

# Characterization and utilization of regulatory variation using ATAC-seq and promoter sequenced mutations

January 15, 2023

Jorge Dubcovsky

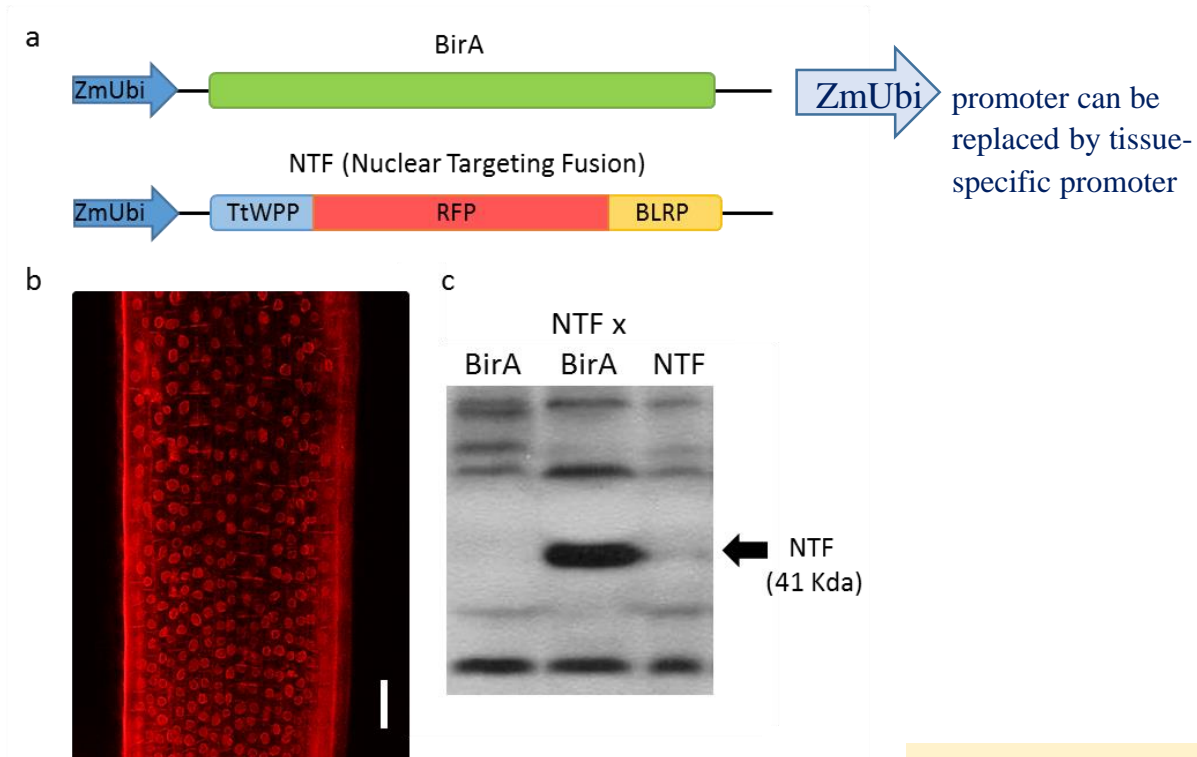


# INTACT technology for high quality ATAC-seq data

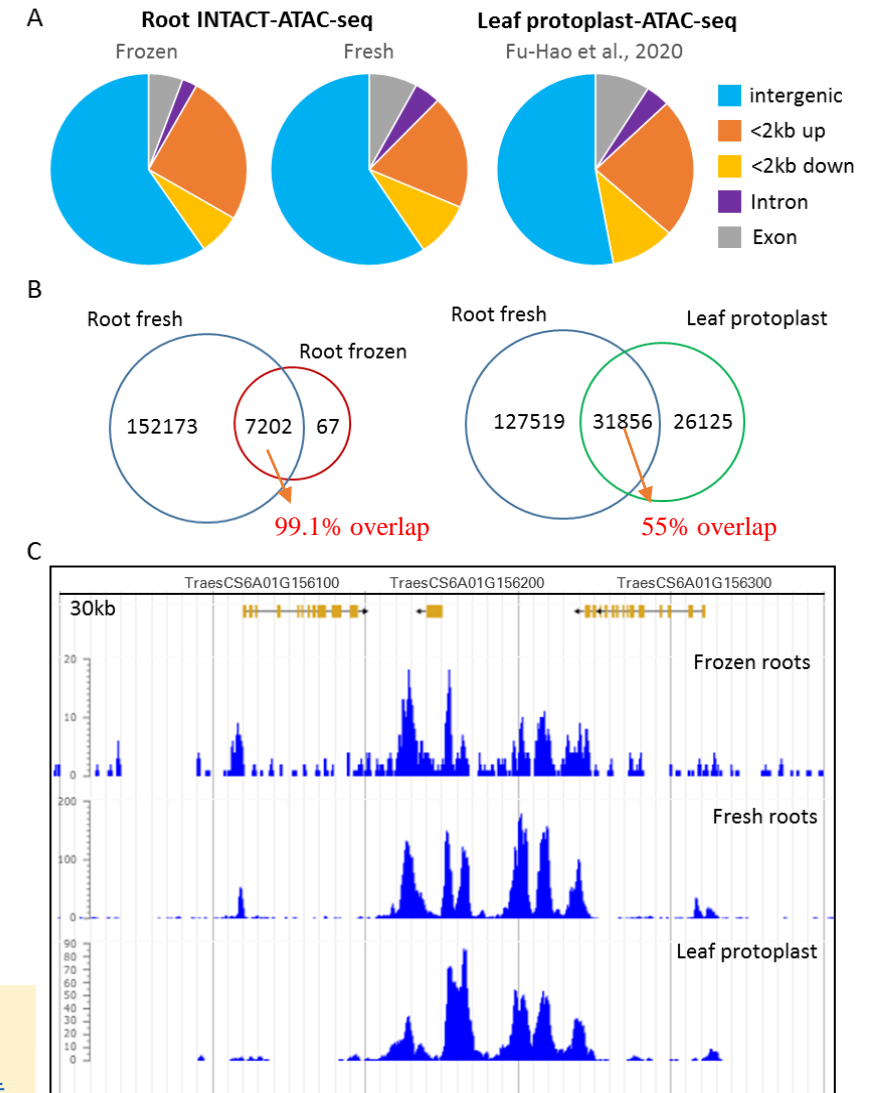
**INTACT:** isolation of nuclei tagged in specific cell types.

