

# Higher Seed Dormancy and ABA Sensitivity Improves Wheat Preharvest Sprouting Tolerance

Shantel A. Martinez, Keiko M. Tuttle, Yumiko Takebayashi,  
Mitsunori Seo, Kimberly Garland Campbell, and Camille M.  
Steber

PAG 2016  
Plant Dormancy Workshop

# Preharvest Sprouting (PHS)

Germination of mature seed on the mother plant when cool and wet conditions occur before harvest



PHS  
Tolerant



PHS  
Susceptible

# Wheat Seed Dormancy

The inability to germinate even under favorable environmental conditions

**Dormant**



**Non-Dormant**

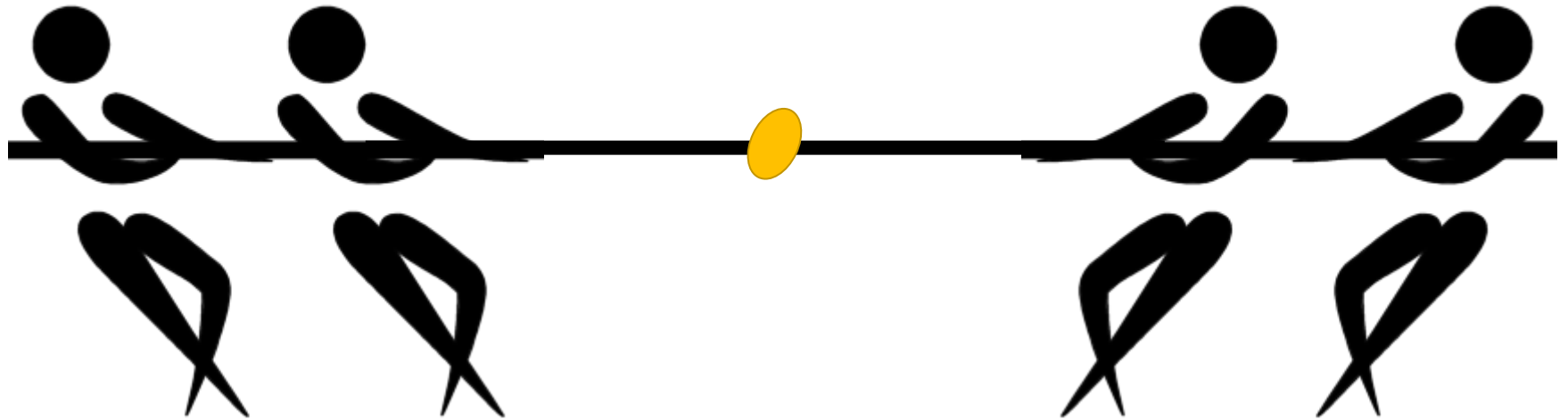
**After-ripening**

**Cold Stratification**

# Germination, a tug-of-war

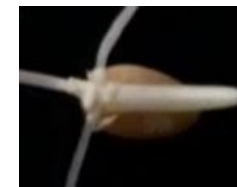
**Abscissic Acid (ABA)**

**Gibberellins (GA)**



**Seed Dormancy**

**Germination**



# Improve Wheat PHS Tolerance

# Improve Wheat PHS Tolerance

Characterizing seed dormancy already present in germplasm in the PNW : Traditional Breeding

