Ancient Grain Genomics to simplify gene discovery and deployment in wheat

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ANCIANT CIVILIZATIONS

EARLY TRADE ROUTS

EMPIRE AND EXPANSION

THE NEW WORLD

INDUSTRIAL REVOLUTION

PRESENT DAY

Wheat cultivation begins 10,000 years ago in the Fertile Crescent, paving the way for ancient civilizations.

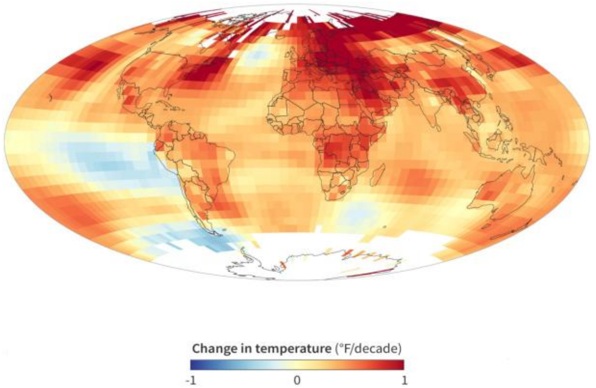
The Roman Empire, with its vast trade network, further expand wheat trade across Asia, North Africa and open the door to Europe.

New technologies during the Industrial Revolution enable mass production of wheat, and more efficiant transportation worldwide

Wheat is the most important crop in feeding the world and sustains billions

Source: https://www.sadotgroupinc.com/wheat-the-staple-crop-that-sustains-billions-worldwide

Global Wheat production faces serious threats from biotic and abiotic stresses and require continuous improvement




Gujhia Weevil (*Tanymecus indicus*)


Termite (*Macrotermes spp*)


Pink Stem Borer (*Sesamea inference*)


Aphid (*Opalsiphum maidis*)


Army Worm (*Mythimna separata*)


Shoot Fly (*Atherigona naqvii*)

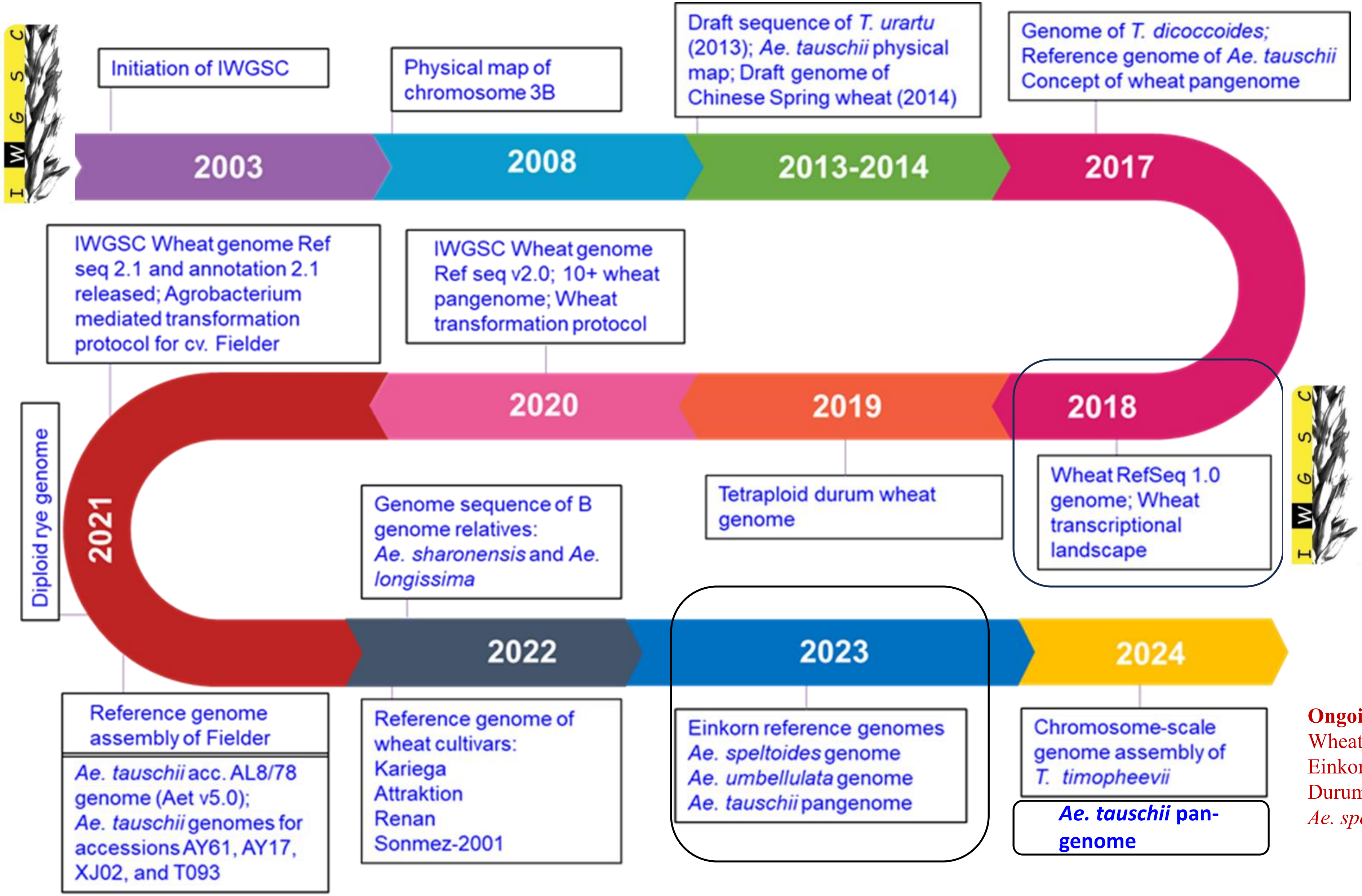
https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2023.1203721/full
https://fertiliserindia.com/major-insect-pests-of-wheat-crop/
https://fertiliserindia.com/major-insect-pests-of-wheat-crop/
https://smallgrains.wsu.edu/looking-ahead-wheat-diseases-2020-21/

Integrated and sustainable solution

Key pillars of wheat production in a circular economy. Original design by L. Muntean

https://www.intechopen.com/chapters/1212234

Advances in wheat genomics in the last 6-7 years



2003

Initiation of IWGSC

2008

Physical map of chromosome 3B

2013-2014

Draft sequence of *T. urartu* (2013); *Ae. tauschii* physical map; Draft genome of Chinese Spring wheat (2014)

2017

Genome of *T. dicoccoides*; Reference genome of *Ae. tauschii* Concept of wheat pangenome

2018

Wheat RefSeq 1.0 genome; Wheat transcriptional landscape

2019

Tetraploid durum wheat genome

2020

IWGSC Wheat genome Ref seq v2.0; 10+ wheat pangenome; Wheat transformation protocol

2021

IWGSC Wheat genome Ref seq 2.1 and annotation 2.1 released; Agrobacterium mediated transformation protocol for cv. Fielder

Diploid rye genome

Reference genome assembly of Fielder

Ae. tauschii acc. AL8/78 genome (Aet v5.0); *Ae. tauschii* genomes for accessions AY61, AY17, XJ02, and T093

2022

Genome sequence of B genome relatives: *Ae. sharonensis* and *Ae. longissima*

Reference genome of wheat cultivars:
Kariega
Attraktion
Renan
Sonmez-2001

2023

Einkorn reference genomes
Ae. speltoides genome
Ae. umbellulata genome
Ae. tauschii pangenome

2024

Chromosome-scale genome assembly of *T. timopheevii*

Ae. tauschii **pan-genome**

Ongoing projects
Wheat pangenome
Einkorn pangenome
Durum pangenome
Ae. speltoides pangenome

Tiwari et al., Trends in Genetics, 2024

Amazing progress in developing tools to manipulate genes and genomes

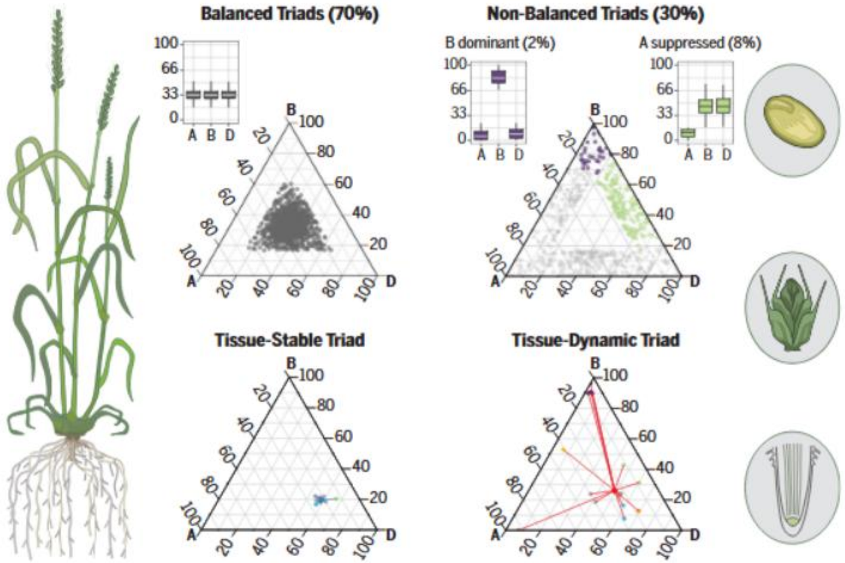
Credit: Google

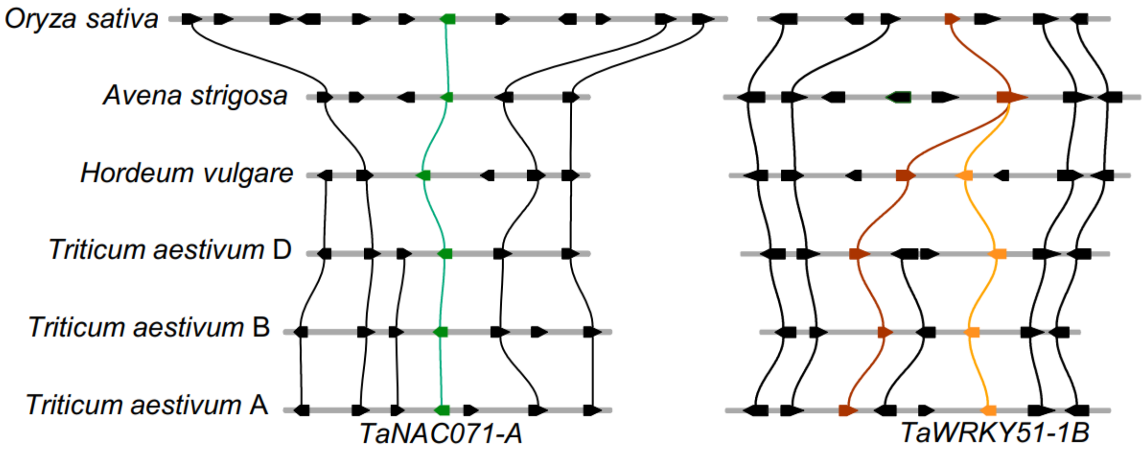
Limitations

Targets: Which genes to edit?

Gene discovery needs to be a fast- paced process

Genome complexity in wheat





Chen et al., 2025

Functional redundancies of the genes and alleles limit the pace of gene discovery in polyploid wheat

https://link.springer.com/article/10.1007/s42994-023-00131-7

Options available in the wheat gene pool

- *Aegilops tauschii (D-genome donor)*
  - Being used extensively in wheat improvement
- *Aegilops speltoides (closest to the B-genome donor)*
  - Being used extensively in wheat improvement
- *Triticum urartu (A-genome donor of wheat)*

Wild species

- *Triticum monococcum*
  - *T. monococcum ssp monococcum (domesticated)*
  - *T. monococcum ssp aegilopoides (wild)*

Einkorn wheat Provides unique advantages due to its cultivated background and common evolutionary history between its wild and domesticated forms

Ancient Grains

Ancient grains are whole grains and seeds that have remained largely unchanged for centuries, offering rich nutrients and unique flavors

[Einkorn](#)
Emmer/Farro
Kamut
Spelt
Sorghum
Teff
Millet
Quinoa
Amaranth
Black barely
Red and black rice
Blue corn
Buckwheat
Wild rice

https://dhwblog.dukehealth.org/ancient-grains-what-are-they-and-why-should-we-eat-them/