Uses 2 transgenic lines expressing:

- A biotinylating enzyme **BirA**
- A chimeric nuclear envelope targeting fusion protein (NTF) including a nuclear binding domain (**TtWPP**), a reporter gene (**RFP**) and a biotin ligase recognition peptide (**BLRP**).



**ATAC-Seq** quality and signal to noise ratio depends on the quality of nuclei extraction.



Research Square Preprint online

<https://doi.org/10.21203/rs.3.rs-2058892/v1>

# Wheat promoter capture mapped to RefSeq v1.0

## NimbleGen promoter design. A. Hall. GigaScience, 8, 2019, 1–13

- 2-kb upstream of 110,790 high-confidence annotated wheat gene.

### Wheat Promoter Capture (RefSeq v1.1)

Collaboration with IAEA and Hong-Chun Xiong

Kronos EMS populations = sequenced 1,513 lines

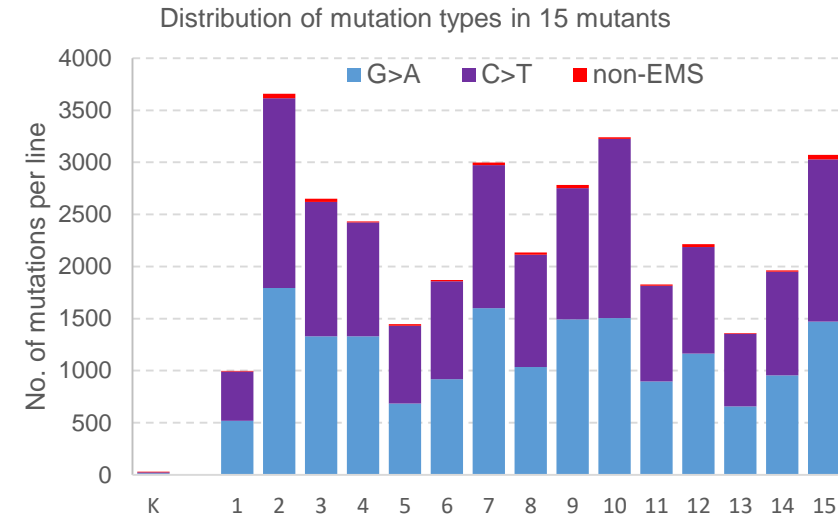
EMS mutations detected = 4,287,361

Error rate = 0.2% (99.8% accuracy)

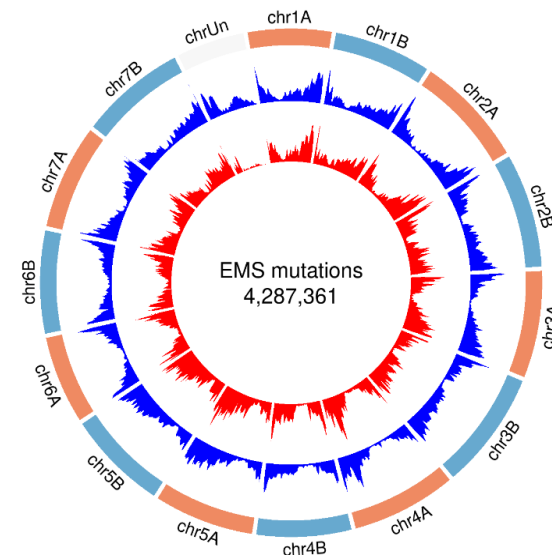
Mutation density = 36 mutations / kb

Data available in

- GrainGenes Genome Browser
- Dubcovsky lab: [https://dubcovskylab.ucdavis.edu/wheat\\_blast](https://dubcovskylab.ucdavis.edu/wheat_blast)



>99% of the mutations are G>A or C>T (caused by EMS)



Blue track: gene density RefSeq v1.1  
Red track: EMS mutation density in promoter capture

# Using ATAC-seq and promoter mutant data to understand and engineer gene expression

Start from gene coordinates or gene name

[https://wheat.pw.usda.gov/GG3/genome\\_browser](https://wheat.pw.usda.gov/GG3/genome_browser)