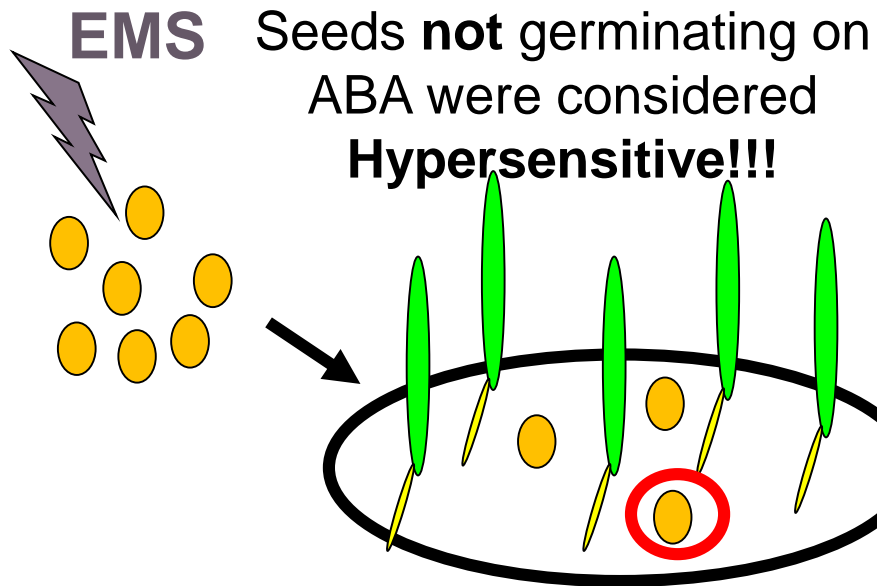
# Improve Wheat PHS Tolerance

Characterizing seed dormancy already present in germplasm in the PNW : Traditional Breeding

Use an ABA sensitive mutant to increase seed dormancy: Mutation Breeding

# Enhanced Response to ABA, ERA8

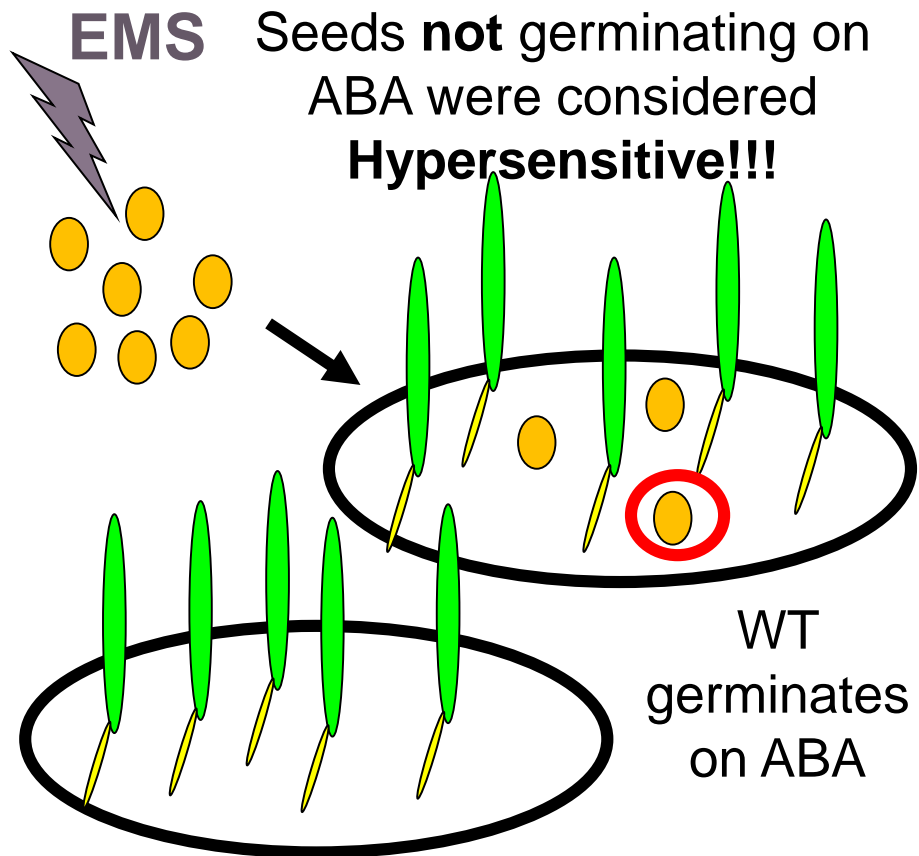
EMS mutagenized in the soft white spring cultivar, Zak.





