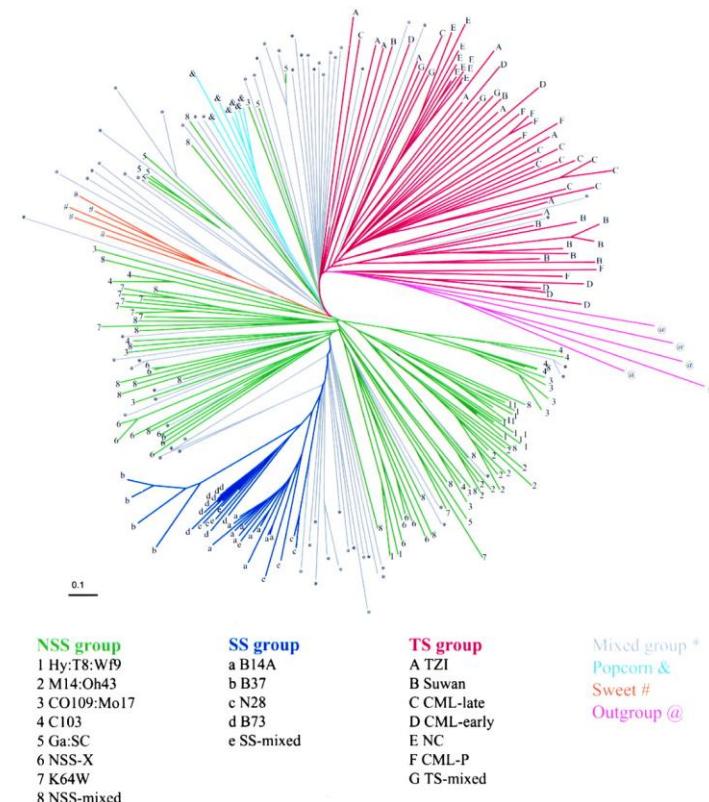
Fig. Breeding funnel of the MAGIC maize population (Dell'Acqua et al., 2015)

Choice of parental lines

- Selection of **8 parental lines** maximizing variation (or incorporating useful traits)
- Effort to maximize **crossability** thanks to uniform phenology

Two main stages:

- 1) **MIXING STAGE:** progenitor lines are intercrossed to produce a foundation population
- 2) **INBREEDING STAGE:** randomly chosen individuals of the foundation population are inbred (by selfing or sibling)

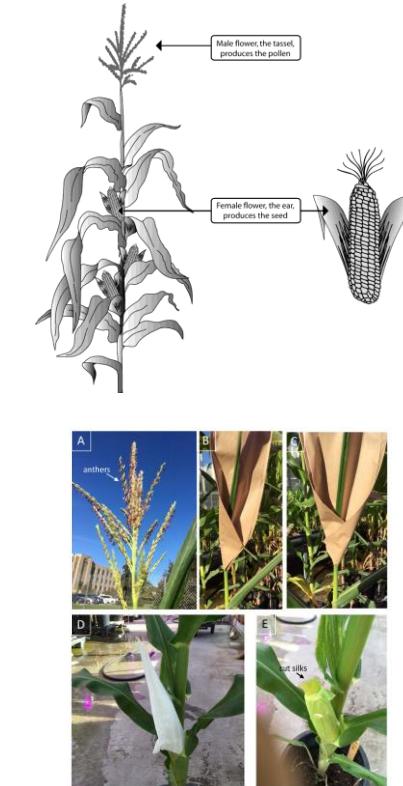


1) Mixing stage

- Production of **2-way hybrids** from every distinct pair of founders, generating a diallel cross
- Ideally, each cross could be the start of a bi-parental population

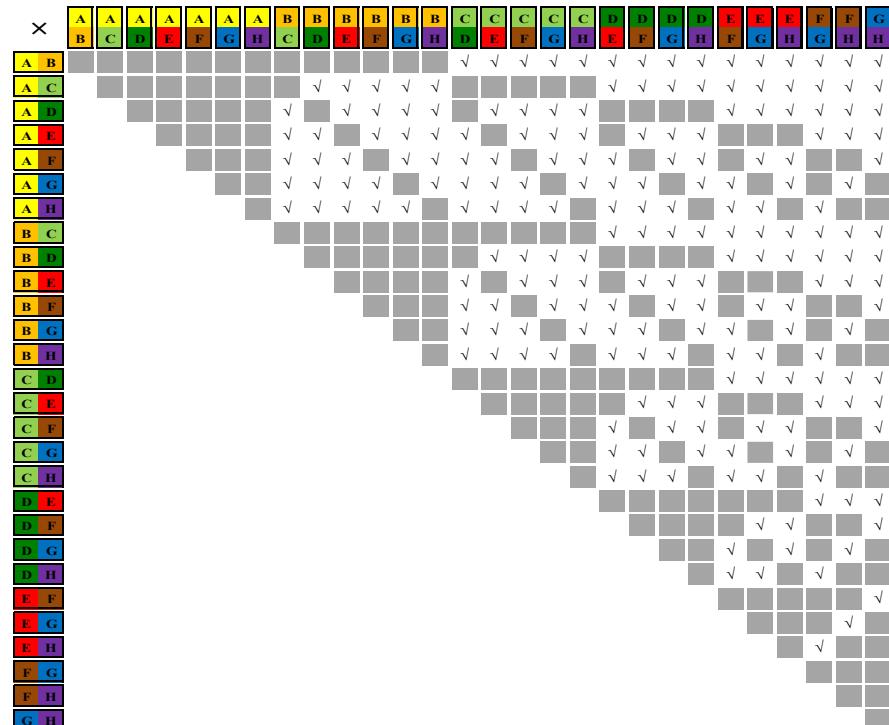
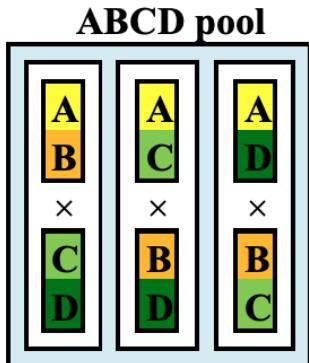
Inbred line	Group	Subgroup	×	A	B	C	D	E	F	G	H	
A632	SS	B14A	►	A		AB	AC	AD	AE	AF	AG	AH
B73	SS	B73	►	B		BC	BD	BE	BF	BG	BH	
B96	TS	Sewan	►	C		CD	CE	CF	CG	CH		
F7	Mixed	-	►	D		DE	DF	DG	DH			
H99	NSS	NSS-mixed	►	E		EF	EG	EH				
HP301	Popcorn	-	►	F		FG	FH					
Mo17	NSS	CO109:Mo17	►	G		GH						
W153R	NSS	NSS-mixed	►	H								

Source: Liu et al. 2003



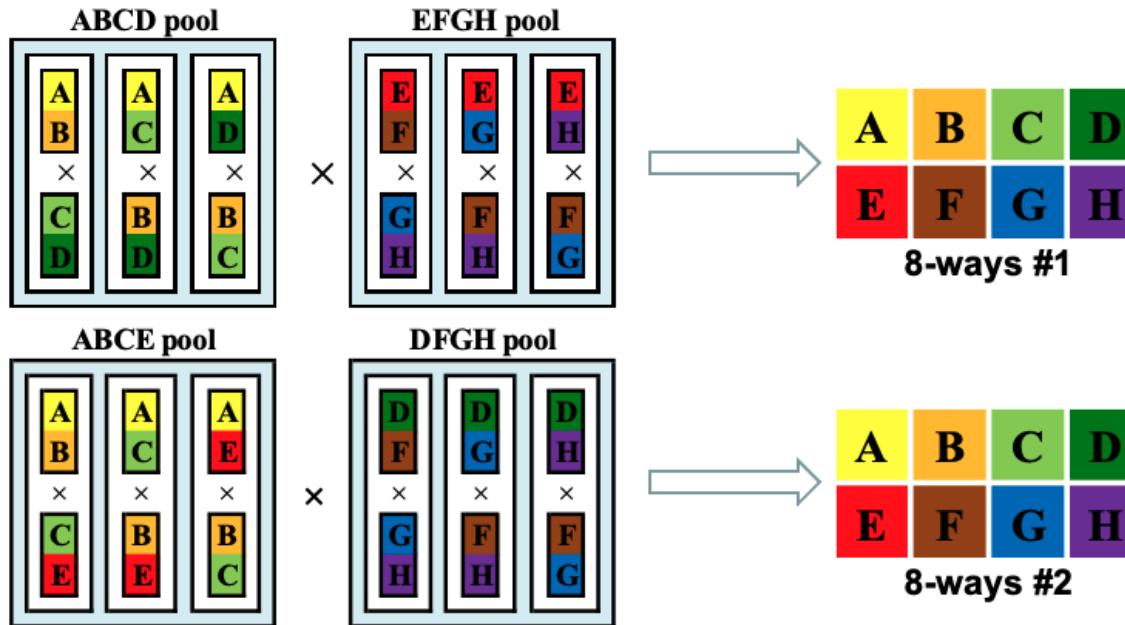
1) Mixing stage

- **4-way hybrids** are then produced
- To reduce complexity, only 2-way hybrids without founders in common were crossed
- **Triplets of 4-way crosses** containing the same parental alleles in different CIS combinations were pooled



1) Mixing stage

- Each **4-way pool** is crossed with its complementary pool to establish **8-way pools**



2) Inbreeding stage

		"genomic" He
Summer	2007 (IT)	F ₁ (100%)
Summer	2008 (IT)	F ₂ (50%)
Summer	2009 (IT)	F ₃ (25%)
Winter	2010 (MM)	F ₄ (12.5%)
Summer	2010 (IT)	F ₅ (6.25%)
Winter	2011 (MM)	F ₆ (3.125%)
Summer	2011 (IT)	F ₆ Sib (3.125%)
Summer	2012 (IT)	PHENOTYPING & GENOTYPING

35 8-ways hybrids pools
(5,000 individuals)

