INITIATIVE ON
Breeding Resources

CGIAR

2024 WEBINAR SERIES

Crops to End Hunger case studies in Africa and beyond: supporting CGIAR partners through genotyping services

Implementation of EiB mid-density genotyping for unlocking the genetic diversity of an Argentinean public temperate maize collection

Professor Rodomiro Ortiz

SLU, Sweden

Introducing:

Dr. Maria L. Federico

CONICET-INTA, Argentina

May 9th, 2024

5 p.m. Nairobi time


SUSTAINABLE DEVELOPMENT GOALS
1 NO POVERTY
2 ZERO HUNGER
3 GOOD HEALTH AND WELL-BEING
4 QUALITY EDUCATION
5 GENDER EQUALITY
6 CLEAN WATER AND SANITATION
7 AFFORDABLE AND CLEAN ENERGY
8 DECENT WORK AND ECONOMIC GROWTH
9 INDUSTRY, INNOVATION AND INFRASTRUCTURE
10 REDUCED INEQUALITIES
11 SUSTAINABLE CITIES AND COMMUNITIES
12 RESPONSIBLE CONSUMPTION AND PRODUCTION
13 CLIMATE ACTION
14 LIFE BELOW WATER
15 LIFE ON LAND
16 PEACE, JUSTICE AND STRONG INSTITUTIONS
17 PARTNERSHIPS FOR THE GOALS
SUSTAINABLE DEVELOPMENT GOALS


GOAL 2
END HUNGER, ACHIEVE FOOD SECURITY AND IMPROVED NUTRITION AND PROMOTE SUSTAINABLE AGRICULTURE
SUSTAINABLE DEVELOPMENT GOALS
More at sustainabledevelopment.un.org/sdgsproposal

Target indicators of Zero Hunger (SDG2) relevant to Crop Improvement

2.1 By 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round

2.2 By 2030, end all forms of malnutrition, including achieving, by 2025, the internationally agreed targets on stunting and wasting in children under 5 years of age, and address the nutritional needs of adolescent girls, pregnant and lactating women and older persons

2.3 By 2030, double the agricultural productivity and incomes of small-scale food producers, in particular women, indigenous peoples, family farmers, pastoralists and fishers, including through secure and equal access to land, other productive resources and inputs, knowledge, financial services, markets and opportunities for value addition and non-farm employment

2.4 By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality

2.5 By 2020, maintain the genetic diversity of seeds, cultivated plants and farmed and domesticated animals and their related wild species, including through soundly managed and diversified seed and plant banks at the national, regional and international levels, and promote access to and fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge, as internationally agreed

Product target

High yielding cultivars with suitable profiles meeting end-user demands

Cultivars with high nutritional quality contributing to diverse healthy diets, particulalry for children, women and elders

Delivering productivity gains through genetically-enhanced seed-embedded technology in released cultivars for farming elsewhere

Climate-resilient, resource-efficient cultivars adapting to global warming and producing more with less in different agro-ecosystems

Freely available core subsets of characterized and evaluated genebank accessions with predicted breeding values as sources of diversity for crop improvement

Plant breeding methods & tools

Genomic prediction of breeding values for selection of parents for crossing block and their offspring for population improvement

Genetic engineering (transgenics, gene editing) and DNA marker-aided breeding

Participatory plant breeding through promising germplasm testing on farm in the cultivar pipeline resulting from selection based on genomic prediction

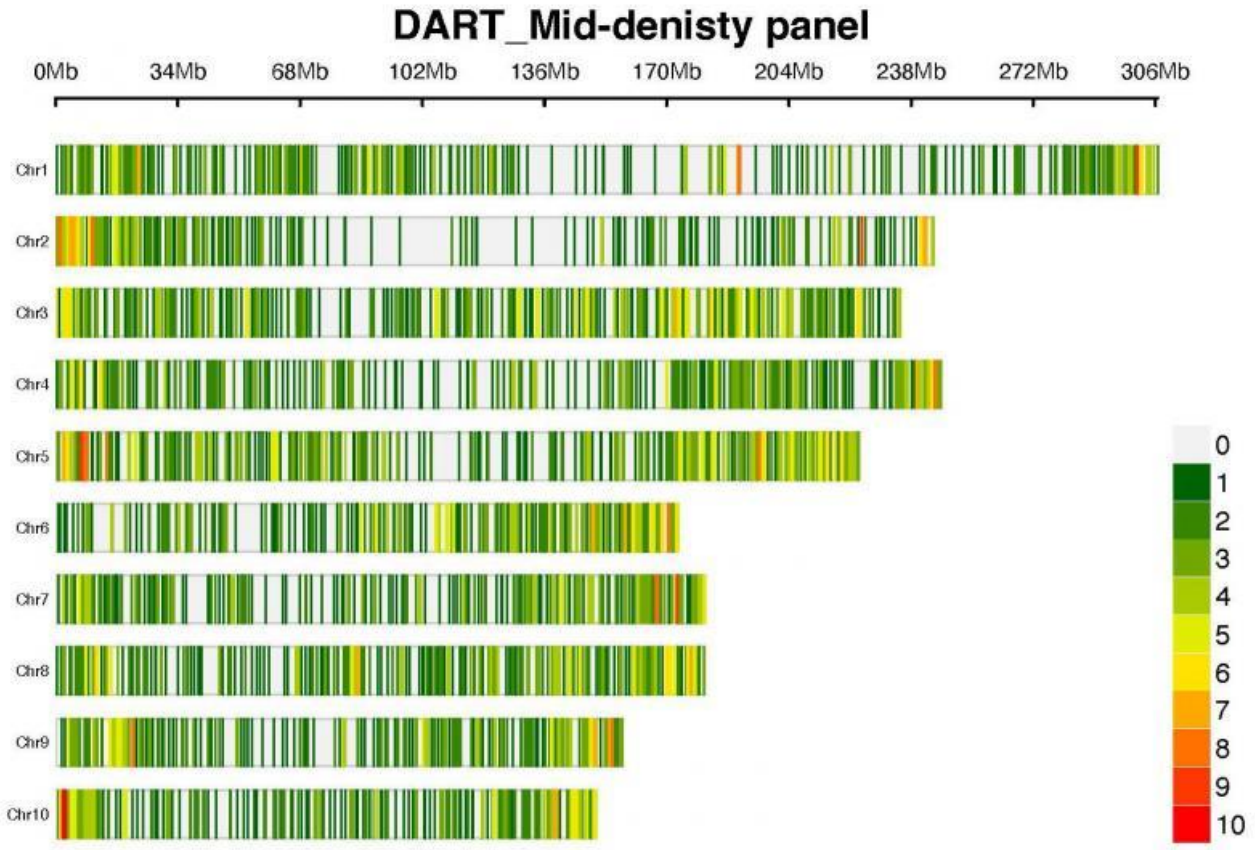
Phenomics for precise phenotyping under stress and enviromics data to include in genomic prediction models to increase their accuracy coupled with a physiology-driven approach

Genomic prediction to identify suitable sources of genetic variation in a core subset defined by DNA diversity analysis and precise phenotyping for target trait(s)

Today	Future
Phenotypic screening for desired characteristic(s) in breeding population or a sample of genebank accessions to identify useful source of variation	Genomic prediction (using phenomics for target traits) of a (mini)core subset (training population) for screening whole genebank (breeding population)
Parents bearing desired characteristic(s) chosen based on pedigree or progeny testing for combining ability	Parents selected according to their genomic estimated breeding values (GEBVs) for desired characteristic(s)
Shuttle breeding (off season nursery), single seed descent or di-haploidy for advancing generations following either a pedigree or bulk selection approach	Speed breeding coupled with genomic prediction for advancing many generations per year that may undergo phenomic-based stress trial(s) in greenhouse
Multisite testing over years or cropping seasons in target population of environments of promising bred germplasm to test their phenotypic stability	Focusing target multisite testing after enviromic-led characterization coupled with genomic prediction of suitable bred germplasm for desired environment(s)
Backcrossing or marker-aided introgression of major gene(s) to elite bred germplasm or desired cultivar(s)	Gene editing desired characteristic(s) in promising bred germplasm or elite cultivar(s)
Crossbreeding or marker-aided selection (MAS) for inbred line development of population improvement	GEBVs “driving” genomic recurrent and reciprocal recurrent selection cycles
Cultivar release after value for cultivation and use (VCU) trial(s) and distinct-uniform-stable (DUS) testing for registration in the national variety list	Deposit of the *de novo* genomic sequence(s) of new cultivar(s) in centralized repository that uses them to trace back contributions and relative importance of loci
Large-scale production of quality seed (after certification) of germplasm included in cultivar catalog	Decentralized community seed enterprises serving local needs use DNA fingerprints for cultivar royalty payments

EiB mid-density genotyping for maize

- **3305 genome-wide markers**
- Developed from sequencing data of > **10000 breeding lines and landraces** from Mexico, Latin America, Africa and Asia
- Whole-genome re-sequencing (**WGS**), genotyping by sequencing (**GBS**), DArTseq genotyping, maize haplotype v3 (**HapMap3**) and Kompetitive Allele Specific PCR (**KASP**) SNPs
- The **average marker density** of the panel is about **1 SNP per 0.72 Mbp**