## The GrainGenes Genome Browsers



Exon/promoter capture and ATAC data are only in RefSeq v1.0



Start from gene sequence

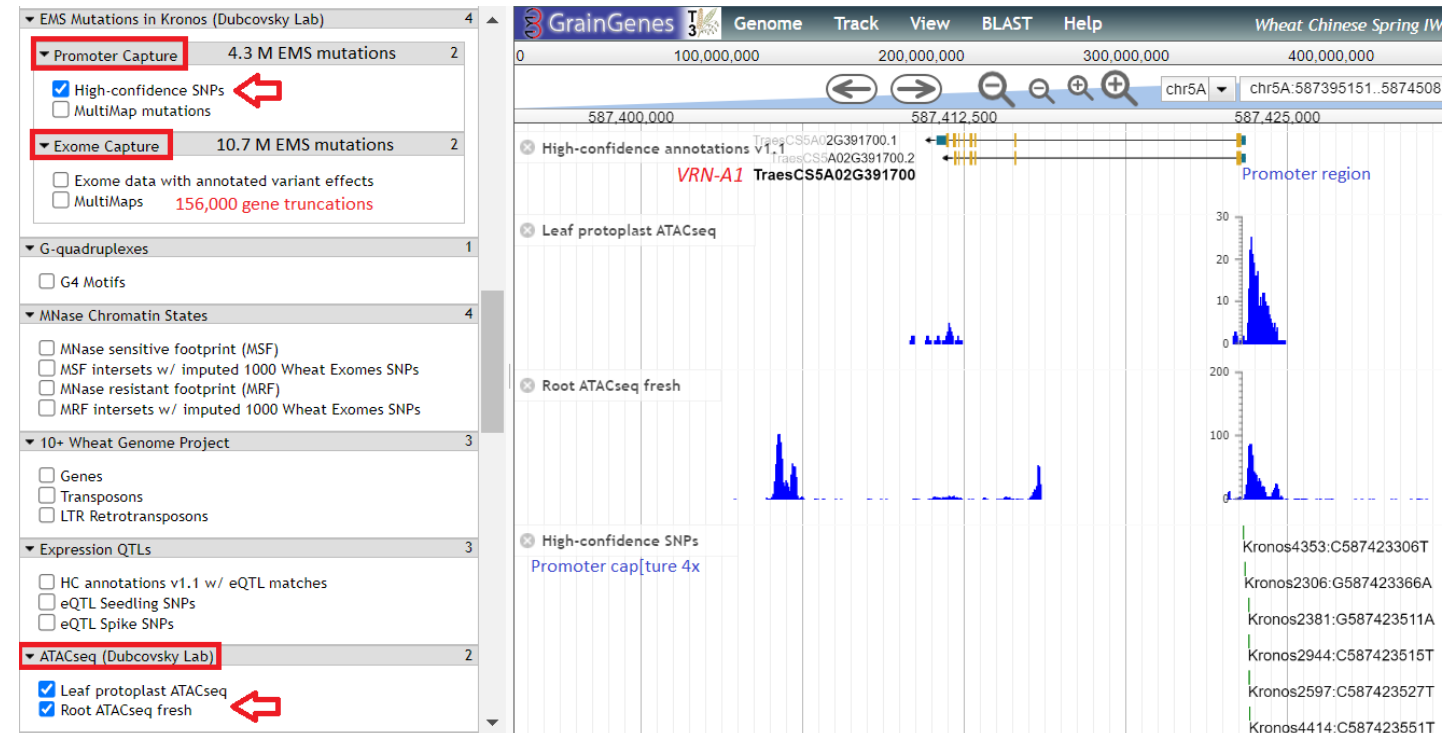
<https://wheat.pw.usda.gov/blast/>



**Note: default BLAST parameters: -max\_target\_seqs 6**

cttgcatacc ttattcggtta ttgaactgg tcaactgtgc atgaagcctt

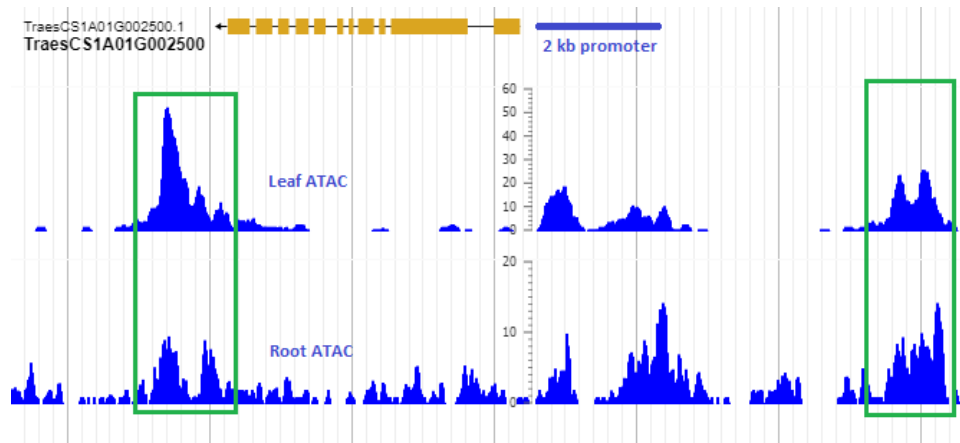
- ☐ 10+ Wheat - Robigus Elv1.1 Ensembl scaffolds (2020)
- ☐ 10+ Wheat - Weebill 1 V1 Ensembl scaffolds (2020)
- ☒  Wheat Chinese Spring IWGSC RefSeq v1.0 genome assembly (2018)
- ☐  Hexaploid Wheat PanGenome, Montenegro et al., 2017
- ☐ Chinese Spring WGA pseudomolecules v1, all data (Oct 2016)



The mutation in the *SPL* binding site in the *VRN1* promoter is associated with a **41% increase in expression** in the early spike (1 cm long).

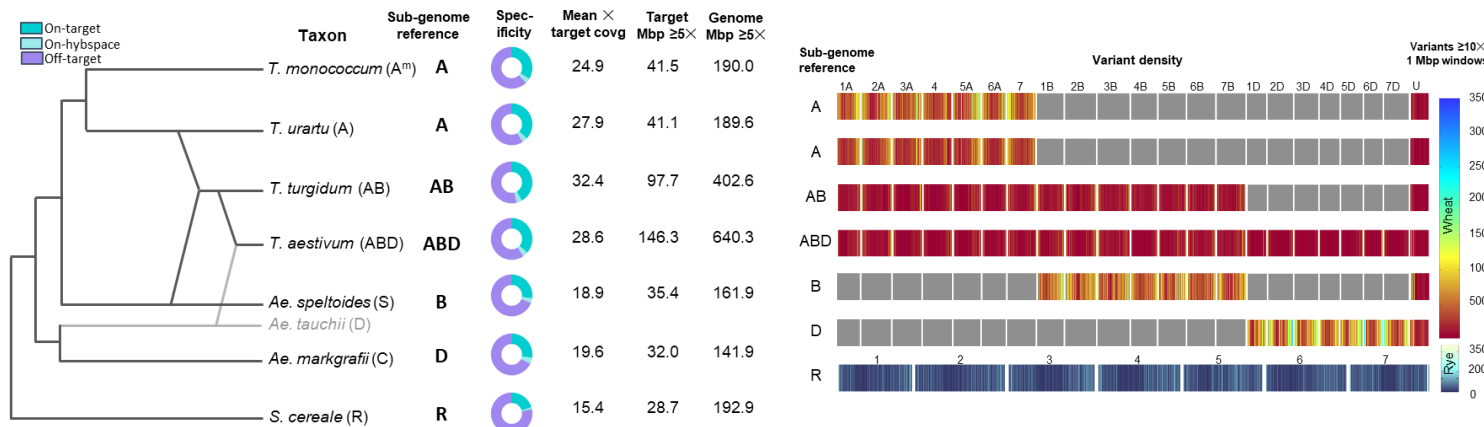
# New: Wheat Regulatory Region Capture with Arbor Biosciences

