Identification of a Locus Corresponding to a Preharvest Sprouting Tolerant Mutant, *ERA8*, in Wheat

Shantel A. Martinez,

Dr. Oluwaseyi Shorinola, Samantha Conselman
Dr. Cristobal Uauy, Dr. Dan Skinner, Dr. Deven See,
Dr. Kimberly Garland Campbell, and Dr. Camille M. Steber

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Preharvest Sprouting

Tolerant ⟷ Susceptible

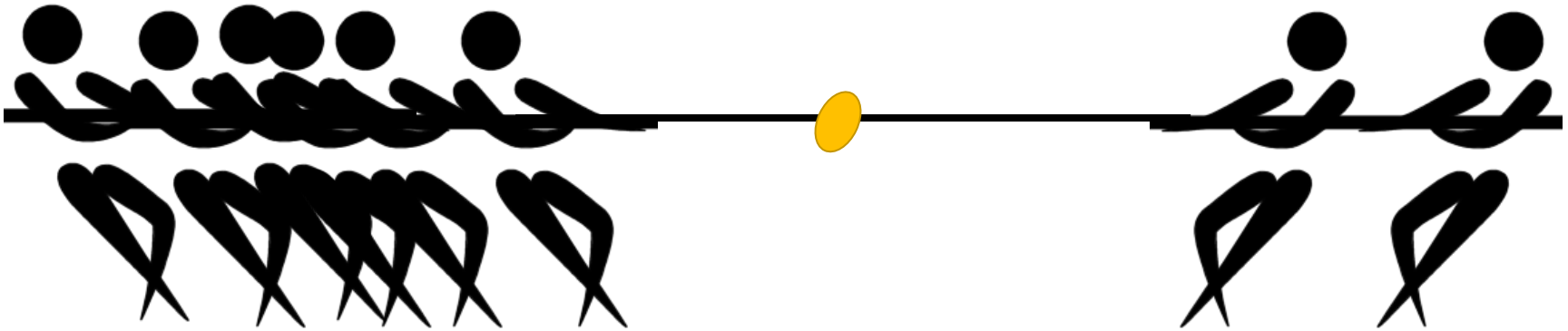
Dormant

Non-Dormant

Germination, a tug-of-war

Abscisic Acid
(ABA)

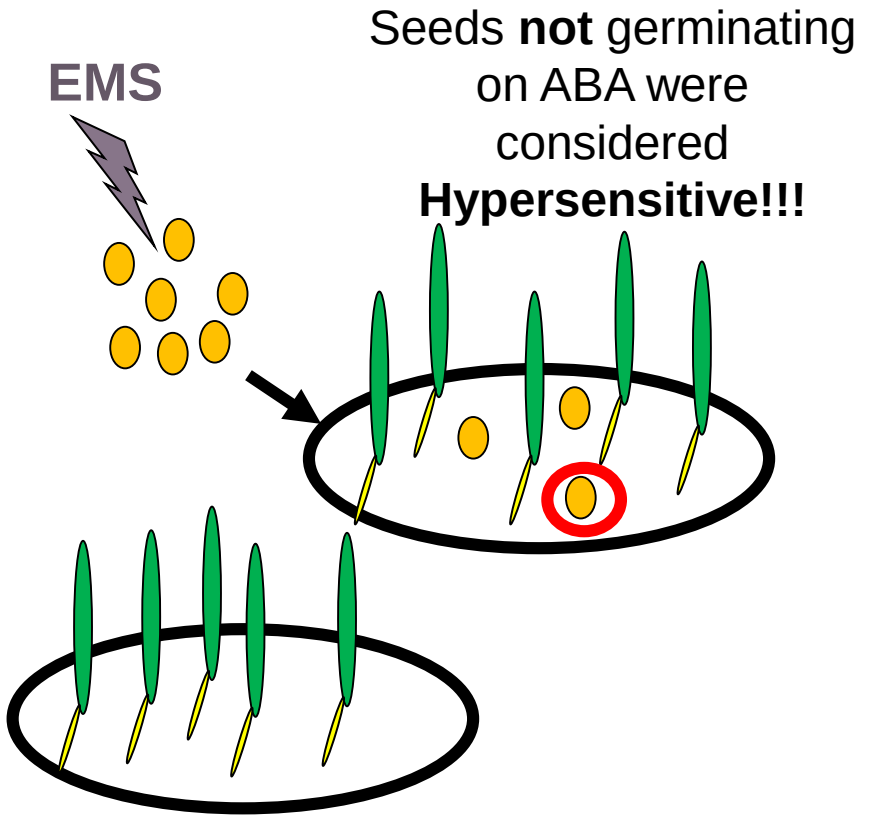
Gibberellins
(GA)