Maria L. Federico

CONICET Consejo Nacional de Investigaciones Científicas y Técnicas

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INVESTIGADORES

FEDERICO MARIA LAURA

- datos académicos
- artículos
- capítulos de libros
- congresos y reuniones científicas

datos académicos

Título/s	Doctor in Philosophy (PhD) Licenciado en Ciencias Biologicas
Categoría	INV ADJUNTO
Disciplina científica	Ciencias Agrarias Bioquímica y Biología Molecular
Disciplina desagregada	AGRONOMIA Y DASONOMIA-FITOTECNIA
Campo de aplicación	Produccion vegetal-Cultivos ind.ex. oleagin Energia-Bioenergia
Especialidad	Mejoramiento molecular de cultivos
Tema	Priorización de genes candidato en loci de carácter cuantitativo asociados a la acumulación de azúcares y producción de biomasa en sorgo (Sorghum bicolor) utilizando un algoritmo de aprendizaje automático y secuenciación masiva de alto rendimiento.
Tema en inglés	PRIORITIZATION OF CANDIDATE GENES IN QTL REGIONS ASSOCIATED WITH BIOENERGY-RELATED TRAITS IN SORGHUM (Sorghum bicolor) USING A MACHINE LEARNING ALGORITHM AND NEXT GENERATION SEQUENCING.
Palabras clave	GENES CANDIDATO // POLIMORFISMOS // BIOENERGIA
Palabras clave en inglés	CANDIDATE GENES // POLYMORPHISMS // BIOENERGY
Lugar de trabajo	[EEA PERGAMINO] ESTACION EXPERIMENTAL AGROPECUARIA PERGAMINO - [CR BUENOS AIRES NORTE] CENTRO REGIONAL BUENOS AIRES NORTE - [INTA] INSTITUTO NACIONAL DE TECNOLOGIA AGROPECUARIA -
Director de :	

BECARIOS

CARRERE GÓMEZ, MANUELA

enviar mensaje

Plant scientist with +10 year-experience leading/conducting research in plant biotechnology and molecular breeding with emphasis on improving host plant resistance, nutritional and agronomical traits (USA, Argentina, Chile). Expertise: plant transformation (monocot and dicot species), double haploid production, trait development (gene and promoter discovery, targeted SNP discovery, metabolic engineering), trait characterization (transgene profiling –DNA, RNA, protein–, phenotyping –greenhouse and field evaluations, HPLC, NIR, microscopy–) and trait integration

Implementation of EiB mid-density genotyping for unlocking the genetic diversity of an Argentinian public temperate maize collection

María Laura Federico, PhD.

CGIAR Seminar Series
May 9th 2024

CONICET

INTA
Instituto Nacional de Tecnología Agropecuaria
Argentina

OUTLINE

- **INTRODUCTION**
  - Maize production in Argentina
  - INTA breeding program
  - INTA temperate IL panel
- **OBJECTIVES**
- **MATERIALS AND METHODS**
- **RESULTS**
- **CONCLUSIONS**

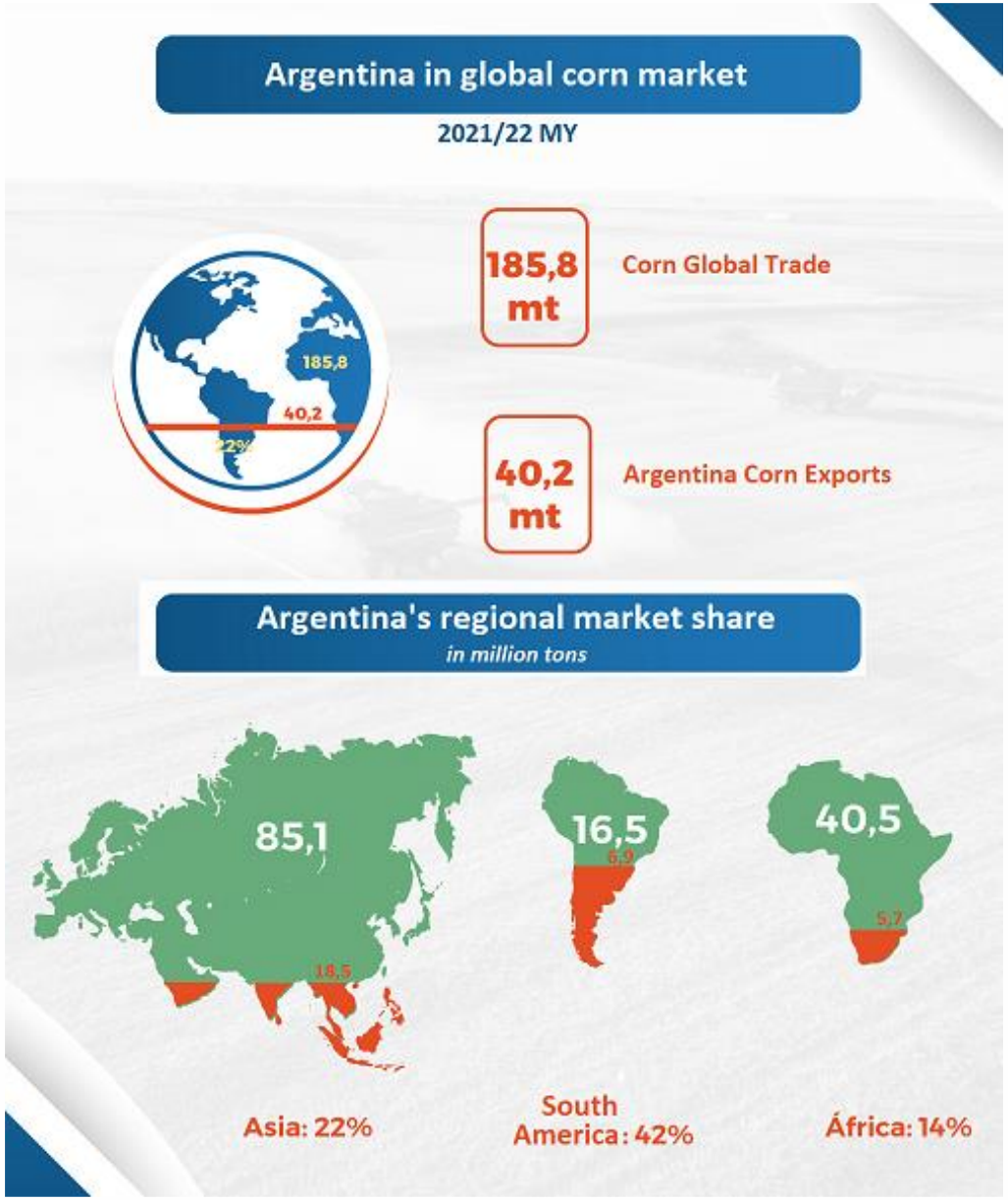
Instituto Nacional de Tecnología Agropecuaria
Argentina

Argentina


© WorldAtlas.com
NORTH AMERICA
CARIBBEAN
North Atlantic Ocean
North Pacific Ocean
Caribbean Sea
CENTRAL AMERICA
EQUATOR
SOUTH AMERICA
TROPIC OF CAPRICORN
N
South Pacific Ocean
Argentina
South Atlantic Ocean
1000 m
1000 km

Argentina contributes to global food security through agricultural exports

- Argentina is the 4th global maize producer (behind the US, China & Brazil).
- Argentina is 2nd global maize exporter behind the US.
- It supplies external demands of other countries.

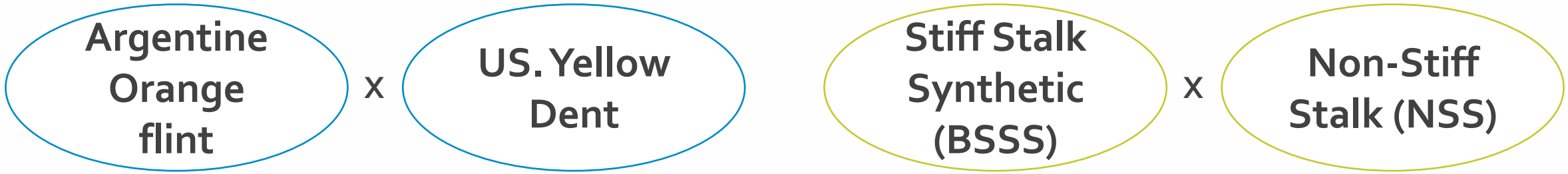


(Sigaudo and Terre, 2022)

Public Maize Breeding in Argentina (1914-present)

- **1914-1956** Ministry of Agriculture
  - "improve yield and help growers"
  - 1949-First doble cross hybrid released.
- **1956-present** INTA
  - 1969 – Germplasm Bank
  - 1960-1980 – the development of orange flint lines, OPVs and doble cross-hybrids laid the foundation for both public and private breeding programs.
  - 1992 –Consortium with private seed companies established.

Common Breeding Strategies



Hybrid

Great abiotic and biotic resistance, suitable for dry milling industry

Hybrid

Good performance , suitable for the wet milling industry

FLINT x FLINT hybrids, depend on the improvement of local flint inbred lines for this purpose (ongoing efforts)

INTA Maize Breeding Program

GERMPLASM DEVELOPMENT: 5 stations

GERMPLASM EVALUATION: 7 units

BIOTECHNOLOGY : 2 laboratories

GERMPLASM BANK

Current INTA Pergamino Temperate Maize IL Panel

- More than 500 public inbred lines mostly adapted to temperate climate conditions.
- They were developed by intermating OP populations (composites and synthetics) and also derived from commercial hybrids.
- This panel encompasses high variation for grain color and texture, maturity, yield and pathogen resistance.