SSD



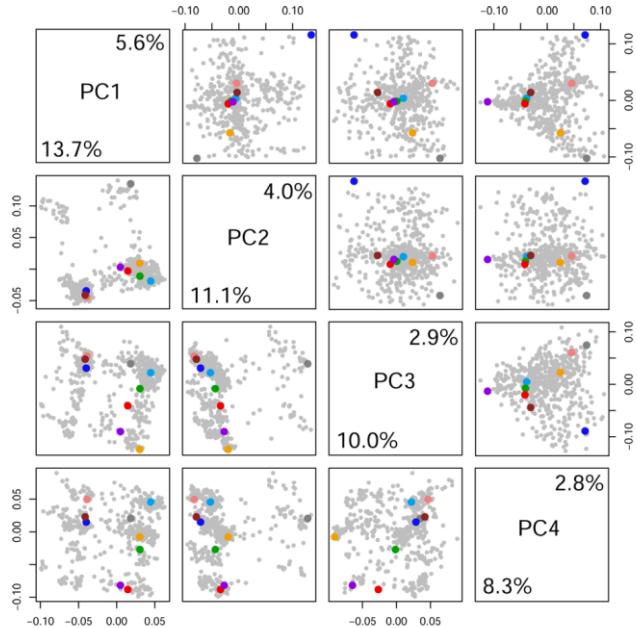
male sterility
female sterility
barreness
human error

1,636 RILs-8W F₆
balanced in 35 families

529 RILs-8W F₆

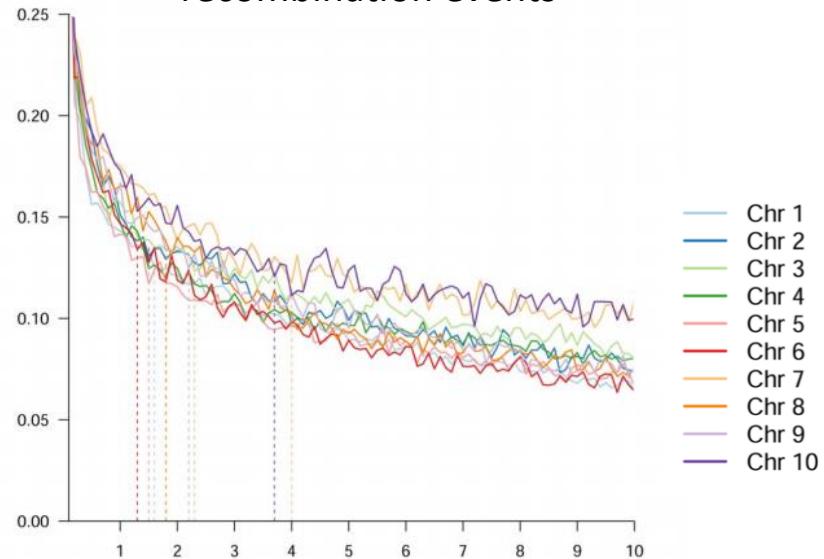
Characteristics of the MAGIC population

- The MAGIC population is **highly diverse**
- Very **low genomic structure**, higher in centromeric regions



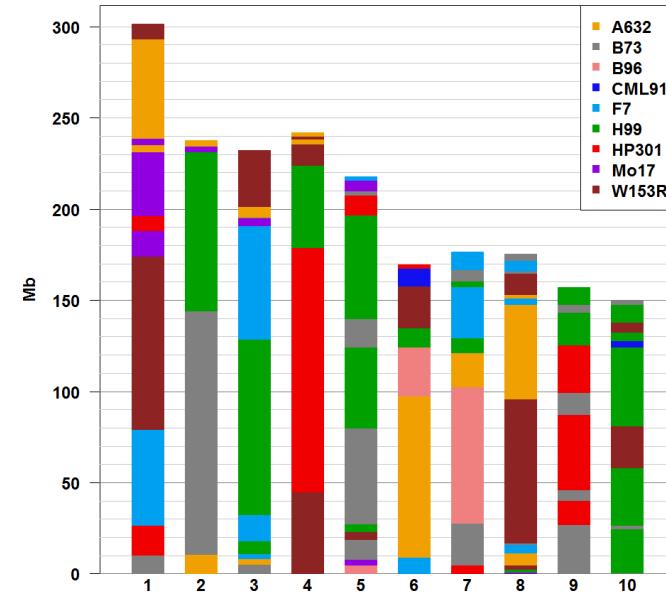
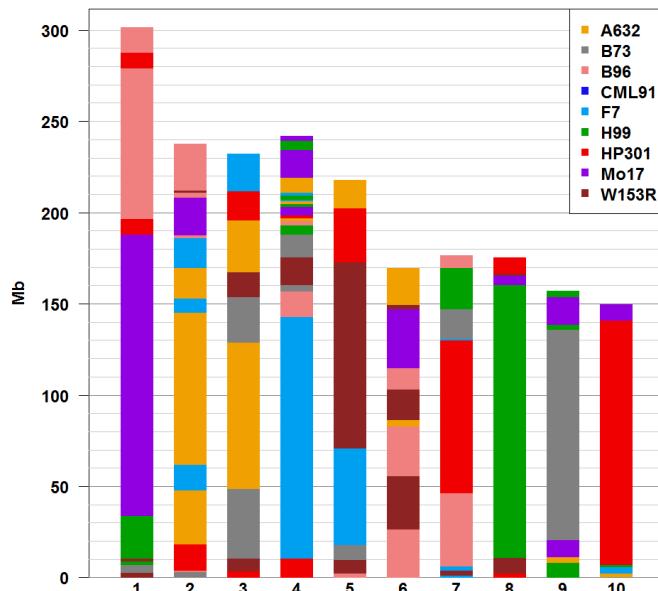
Telomeric regions don't show structure in relation to founders lines

- LD decays fast, indicating high density of recombination events



RIL genomes

RIL genomes are reconstructed via **hidden Markov model (HMM)** and founder haplotypes are assigned to each line accordingly



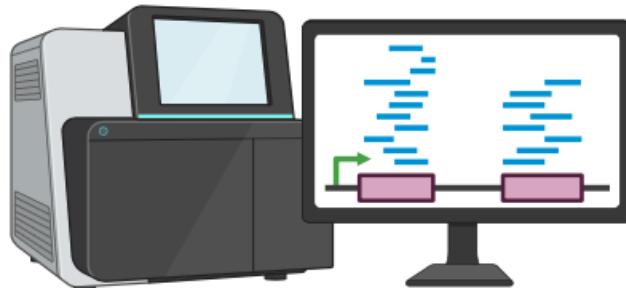
Genomic data available

DNA



Whole genome sequencing produced for:

A632, F7, H99, HP301, and W153R (Mo17 and B73 were already available)



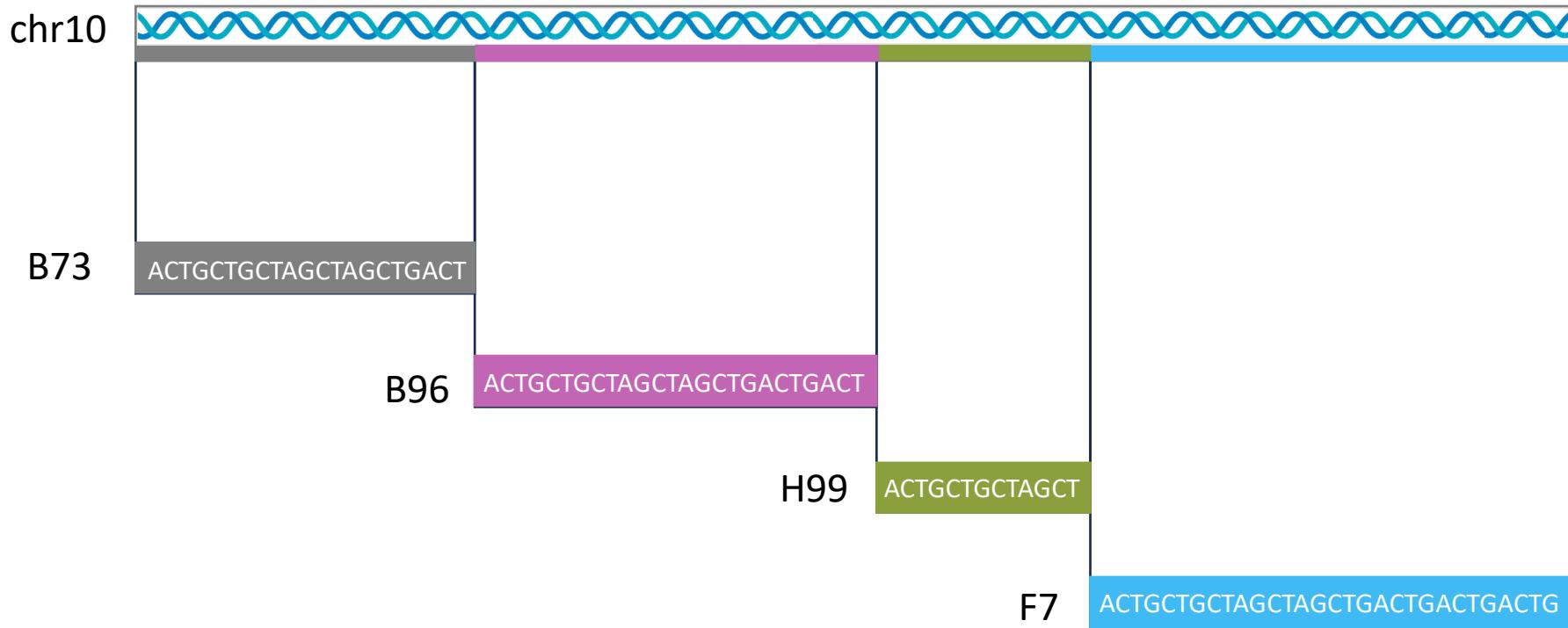
RNA



Transcriptomics data at the fourth leaf stage were produced for:

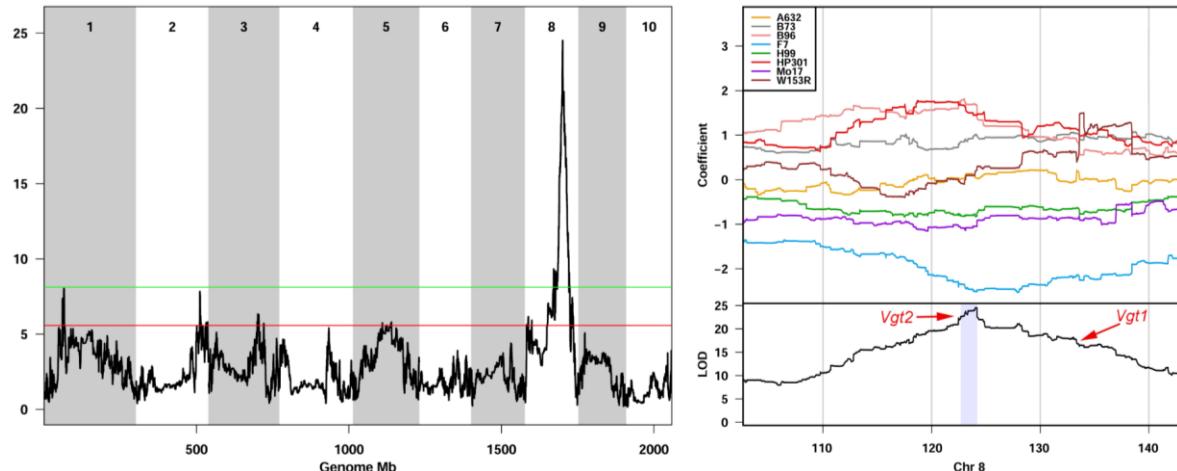
A632, B73, F7, H99, HP301, Mo17, W153R

We can project this information on RIL reconstructed genomes



MAGIC maize as platform for QTL mapping

- QTL mapping can then be performed testing co-inheritance of **parental haplotypes** and **measured traits**
- Once a QTL is identified, the local effect of founder haplotypes can be estimated accordingly
- Data generated on parental lines (DNA seq, RNA seq, metabolomics, proteomics, ...) can be **projected on parental haplotypes**



Current work on the MAGIC maize

- Broad, untargeted phenotypic diversity
- Oxford Nanopore Technology pangenome of parental lines
- Reconstruction of pan-transcriptome
- Integration of structural variation in forward genetics



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Application of the MAGIC Maize Population to Uncover the Genetic Determinants of Drought Tolerance



Maize and drought



Global crop: staple food, feed and bio-based products



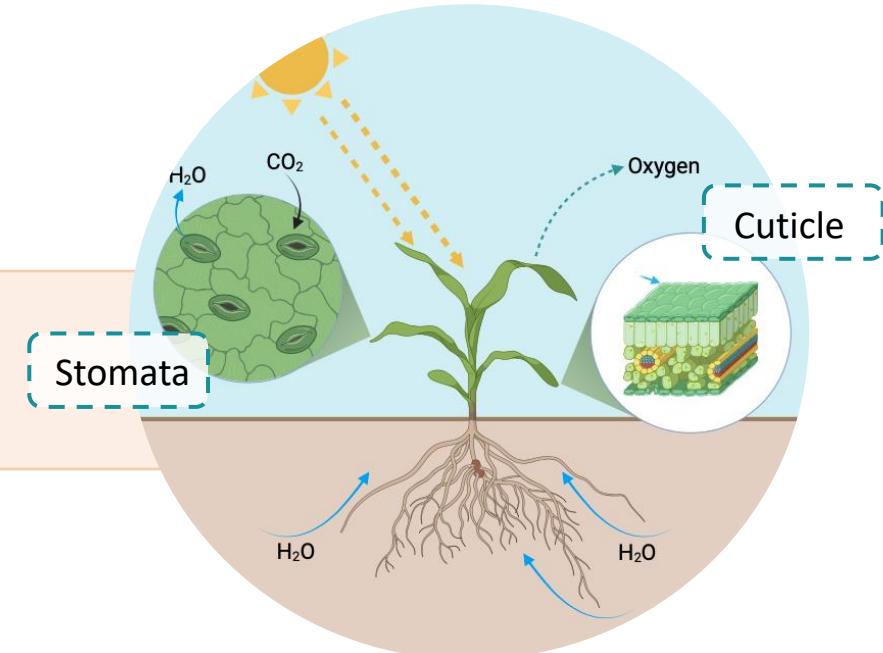
Genetic model: study of complex traits



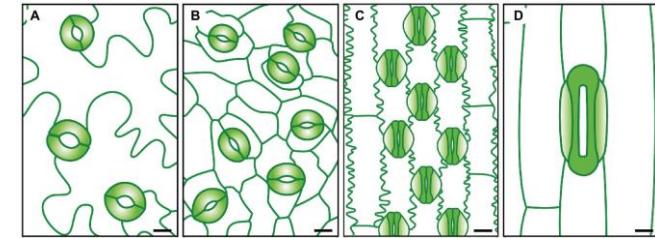
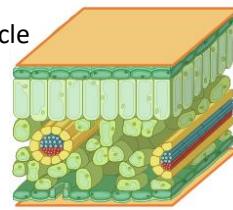
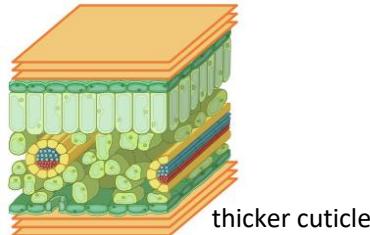
Drought impact: up to 15% global yield loss with effects on several agronomic traits

How can maize cope
with this challenging
environment?

Physiological and
structural components
traits of adaptation



Cuticle and stomata



Bertolino LT et al. 2019

Cuticle: multi-layered hydrophobic structure **limiting** water loss and **protecting** against dehydration

Cutin and cuticular waxes: fatty acids

Stomata: tiny pores on leaf surface that **regulate** gas exchange and **minimise** water loss

Stomatal Density: n° of stomata / unit area (stomata/mm²)

Affecting leaf permeability: thicker cuticle and decreased stomatal density can **reduce the leaf permeability** and the water loss



Working hypothesis: **natural allelic variation** in maize for cuticular and stomatal traits can be used **to identify** **potential candidate genes** that are crucial for developing **maize varieties more adapted to drought**

The Multi-parent Advanced Generation Inter-Cross (MAGIC) maize population

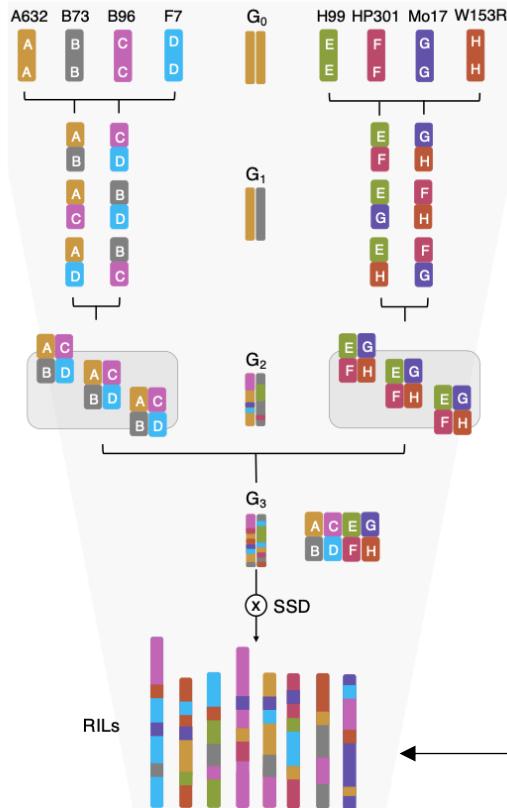


Fig. Breeding funnel of the MAGIC maize population (Dell'Acqua et al., 2015)

Multi-parent population developed following a MAGIC crossing scheme inter-crossing **8 diverse lines** (founders)



Fig. Founders lines of MAGIC population

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The Multi-parent Advanced Generation Inter-Cross (MAGIC) maize population

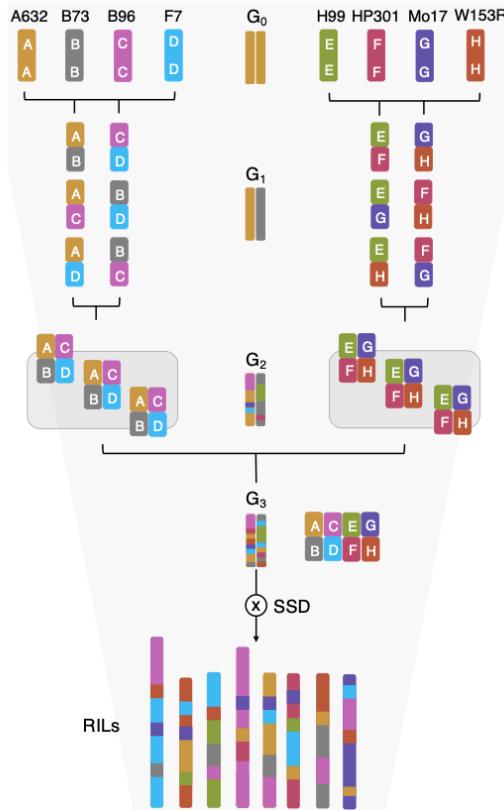


Fig. Breeding funnel of the MAGIC maize population (Dell'Acqua et al., 2015)

Data used in the experiment:

- ✓ 285 RILs
- ✓ 75,000 SNPs markers from sequencing
- ✓ Oxford Nanopore Technology **pangenome** of founders
- ✓ **RNA-seq** in the growth chamber and in the field of founders and RILs