- **NimbleGen promoter design was Discontinued 12/2019**
- 2-kb promoter high confidence genes in RefSeq v1.0 minus repetitive elements (>99% identity eliminated).
- Masked regions with high 17 kmer frequencies (>100) and/or high coverage in previous design.
- Complemented with open chromatin ATAC peak regions from leaves and roots outside the promoter region (30 Mb)



Green rectangles are open chromatin outside 2kb promoter region

**30 Mb open chromatin + 150 Mb promoter = 180 Mb**



- Good coverage in all genomes
- It also works well for rye
- 61% increase in read-pairs on target
- 3-fold more coverage target region
- The Plant Genome (minor corrections)



# Allele mining for improving grain yield potential

January 15, 2023

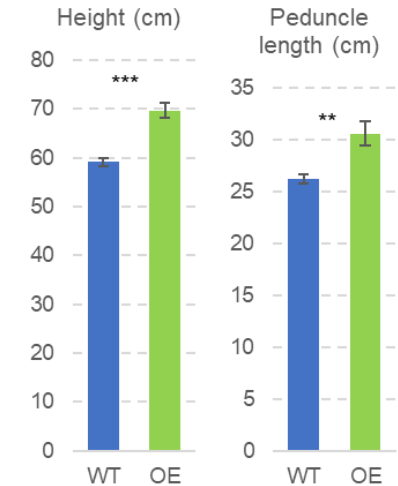
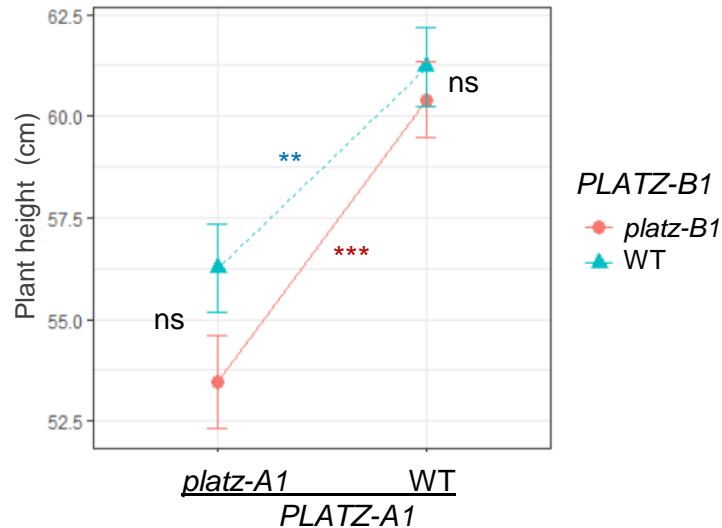
Jorge Dubcovsky



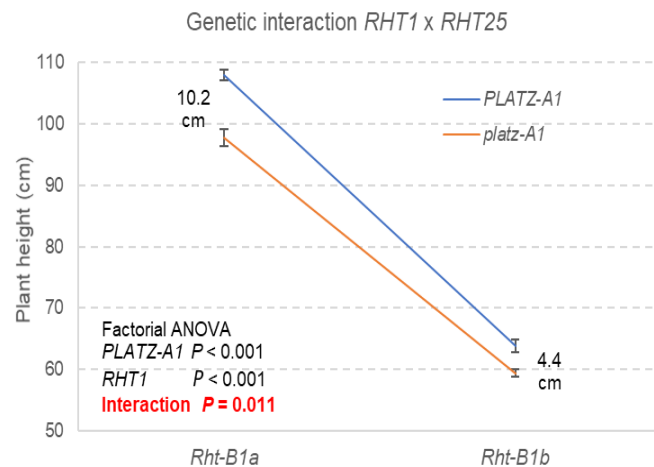


# *RHT25* encodes a PLATZ transcription factor that interacts with *RHT1*

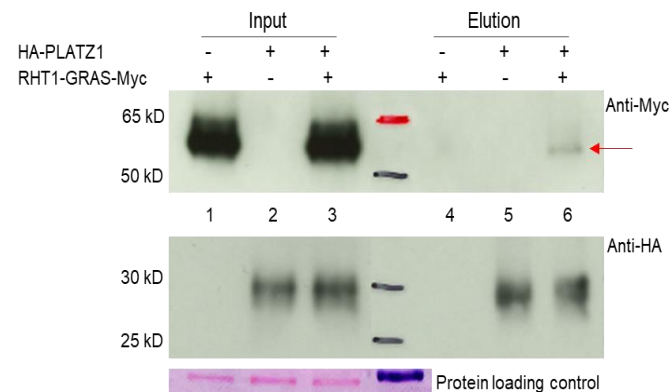
PLATZ1 encodes a **p**lant-specific **A**T-rich sequence- and **z**inc-binding protein



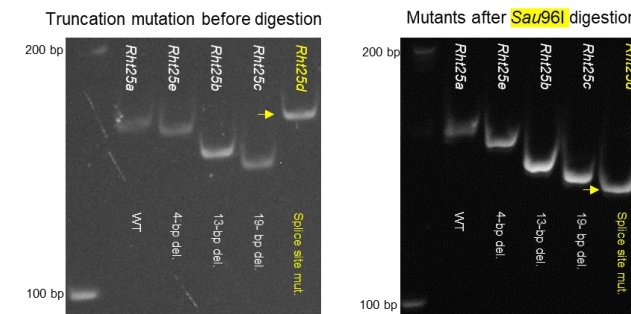
*PLATZ-A1* interacts genetically with *RHT1*



*PLATZ-A1* interacts physically with *RHT1*

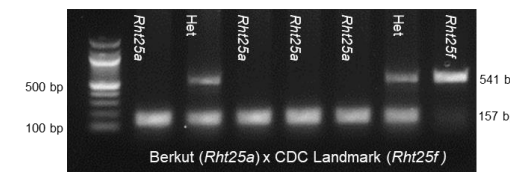


*PLATZ-A1* natural mutants: 0% in 4x and 30% in 6x



- 4 loss-of-function alleles**  
(1 pair of primers!)
- 4-bp deletion
  - 13-bp deletion
  - 19-bp deletion
  - Splice site (*Sau96I* dig.)