# Enhanced Response to ABA, ERA8

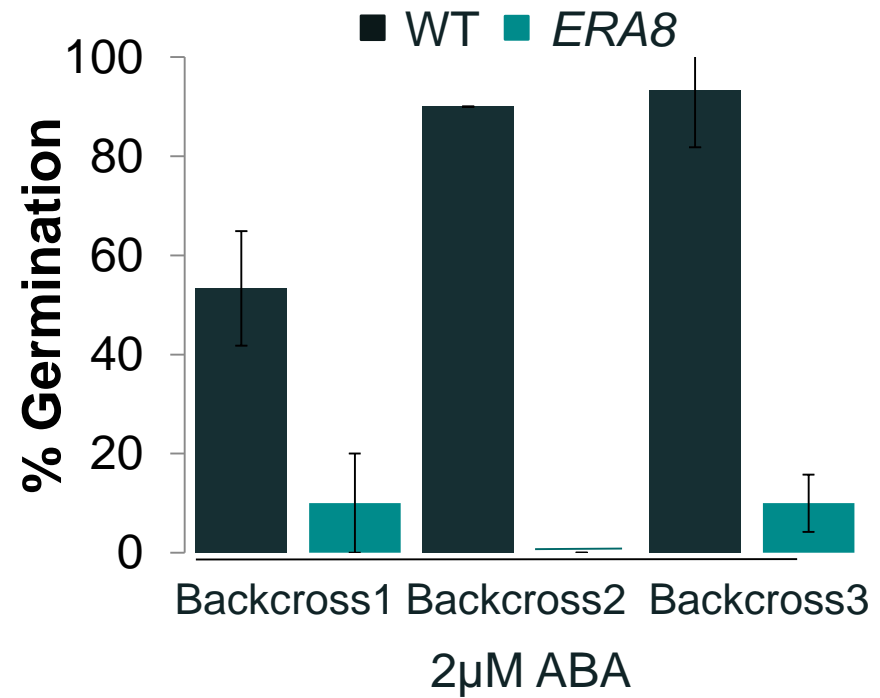
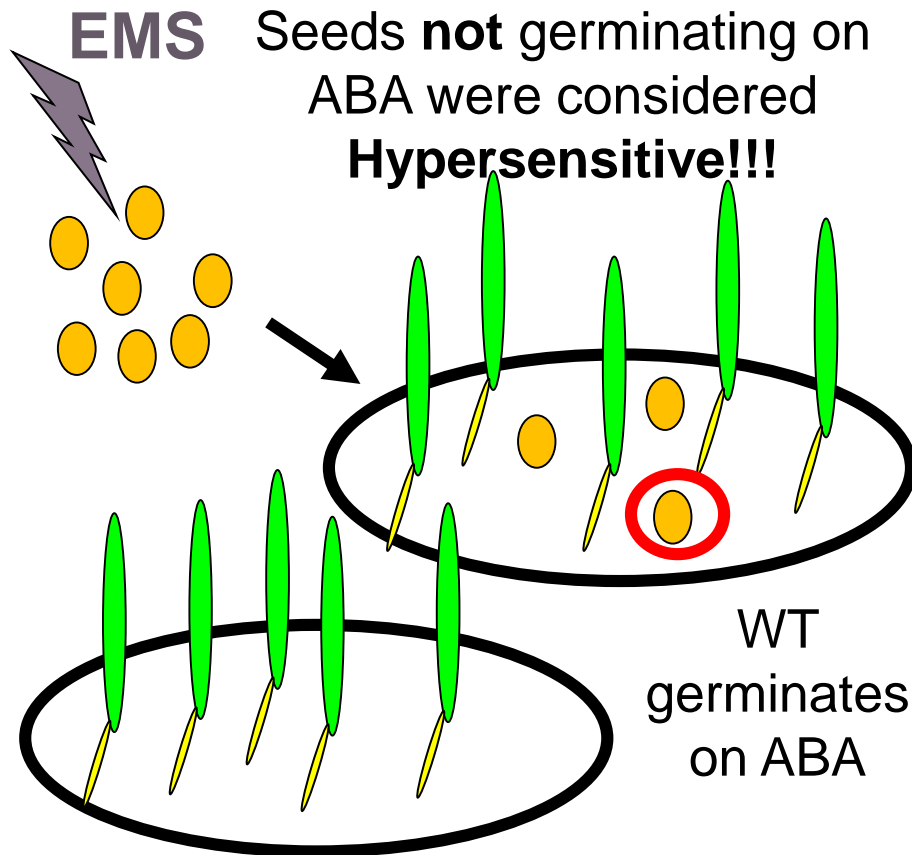
EMS mutagenized in the soft white spring cultivar, Zak.