Data further available: 240 more RILs characterised



Fig. Founders lines of MAGIC population



Fig. Seed material of MAGIC population

Experiment in greenhouse October-December 2024

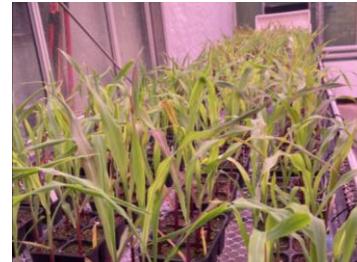
285 RILs + 8 founders = 293 genotypes

Up to 6 replicates for each genotype

Augmented block design: 4 batches with 4 blocks each one

Environmental controlled conditions

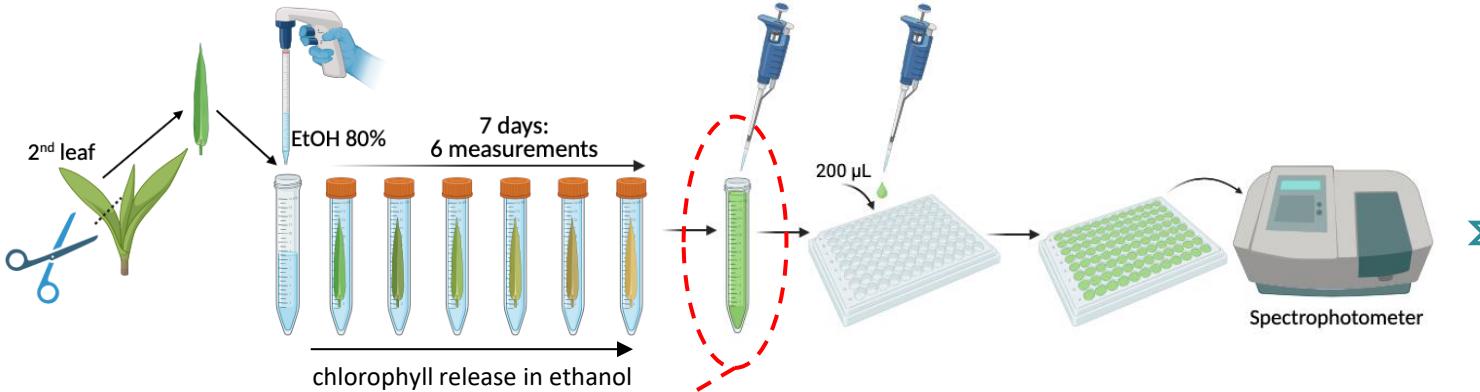
BATCH 1	Block 4	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
	Block 3	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
	Block 2	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	Block 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24



Cuticle-stomata high-throughput phenotyping pipeline

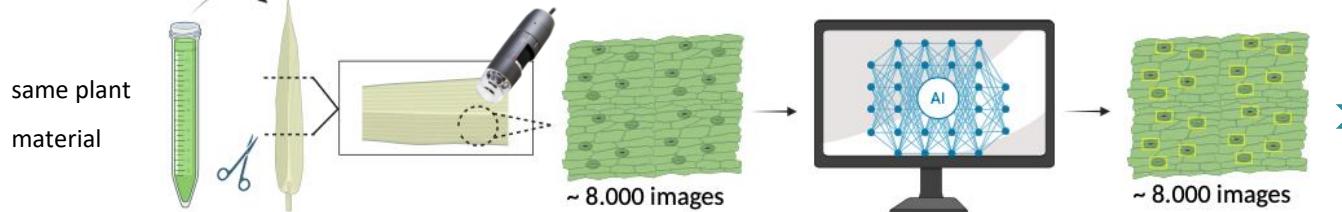
1

Cuticle permeability by chlorophyll leaching assay:



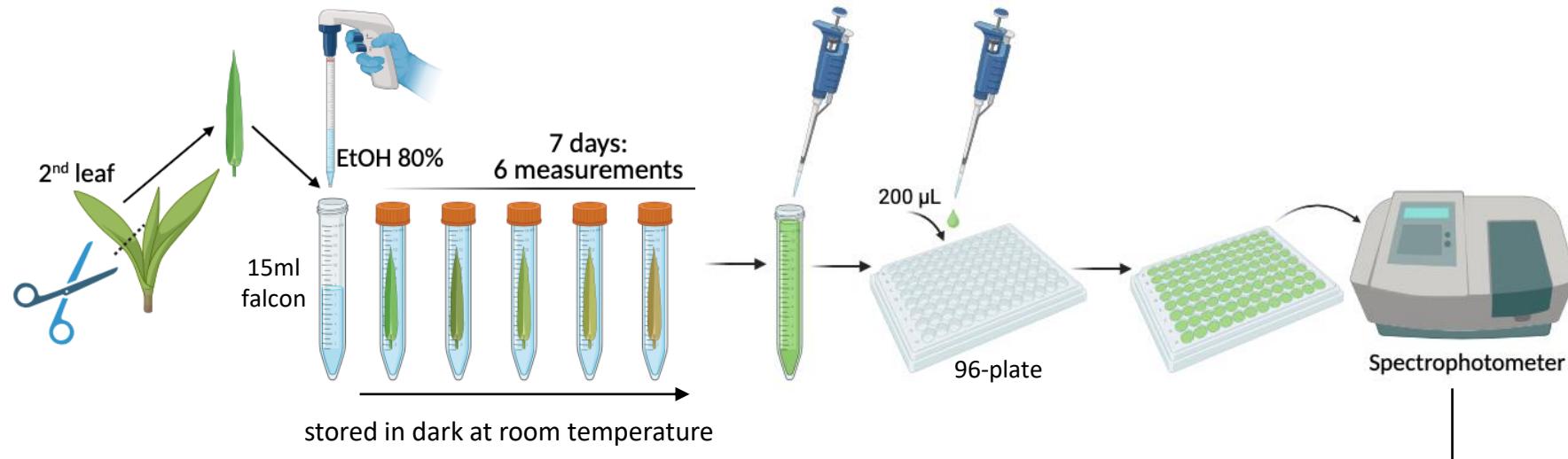
2

Imaging and stomata detection:



stomatal density and other traits

Cuticle permeability by chlorophyll leaching assay:



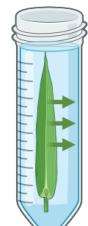
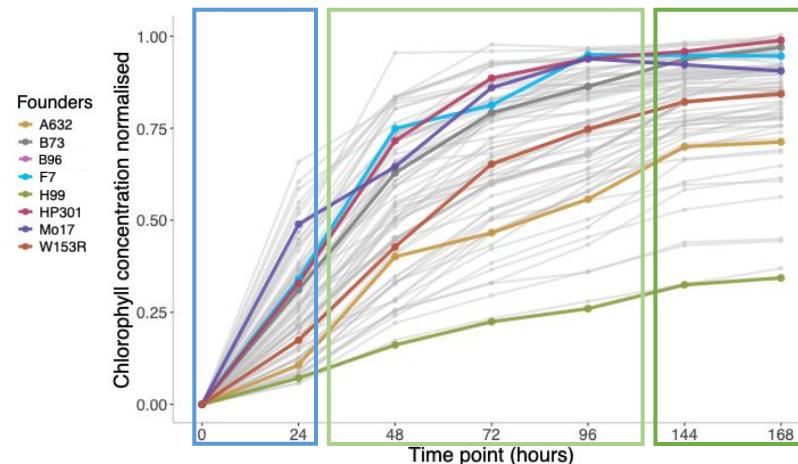
normalised per gram of
fresh weight

$$\text{chlorophyll concentration } (\mu\text{M}) = (7.93 * A_{664}) + (19.53 * A_{647})$$

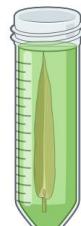
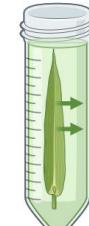
absorbance 664 nm
absorbance 647 nm

Cuticle permeability

Rate of the chlorophyll release:



chlorophyll ethanol

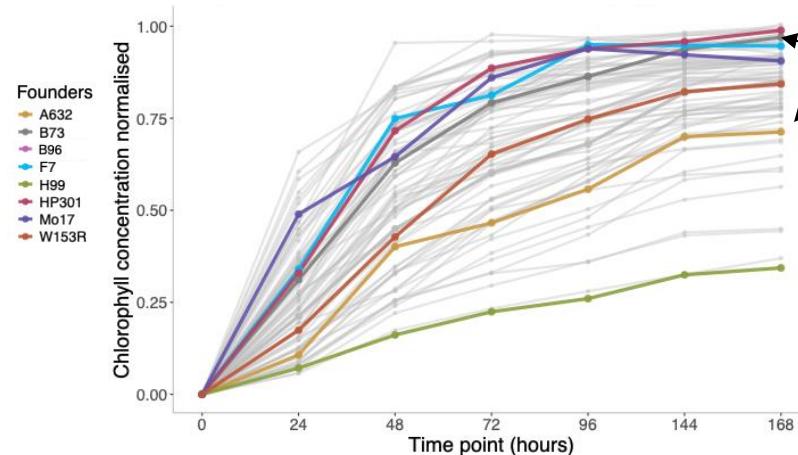


= saturation state (plateau)

Cuticle permeability

Rate of the chlorophyll release:

$$C(t) = C_{\max} \times (1 - e^{-k \times t})$$



C_{max} = maximum chlorophyll concentration released

How much chlorophyll a genotype can release

k = rate constant

How fast a genotype releases chlorophyll



$$\text{Cuticle leaf permeability} = \text{Speed}(t) = \frac{dC(t)}{dt} = C_{\max} \times k \times e^{-k \times t}$$

Speed at different time points → how fast different genotypes release chlorophyll over time

Traits for QTL mapping

Deriving the trait(s) for mapping



Extraction of k and C_{max} for each genotype

Fitting a **non-linear mixed effects model**:

- fixed effects = C_{max} and k
- random effect = genotype

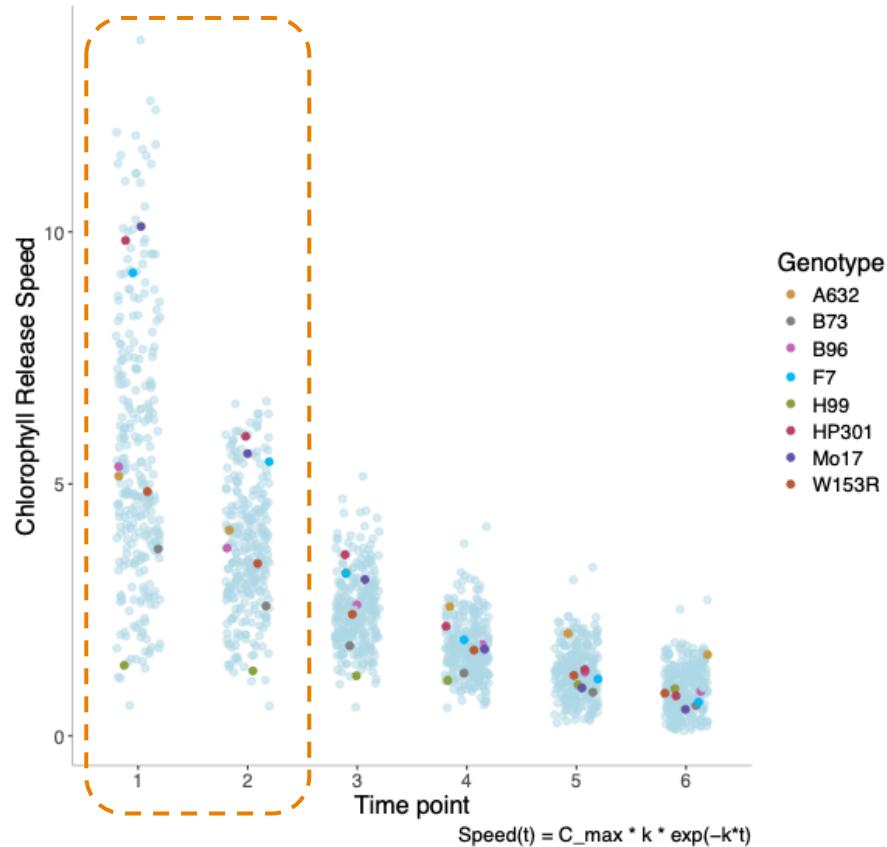


Speed of release of chlorophyll across timepoints $\rightarrow t$ = from 0 to 6

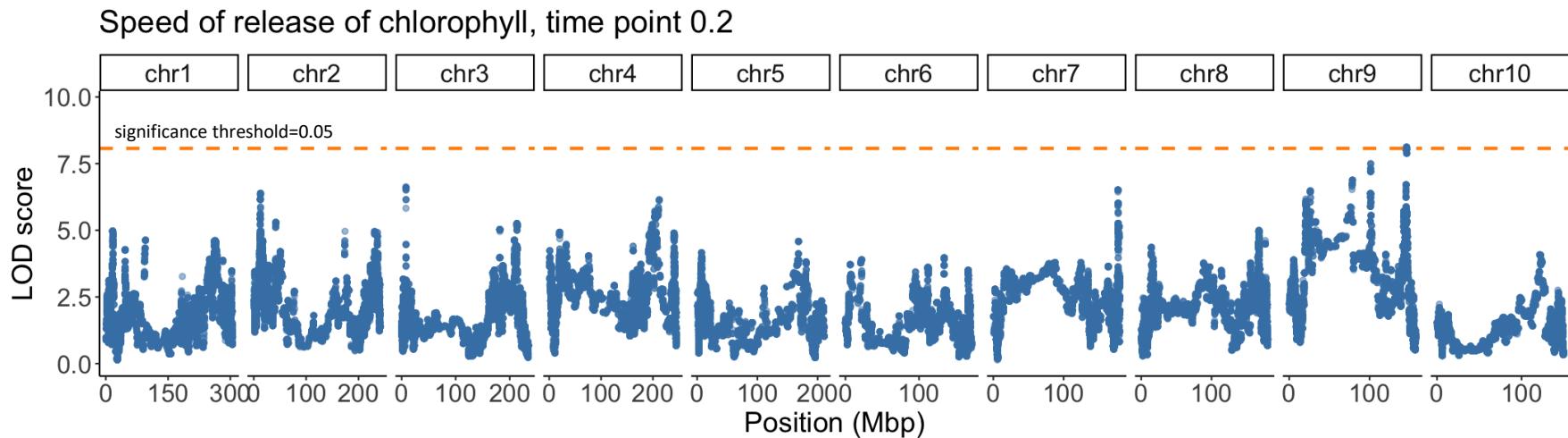
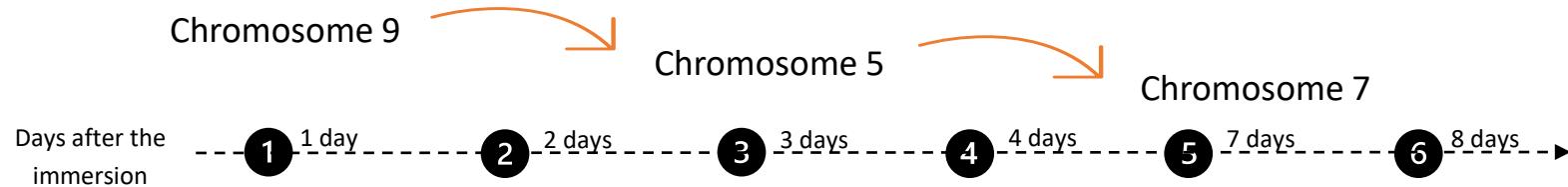
Traits for mapping:

- k
- C_{max}
- Speed of chlorophyll release for 6 timepoints

Distribution of chlorophyll release speed



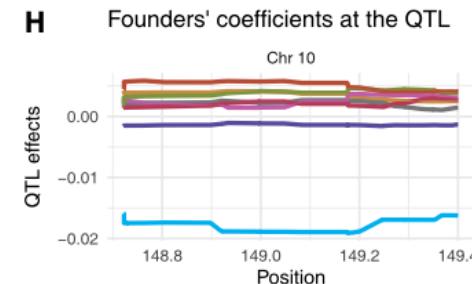
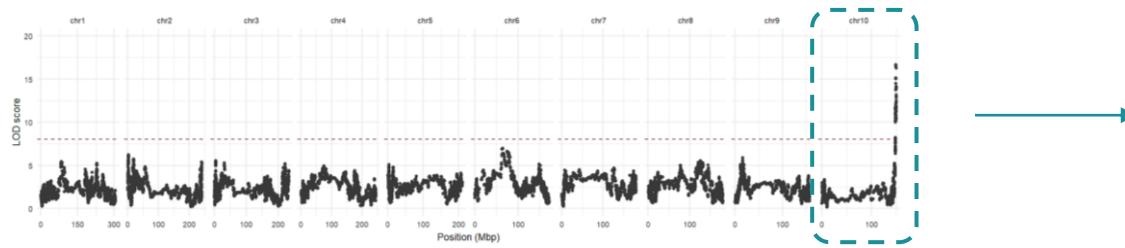
QTL mapping



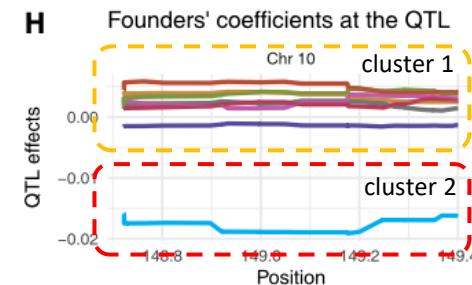
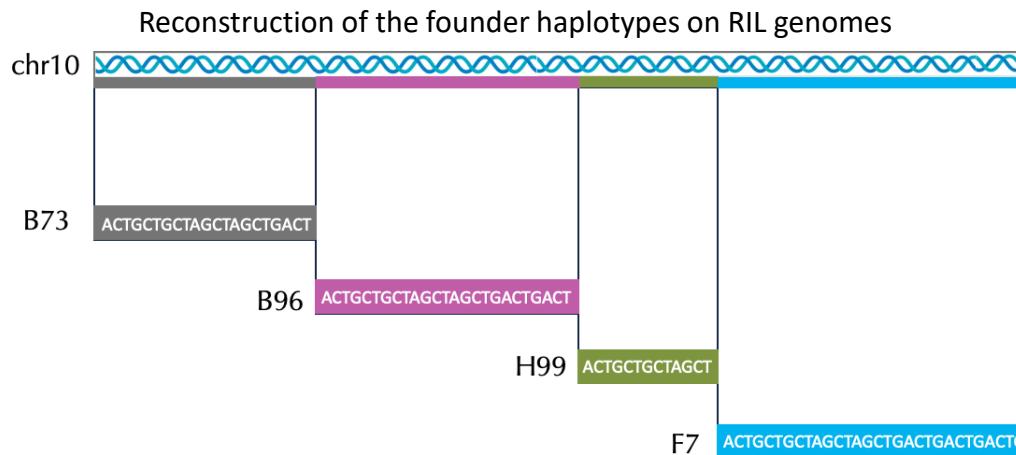
Genotypic data: 75,000 SNPs