Promoter insertion in (*Rht25f*) F<sub>2</sub>

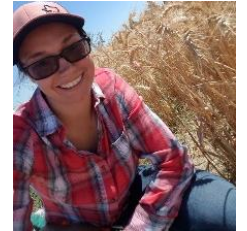


- 1 reduced expression allele**
- 384 bp promoter insertion
  - Smaller effect on height



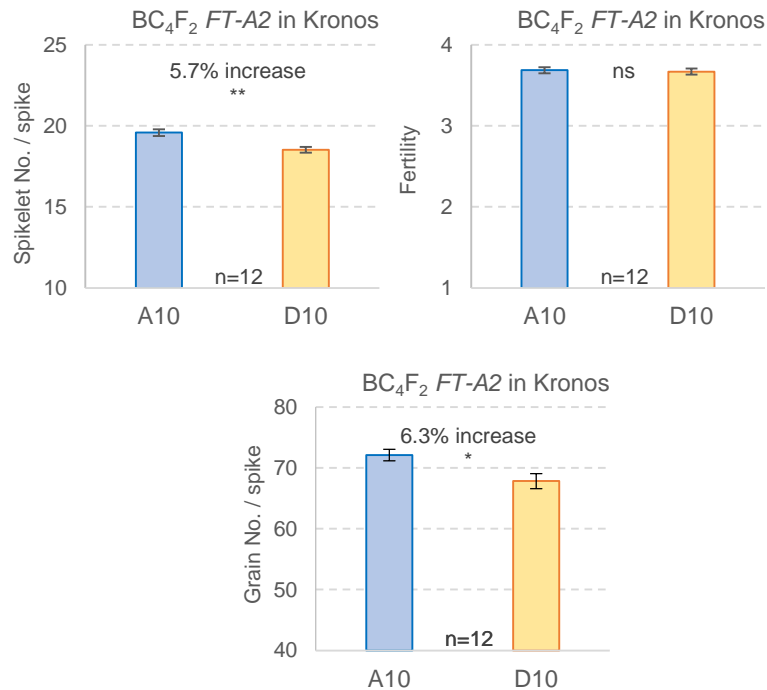
# A natural variant in *FT-A2* (Chr 3AS) with beneficial effect on SNS and GNS

- Loss-of-function mutations in *FT2* increase SNS but decrease fertility (similar GNS)
- Natural Polymorphism in *FT-A2* at position 10 from Asp to Ala (**D10A**) associated with higher SNS in our spring panel
- D10 is the ancestral allele conserved in other grasses
- A10 allele is present in **1% of a durum** collection but in **60 % of our spring common wheat panel** suggesting positive selection
- High-density map located SNS QTL to a 5.2-Mb region in 3AS including *FT-A2* (>6000 gametes)
- 28 genes but only *FT-A2* has non-synonymous polymorphisms in both mapping populations

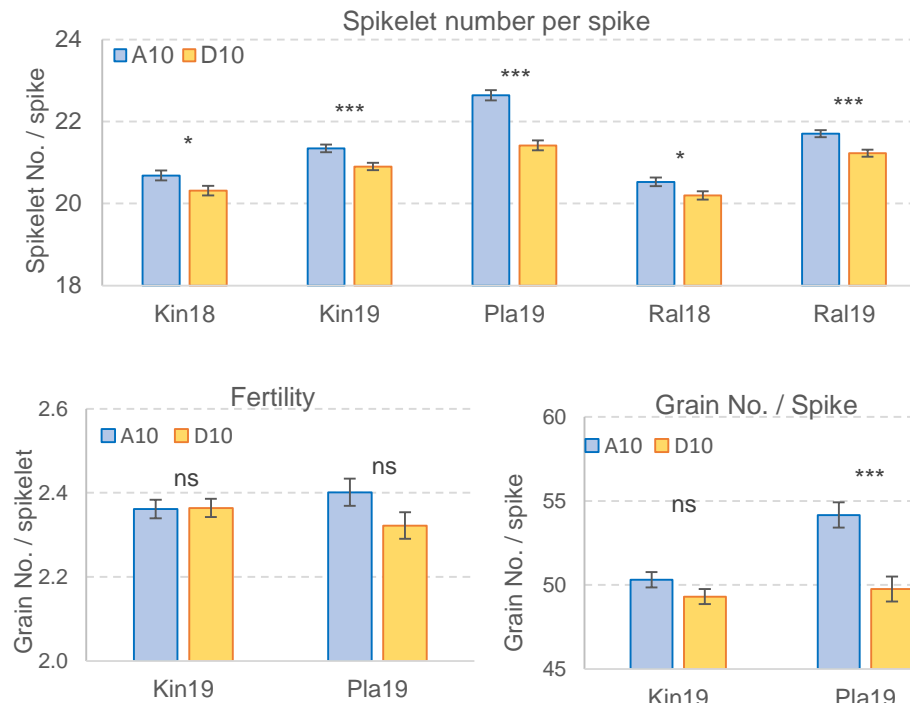


Priscilla Glenn  
TAG (2022) 135:679

Field exp. in BC<sub>4</sub>F<sub>2</sub> sister lines in tetraploid wheat (UCD)