# Enhanced Response to ABA, ERA8

EMS mutagenized in the soft white spring cultivar, Zak.

Consistently ABA Hypersensitive



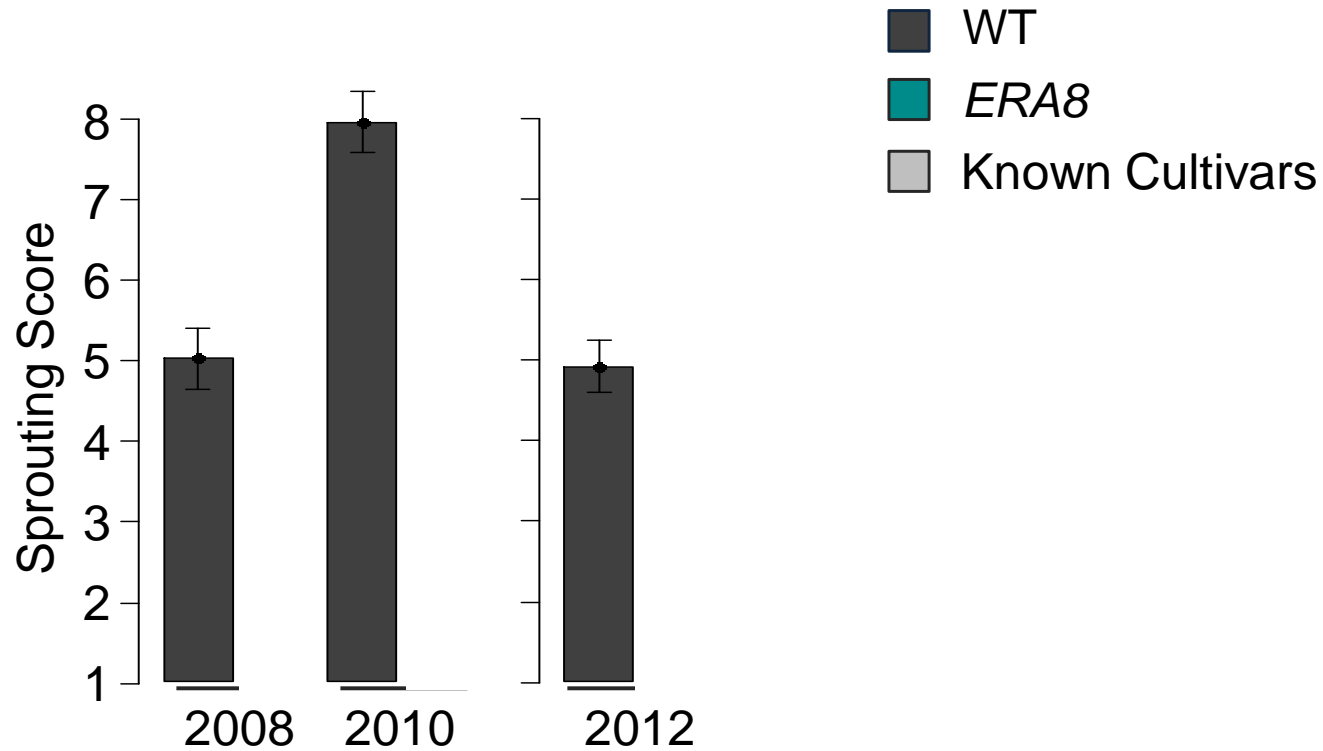
# Spike Wetting Tests

Field Grown  
Harvested at PM  
AR for 5 days  
Misted 6 sec / min

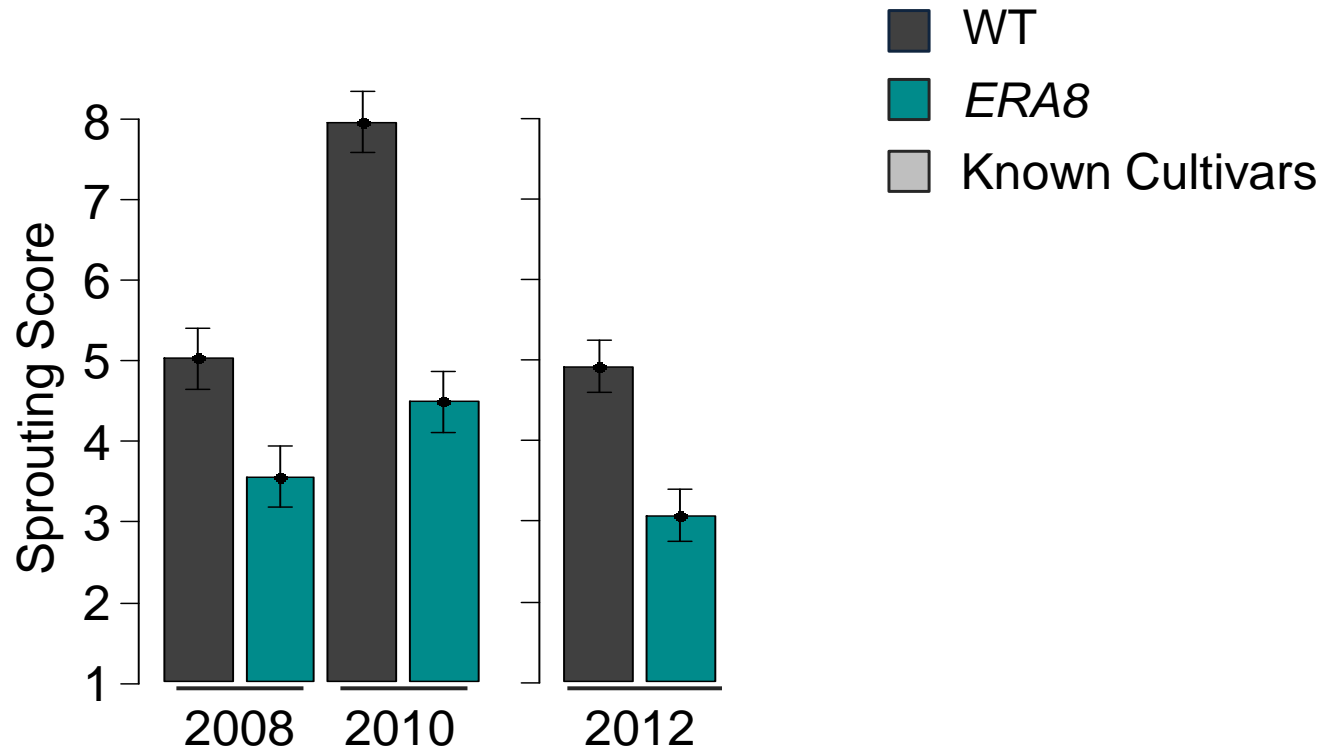