Contrasting *Fusarium* ear rot disease severity in 3 panel ILs

Phenotypic Diversity (Ignacio Torrent PhD dissertation)

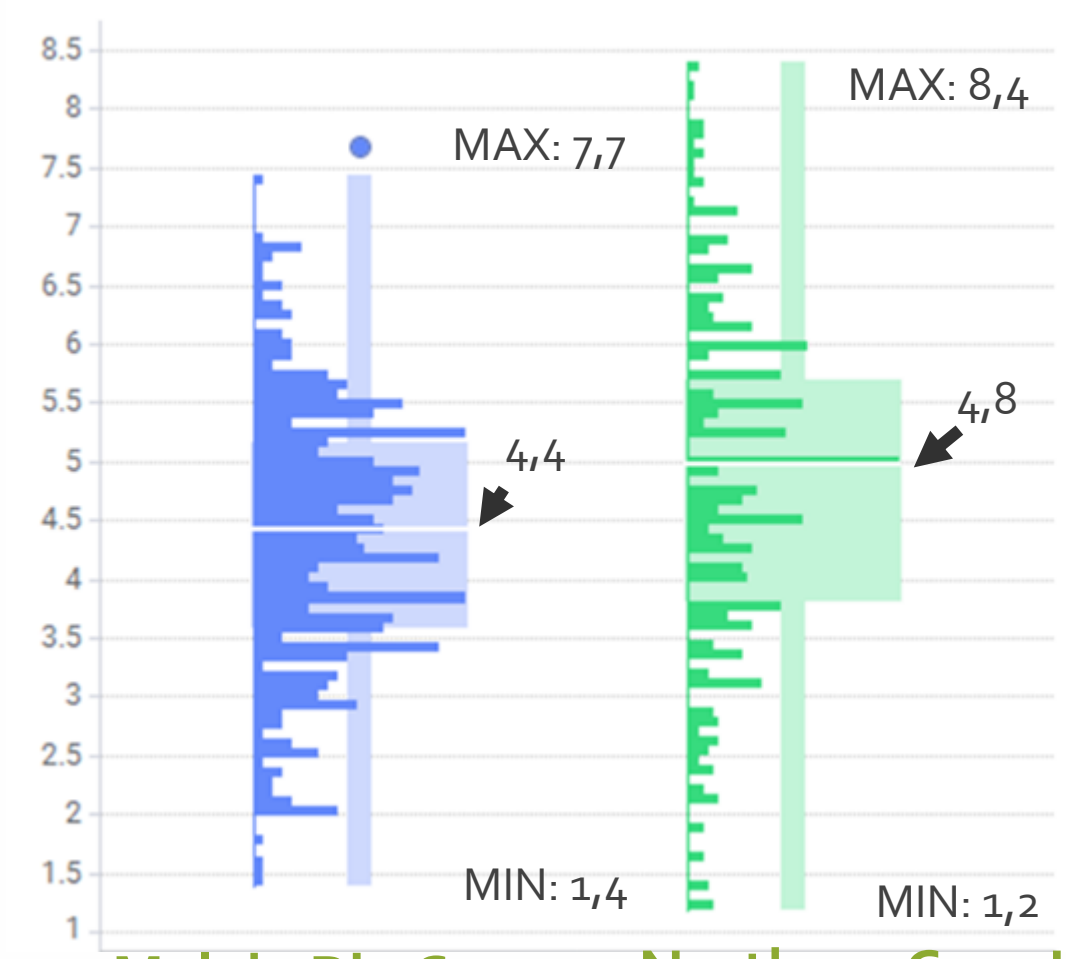
- A set of 484 lines has been evaluated for 3 relevant diseases during 3 years in replicated trials.

Mal de Rio Cuarto (MRC)

Northern Corn Leaf Blight (NCLB)

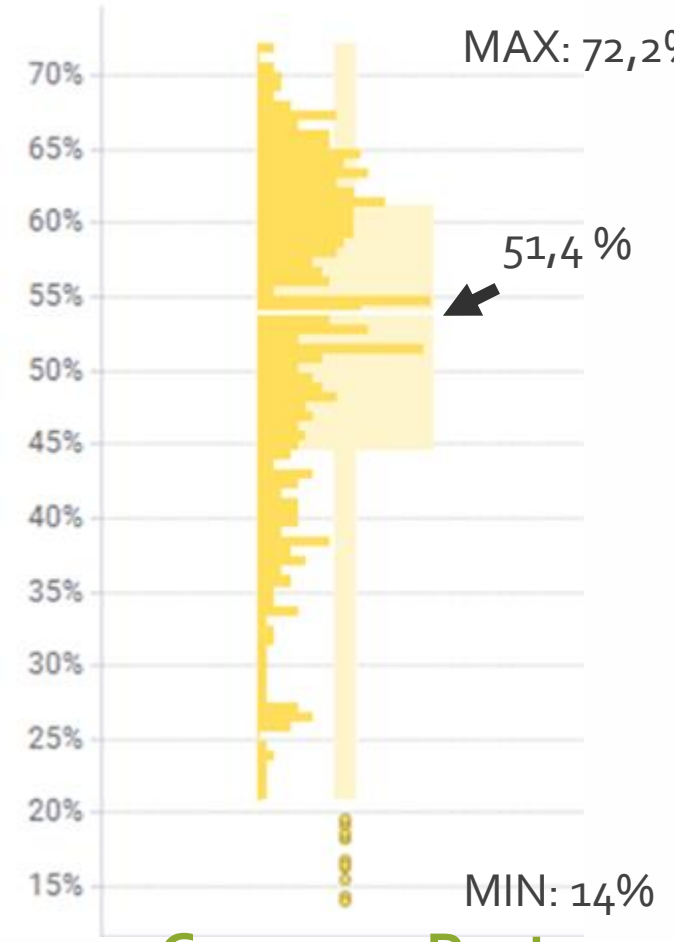
Common Rust (CR)

Disease Response BLUPs of 484 ILs (Ignacio Torrent PhD dissertation)



Mal de Rio Cuarto (MRC)

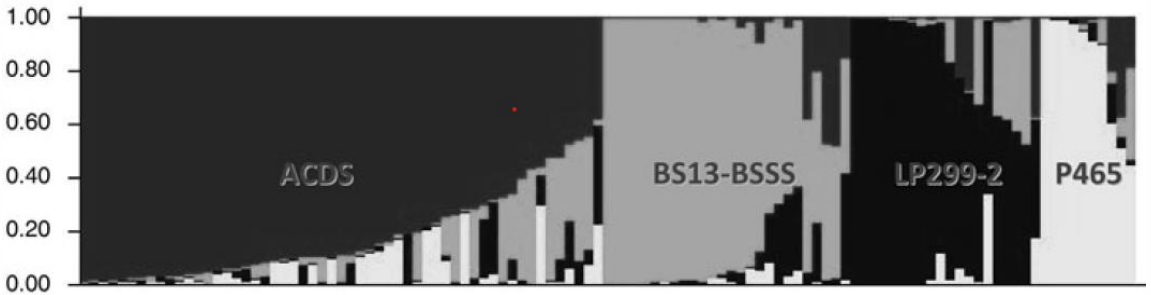
Northern Corn Leaf Blight (NCLB)



Common Rust (CR)

Previous studies on INTA- temperate maize ILs

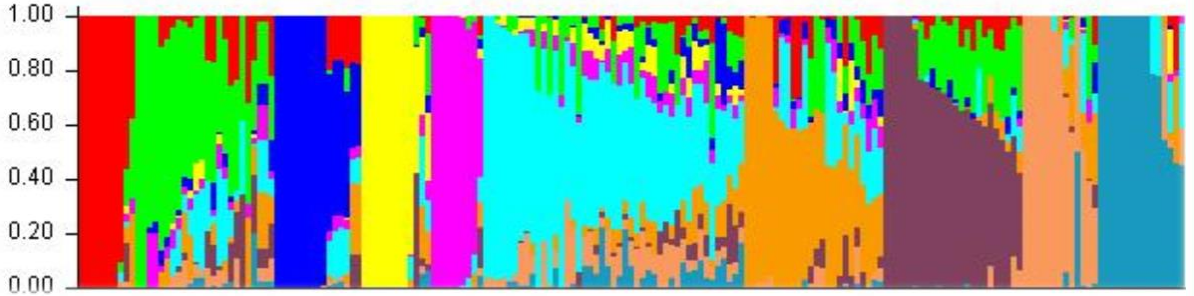
- 111 inbred lines were genotyped with 74 SSRs.



- K=4

(Olmos et al. 2014, 2016)

- 191 inbred lines were genotyped with Illumina MaizeSNP50 BeadChip.