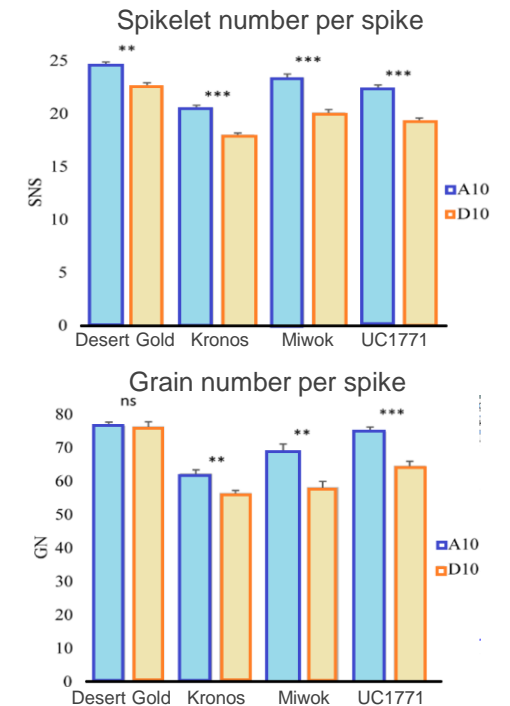


Field exp. in winter 6x wheat pop. SS-MVP57 (A10) x LA95135 (D10) (F<sub>5</sub> RILs)



Model included *FT2* *RHT1*. *PPD1* and *WAP01* (increase yield per spike)

Field exp. UCD isogenic 4x 2022

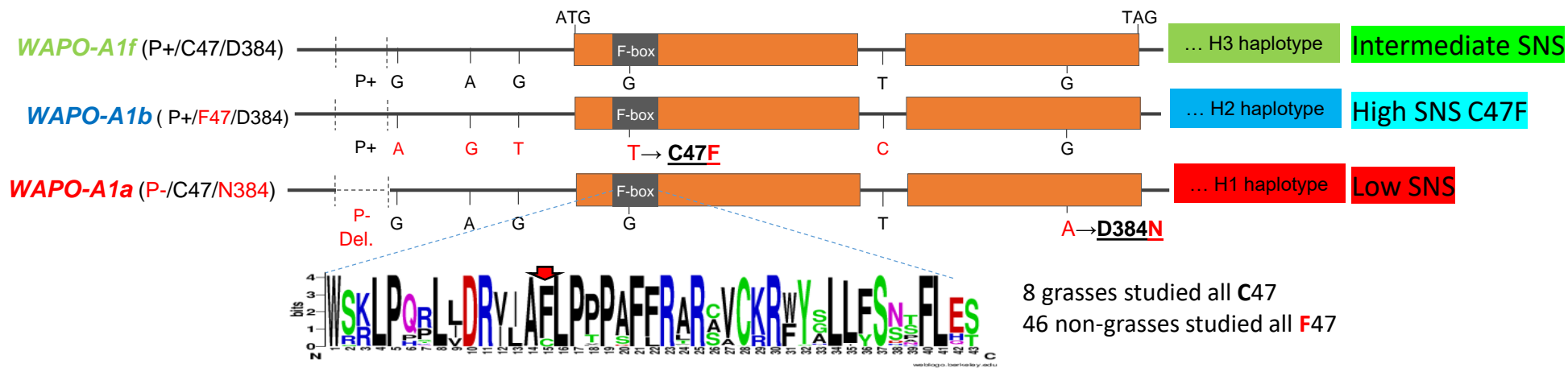


Split plot 8 reps, small plots  
No significant increase in yield!

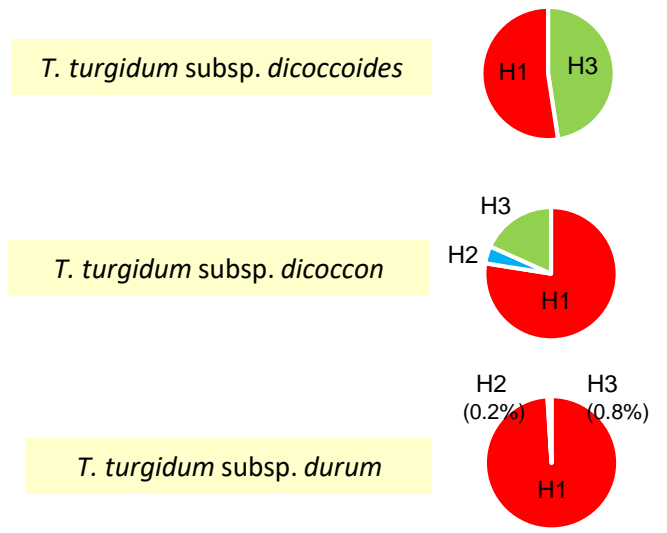
# A natural variant in *WAPO-A1* (Chr 7AL) with beneficial effect on SNS and GNS