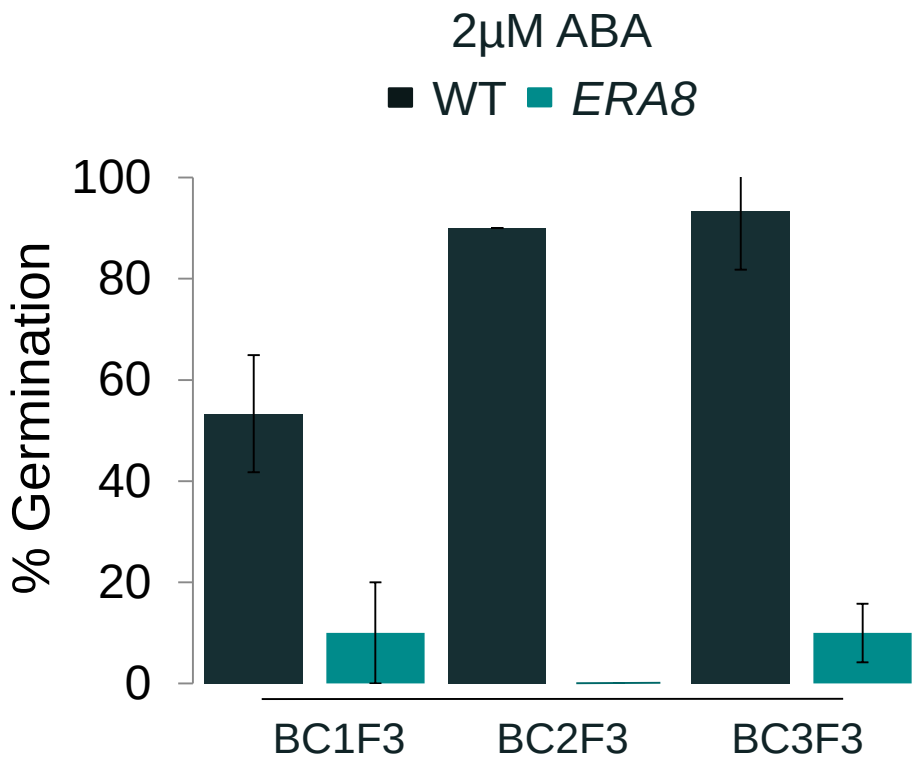


Dormant

Non-Dormant

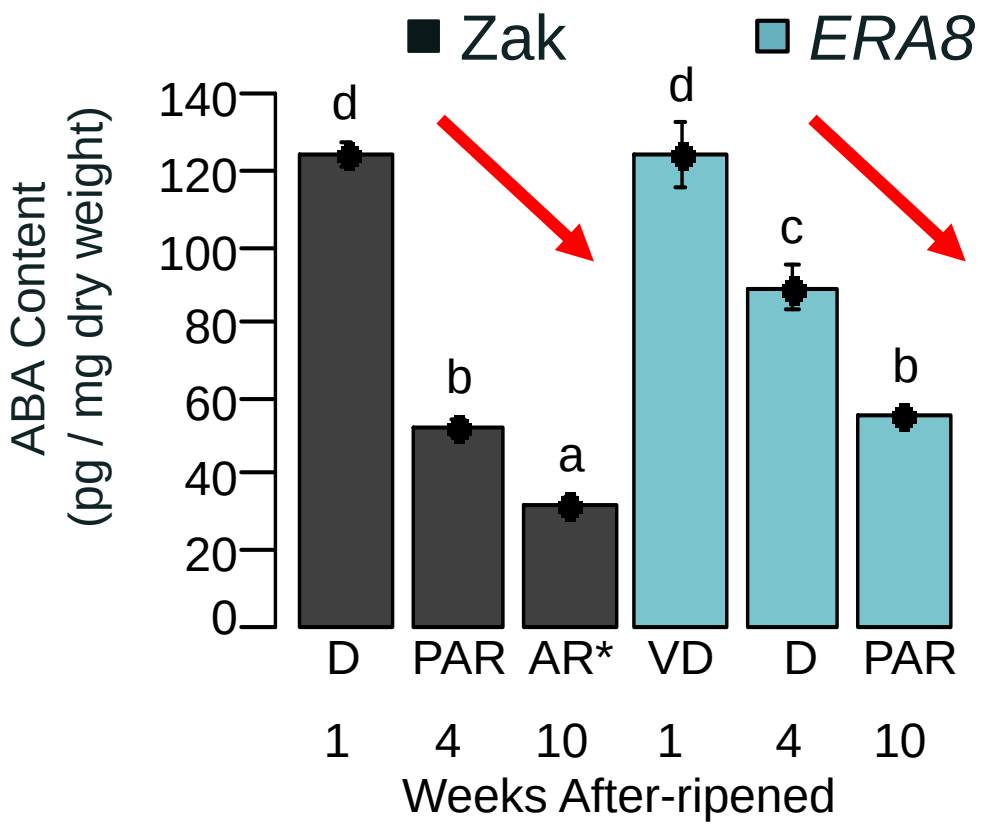
Semi-dominant *ERA8* has an Enhanced Response to ABA





Schramm et al., 2013; Martinez et al., 2016; T. Harris

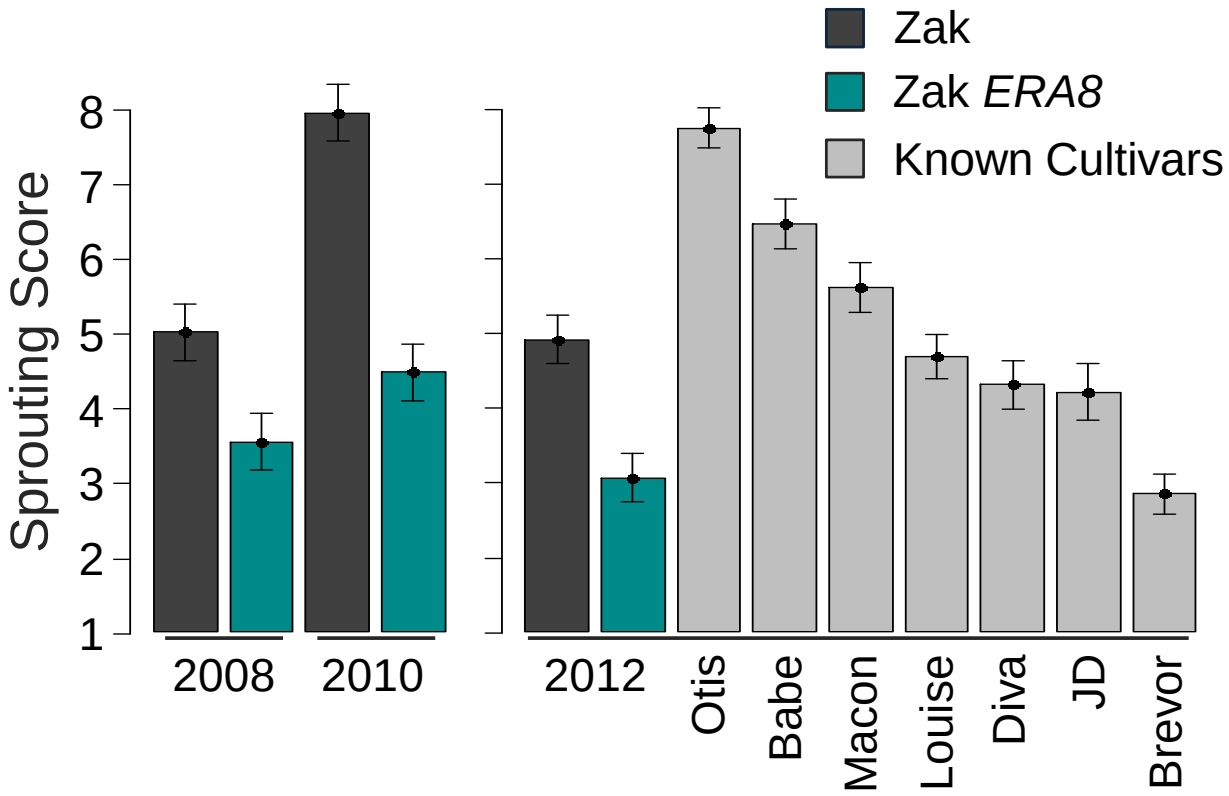
ABA decreases with after-ripening in *ERA8*