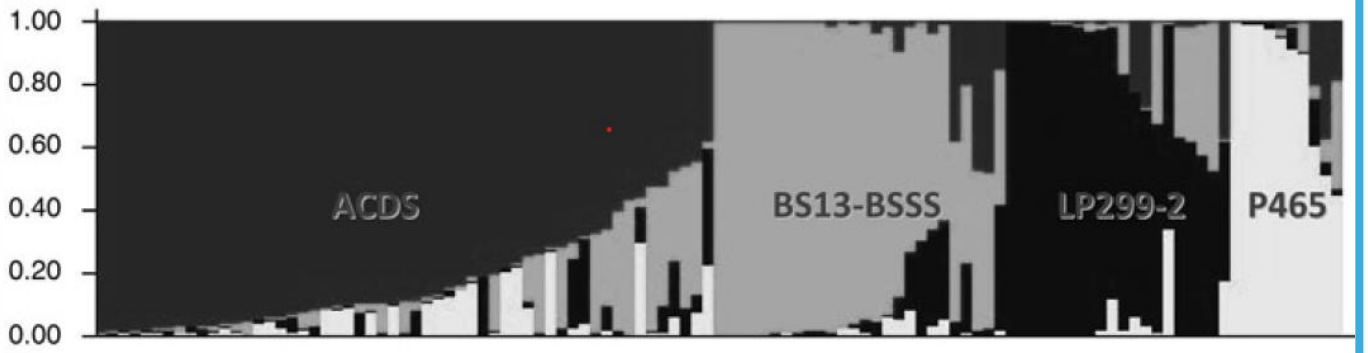


- K=9-10

(Torrent et al. 2018, Perdomo et al. 2022)

4 subpopulations

- 70 lines were assigned to 4 subpopulations (Q>0,7)

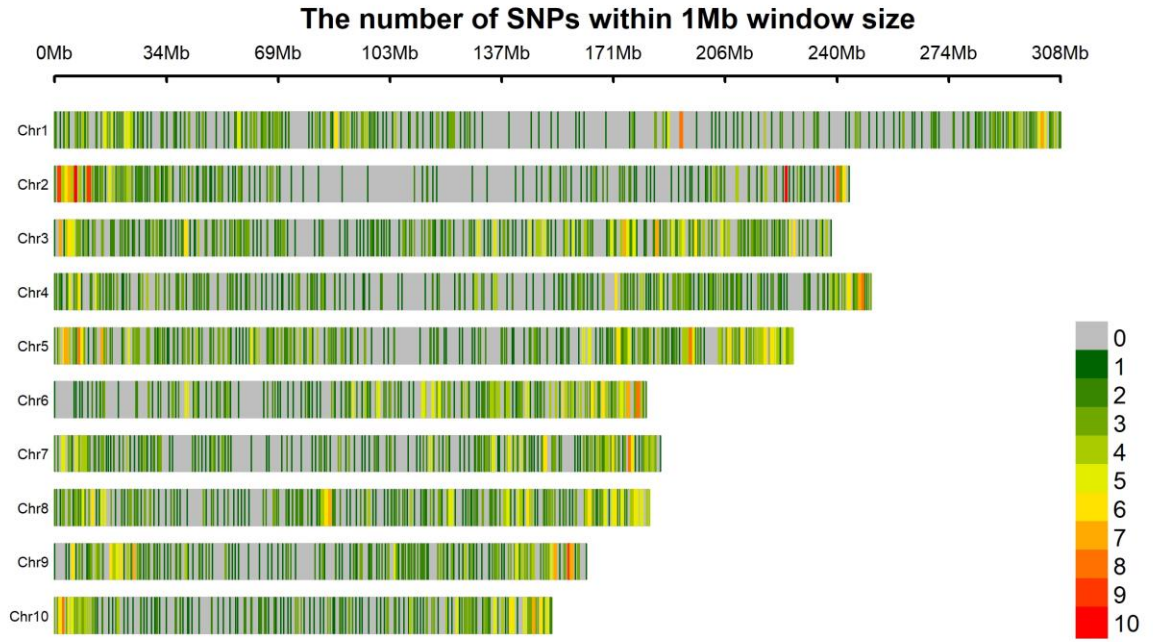


- **ACDS** (Argentine x Caribbean Derived Stock, flints, with variable or unknown combining ability)
- **P465** (Orange flints, mainly derived from an Argentine landrace, good combining ability with US dents)
- **BS13-BSSS** (Iowa Stiff Stock Synthetic background, dent or semi-dent)
- **LP299-2** (Local inbreds derived from commercial hybrids, flint combining ability)

(Olmos et al. 2014, 2016)

Implementation of Eib maize mid-density genotyping

- Access to the EiB mid-density SNP panel let us include more lines not previously genotyped and unify marker sets.
- A first set of 376 ILs was genotyped on 2023-remaining lines will hopefully be genotyped soon.
- Future implementation of genomic prediction and genomics-assisted breeding (eg. germplasm organization, trait introgression).



3305 genome-wide SNP markers

CGIAR Genetic Innovation TOOLBOX

Objective

- Assess the genetic diversity, degree of linkage disequilibrium and genetic structure of an extended panel of INTA temperate ILs (n=376).

Ultimate Goal

- Organize INTA maize breeding program through data-driven decisions (eg. establishing heterotic patterns, exploiting and maintaining genetic diversity).

Materials and Methods

- Five seeds from each of the 484 temperate ILs were sown in sand and germinated in a growth chamber at 25C.
- Ten day old seedlings were used to collect 4 leaf punches per genotype.
- Freeze-dried samples were sent to Intertek Australia for DNA extraction and genotyping with the 3.3K maize DArtTAG SNP panel.

Materials and Methods

- **Molecular Data Analysis:**

-ASRgenomics (Gezan et al. 2024)

-snpREADY (Granato &Fritsche-Neto, 2018)

-TASSEL 5.0 (Bradbury et al. 2007): for kinship , linkage disequilibrium, Roger´s genetic distance estimation and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendograms.

- **Population Structure Analysis:**

- Admixture model-based clustering method using the software Structure V2.3.3 (Hubisz et al. 2009) and Structure Harvester (Dent et al. 2012).
- Principal component analysis (PCA) was implemented in TASSEL 5.0.

- **Graphs:**

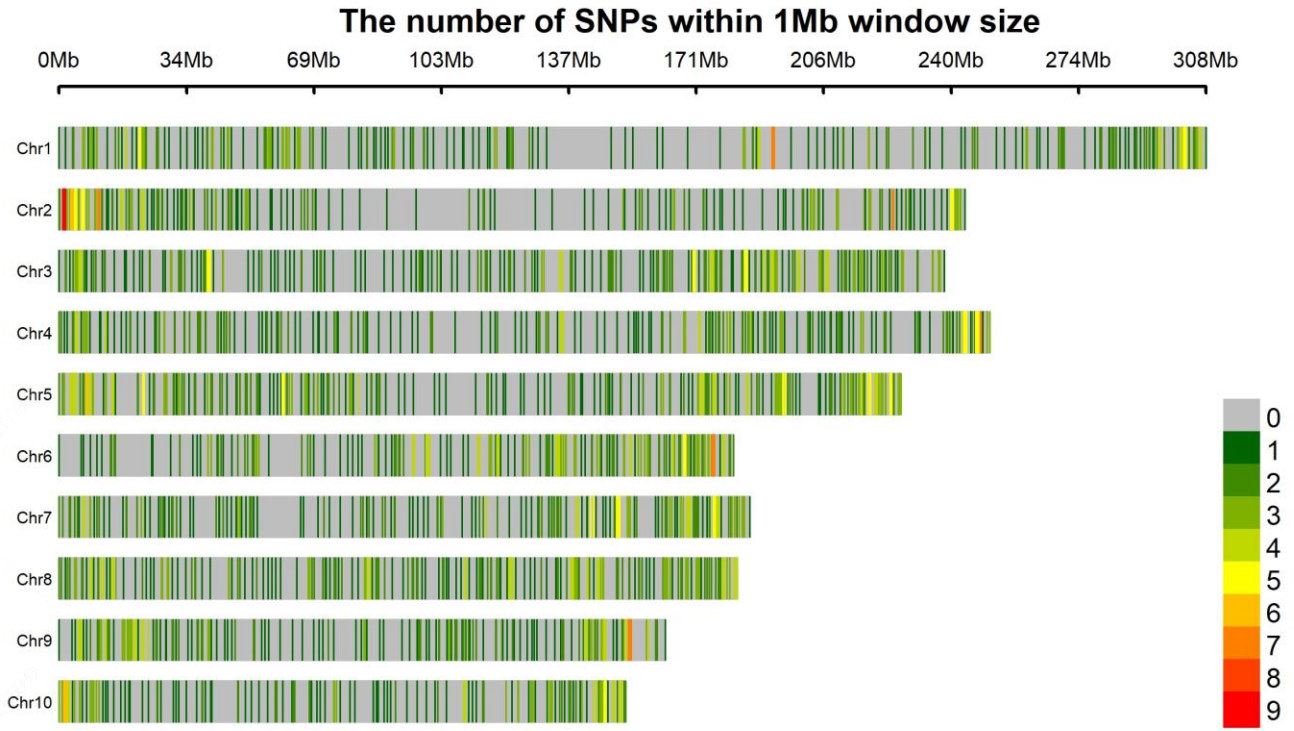
- Structure Plot v2.0 (Ramasamy et al. 2014)
- iTOL v6 (Letunic & Bork, 2024)