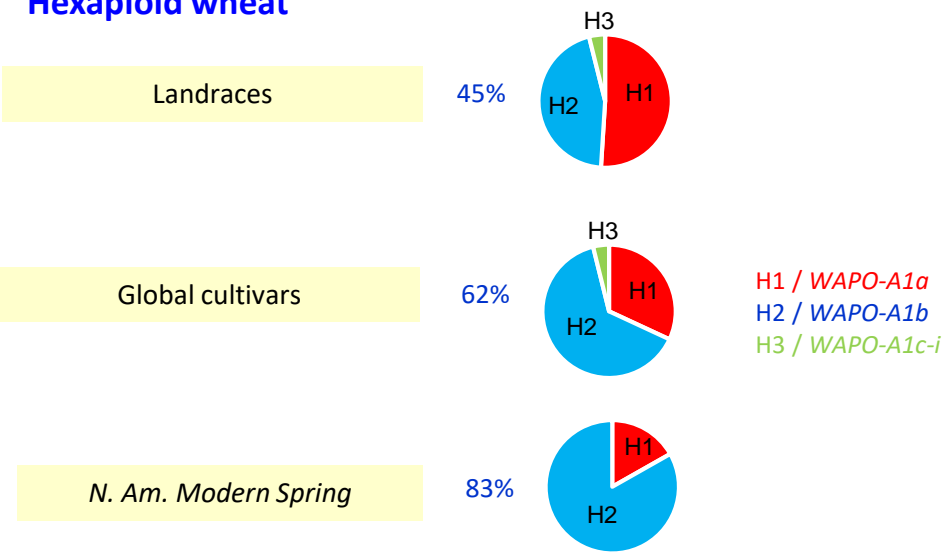
Saarah Kuzay  
TAG 2019 132:2689



## Tetraploid wheat

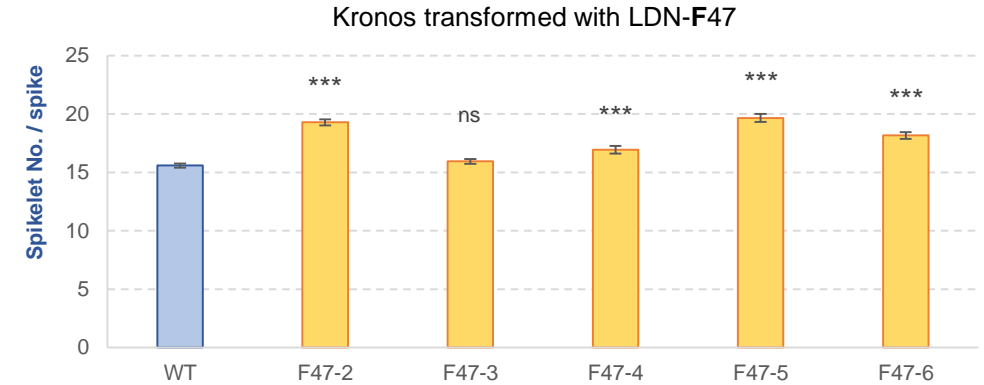
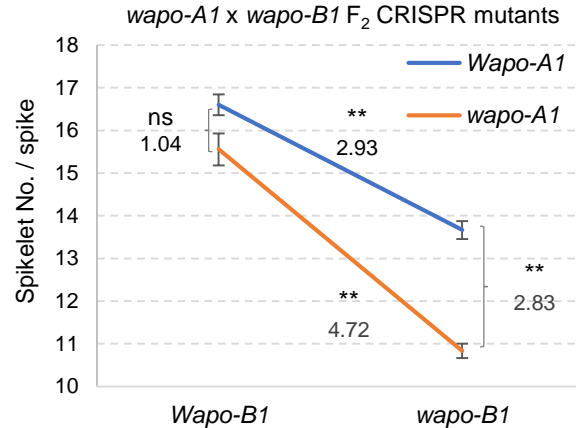
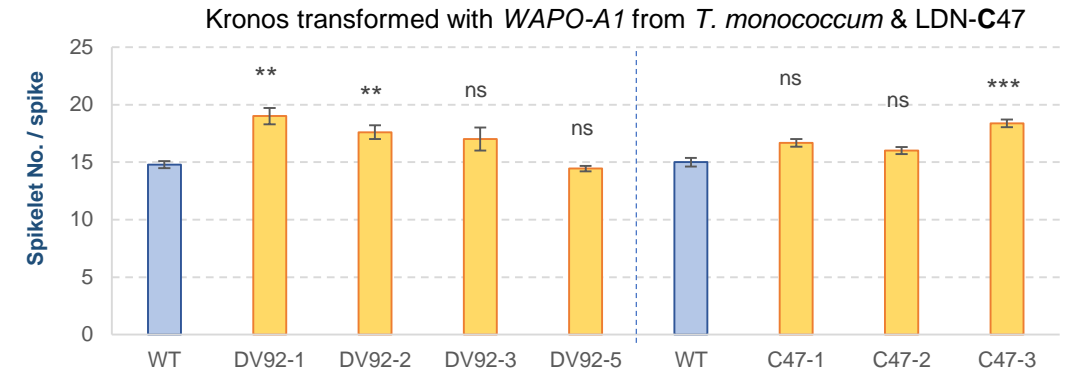
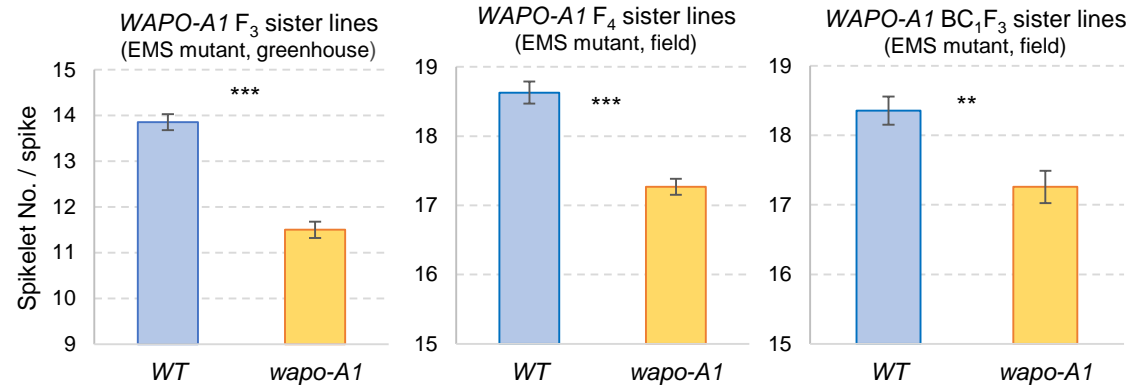