Embryo Tissue
18 Hours
Imbibed

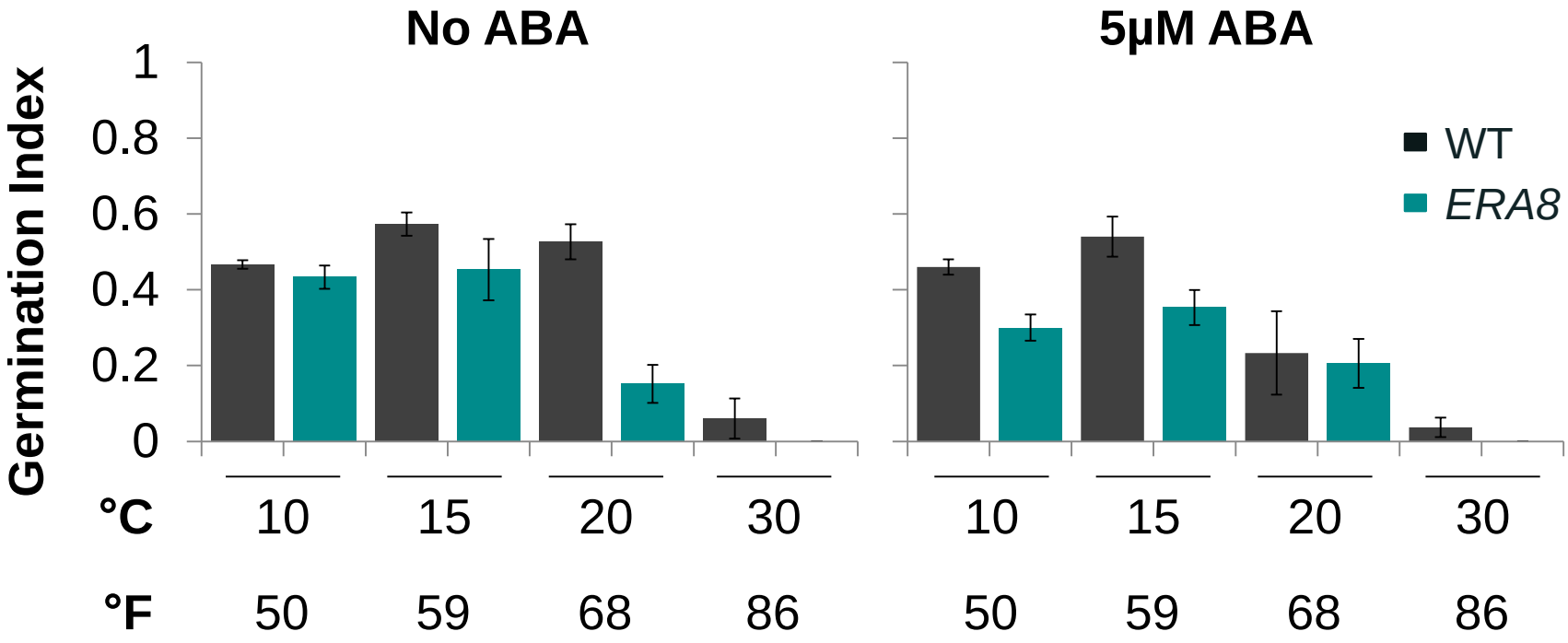
Martinez et al., 2016

ERA8 shows increased PHS tolerance than WT



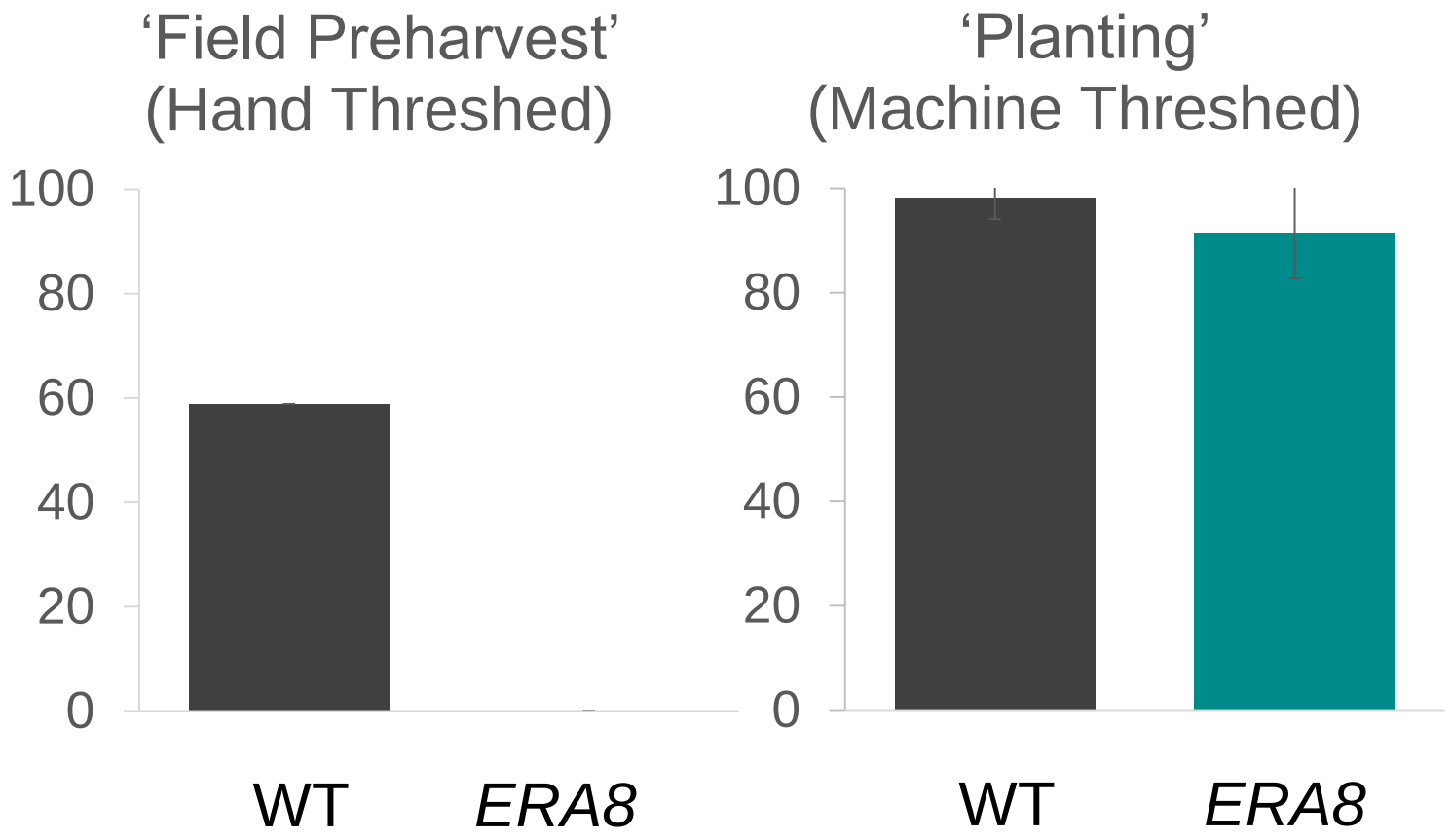
Martinez et al., 2016

ERA8 is still ABA sensitive at lower temperatures



Martinez et al., 2016