Results / SNP markers data processing

IL
- Heterozygocity < 0,15
- Call Rate >0,5

SNP
- MAF >0,05
- Call Rate>0,8

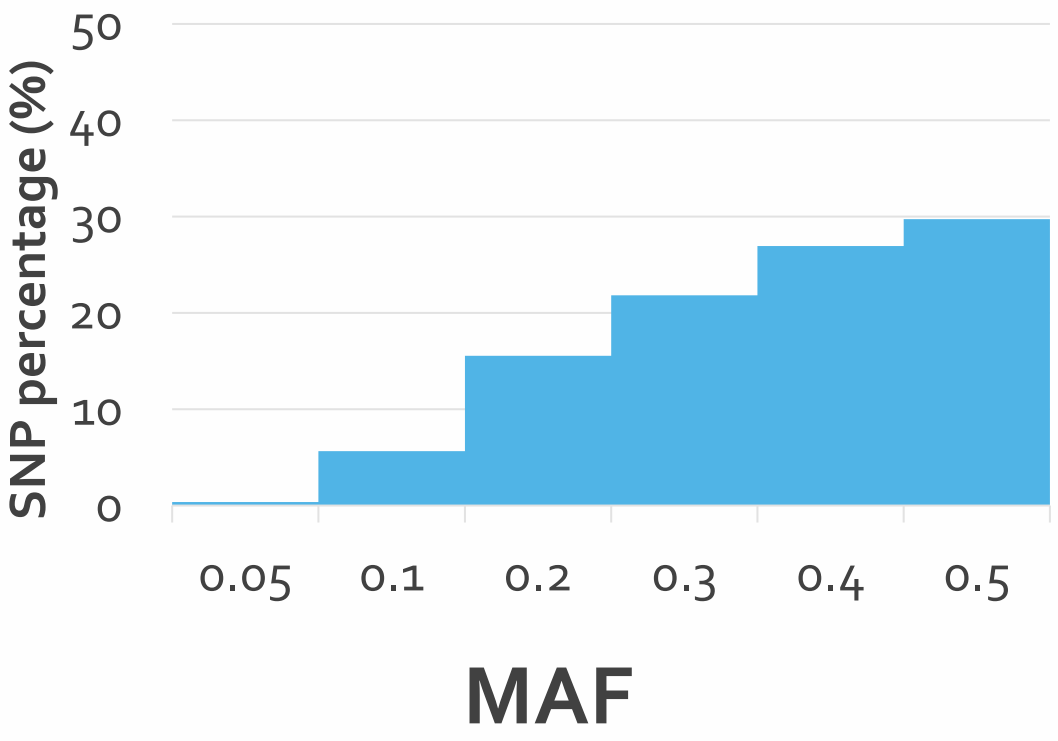
363 inbred lines (ILs)

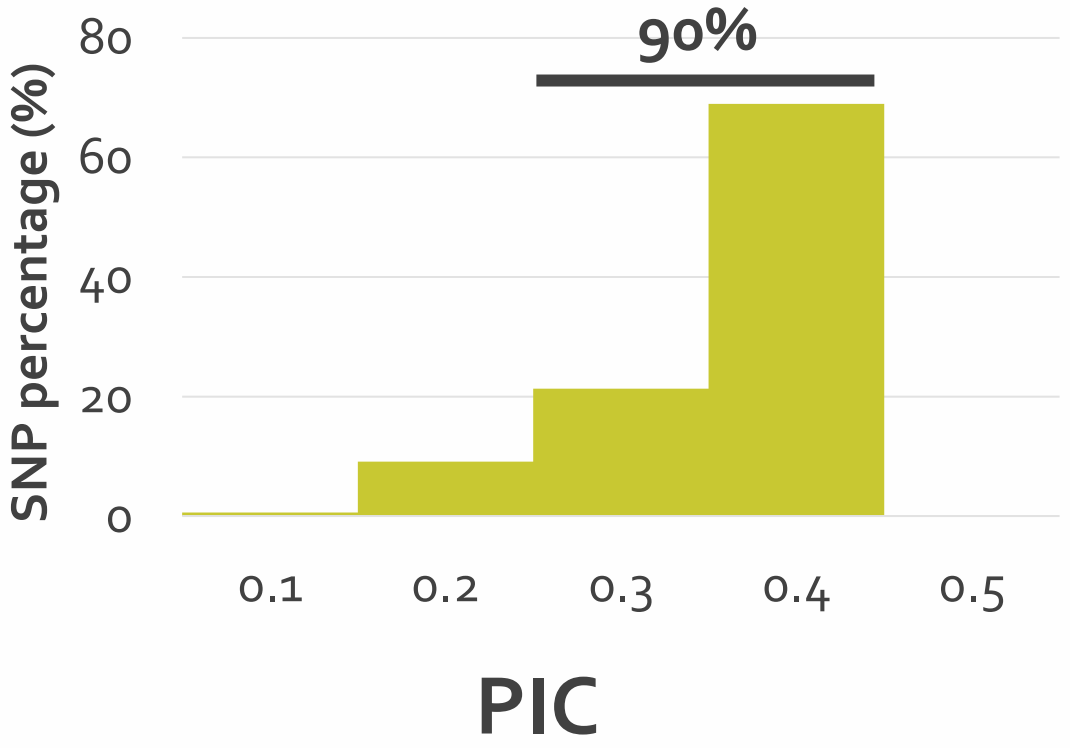


Density Plot
(2187 genome-wide SNP markers)

High proportion of informative SNPs

	Average	Min	Max
PIC	0.32	0.09	0.38
MAF	0.32	0.05	0.5
Miss (%)	2.55	0	20



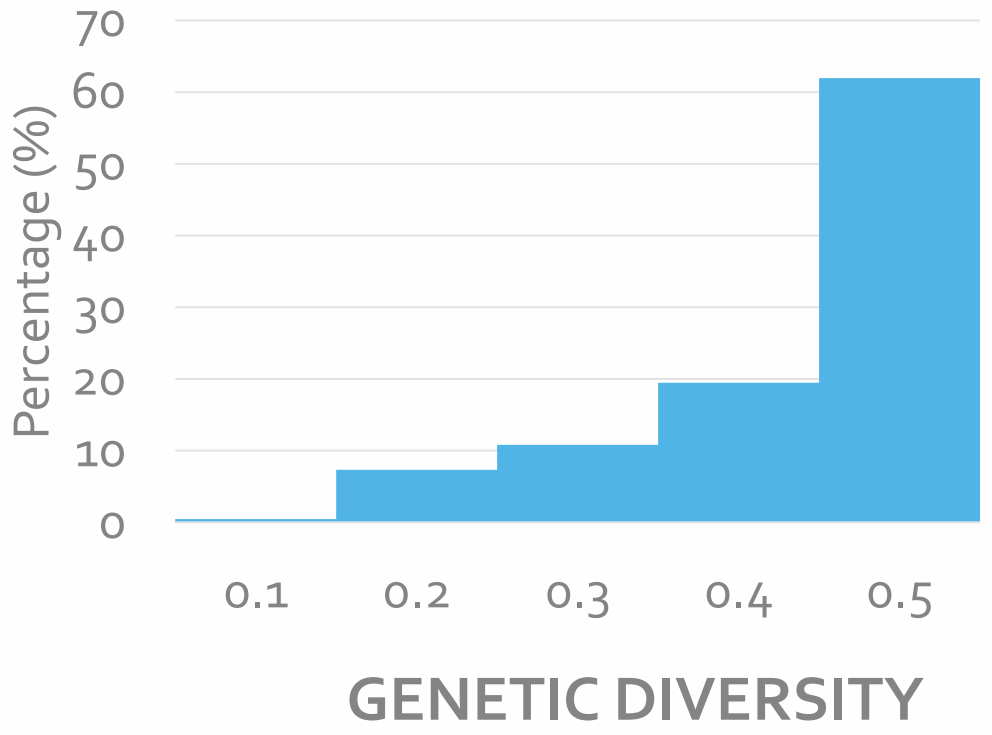


The 363 ILs exhibit low levels of heterozigocity and are genetically diverse as revealed by GD estimates.

	Average	Min	Max
GD	0.4	0.1	0.5
Ho	0.02	0	0.15
F	0.96	0.62	0.99

Ho= observed heterozygosity per IL in all loci (2187 SNP)

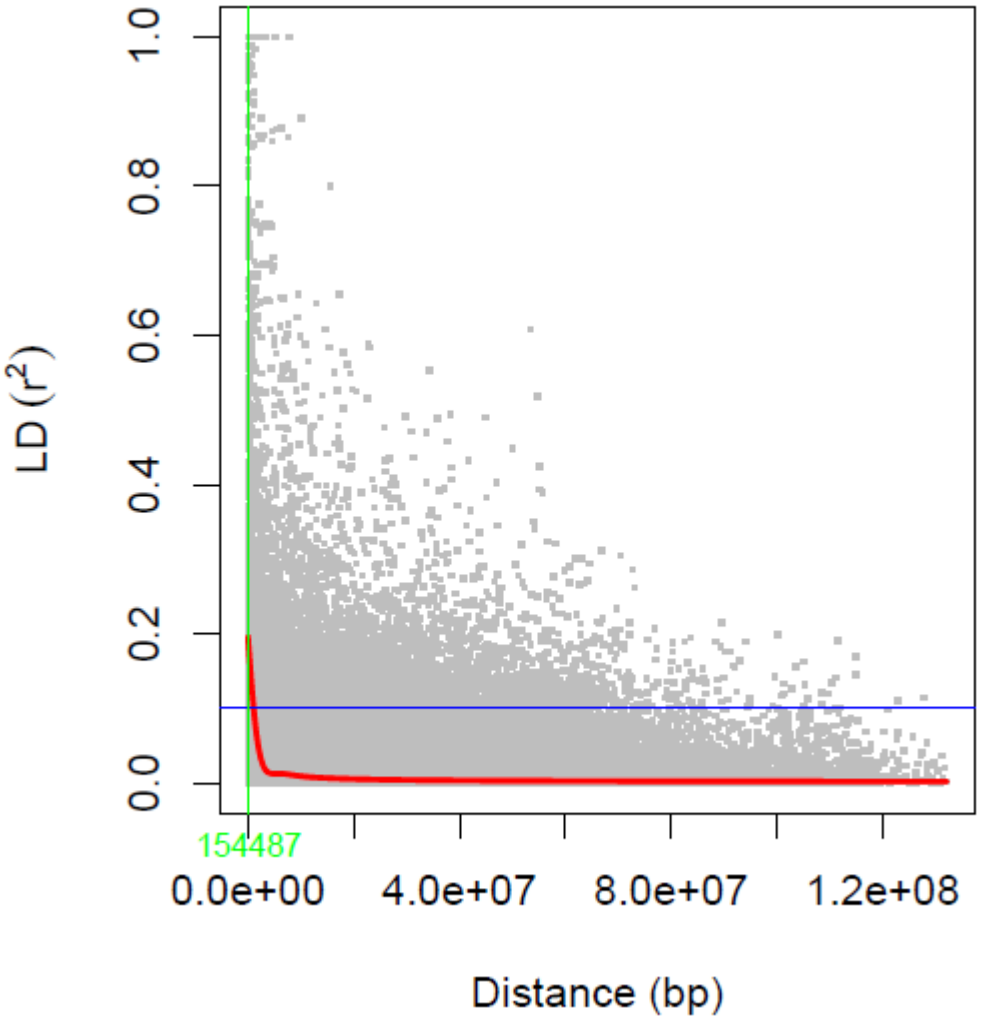
F= Inbreeding coefficient per IL across all loci (2187 SNP)