Covariate: initial chlorophyll concentration inside the leaf

Identification of candidate genes

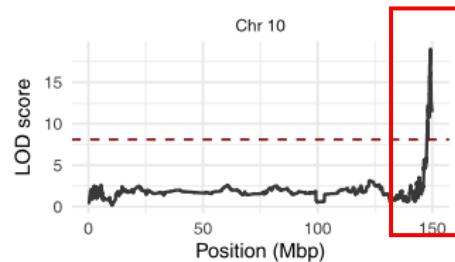


(2) Calculate founder allele effects on QTL

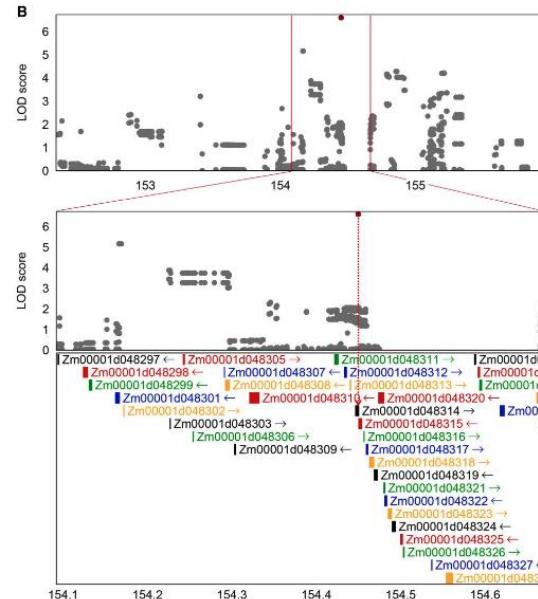


(3) Cluster founders by similarity of the effects (haplotype groups)

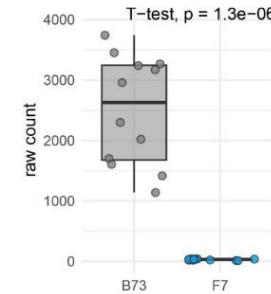
Identification of candidate genes



(4) Extract genes in the QTL interval



(5) Integration of RNA-seq data to test the genes for differential expression matching founder effects



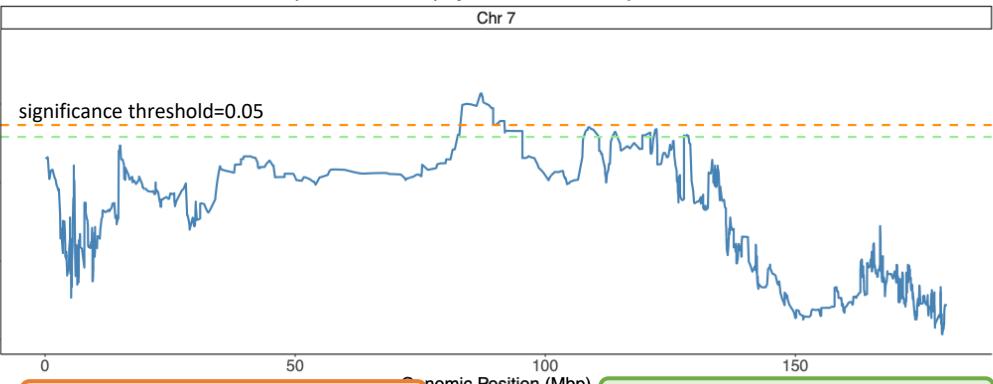
Candidate gene on chr7

1

Speed of chlorophyll release at timepoint 5.8

Chr 7

LOD score



From 135 genes on this QTL to 2 candidates

glossy1 - gl1

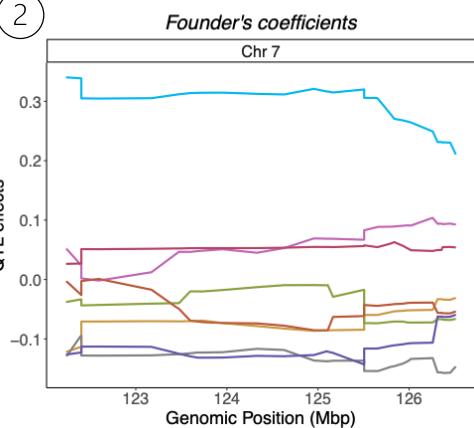


2

Founder's coefficients

Chr 7

QTL effects



3

Differential expression of Zm00001d020557

normalised count

founders

Founder

A632

B73

B96

F7

H99

HP301

Mo17

W153R

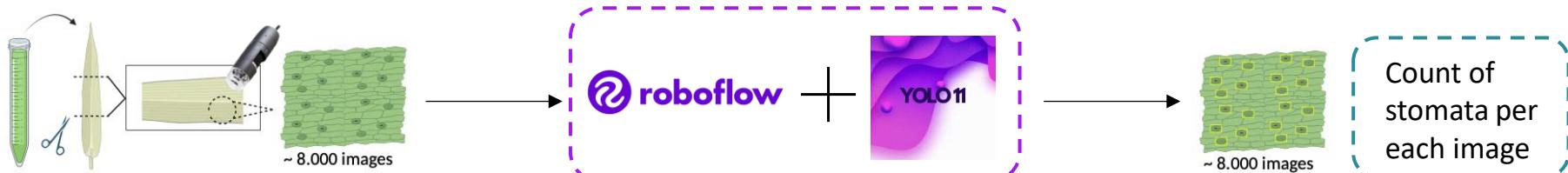
Plant Physiology®

JOURNAL ARTICLE

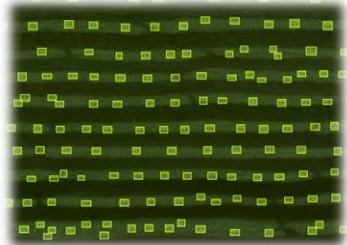
Cloning and Characterization of *GLOSSY1*, a Maize Gene Involved in Cuticle Membrane and Wax Production [Get access >](#)

Monica Sturaro, Hans Hartings, Elmon Schmelzer, Riccardo Velasco, Francesco Salamini, Mario Motto

Stomata imaging and detection with AI



- ✓ 500 images manually annotated
- ✓ Training Yolo11 algorithm on the 500 images
- ✓ Creating workflow for the stomata detection
- ✓ Using the model to count the stomata on each image
- ✓ Creating the dataset with stomata count



Count of
stomata per
each image

YOLO
Running

MARTINA P WOR...

Merge Final Object Detection

DATA

- Upload Data
- Annotate
- Dataset 500
- Versions Train
- Analytics
- Classes & Tags

MODELS

- Models
- Visualize

DEPLOY

- Deployments

Model URL: merge-final-zwtxs/12

Checkpoint: merge-final-zwtxs/2

Dataset Version: Roboflow 3.0 Augmentations ↗

Updated On: 04/09/25, 12:34

Model Type: Roboflow 3.0 Object Detection (Fast)

Metrics ⓘ

Valid Set	External ⓘ
mAP@50 90.2%	Precision 85.3%
	Recall 88.5%

Preview Model

Samples from Test Set



View Test Set ↗

Upload Image or Video File

Drop file here or

Image URL

Paste a link...

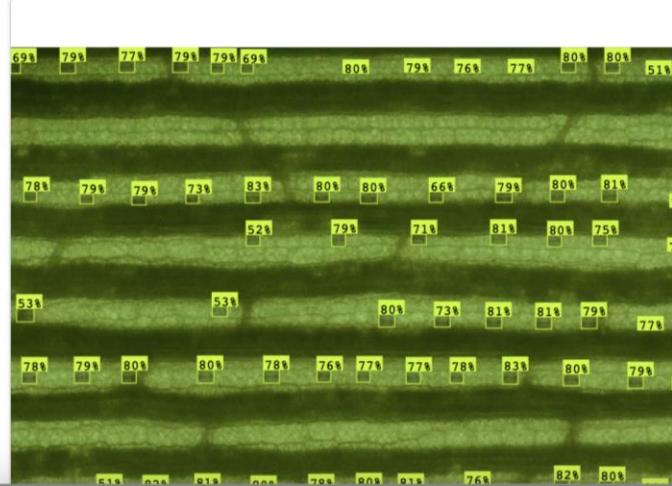
Confidence Threshold: 50%

Overlap Threshold: 50

Opacity Threshold: 75

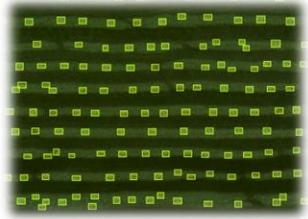
Label Display Mode:

Draw Confidence

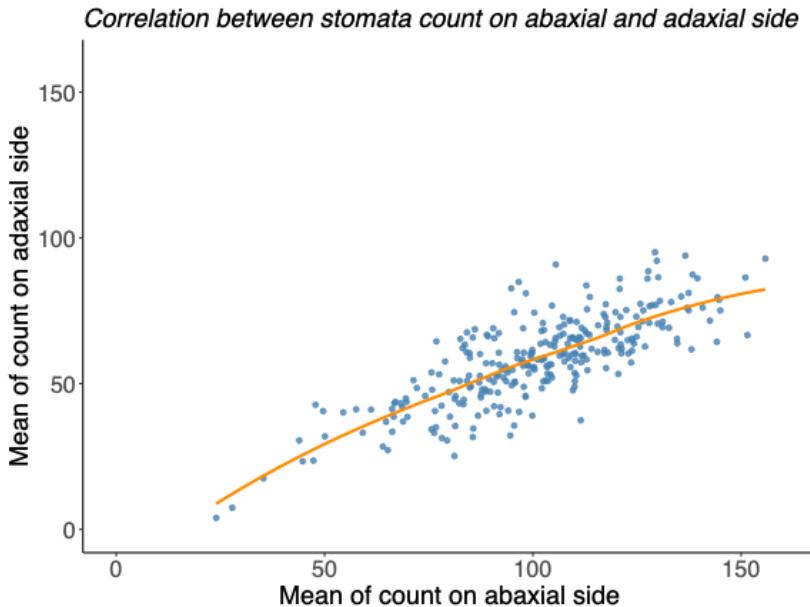
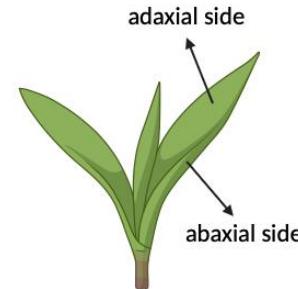
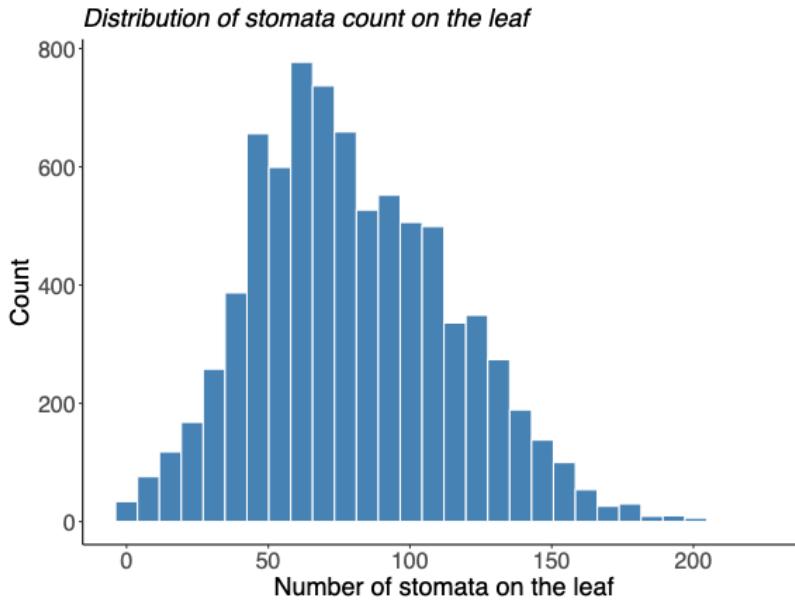