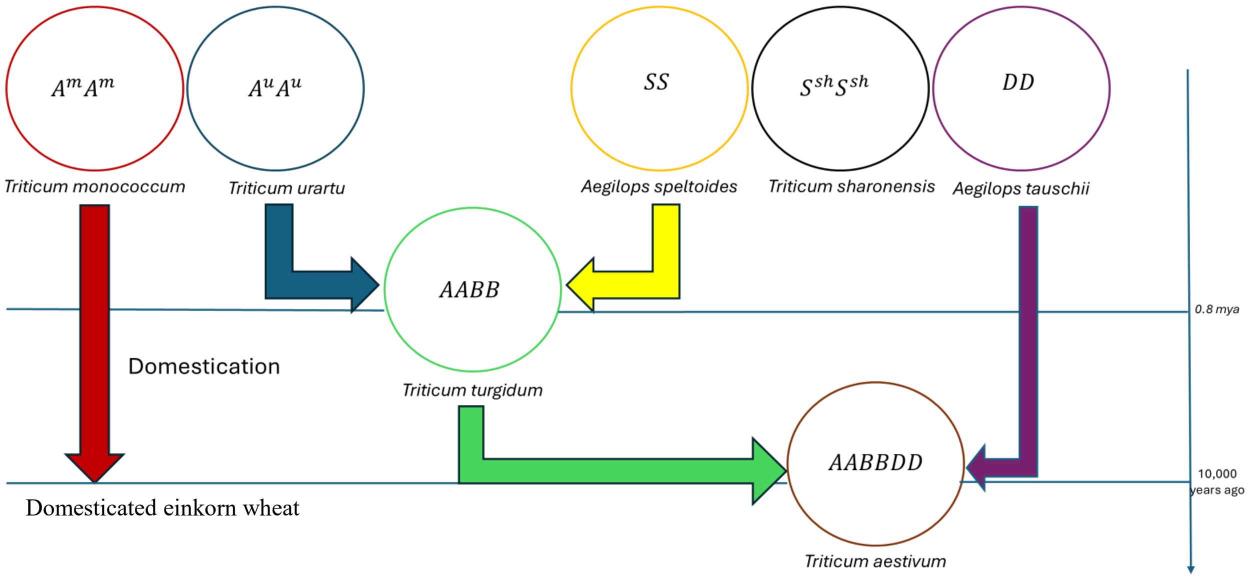
Einkorn Wheat: *Triticum monococcum*

$2n=2x=14$;
Genome size ~5.0 Gb

- Ancient and pure: Einkorn is the original form of wheat, which has never been hybridized or genetically modified
- Nutritional value: It is higher in protein, vitamins, and minerals than modern wheat
- Flavor and texture: Einkorn has a rich, nutty, and buttery flavor and a silky texture
- Gluten content: It has weaker gluten than modern wheat, which can make it easier to digest

Evolution and domestication

- Einkorn is one of the oldest cultivated forms of wheat, dating back over 10,000 years
- Its name means “single grain,” referring to the single kernel per spikelet characteristic
- Close to the A-genome donor of the bread wheat



Paulson, 2025; IWGSC, 2018

Einkorn wheat is a cornerstone of crop domestication

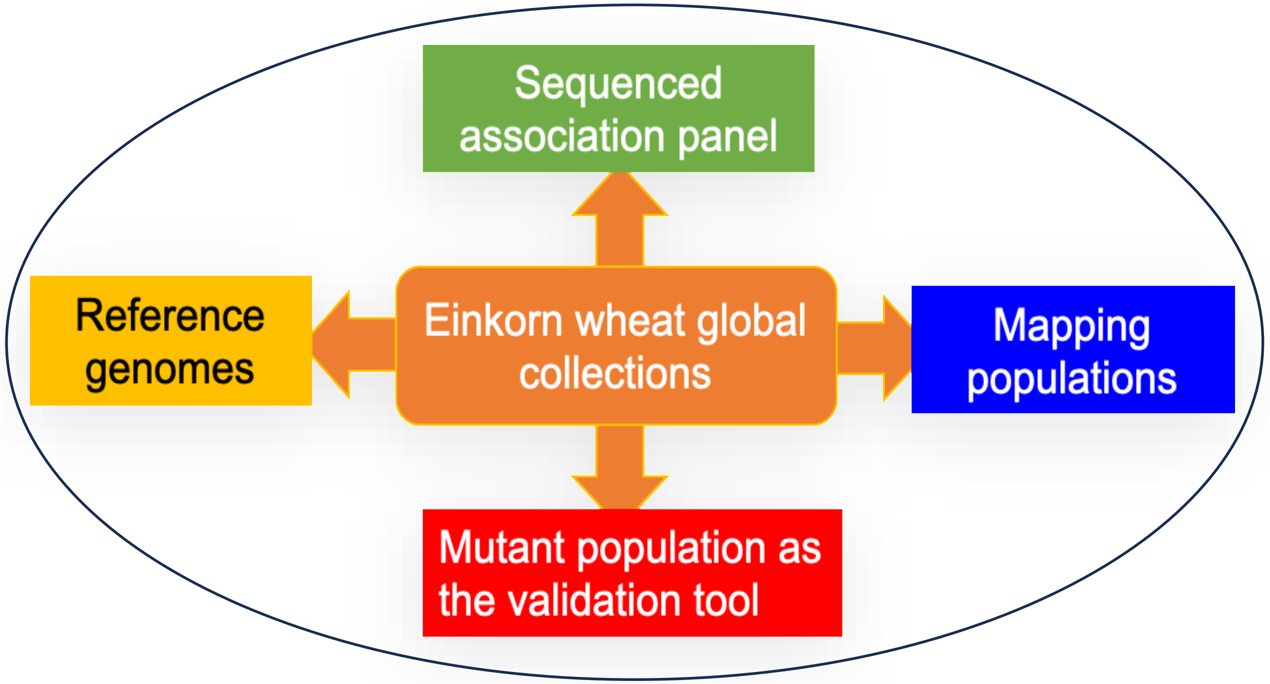
Göbekli Tepe; *Credit: Vincent J. Musi*

Some of the earliest evidence for plant domestication comes from einkorn wheat grains recovered from a site near Göbekli Tepe that are subtly different in shape and genetics from wild varieties.

At Göbekli Tepe grains look wild, suggesting that domestication hadn’t taken place or was in its earliest stages.

Einkorn wheat was domesticated in what is now Turkey (on the northern edge of the Fertile Crescent) from wild einkorn wheat (Triticum monococcum subspecies aegilopoides), approximately 10,000 years ago1.

Einkorn wheat present a simple and elegant system for gene discovery in wheat



Sequenced association panel

Reference genomes

Einkorn wheat global collections

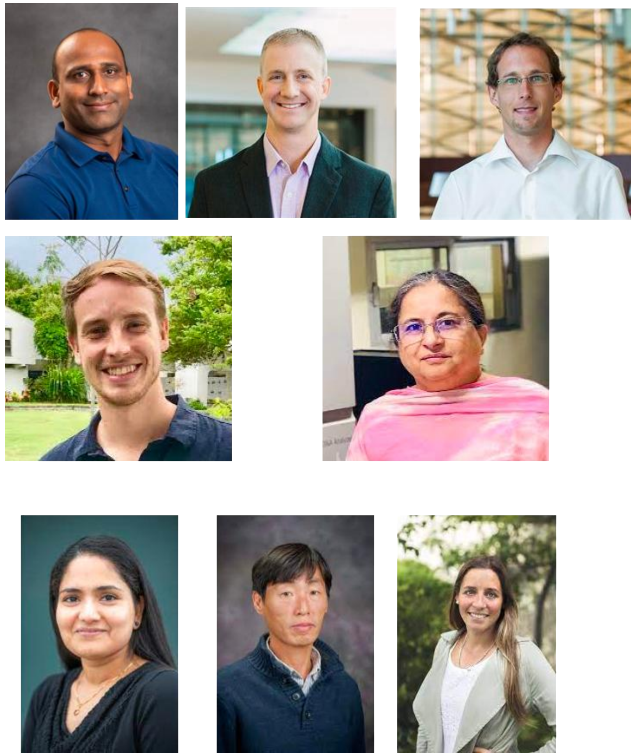
Mapping populations

Mutant population as the validation tool

- ✓Spring wheat habit
- ✓ Up to four generations in a year
- ✓ Simple diploid genome
- ✓ Sequenced GWAS panel
- ✓ Mutant populations
- ✓ Transformation amenable

PLANT BREEDING PARTNERSHIP: Accelerating Genomics Assisted Wheat Improvement by Utilizing Genetic Diversity of the Ancient Einkorn Wheat

Vijay Tiwari	UMD (PI)
Jesse Poland	KAUST
Simon Krattinger	KAUST
Parveen Chhuneja	PAU, India
Paula Silva	INIA, Uruguay
Nidhi Rawat	UMD
Dal-Hoe Koo	WGRC,K-State
Grey Monroe	UC Davis

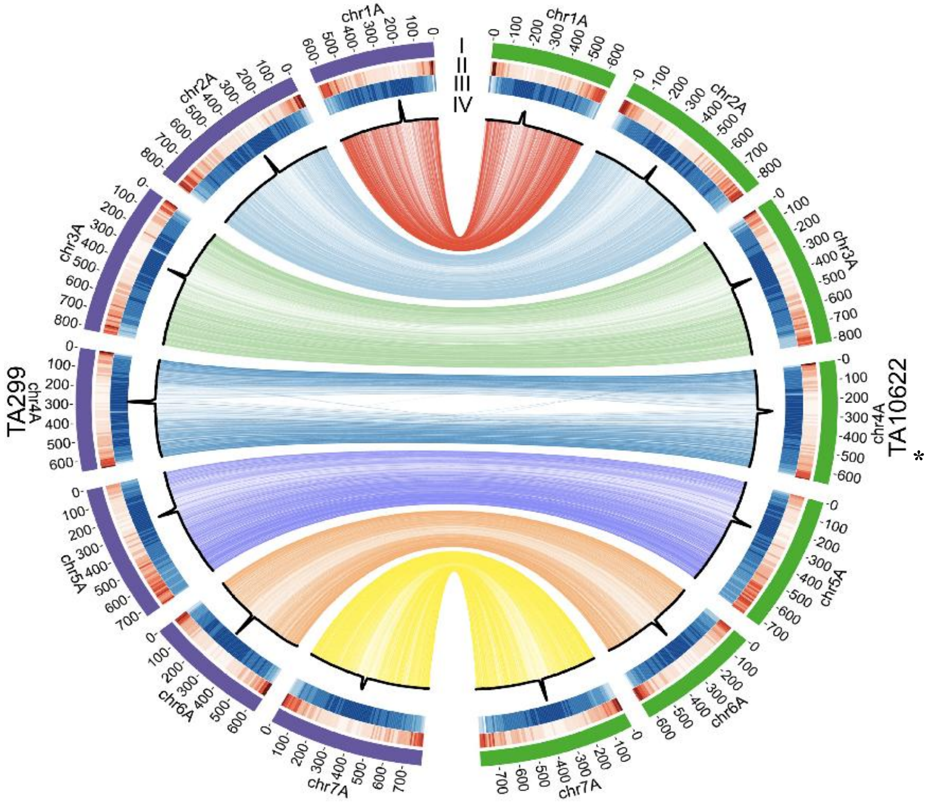


Key Collaborators

Beat Keller,
Javier Sanchez
James Kolmar
Matthew Rouse
Xianming Chen
Paul Nicholsan
John Raupp
Jorge Dubcovsky

Objective 1: Generate high-quality assemblies of selected einkorn accessions
Objective 2: Whole-genome sequencing of Tm-GWAS panel
Objective 3: Phenotying & GWAS to identify important genes and alleles for wheat improvement
Objective 4: Develop, characterize, and deploy introgressions from *T. monococcum in wheat*