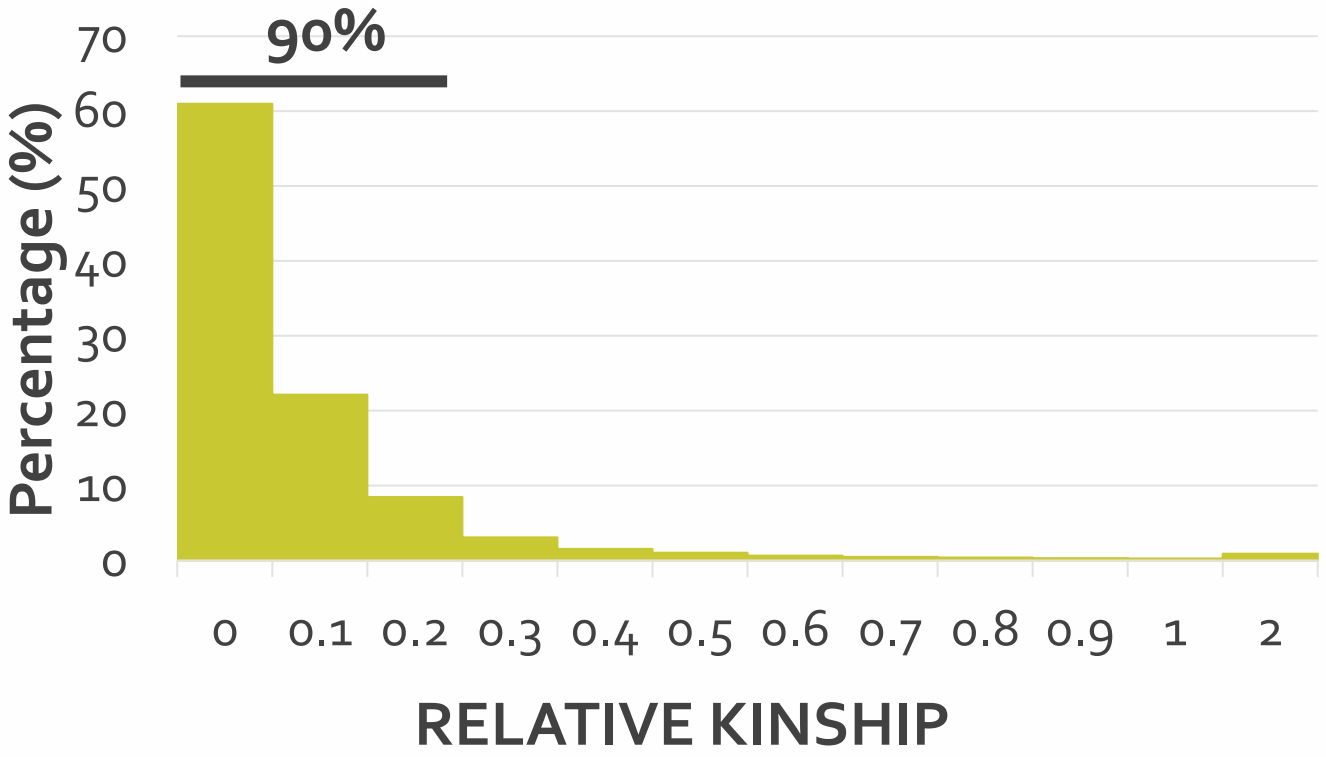
Linkage Disequilibrium (LD)

- The genome-wide LD-decay distance in the 363 temperate INTA panel was determined to be 154.5 Kb at $r^2 = 0.1$.

- Rapid LD decay is expected in maize, with distances 2 to 10 times higher in temperate (10-100Kb) compared to tropical germplasm (5-10 kb) (Lu et al. 2011).



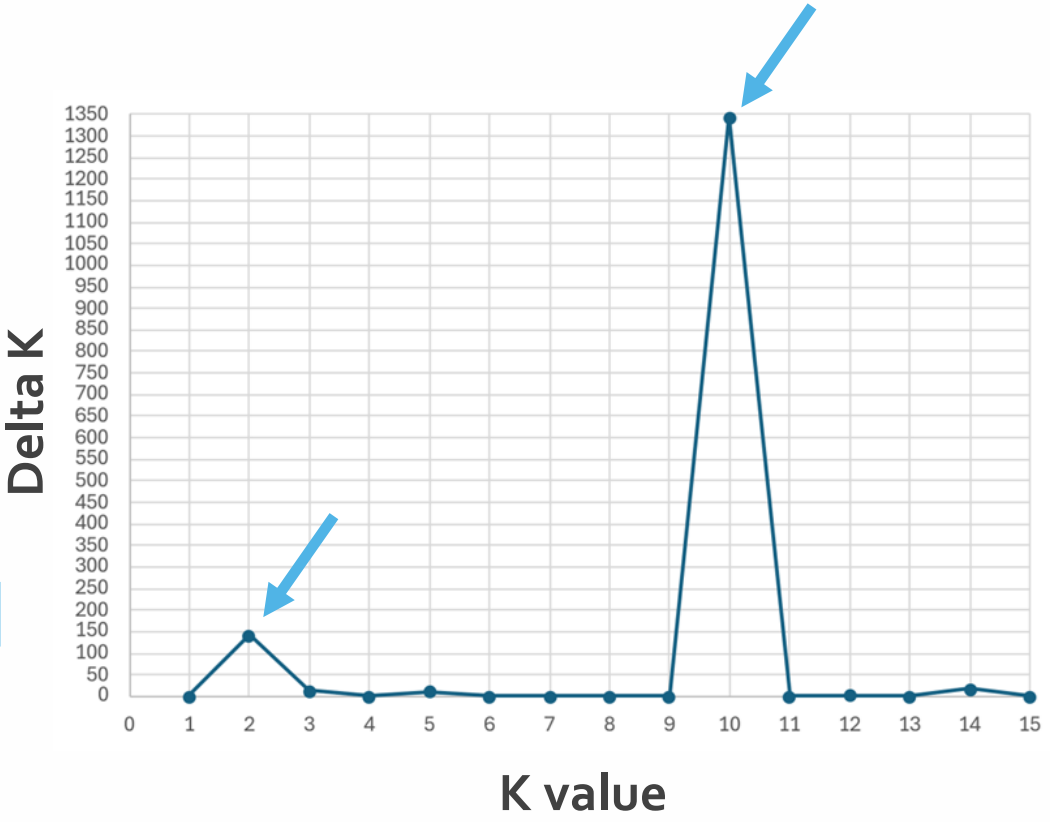
Pairwise relative kinship distribution for 363 ILs estimated with 2187 SNPs



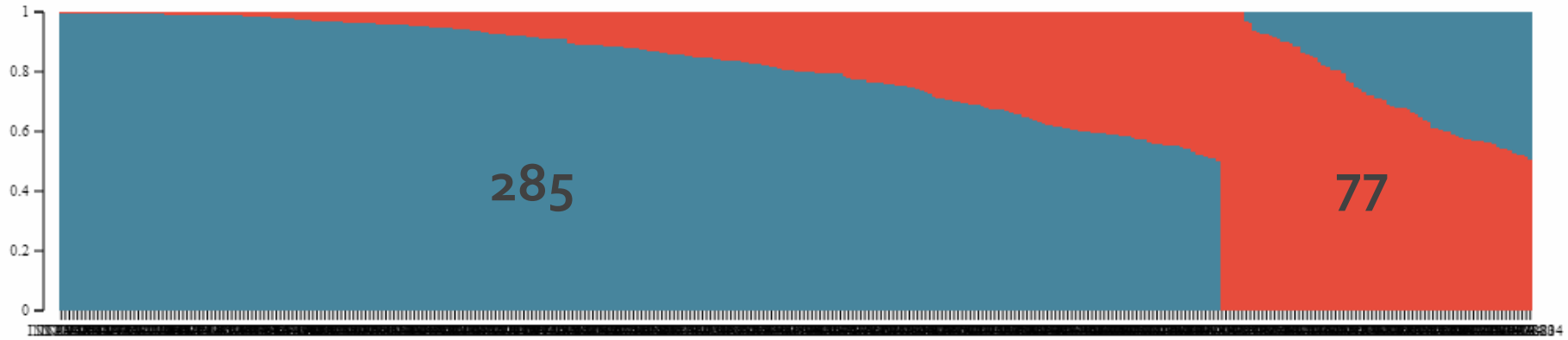
Most of the lines in the 363-panel are either not related or only weakly related to each other, with only just a few lines showing strong similarity among them.