## Hexaploid wheat





# Validation of *WAPO-A1* as the causal genes of the SNS QTL on 7AL



The functional *WAPO1* gene is necessary to increase SNS

The functional *WAPO1* gene is sufficient to increase SNS

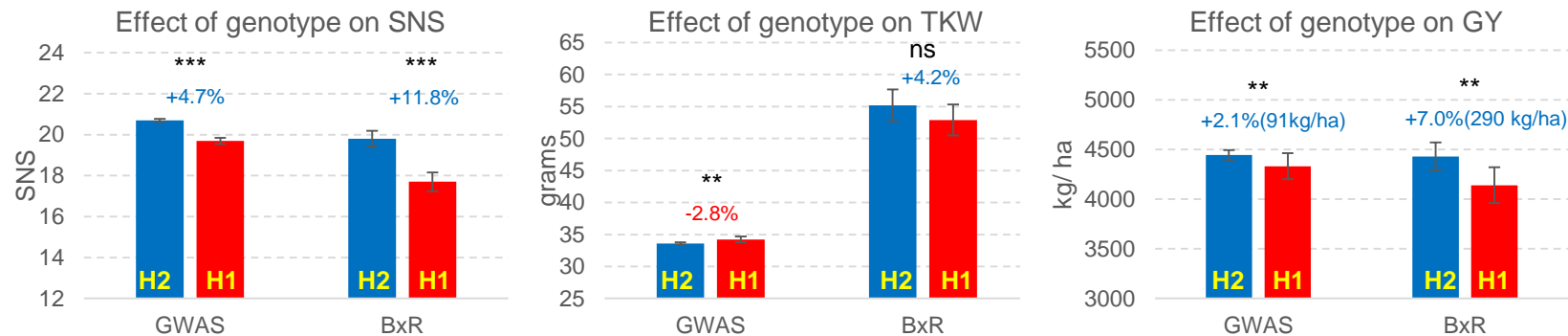
***WAPO-A1* is the causal gene for the 7AL QTL for SNS**



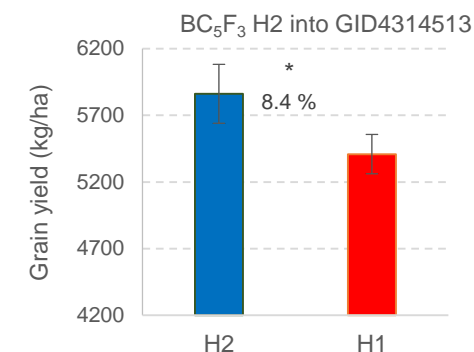
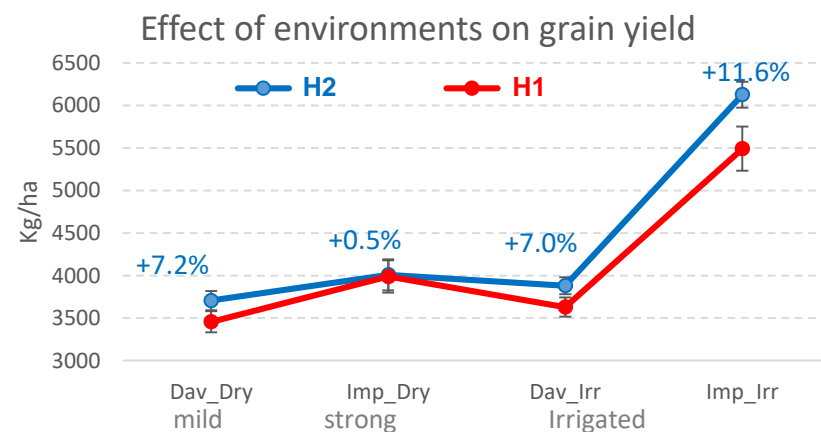
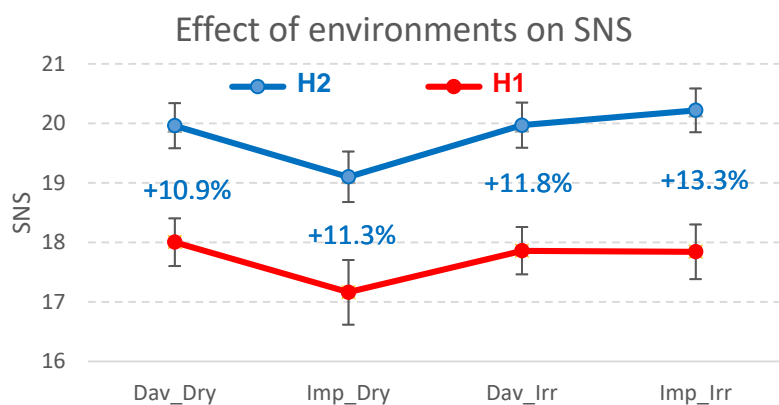
Saarah Kuzay et al.  
PLOS Genetics 2022)

# Effect of *WAO-A1* alleles on SNS, TKW and grain yield

Comparison between **H1** and **H2** haplotypes in a GWAS panel of 238 photoperiod insensitive spring wheats and in 75 biparental RILs **Berkut** (**H2**) x **RAC875** (**H1**)



Some genotypes were able to translate a higher proportion of the increase in SNS into increases in total grain yield. We hypothesize that those genotypes have a larger “source”.



Limiting environmental conditions (terminal drought) reduced the proportion of the increases in SNS that were translated in increases in grain yield. We hypothesize that those conditions limited the “source”.



UC Davis is looking for a Tenure Track Assistant Professor to leadership the small grains breeding program.

## Assistant Professor of Molecular Breeding for Small Grains Crops

Apply now

Job #JPF05419

• PLANT SCIENCES / COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES / UC Davis <https://recruit.ucdavis.edu/JPF05419>

### APPLICATION WINDOW

**Open date:** December 7, 2022

**Next review date:** Friday, Feb 24, 2023 at 11:59pm (Pacific Time)

Apply by this date to ensure full consideration by the committee.

**Final date:** Wednesday, Dec 6, 2023 at 11:59pm (Pacific Time)

Applications will continue to be accepted until this date, but those received after the review date will only be considered if the position has not yet been filled.

### POSITION DESCRIPTION

As part of UC Davis' commitment to hire leading research faculty with an outstanding commitment to teaching, research and service that will promote the success of historically underrepresented and marginalized student communities and address the needs of our increasingly diverse state and student population, the Department of Plant Sciences in the College of Agricultural and Environmental Sciences at the University of California, Davis is recruiting an Assistant Professor of molecular breeding of small grains crops. This is an academic-year (9-month), tenure-track position with teaching, research, outreach/engagement, and service responsibilities. The appointee is expected to conduct mission-oriented research and outreach/engagement activities relevant to the California Agricultural Experiment Station (<https://caes.ucdavis.edu/research/aes>). This is an outstanding opportunity to lead the prestigious small grains breeding program of the University of California. We seek an exceptional candidate for this role, from either academia or industry, who is committed to developing and deploying cutting-edge plant breeding technology to ensure the continued development of successful commercial varieties with enhanced quality, yield, disease resistance and resilience to the changing climate.