Einkorn genome and applications: Enriching genomic resources for einkorn wheat

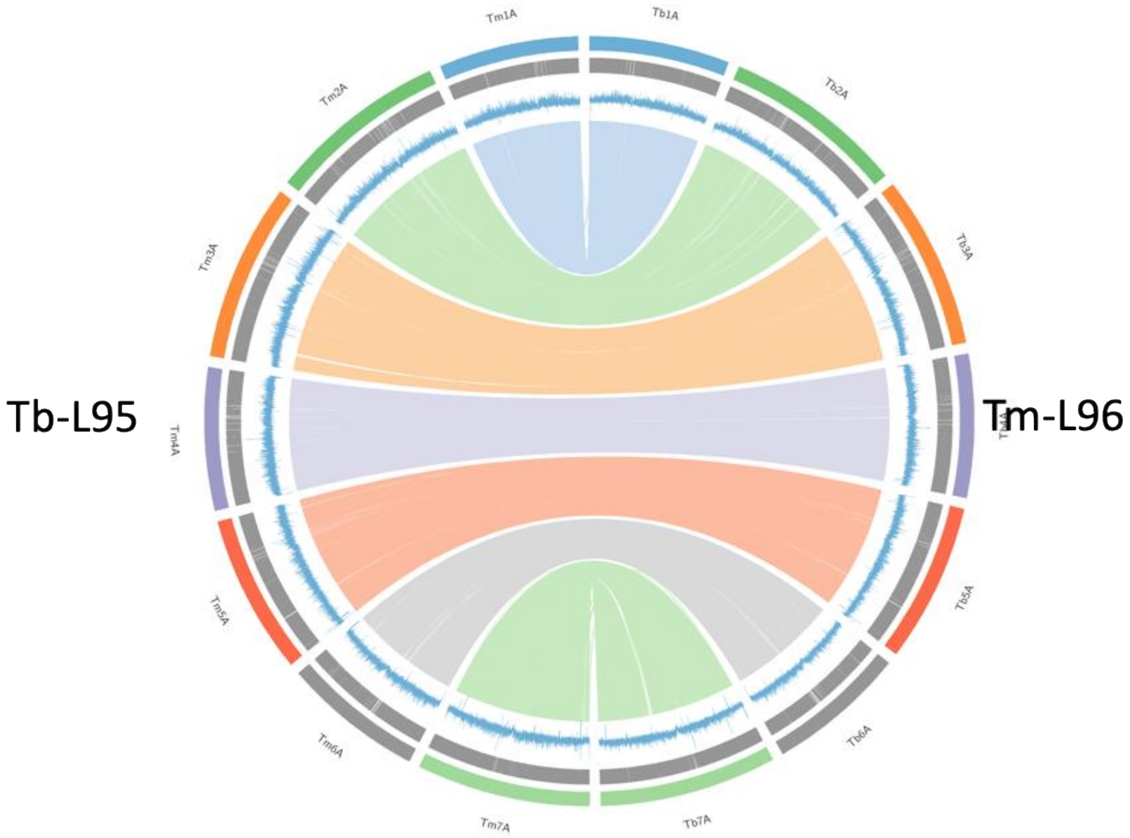


*Hanin Ahmed

*Matthias Heuberger

*Adam Schoen

We assembled two more einkorn genomes

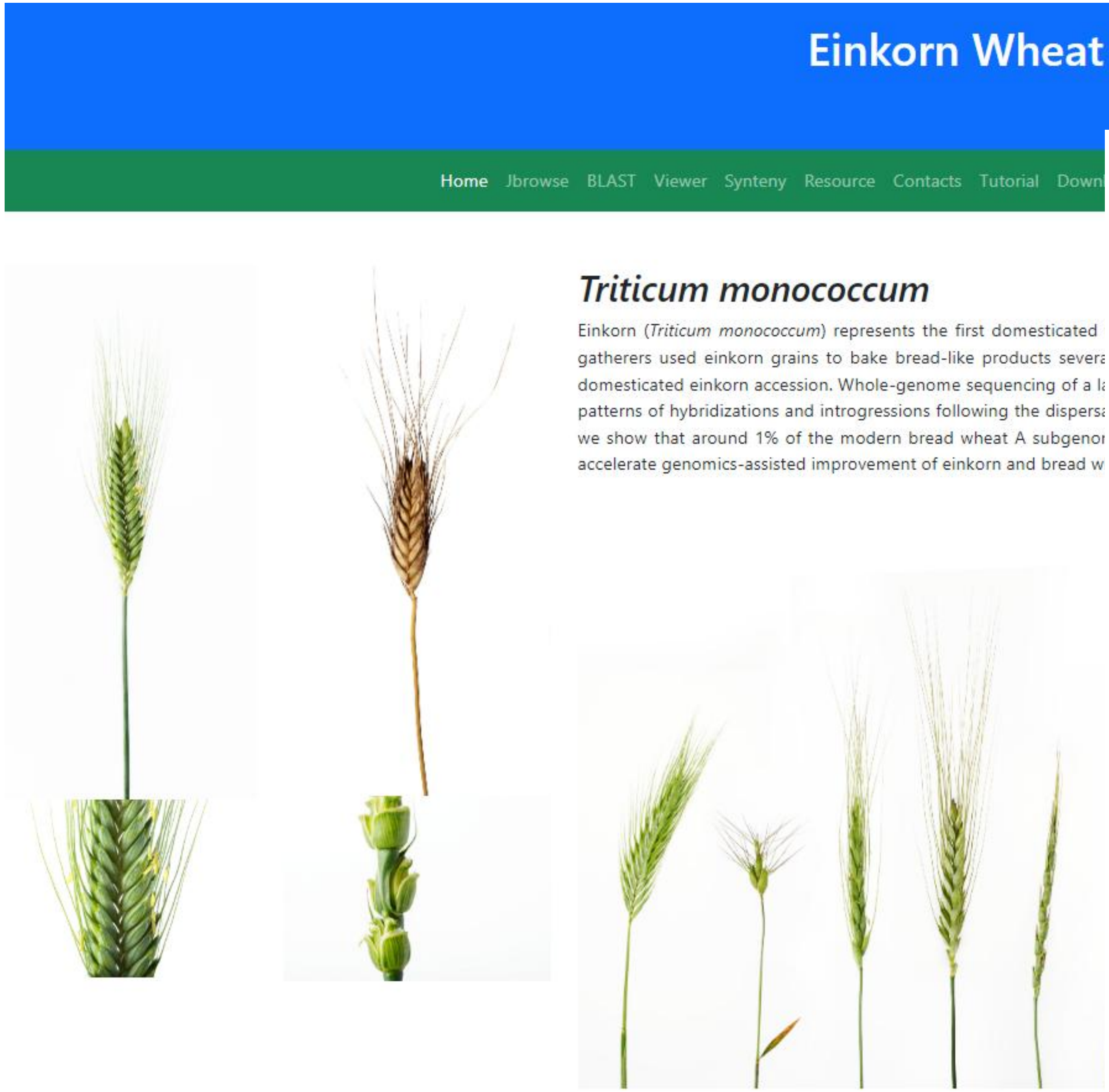


Yadav et al., unpublished results

Best reference assembly in Triticeae

First insight about centromeric evolution in monocots

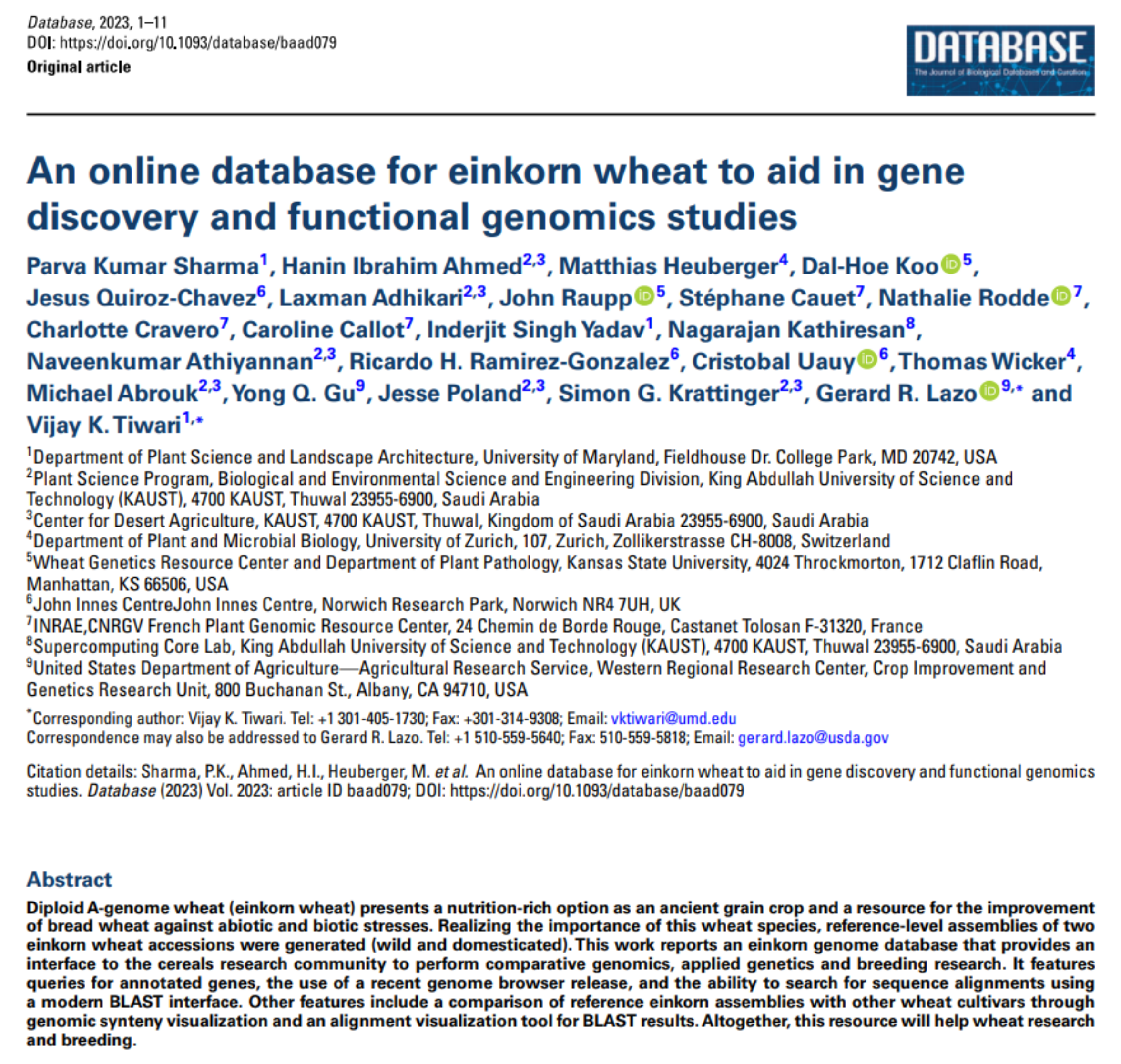
Hanin*, Heuberger*, Schoen* et al; Nature



Einkorn Wheat Resource Database

Triticum monococcum

Einkorn (*Triticum monococcum*) represents the first domesticated
gatherers used einkorn grains to bake bread-like products severa
domesticated einkorn accession. Whole-genome sequencing of a la
patterns of hybridizations and introgressions following the dispersa
we show that around 1% of the modern bread wheat A subgenor
accelerate genomics-assisted improvement of einkorn and bread w



Database, 2023, 1–11
DOI: https://doi.org/10.1093/database/baad079
Original article

An online database for einkorn wheat to aid in gene discovery and functional genomics studies

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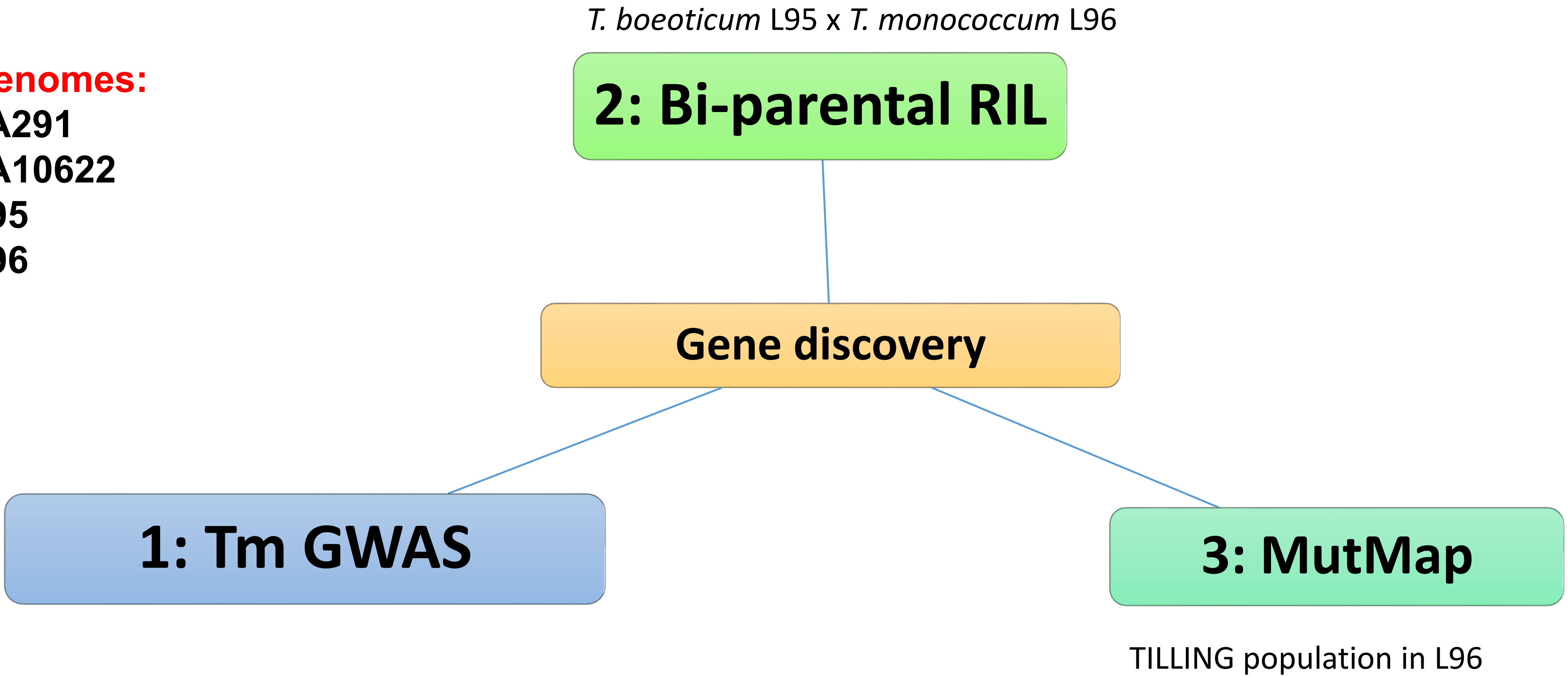
Abstract

Diploid A-genome wheat (einkorn wheat) presents a nutrition-rich option as an ancient grain crop and a resource for the improvement of bread wheat against abiotic and biotic stresses. Realizing the importance of this wheat species, reference-level assemblies of two einkorn wheat accessions were generated (wild and domesticated). This work reports an einkorn genome database that provides an interface to the cereals research community to perform comparative genomics, applied genetics and breeding research. It features queries for annotated genes, the use of a recent genome browser release, and the ability to search for sequence alignments using a modern BLAST interface. Other features include a comparison of reference einkorn assemblies with other wheat cultivars through genomic synteny visualization and an alignment visualization tool for BLAST results. Altogether, this resource will help wheat research and breeding.