Is the Increased Seed Dormancy Going to be a Problem with Planting?

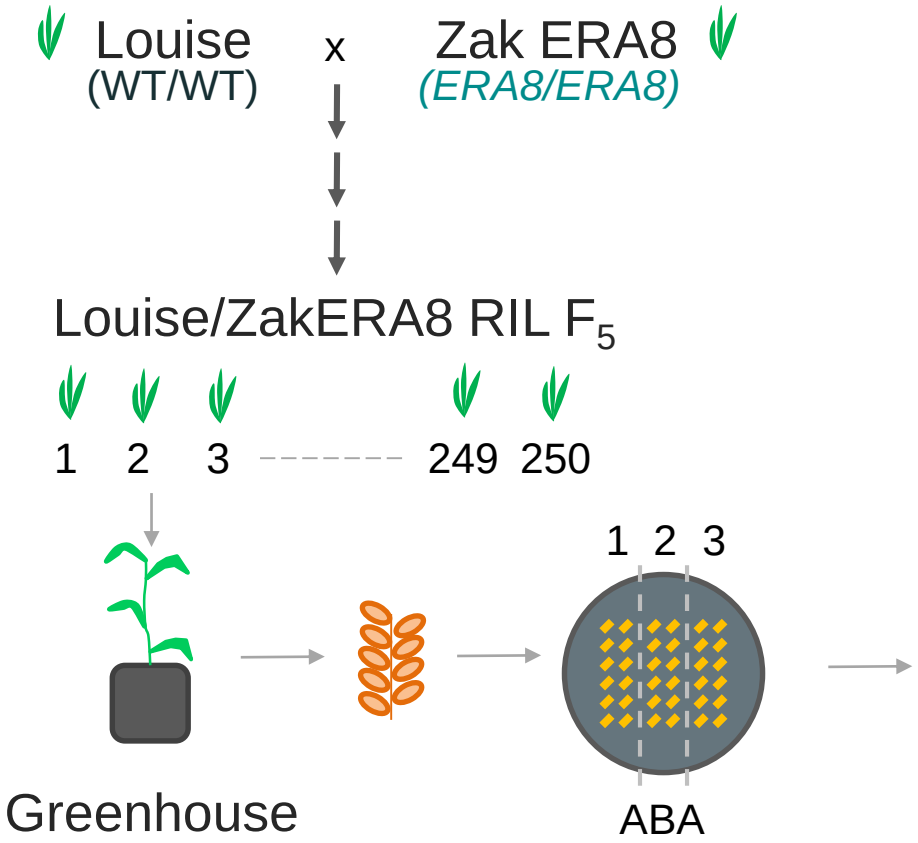


Finding the *ERA8* locus

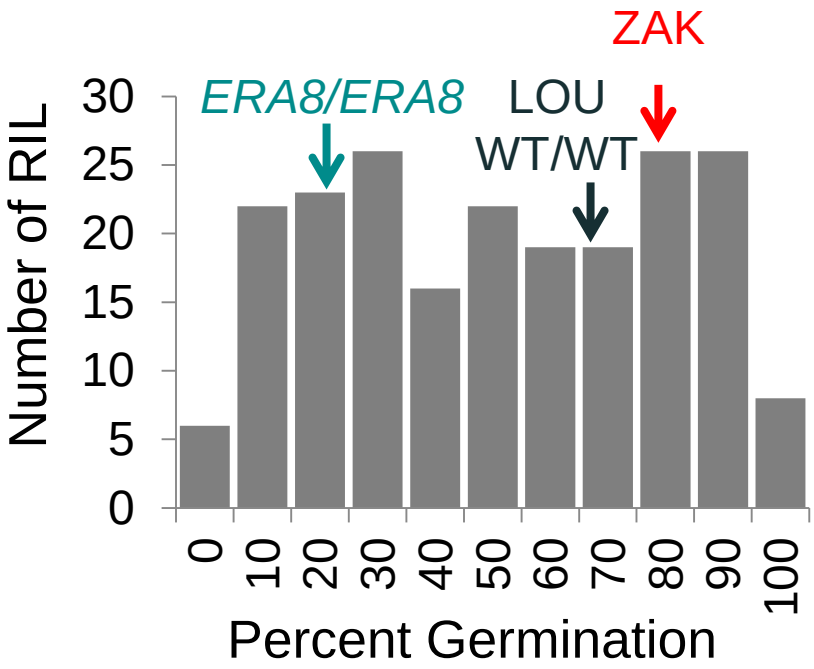
Using a bi-parental Recombinant Inbred Line (RIL) population to map loci associated with ABA sensitivity