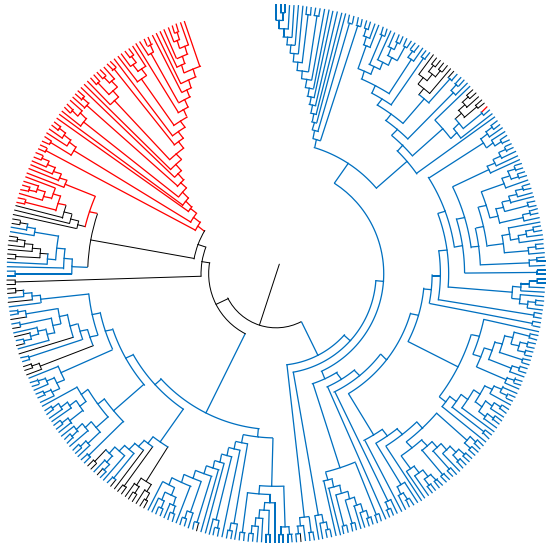
Population structure of 363 temperate ILs estimated with 2187 SNPs –Bayesian clustering.

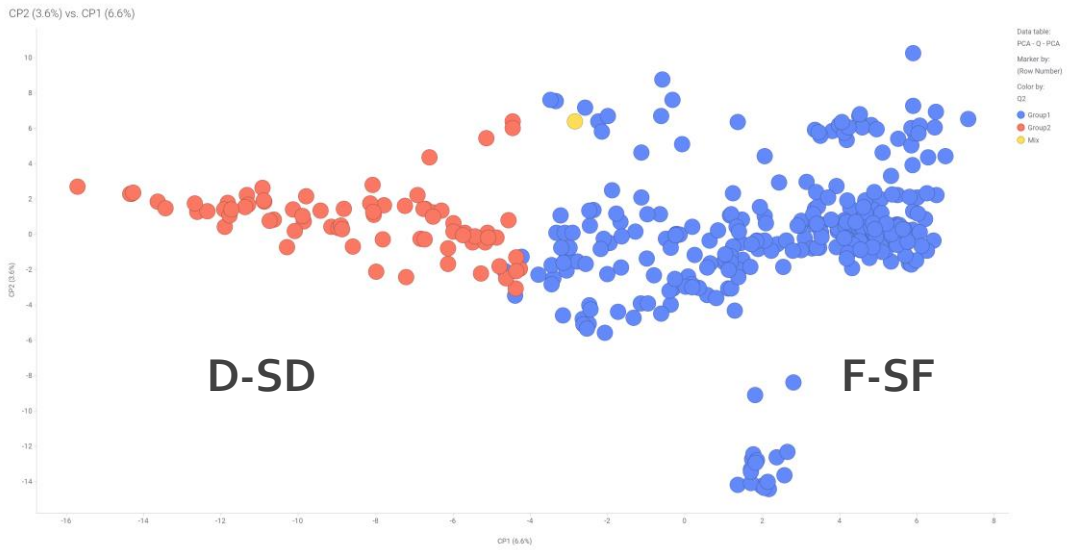
# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-496,341.1	26.9	NA	NA	NA
2	5	-471,666.8	71.2	24,674.3	10,097.4	141.9
3	5	-457,089.9	252.7	14,576.9	3,411.0	13.5
4	5	-445,924	1,109.5	11,165.9	1,362.5	1.2
5	5	-436,120.6	231.7	9,803.5	2,453.7	10.6
6	5	-428,770.8	1,587	7,349.7	367.2	0.2
7	5	-421,053.9	1,359.7	7,717.0	937.8	0.7
8	5	-414,274.7	757.6	6,779.2	1,036.7	1.4
9	5	-408,532.2	1,852.4	5,742.5	1,242.5	0.7
10	5	-401,547.3	916.9	6,984.9	1,232,595.8	1,344.3
11	5	-1,627,158.2	2,753,314.6	-1,225,610.9	623,925.5	0.2
12	5	-3,476,694.5	3,372,416.5	-1,849,536.4	6,091,369.7	1.8
13	5	-11,417,600.6	12,871,189.8	-7,940,906.1	18,270,081.6	1.4
14	5	-1,088,425.1	1,379,430.1	10,329,175.5	23,616,453.6	17.1
15	5	-14,375,703.1	16,023,220.6	-13,287,278	NA	NA