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{ "predictions": [ { "x": 952.5, "y": 630.5, "width": 29, "height": 25, "confidence": 0.829, "class": "stomata", "class_id": 0, "overlap": 0.53 } ] }
```

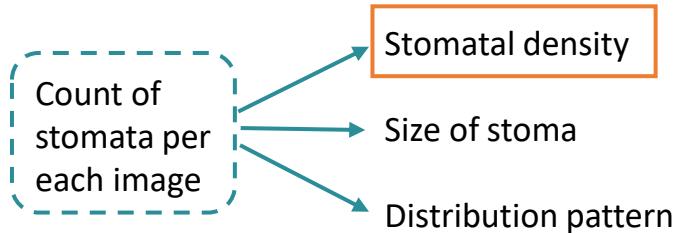
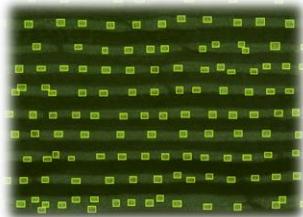
Stomata imaging and detection with AI



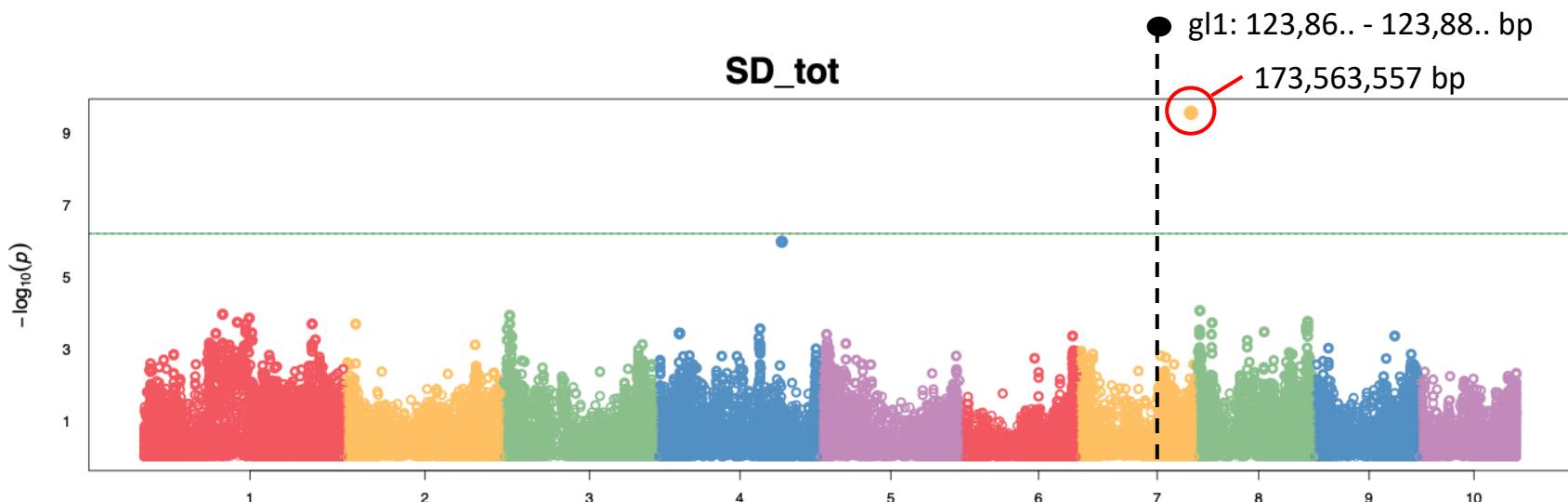
Count of
stomata per
each image



Preliminary results on stomatal density

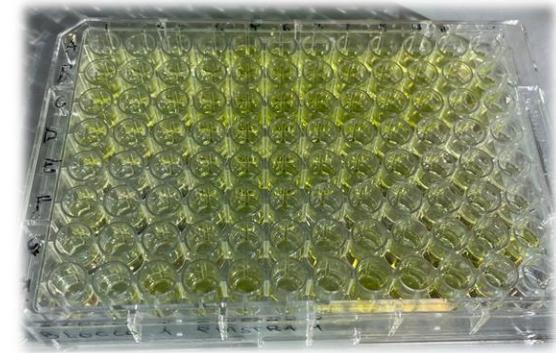


➡ **GWAS** on stomatal density (75,000 SNPs):



Next steps

- ✓ Improvement of the model through **machine learning approaches** to derive other stomatal traits as size and distribution pattern
- ✓ **QTL mapping** of SD and other stomatal traits
- ✓ Integration of the **pangenome** data on other candidate genes
- ✓ Consolidation of the candidate genes through ***Arabidopsis thaliana* mutants**
- ✓ Test the effective **drought tolerance** on the MAGIC population



Relevance for breeding

Identifying the ***glossy1* gene** and other **candidate genes** could guide research on developing **new maize varieties** that have **lower permeability** and are **more drought-tolerant** through gene editing or molecular breeding techniques

Take home messages:

- Modern breeding requires genomics + genetics + diversity
- Multi-parental populations are a powerful and enduring platform to combine agrobiodiversity and big data to support candidate gene mapping

Acknowledgments



TRANSLATIONAL
PLANT GENOMICS

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Dr. Leonardo Caproni



Prof. Matteo Dell'Acqua

Dr. Afewerki Yohannes Kiro



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Answer&Question

Deriving the kinetics of the chlorophyll release:

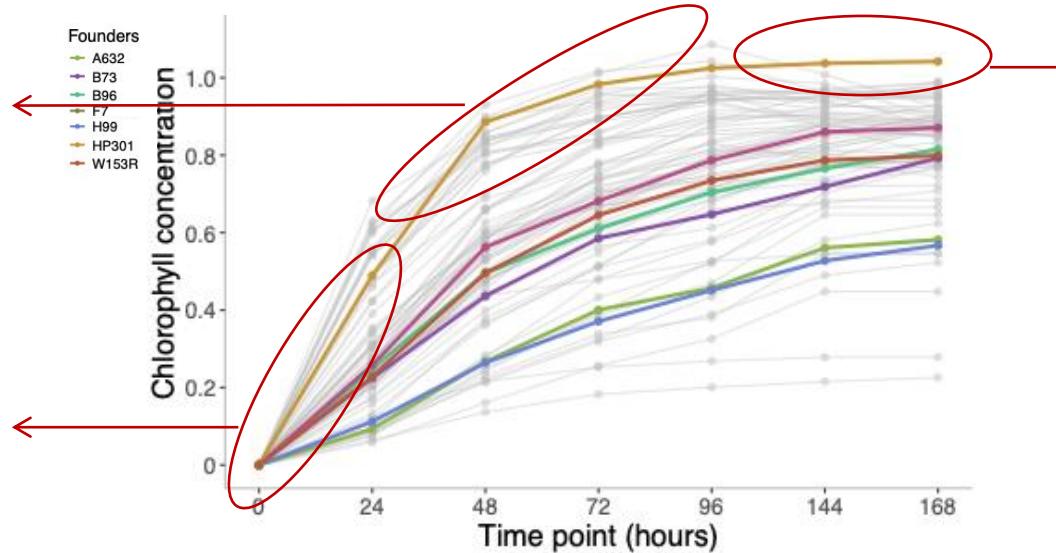
The curve of the chlorophyll release is a **kinetics of a release** and follows a **non-linear model**

$$\text{Speed}(t) = \frac{dC(t)}{dt} = C_{\max} \times k \times e^{-k \times t}$$

As t increases, the speed decreases because of e^{-kt}

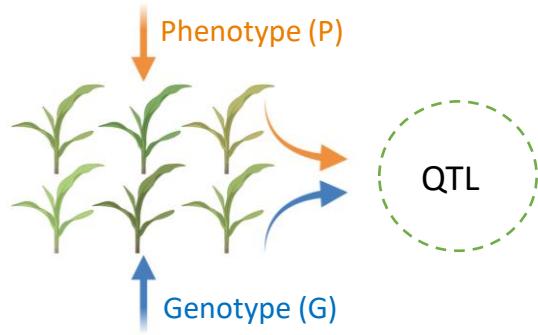
$0 < t < 1$, speed is highest

At very large t , the speed approaches zero: no more chlorophyll is being released



Calculating the speed at different time points → **how fast different genotypes release chlorophyll over time**

Quantitative Trait Loci (QTL) mapping



Genotype: 79,000 SNPs (founder and RILs)

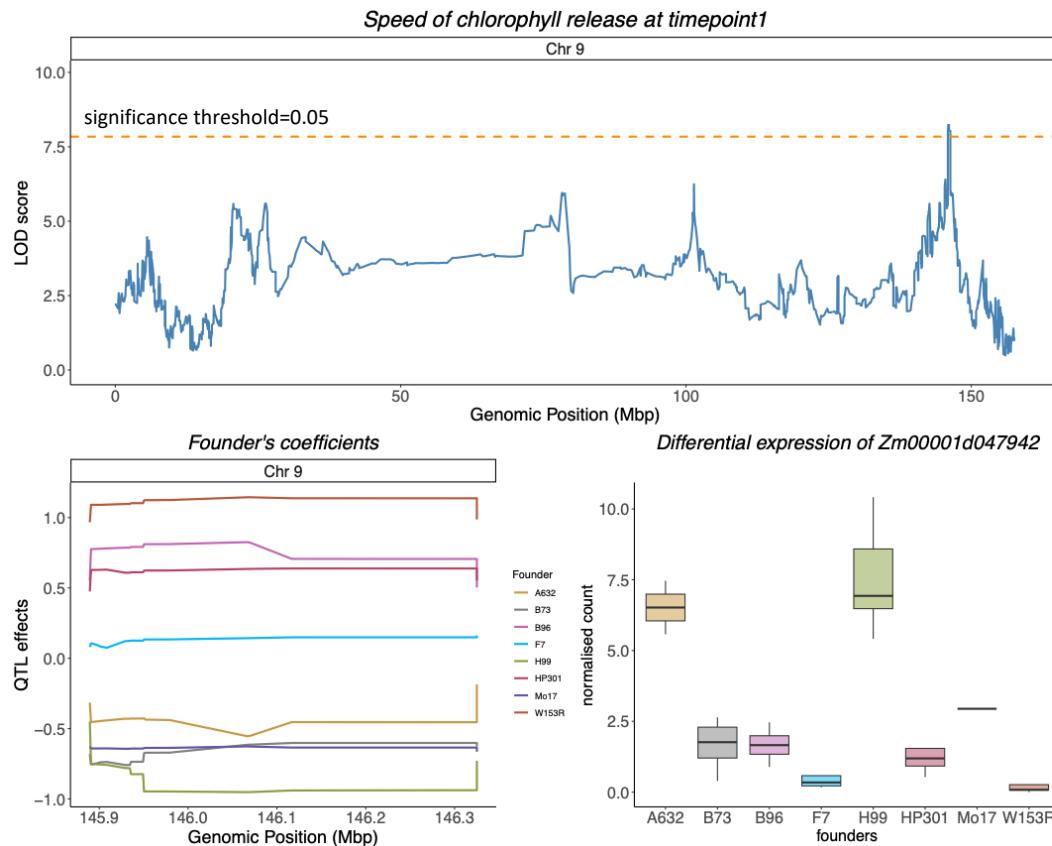
Phenotype: k , C_{max} , speed of release $t(1-6)$

Environment: controlled conditions

Peaks on different chromosomes

lodcolumn	chr	pos	lod	ci_lo	ci_hi
k	9	20.524041	7.83446998796123	20.173237	23.636085