Use a backcross population in Bulk Segregant Analysis (BSA) DNA sequencing to fine map *ERA8*

Mapping the loci contributing to the ABA sensitivity phenotype

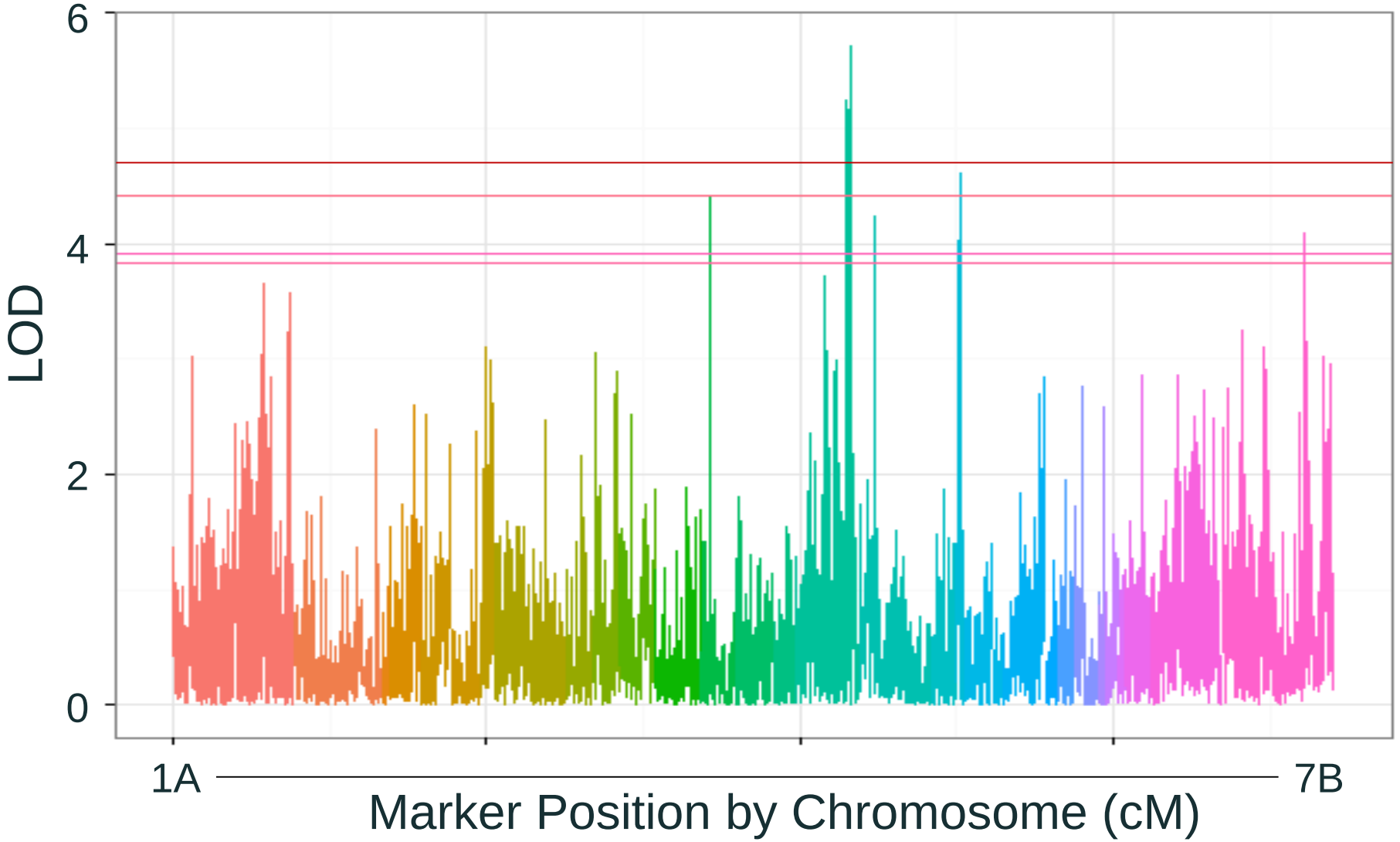


Genetic Cultivar differences in addition to the *ERA8* gene.