Population structure, K=2

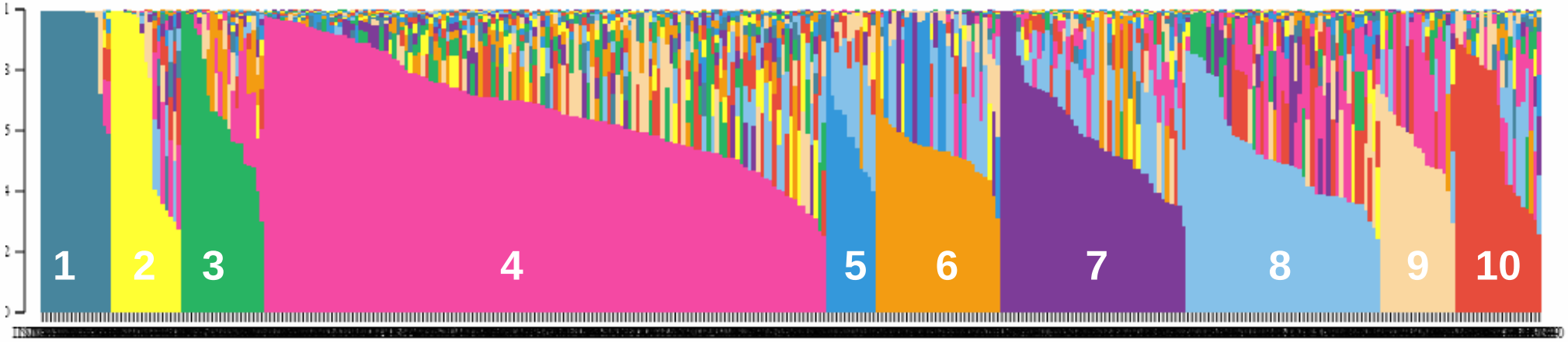


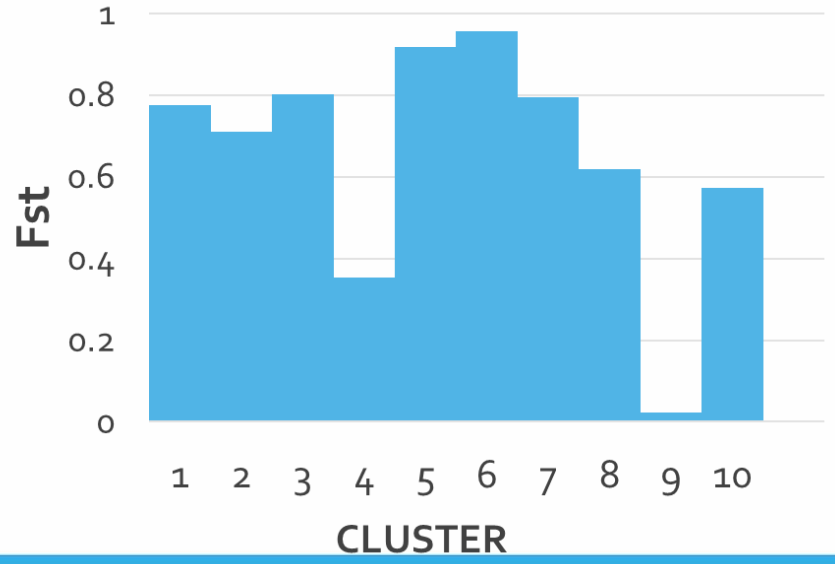




K=2

Population structure, K=10



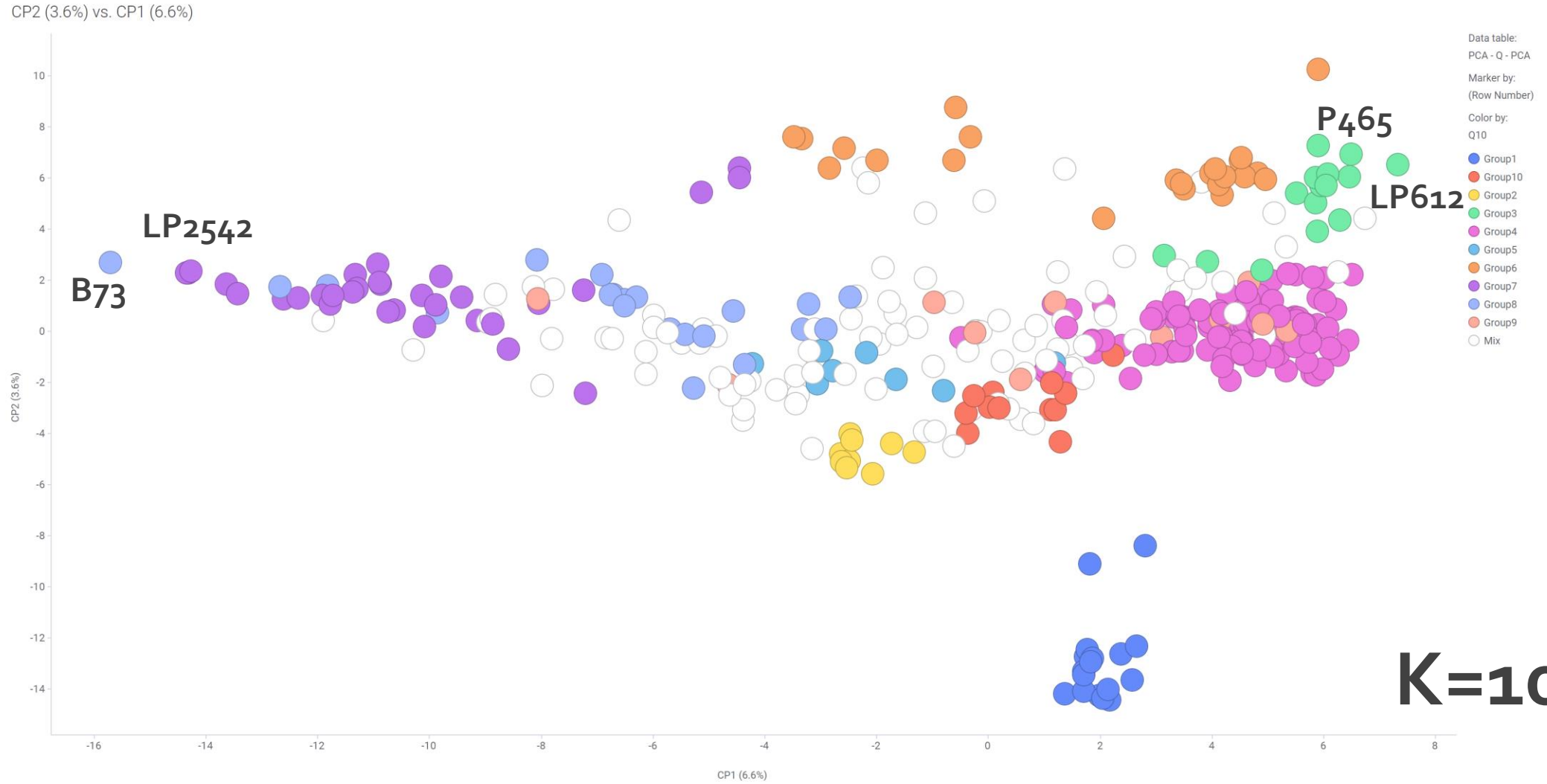


- The panel of 363 ILs was divided into 10 subpopulations. With a threshold level of Q>0.5, 98 IL were considered as admixture.
- Fst values indicate highly differentiated subpopulations, except for cluster 9.

Description of subpopulations

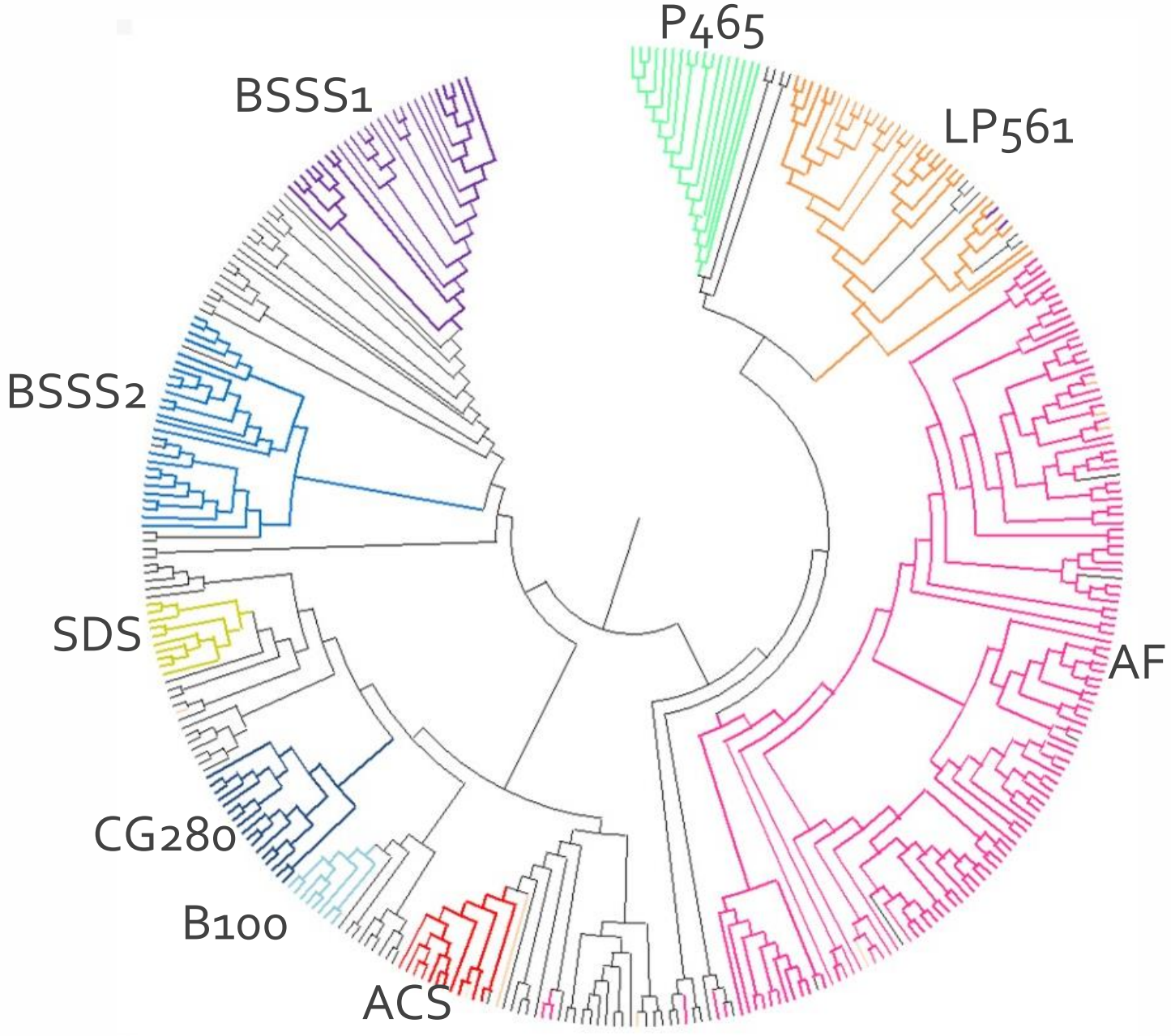
- **1-CG280**: 17 ILs, derived from Cargill280. Orange, SF.
- **2-SDS:** 10 ILs, semident synthetics and lines derived from Pioneer hybrids.
- **3-P465:** 15 ILs, derived from an Orange Flint landrace (P465).
- **4-AF**: 115 ILs, orange argentine flints of different origins.
- **5-B100:** 8 ILs, B100 and lines derived a cross of B100 and a commercial hybrid.
- **6-LP561:** 23 ILs, mainly derived from LP561, a red flint (R4).
- **7-BSSS_1**: 32 ILs, derived from a synthetic population (BSSS heterotic group) and other crosses of B110 and commercial hybrids.
- **8-BSSS_2**: 22 ILs, B73 and lines derived from commercial hybrids.
- **9-SCR:** 11 ILs, not clear origen, some red flints with short cycle.
- **10-ACS:** 12 ILs, Arg-Canada synthetics.

PCA


CP2 (3.6%) vs. CP1 (6.6%)
Data table:
PCA - Q - PCA
Marker by:
(Row Number)
Color by:
Q10
Group1
Group10
Group2
Group3
Group4
Group5
Group6
Group7
Group8
Group9
Mix
P465
LP612
LP2542
B73
CP2 (3.6%)
CP1 (6.6%)
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8

K=10

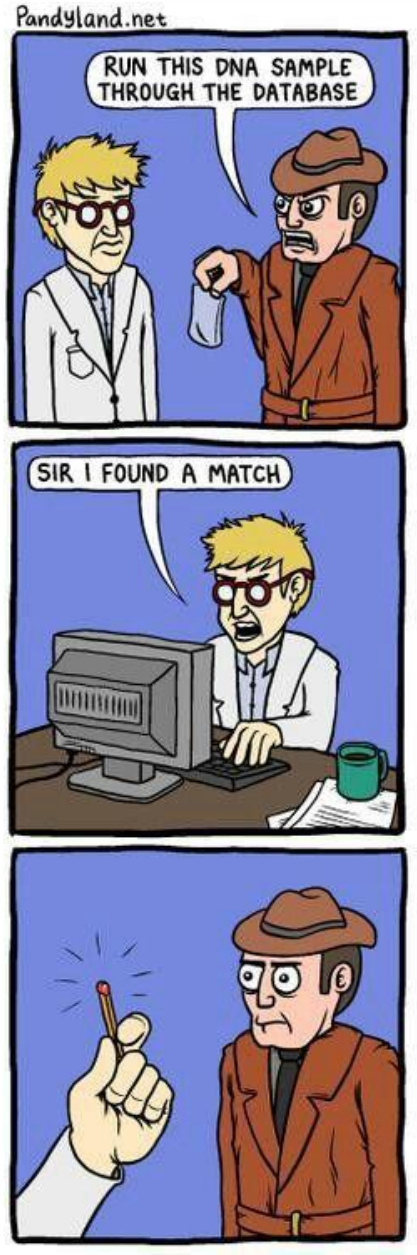
UPGMA



K=10

Conclusions – Final Remarks

- Implementation of Eib mid density SNP genotyping has allowed us to evaluate the genetic diversity, degree of LD and genetic structure of INTA's temperate IL panel.
- The panel exhibits high genetic diversity and a complex population structure. Most of the ILs are either not related or only weakly related to each other.
- These results constitute a first step towards a better understanding and reorganization of the existing germplasm which, in turn, will facilitate breeding decisions.
- This study also paves the way for marker-assisted backcrossing and developing genomic prediction models in the near future.



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