Gene Discovery: Three independent approaches

Genomes:
TA291
TA10622
L95
L96



T. boeoticum L95 x *T. monococcum* L96

2: Bi-parental RIL

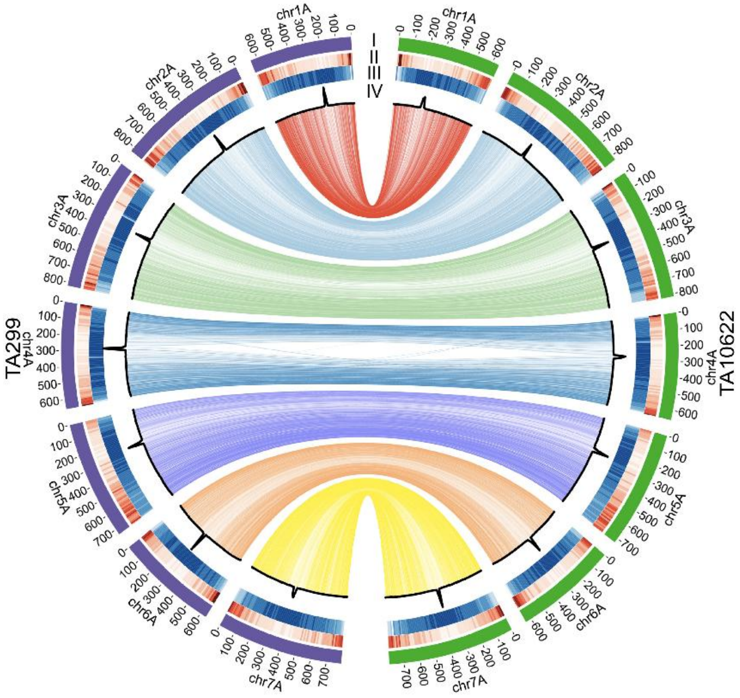
Gene discovery

1: Tm GWAS

3: MutMap

TILLING population in L96

The first einkorn genomes



	Scaffolds		
	CS RefSeq v.1.0	Einkorn TA299	Einkorn TA10622
N50-length (Mb)	7.01	640.55	522.92
Maximal Length (Mb)	45.79	823.64	800.70
Gaps (%)	1.80	0.02	0.10

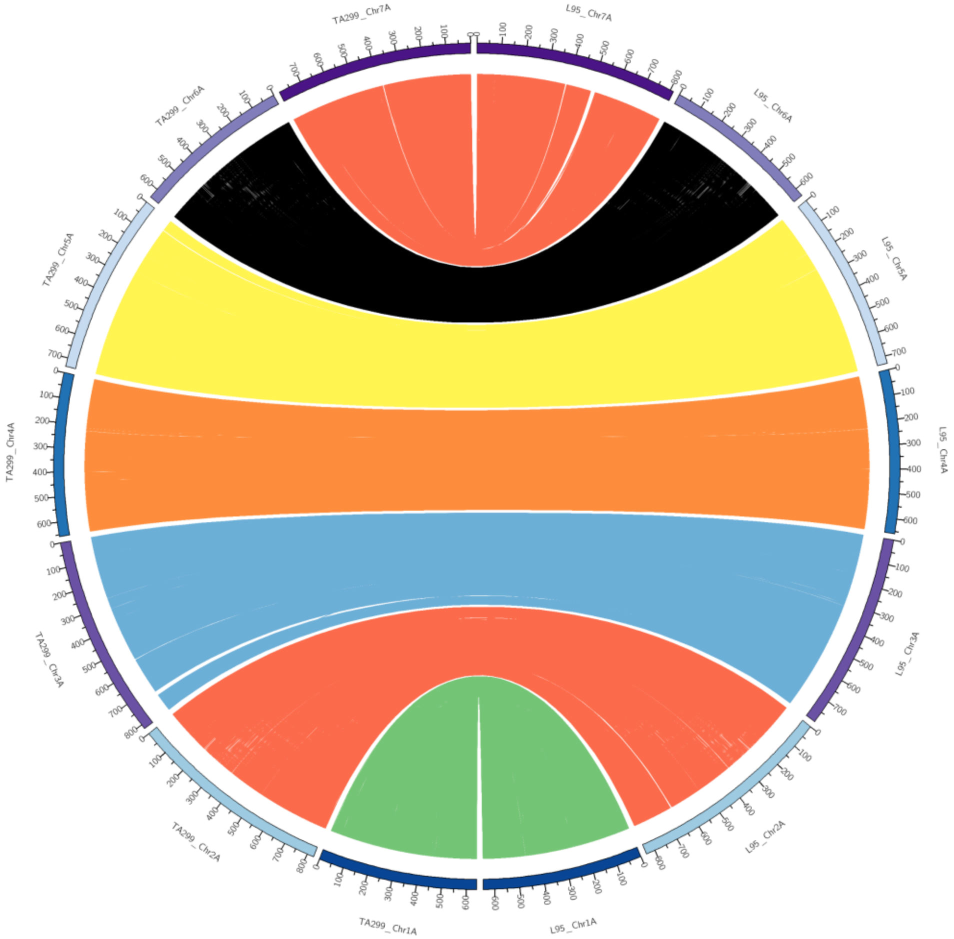

*Hanin Ahmed


*Matthias Heuberger

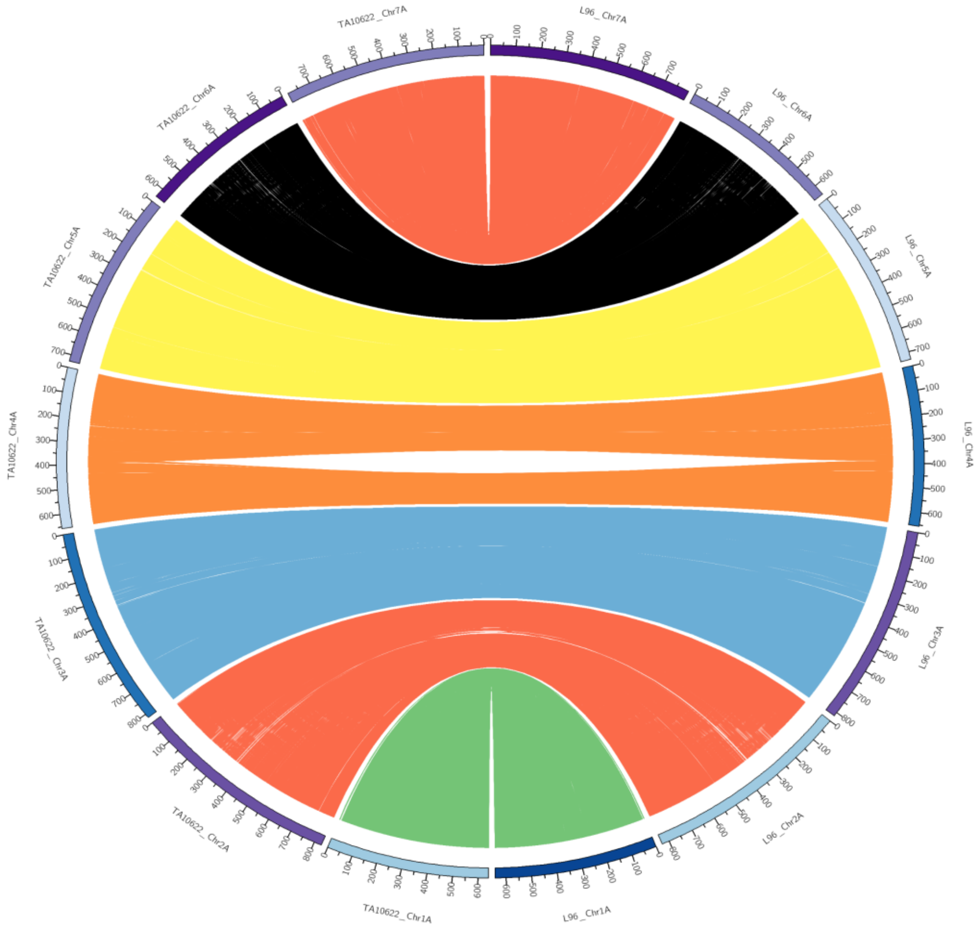

*Adam Schoen

Hanin*, Heuberger*, Schoen* et al; Nature, 2023

Additional two fully annotated genomes



Wild einkorn acc: L95 vs TA299



Domesticated einkorn acc. L96 vs TA10622

Sequence Indexed RIL population

Wild einkorn L95 x Domesticated einkorn L96

F1

F2

F3

⋮

812 RILs **Skim sequenced**

Parental lines show contrasting phenotype for several diseases, and agronomically important traits

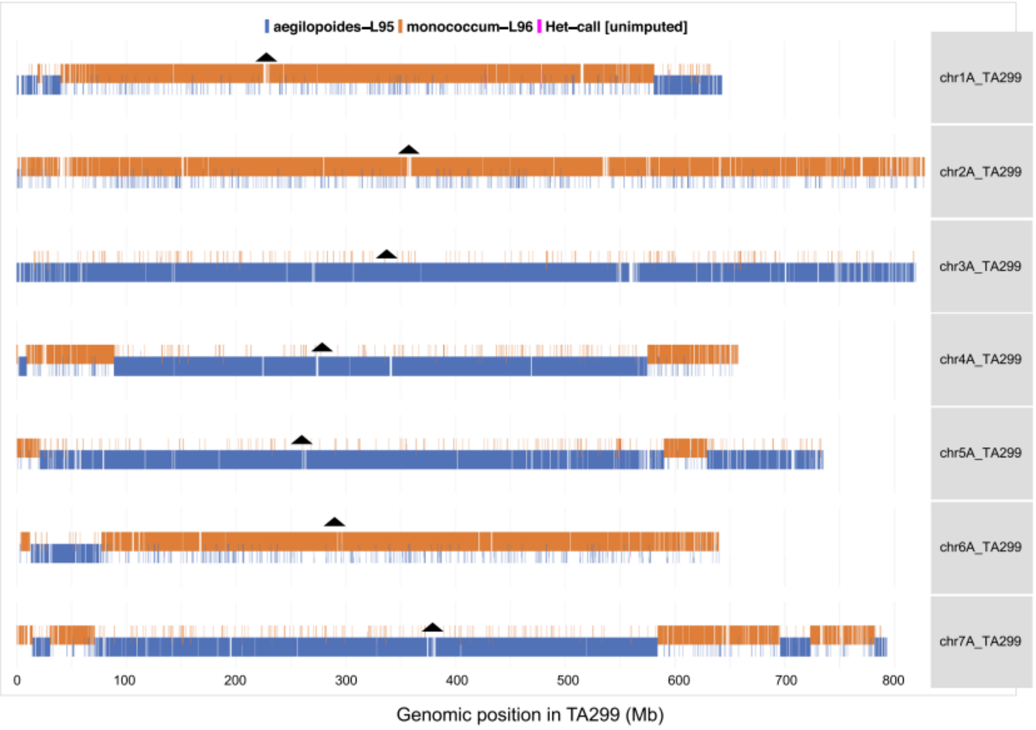
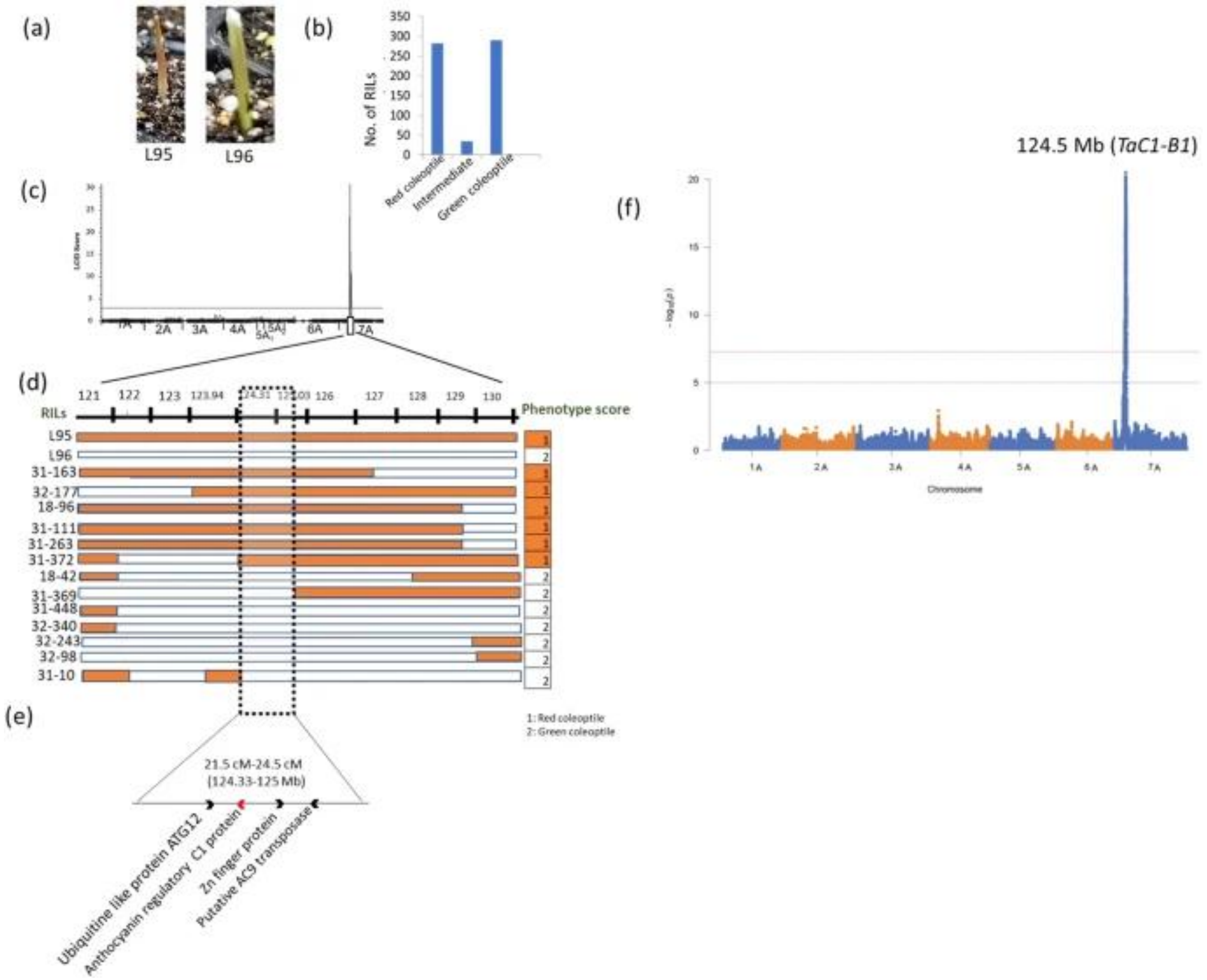


Fig. 2 A recombinant inbred line (2013-31-153) showing distribution of wild (L95) and cultivated (L96) parental alleles. The orange bars are individual variant calls matching parent L96, in blue for matching parent L95 and a few magenta bars represent the heterozygous. The black triangle displays the centromeric positions in each of the TA299 chromosomes.