S. Beck

Multiple QTL found for increased ABA sensitivity



D. Skinner and D. See

Mapping *ERA8* in a backcross population

Only mutagen induced differences

Zak ERA8 x Zak WT

↓

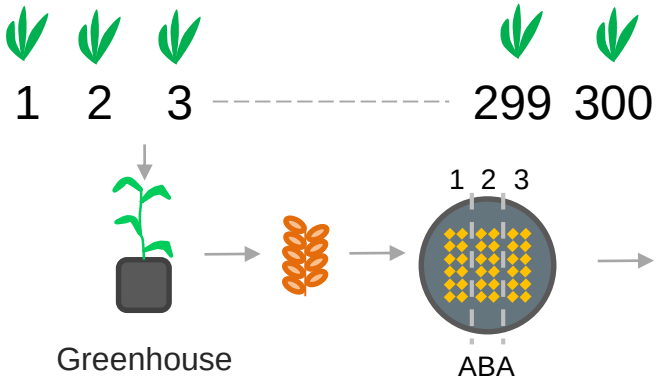
Zak ERA8 BC1 x Zak WT

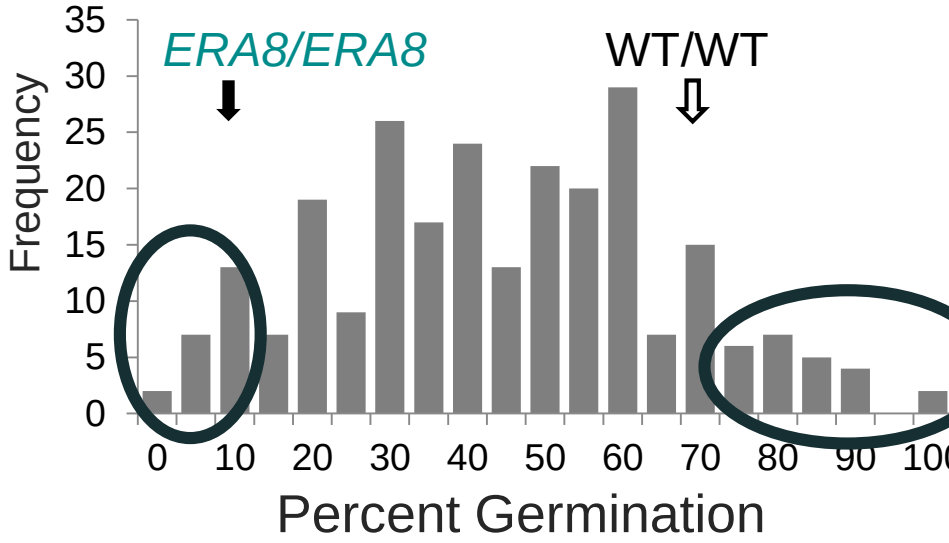
↓

Zak WT (WT/WT) x Zak ERA8 BC2 *(ERA8/ERA8)*

↓

Zak/Zak ERA8 BC3





15
***ERA8*-like**

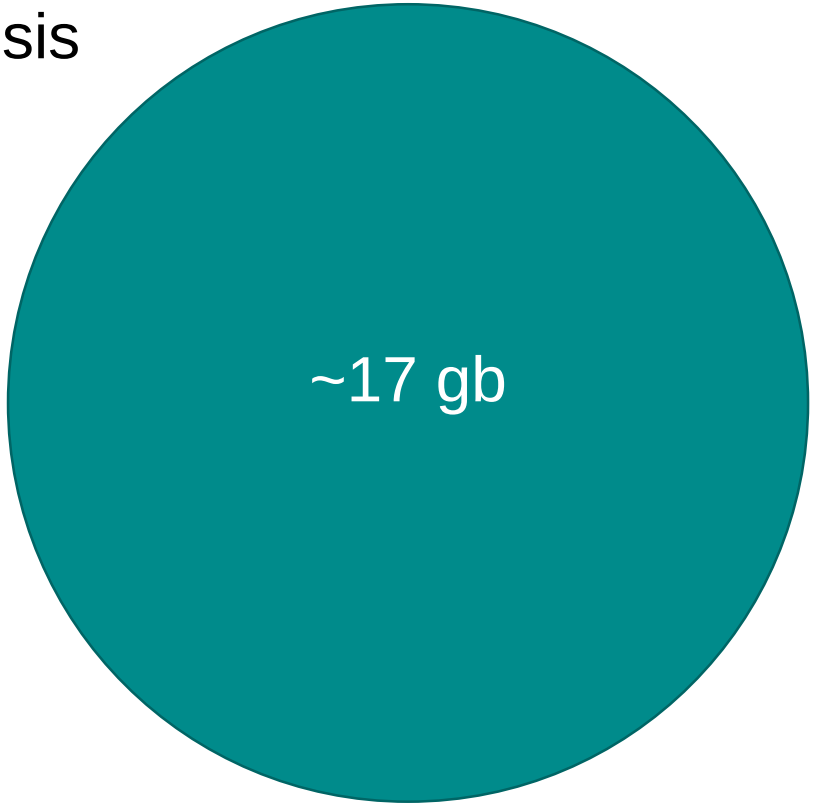
17
Zak-like

Mapping *ERA8* in a backcross population

Human

- Arabidopsis
- Rice

Wheat



Mapping *ERA8* in a backcross population

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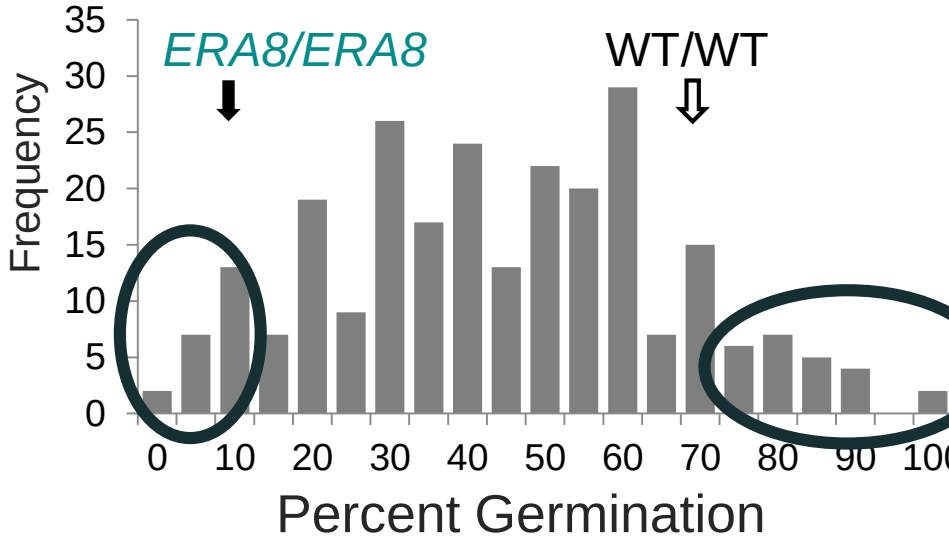

15
***ERA8*-like**

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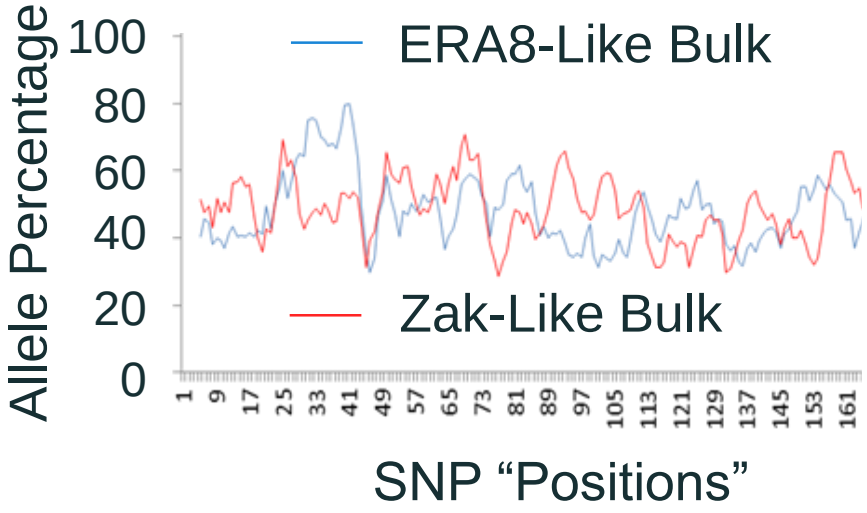
Zak-like