Scored every 24 hrs for 7 days



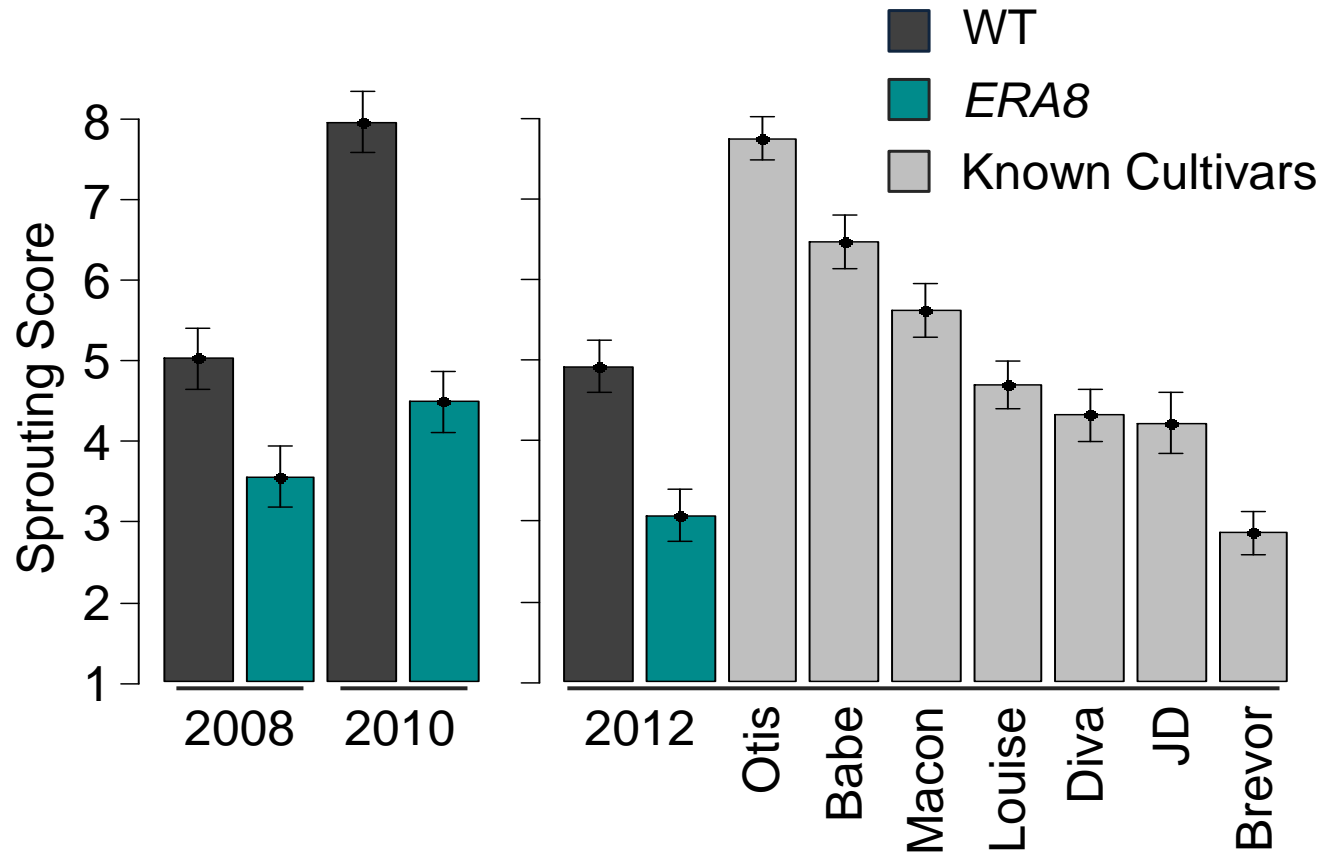
# *ERA8* shows increased PHS tolerance than WT