- ✓ 15,919 crossover breakpoints delimited to a median and average interval of 114 Kbp and 219 Kbp, respectively
- ✓ High-resolution mapping resource enables us to perform fine-scale mapping of targeted traits

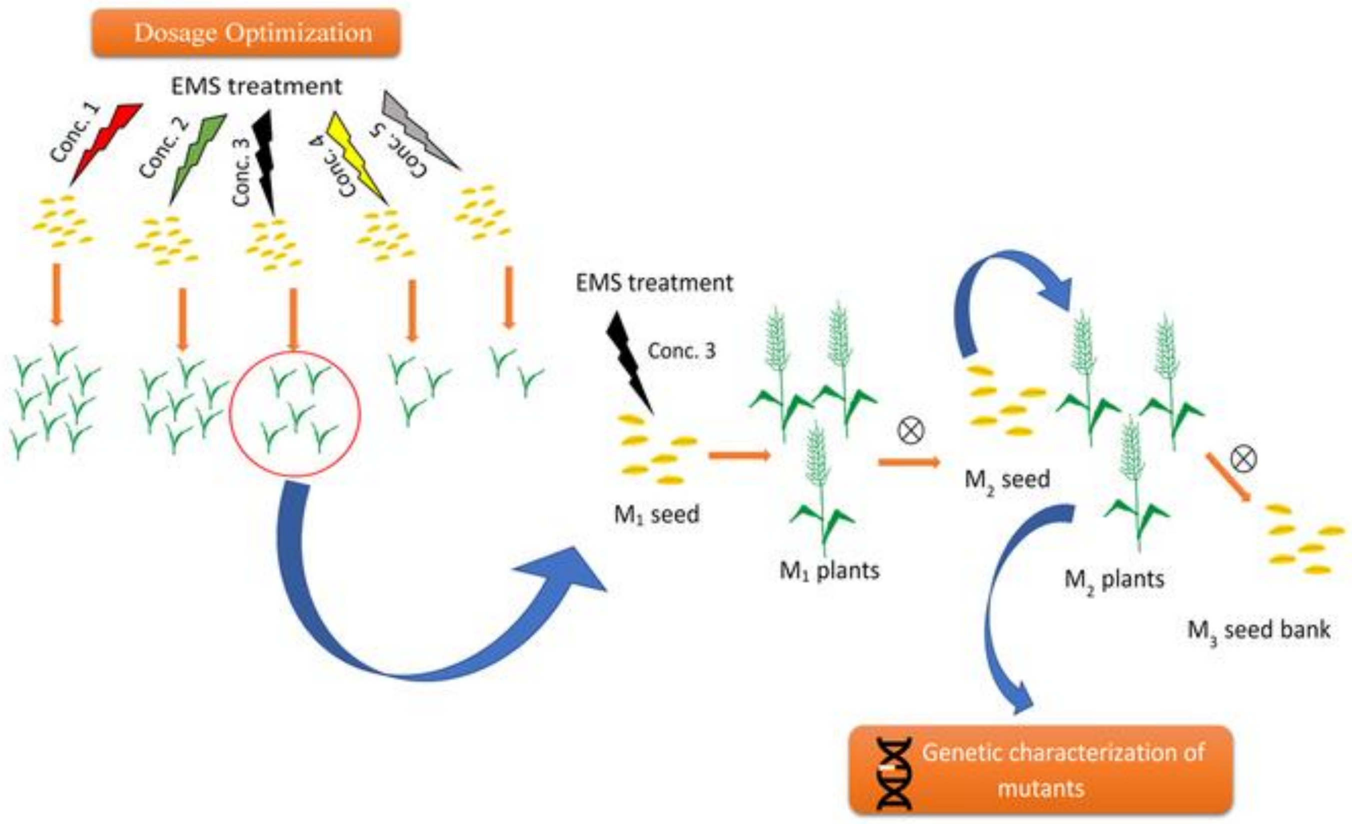
Trait mapping using sequence indexed RIL population

- ✓ We used coleoptile color to test the mapping potential of the mapping panel
- ✓ Genetic mapping delineated the physical region to a narrow 670Kb interval with a mapping resolution of ~225 kb
- ✓ With 4 candidate genes
- ✓ Candidate gene was validated



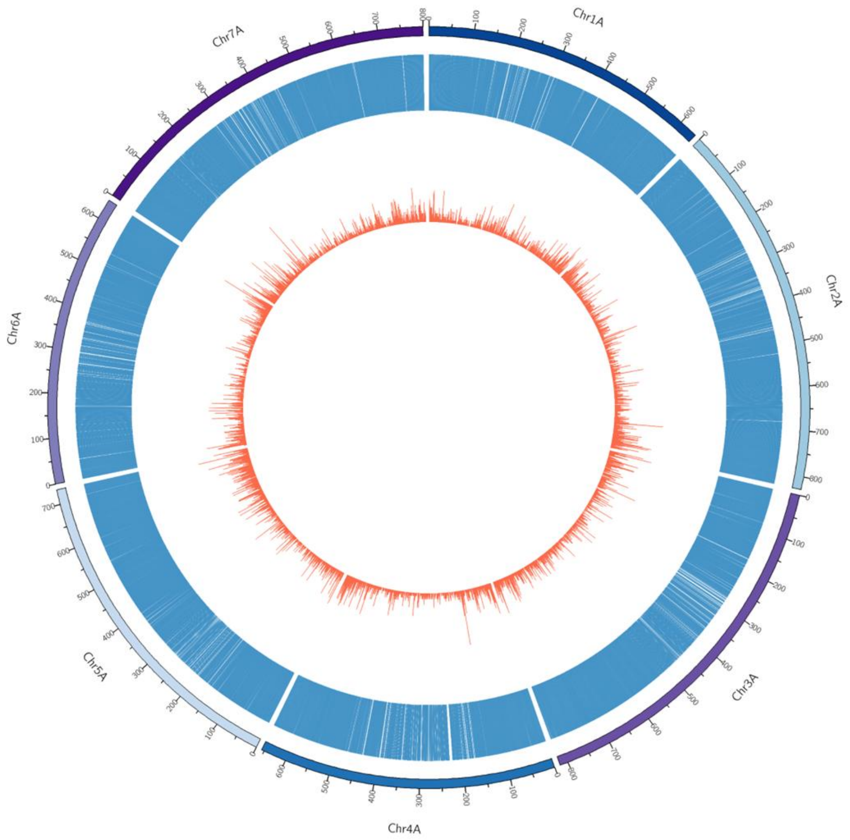
Einkorn TILLING Population

- 5000 M2 plants
- Mutation frequency 1/100Kb
- Sequencing of this useful resource will fast-track gene discovery and validation in wheat
- **Fix a missing link between 2x, 4x, and 6x sequenced mutant populations**



Whole genome sequencing of some Einkorn EMS mutants

12 randomly selected M_2 plants from the einkorn TILLING population were using WGS (10x)



Types of EMS mutations	Number of SNPs	%
Total number of G/A or C/T SNPs / plant	58,000	-
Total number of genic SNPs/ per plant	1103	1.9
Total number of intergenic SNPs per plant	56,897	98.1

WGS of TILLING population can build an exciting resource:

1100 Genic mutations per plant

WGS of 2000 independent M2 plants = 1100 x 2000 genic mutations

Number of genes in diploid einkorn wheat=32,000

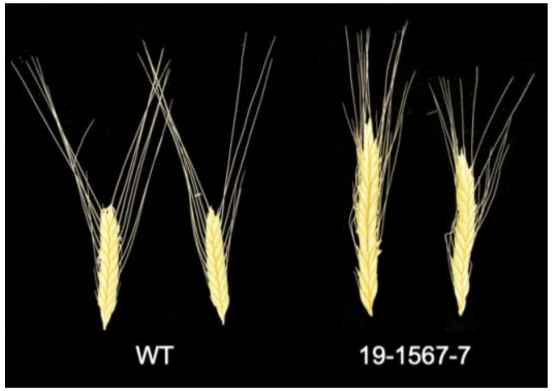
About 70 mutations per gene

Genome-wide positions of identified mutations in the sequenced individuals based on einkorn genome L96. The blue track indicates gene density (based on Einkorn genome L96.v1), the orange track indicates EMS mutation density, and the blue track indicates gene distribution and outer track represents einkorn wheat chromosomes.

Induced variations in einkorn

- ✓ ***Positional cloning of tin3*** **was a successful case but can we routinely use MutMap approach to identify causal SNPs**
- ✓ **How long it will take to get to a gene?**

Spikelet Number



Grain Size

Early Flowering

Late Flowering

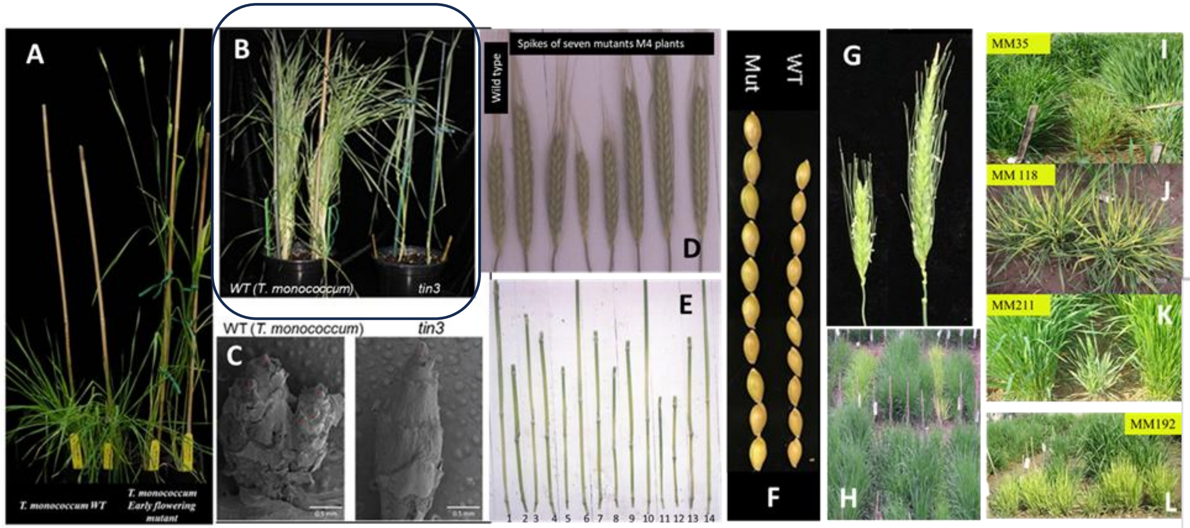
Dwarf and High Tiller

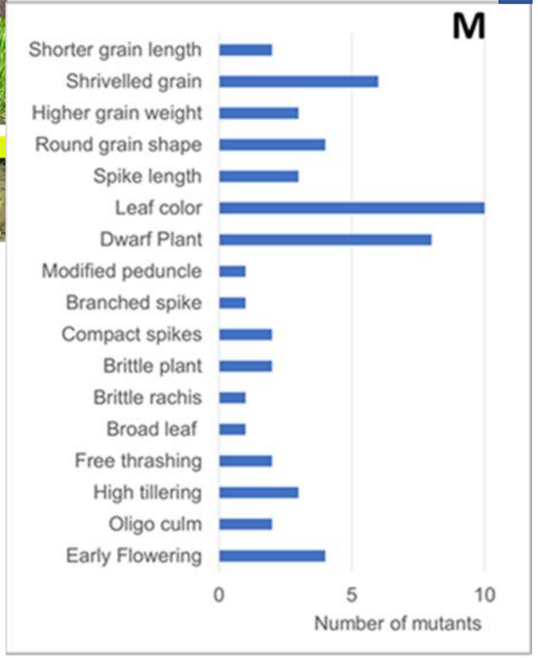
Dwarf

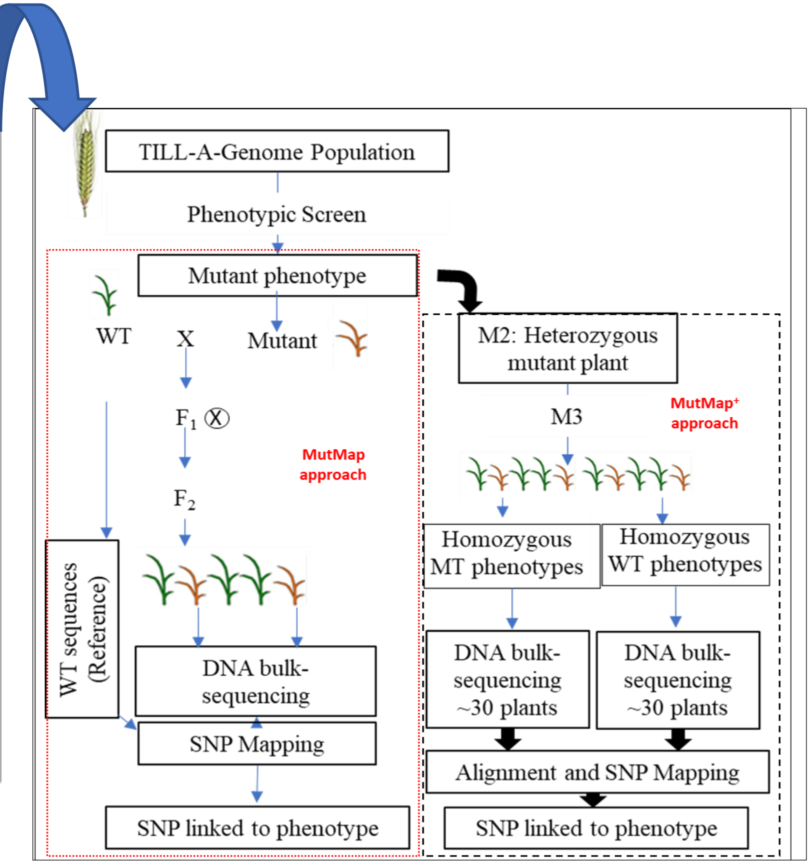
Free Threshing

Forward Genetics Approach for Gene Discovery

More than 80 phenotypic mutants identified from the forward genetics screen of a subset of Einkorn TILLING population