Exome Capture

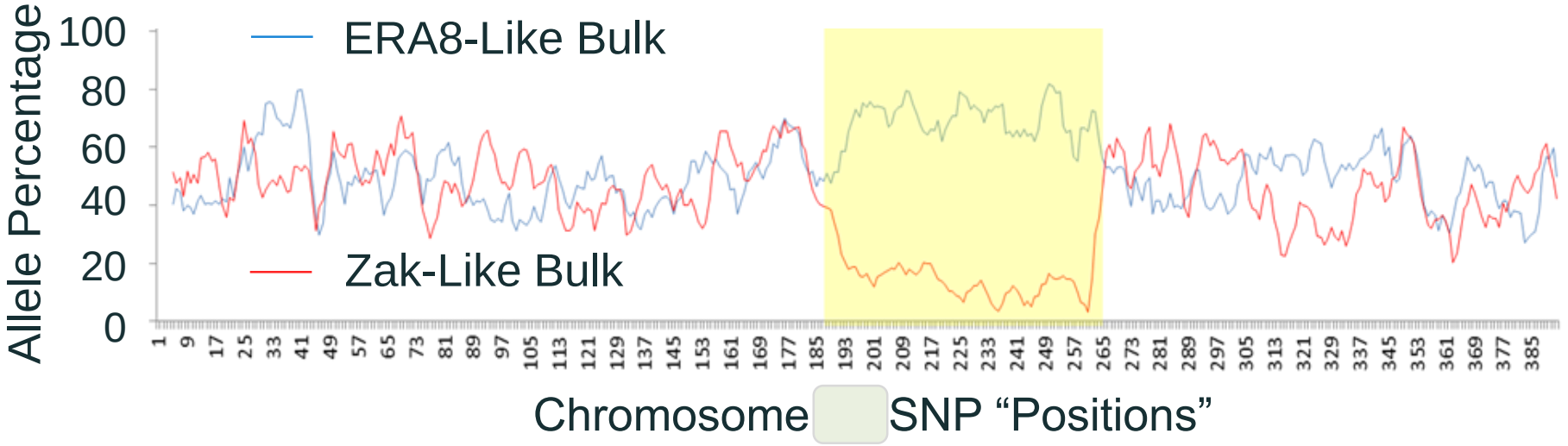
Sequenced

Chromosome region NOT linked to *ERA8*



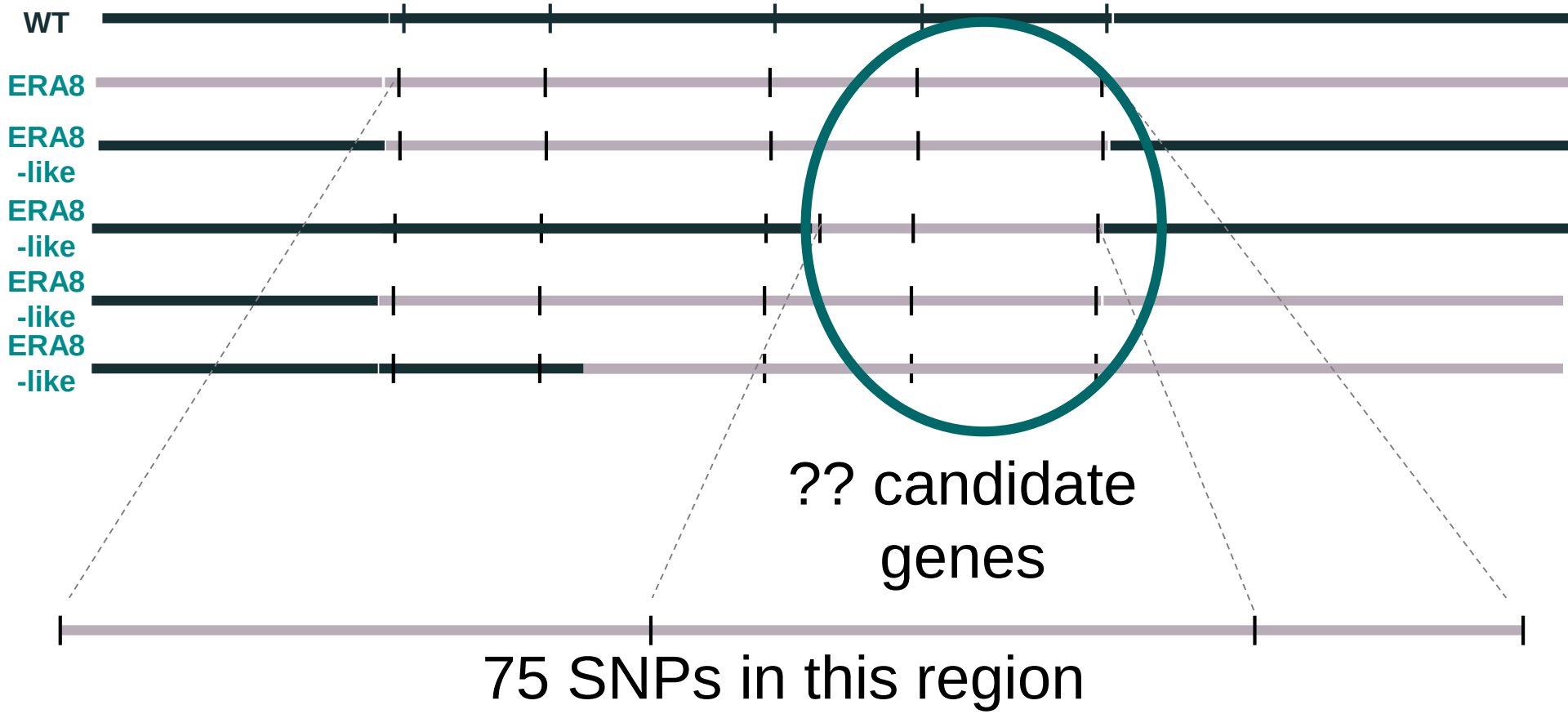
O. Shorinola and C. Uauy

Chromosome region LINKED to *ERA8*



Significant region on chromosome , again!

Finding more Zak/Zak*ERA8* recombinants in the region



IF *ERA8* is an ABA hypersensitive mutant, what could it be?