k	9	26.23272	8.67548984873511	25.252052	26.661695
k	9	78.774982	9.87153723916841	32.620035	79.552722
k	9	101.377009	8.39956209475854	100.458234	146.070386
V_release_T0.4	9	146.066907	8.19872894141161	20.448291	146.766055
V_release_T0.6	9	146.066907	8.24842817826664	20.677708	146.759044
V_release_T0.8	9	146.066907	8.25744246004469	78.303794	146.432785
V_release_T1	9	146.066907	8.21238687538926	145.890735	146.325138
V_release_T1.2	9	146.066907	8.09810728512475	145.890735	146.325138
V_release_T1.4	9	146.066907	7.89884451773214	145.890735	146.325138
V_release_T3	5	50.23289	8.2478674937375	45.070079	60.080864
V_release_T3.2	5	50.23289	8.47364612208466	45.070079	59.686499
V_release_T3.4	5	50.23289	8.48430354022341	45.070079	55.894742
V_release_T3.6	5	50.451209	8.32292436168174	45.070079	55.894742
V_release_T5.4	7	87.03816	8.65052897370506	0.110398	128.990996
V_release_T5.6	7	87.03816	9.04252685501592	15.005919	128.990996
V_release_T5.8	7	87.249434	9.40556410694887	82.537038	128.614711
V_release_T6	7	87.249434	9.7347113666752	83.408208	93.518512
V_release_T6	7	108.770254	8.40832046175901	107.532652	110.984314
V_release_T6	7	122.234996	8.30564508528347	113.103042	128.990996

Candidate gene on chr9



High speed=high permeability

From 16 genes on this QTL to 1 candidate

Actin-depolymerizing factor 5

ADF5 involved stomatal closure and drought stress response (*A. thaliana*)

Journal of Experimental Botany, Vol. 70, No. 2 pp. 435–446, 2019
doi:10.1093/jexbot/ezz355. Advance Access publication 24 November 2018
This paper is available online free of at access charges (see <https://academic.oup.com/jexb/pages/openaccess> for further details)



RESEARCH PAPER

Arabidopsis ADF5 promotes stomatal closure by regulating actin cytoskeleton remodeling in response to ABA and drought stress

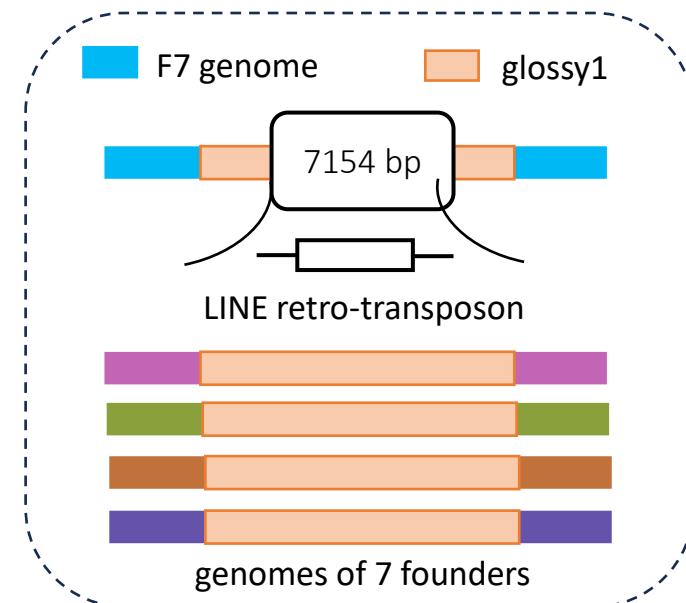
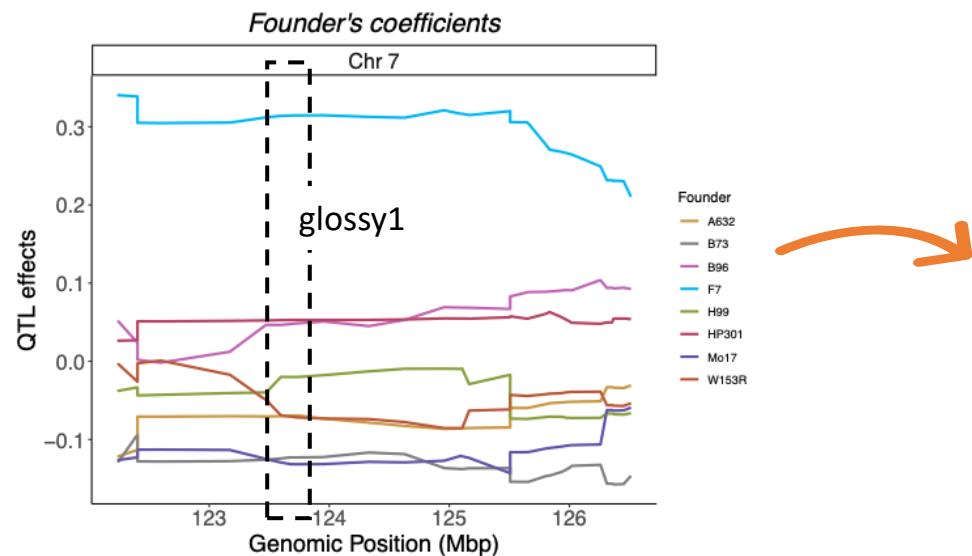
Dong Qian¹, Zhe Zhang¹, Juanxia He¹, Pan Zhang¹, Xiaobin Ou², Tian Li¹, Lipan Niu¹, Qiong Nan¹, Yue Niu¹, Wenliang He¹, Lizhe An¹, Kun Jiang¹, and Yun Xiang^{1*}

Pangenome of MAGIC founders

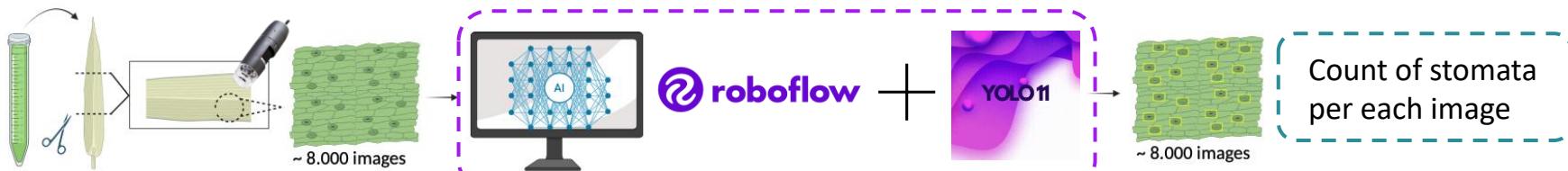
De novo genome assemblies: ONT long reads (poster 1.25)

Pairwise alignment of each founder on F7: **unique F7 insertion** identified

TE annotation of the pangenome (PanETDA) : **LINE retro-transposon**



Stomata detection



- ✓ 500 images manually annotated (+ oriented annotation)
- ✓ Training Yolo11 algorithm on the 500 images
- ✓ Creating workflow for the stomata detection
- ✓ Using the model to count the stomata on each image
- ✓ Creating the dataset with stomata count