TILL-A-Genome Population

Phenotypic Screen

Mutant phenotype

WT

X

Mutant

F_1 ⊗

F_2

MutMap approach

WT sequences (Reference)

DNA bulk-sequencing

SNP Mapping

SNP linked to phenotype

M2: Heterozygous mutant plant

M3

MutMap⁺ approach

Homozygous MT phenotypes

Homozygous WT phenotypes

DNA bulk-sequencing ~30 plants

DNA bulk-sequencing ~30 plants

Alignment and SNP Mapping

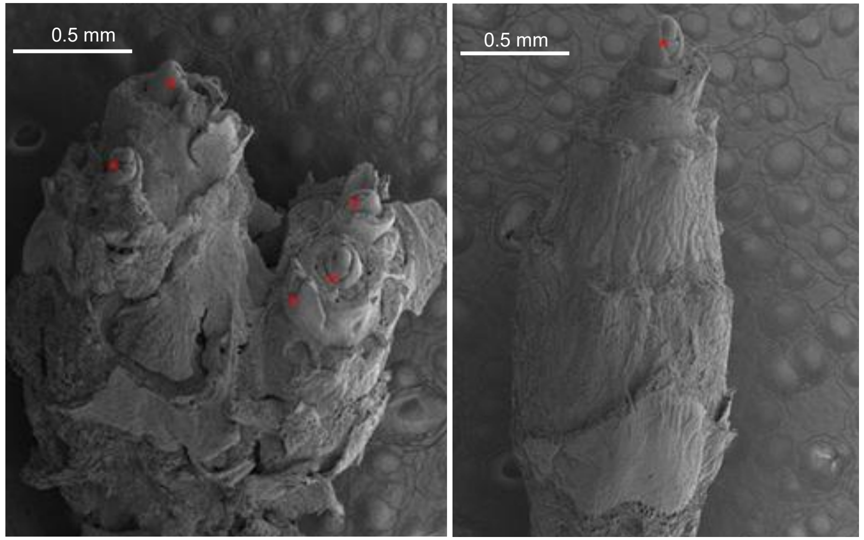
SNP linked to phenotype

Adapted from Abe et al., 2012

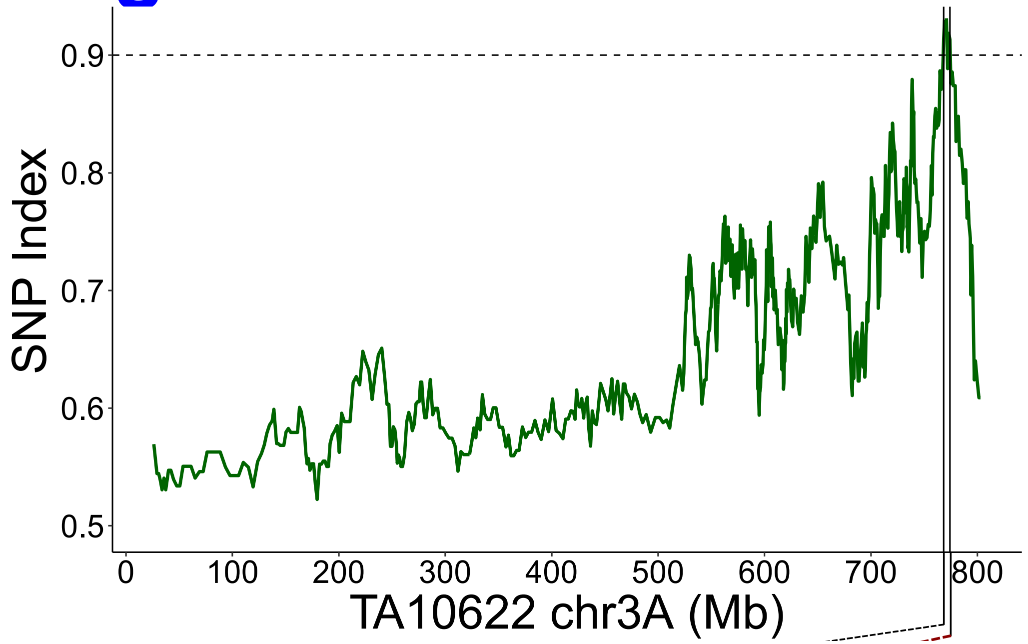
*Adam Schoen

An example: Cloning of *tin3 locus*

T. monococcum (wt) *T. monococcum (tin3)*



T. monococcum (wt) **X** *T. monococcum (tin3)*

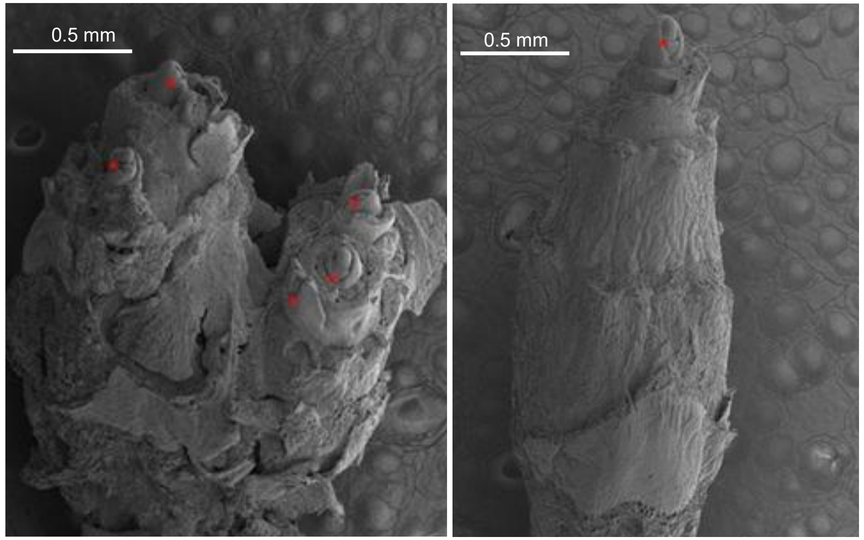


Kuraparthy et al., 2007

Cloning of *tin3* using high-quality *T. mono* assembly

*Adam Schoen

T. monococcum (wt) *T. monococcum (tin3)*



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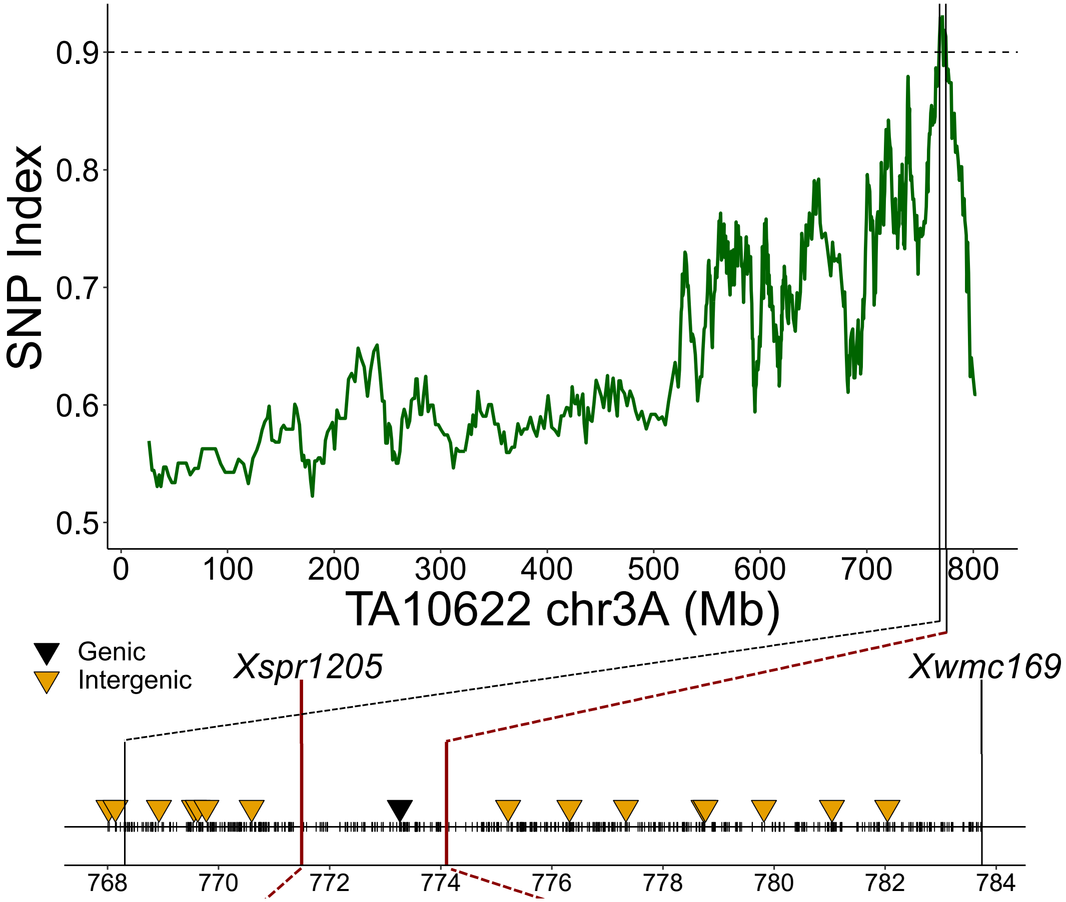

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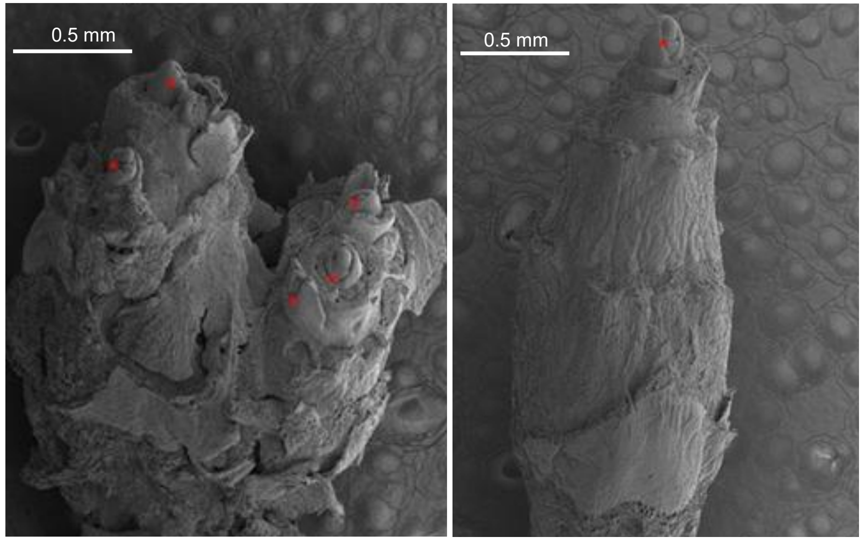
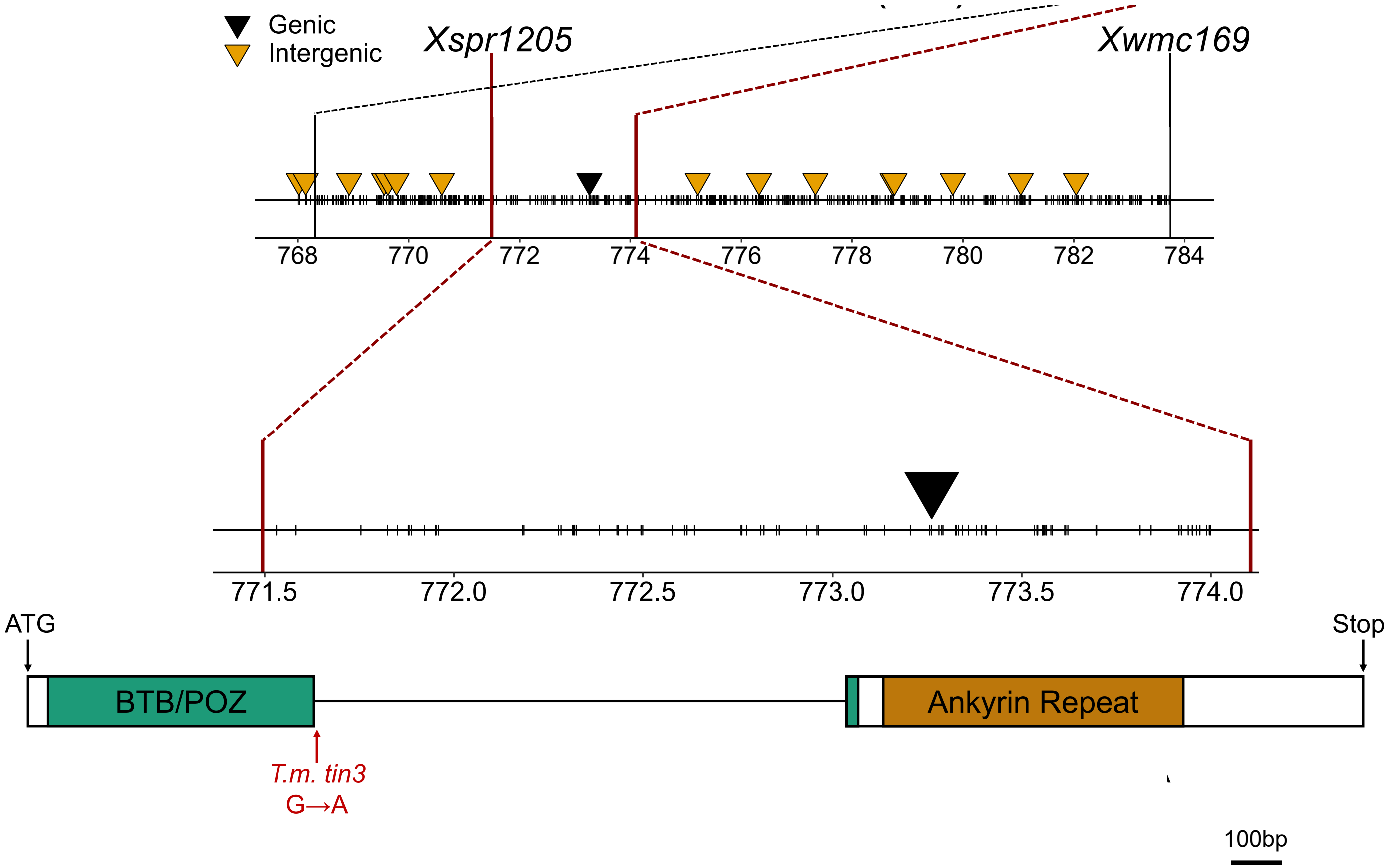