ERA8 is a gain-of-function semi-dominant mutant

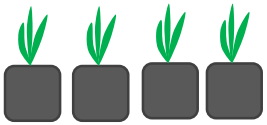
3 possibilities

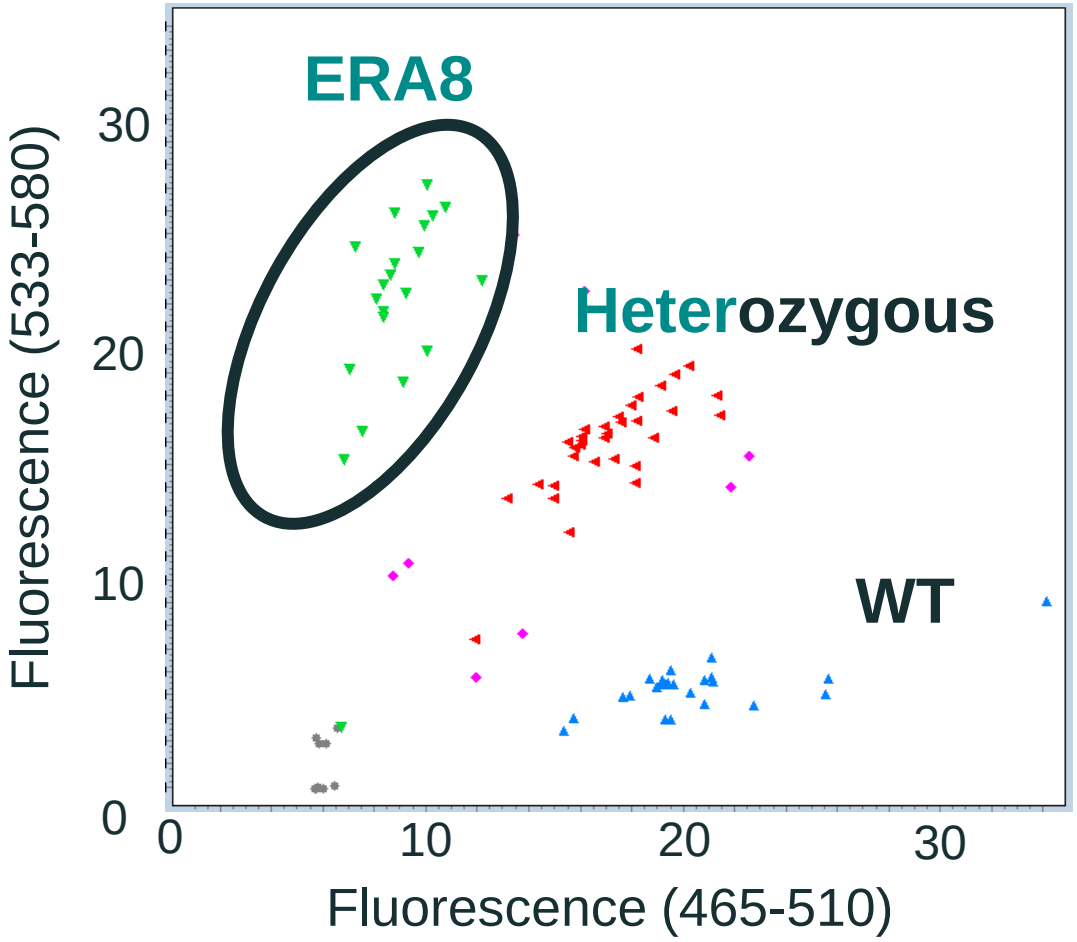
- ABA overaccumulation
- ABA transport
- ABA signaling

Annotation
B0403H10-
Metal transporter protein
S-acyltransferase
Nup133 nucleoporin family protein, expressed, protein, Uncharacterized protein
Trehalose-6-phosphate synthase 4
microtubule-associated protein 65-7
Calcium-binding EF-hand family protein
Disease resistance protein RPS2, Uncharacterized protein
phosphoenolpyruvate carboxykinase 1
Importin-4, Uncharacterized protein
Cysteine-rich receptor-like protein kinase 10, Uncharacterized protein
ADP-ribosylation factor GTPase-activating protein
Kinesin-like protein
Glutathione-S-transferase
DUF810 family protein, Uncharacterized protein
Protein EFR3-like protein A, Uncharacterized protein
AP2-like ethylene-responsive transcription factor AIL5, Uncharacterized protein
ALG6, ALG8 glycosyltransferase family protein
Myb family transcription factor APL, Uncharacterized protein
vacuolar sorting receptor homolog 1
Zinc knuckle family protein
Retrotransposon protein, putative, Ty3-gypsy subclass
Pectinesterase
DEAD-box ATP-dependent RNA helicase 20, Uncharacterized protein
Phototropic-responsive NPH3 family protein
Methyl esterase 17
O-fucosyltransferase family protein
DEAD/DEAH box RNA helicase family protein

Breeding for *ERA8*

F2 leaf tissue





Line	Allele
Zak ERA8	ERA8
Zak	WT
Louise	WT
Jasper	WT
Brevor	WT
Bruehl	WT
14x1070	WT
2006X121-0-47C	WT
X010263-10-3C	WT
X06132-36C	WT
X06141-22C	WT
ARS990077-1C	WT
HS00293-2C-1	WT
X010746-4C?	WT
070048-0-0-45L	WT
X060192-0-21C	WT
070051-0-0-20C	WT
X010301-4-2C	WT
ARS010251-5C	WT
X06136-59C	WT
2006X123-0-16C	WT
X06134-12C	WT
070056-0-0-10C	WT
X06137-2C	WT
X010662-2C	WT

Conclusion

ERA8 mapped to Chrm both by conventional QTL analysis and BSA DNAseq

We are able to use BSA DNAseq to fine map genes in wheat

ERA8 KASP markers can be used for selection in breeding programs

Future Work

See if we can clone *ERA8* using additional BC3F2 lines

Determine if candidate genes are differentially expressed

Collaborators

USDA ARS Wheat Health, Genetics, and Quality Research Unit

C.M. Steber

K.A. Garland Campbell

D. Skinner

D. See

John Innes Centre
Unlocking Nature's Diversity

O. Shorinola

Washington State University

S. Conselman

C. Uauy

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Questions