# *ERA8* shows increased PHS tolerance than WT

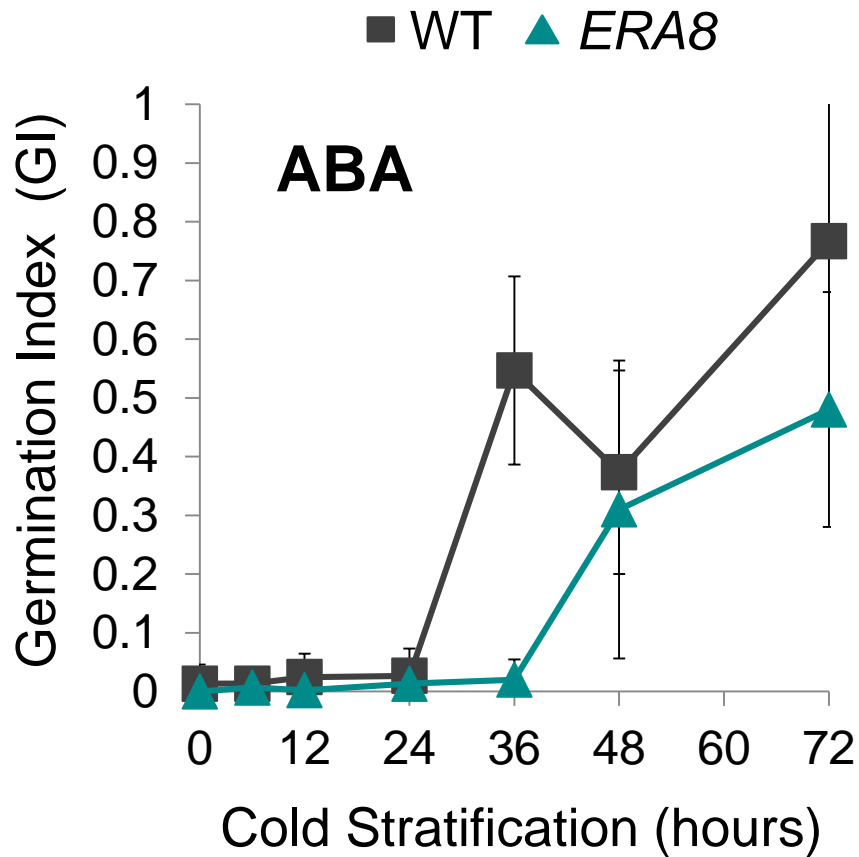


# *ERA8* shows increased PHS tolerance than WT



1. Does the *ERA8* mutant lose sensitivity to ABA more slowly with dormancy breaking treatments?  
*(cold stratification & after-ripening)*
2. Does *ERA8* show any difference in response to GA rescue of seed germination?

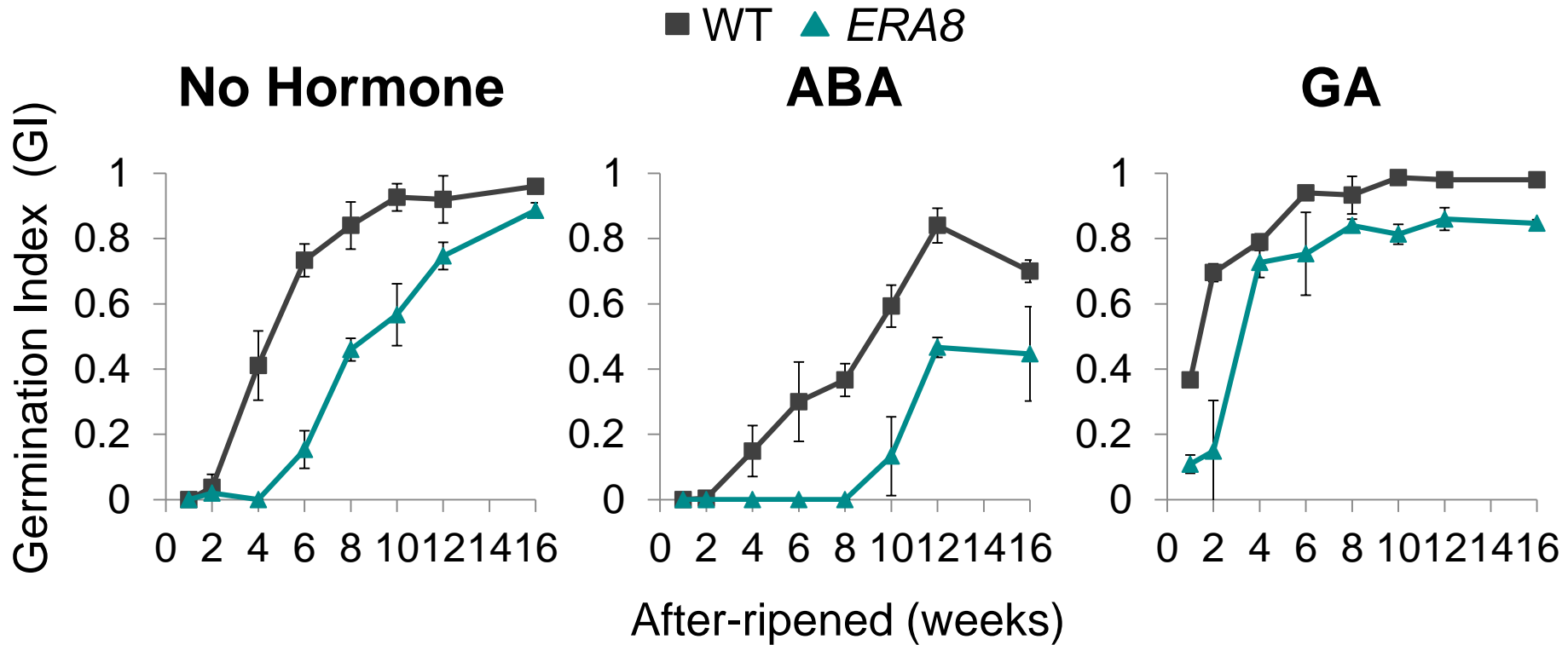
# Exogenously applied hormones over a cold stratification time course