T. monococcum (wt) **X** *T. monococcum (tin3)*



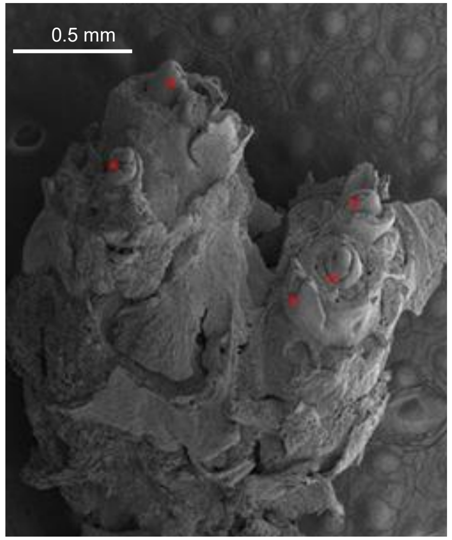
Ahmed*, Schoen*, Heuberger* et al., *Nature* 2023

*Adam Schoen

Cloning of *tin3* using high-quality *T. mono* assembly

T. monococcum (wt)

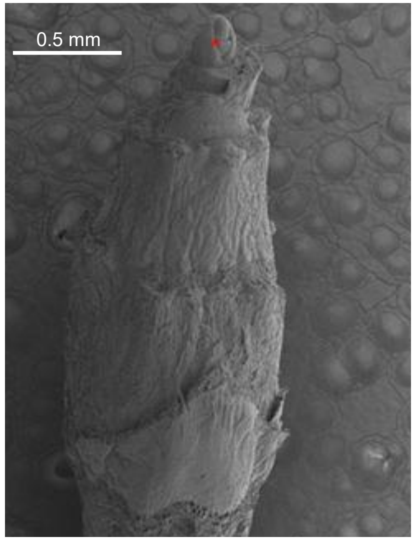
T. monococcum (tin3)



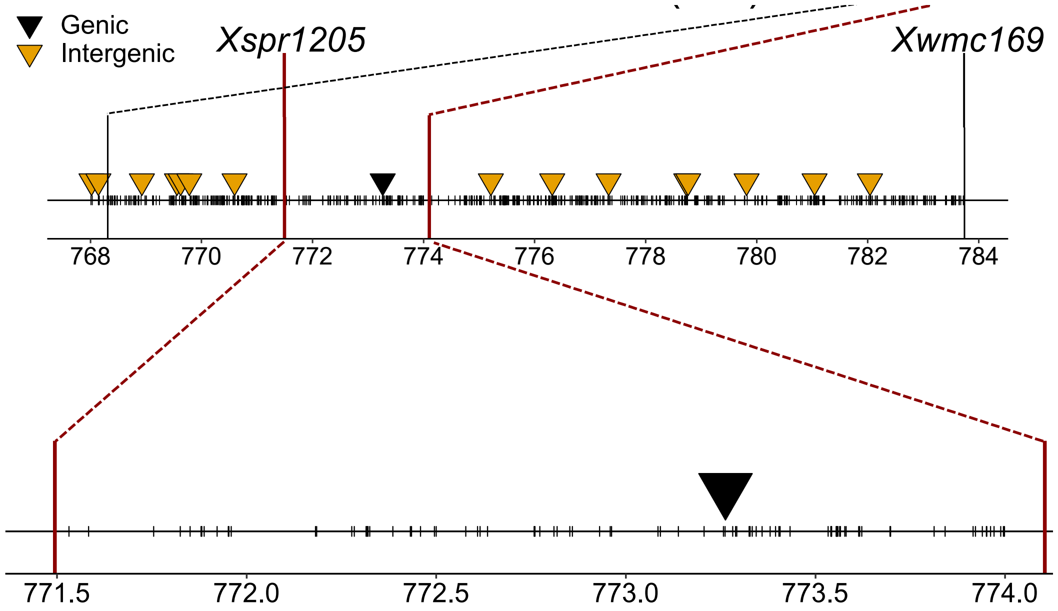
T. monococcum (wt)

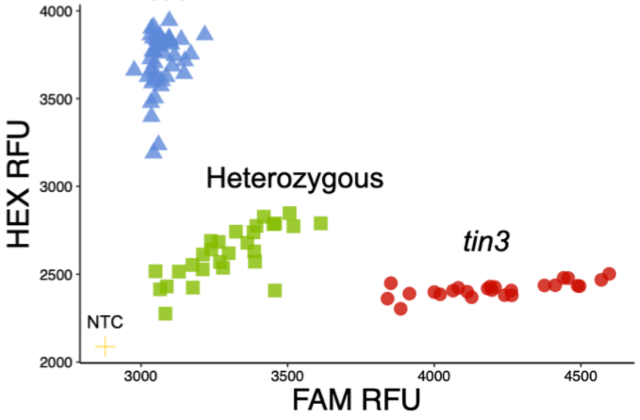
X

🟩



T. monococcum (tin3)

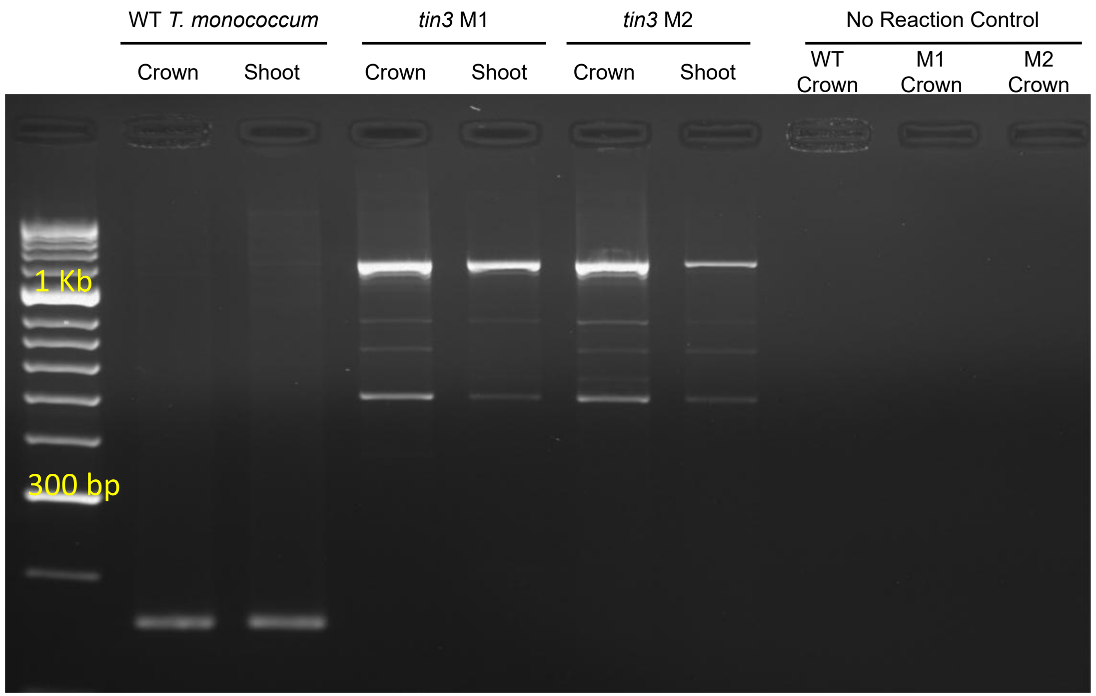


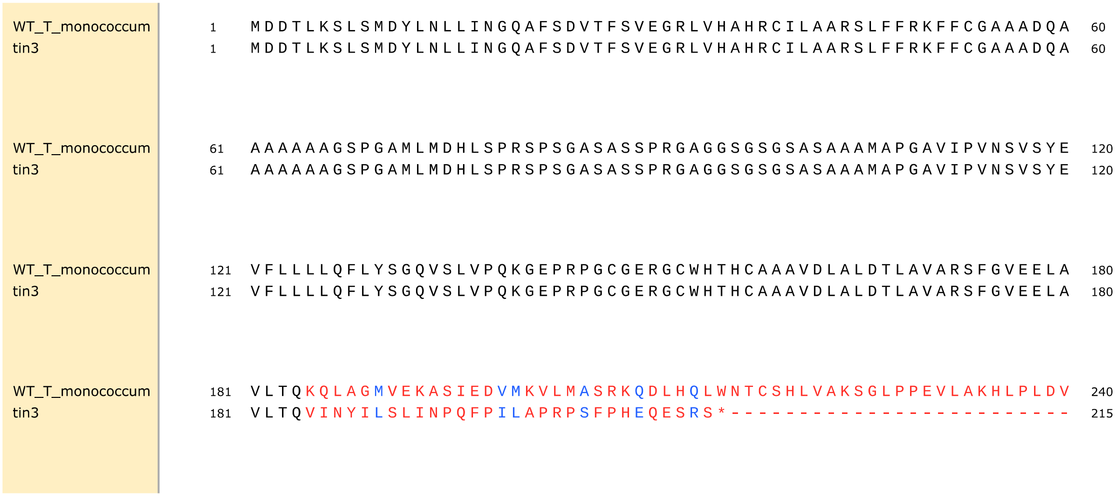


Ahmed*, Schoen*, Heuberger* et al., *Nature* 2023

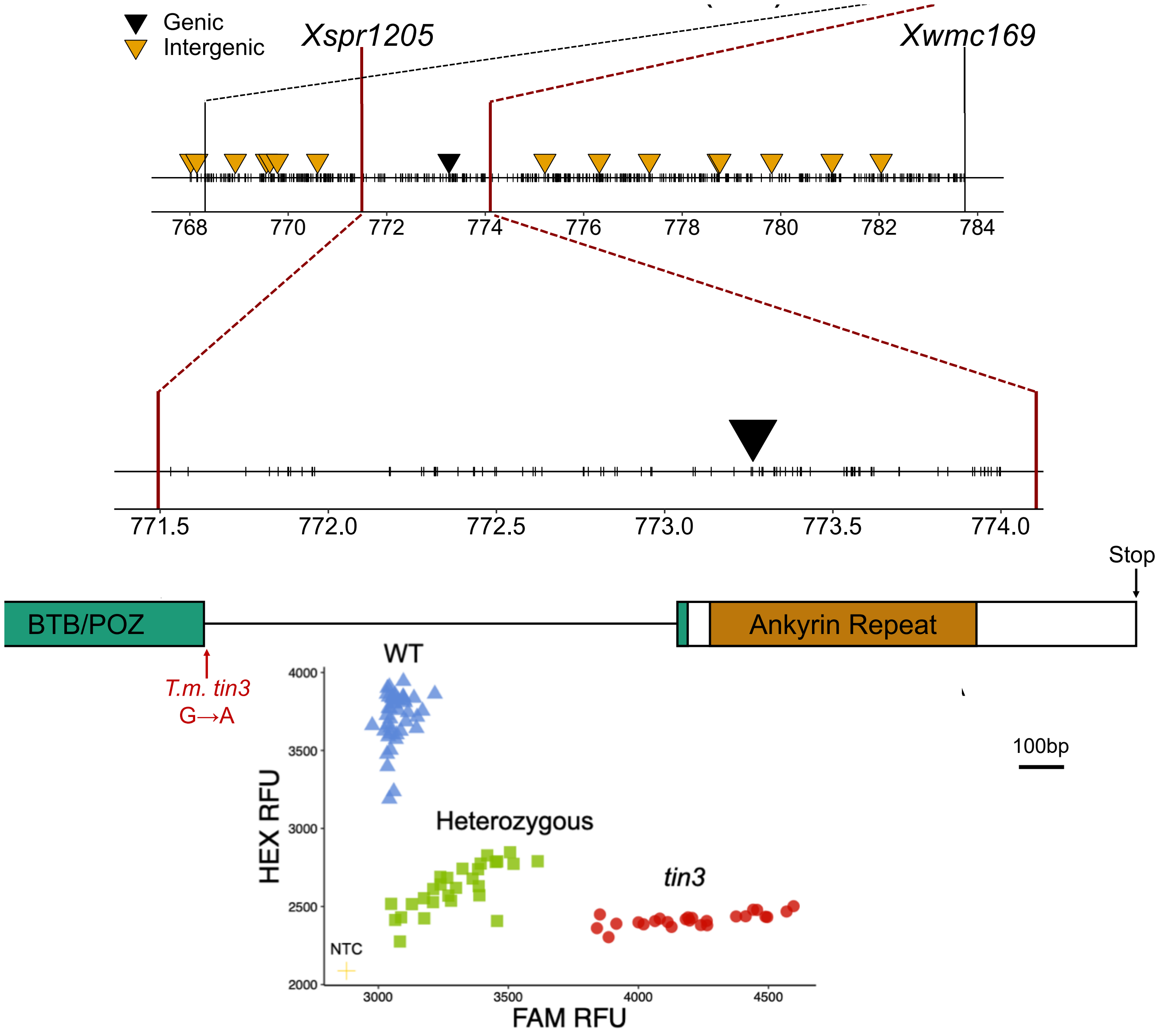
*Adam Schoen

Cloning of *tin3* using high-quality *T. mono* assembly





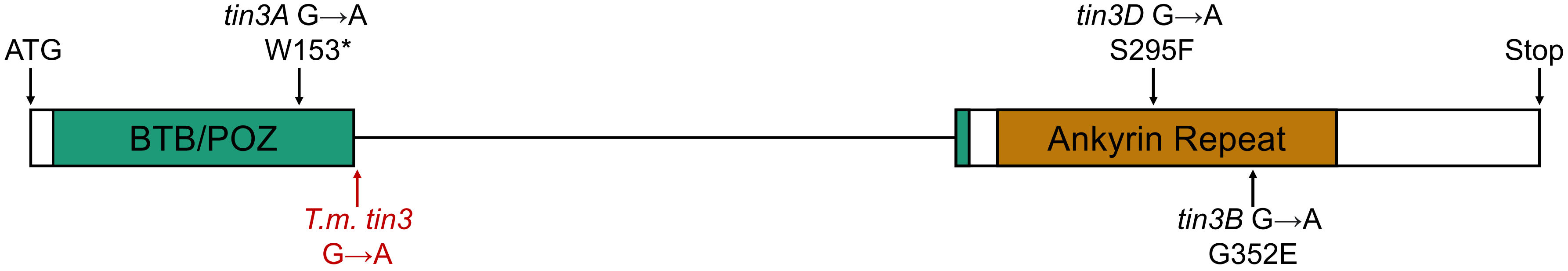
```
WT_T_monococcum   1    MDDTLKSLSMDYLNLLINGQAFSDVTFSVEGRLVHAHRCILAARSLFFRKFFCGAAADQA   60
tin3              1    MDDTLKSLSMDYLNLLINGQAFSDVTFSVEGRLVHAHRCILAARSLFFRKFFCGAAADQA   60

WT_T_monococcum   61   AAAAAAGSPGAMLMDHLSPRSPSGASASSPRGAGGSGSGSASAAAMAPGAVIPVNSVSYE   120
tin3              61   AAAAAAGSPGAMLMDHLSPRSPSGASASSPRGAGGSGSGSASAAAMAPGAVIPVNSVSYE   120

WT_T_monococcum   121  VFLLLLQFLYSGQVSLVPQKGEPRPGCGERGCWHTHCAAAVDLALDTLAVARSFGVEELA   180
tin3              121  VFLLLLQFLYSGQVSLVPQKGEPRPGCGERGCWHTHCAAAVDLALDTLAVARSFGVEELA   180

WT_T_monococcum   181  VLTQKQLAGMVEKASIEDVMKVLMASRKQDLHQLWNTCSHLVAKSGLPPEVLAKHLPLDV   240
tin3              181  VLTQVINYILSLINPQFPILAPRPSFPHEQESRS*-------------------------   215
```



Ahmed*, Schoen*, Heuberger* et al., *Nature* 2023

Confirmation in Bread Wheat

*Adam Schoen



tin3A G→A
W153*

ATG

BTB/POZ

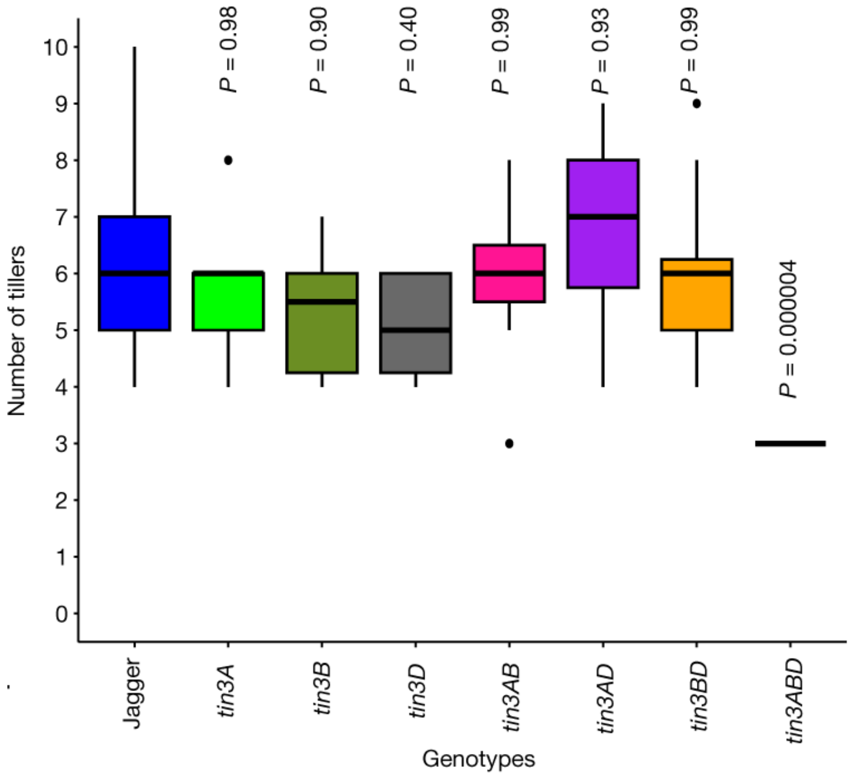
T.m. tin3
G→A

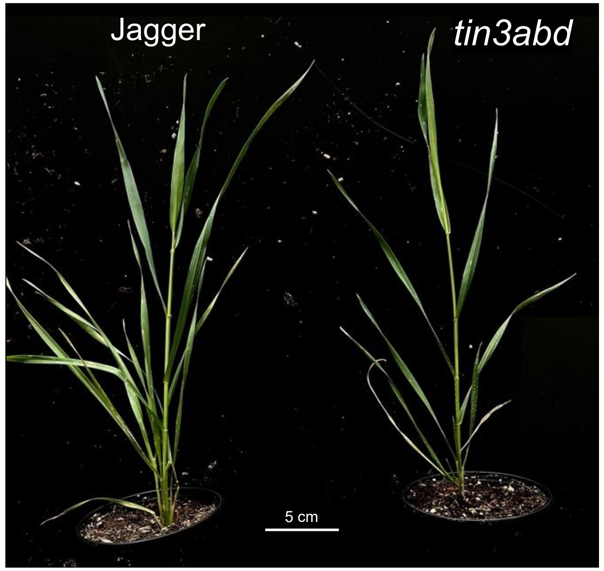
tin3D G→A
S295F

Stop

Ankyrin Repeat

tin3B G→A
G352E





Ahmed*, Schoen*, Heuberger* et al., *Nature* 2023

MutMap approach

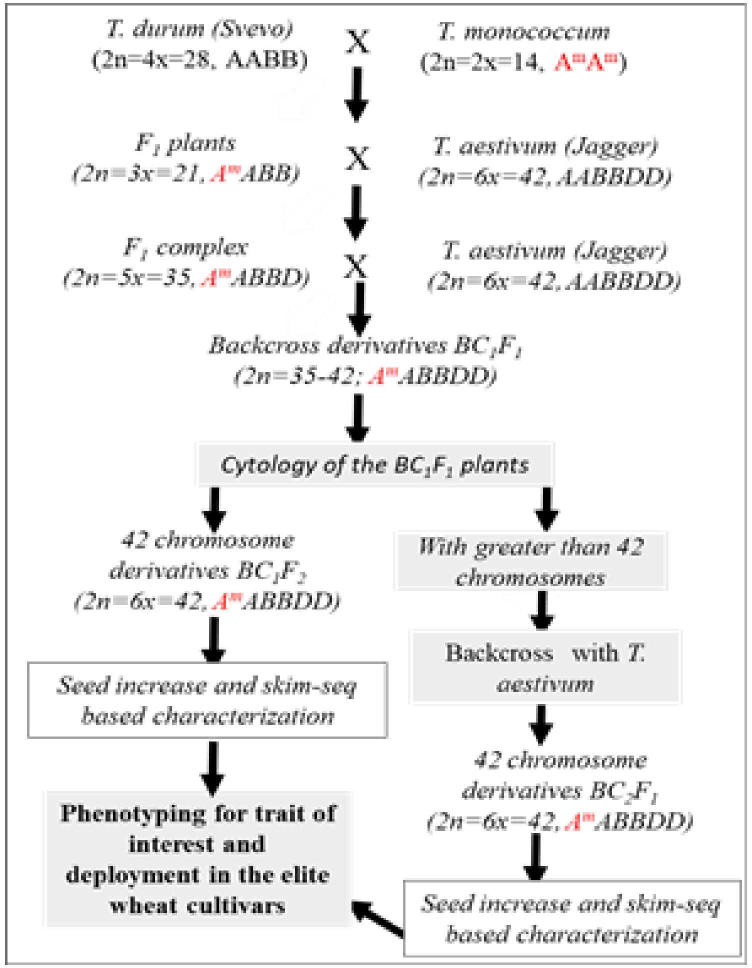
Phenotypes in a free thrashing and two dwarfing gene MutMap populations

Developing introgressive germplasm in bread wheat background

TA299
TA10622
→
HRWW
@ K-State

TA391 (L95)
TA10858 (L96)
→
SRWW
@ UMD#

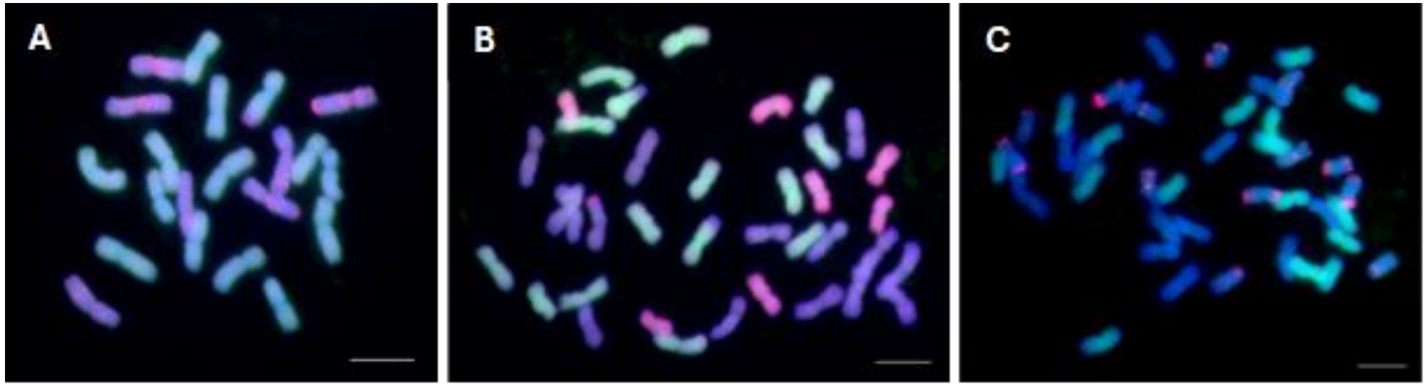
DV92
→
Durum wheat
@ UCD*



Dal-Hoe Koo
WGRC, K-State

*Developed by Dr. Jorge Dubcovsky

Back crossed introgression derivatives in the bread wheat cultivars



ph1b mutant-based crosses to transfer einkorn traits in wheat

Characterization of einkorn introgressions in wheat

About 400 BC3F2 germplasm using L95 and L96 accessions

BC2F1 plants are in progress with TA299

DV92 introgression in durum wheat

GBS / skim-sequencing based identification of introgression fragments

Einkorn Pangenome Initiative

Einkorn

Image Credit: Kimbra Cutlip

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…………………

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Suggested Reading

1. https://link.springer.com/article/10.1186/1471-2229-12-205
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