*ERA8* remains sensitive to ABA longer in the cold than WT but is able to break dormancy eventually.

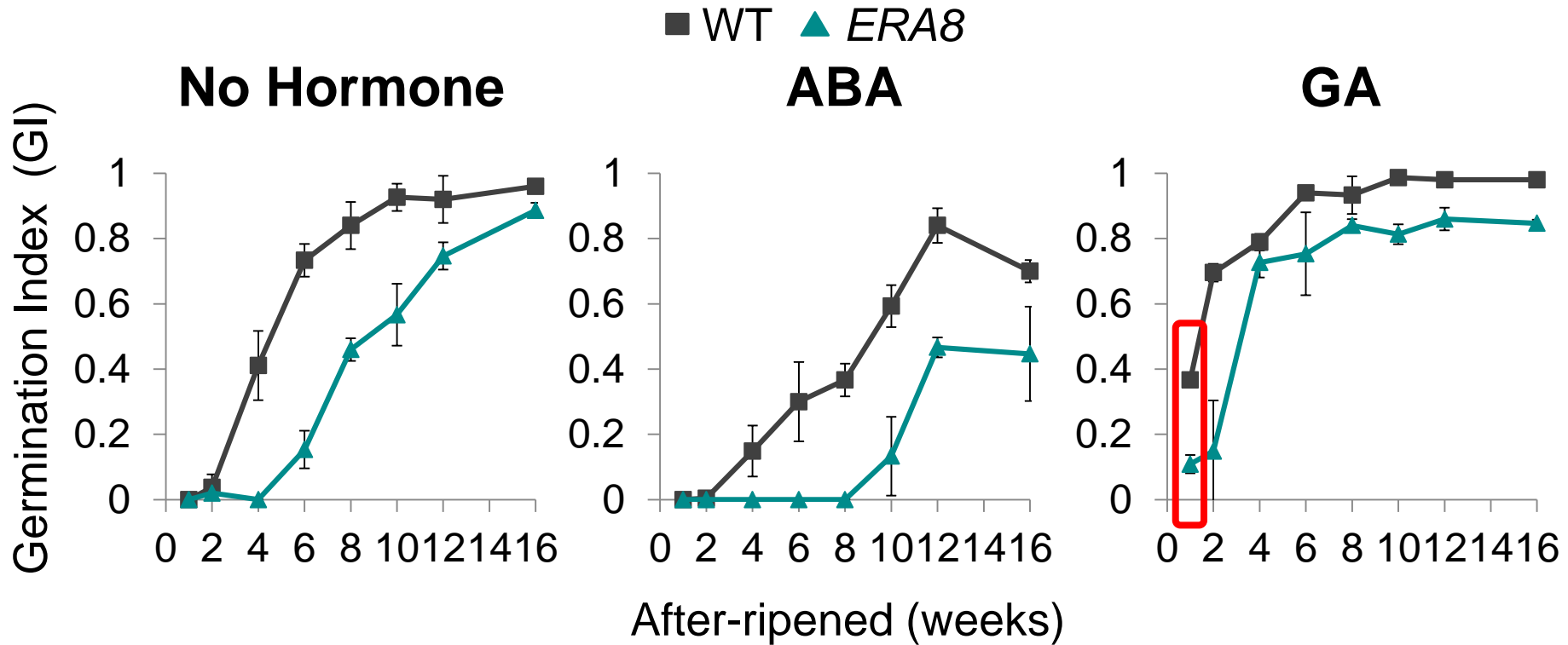


# *ERA8* after-ripens more slowly than WT



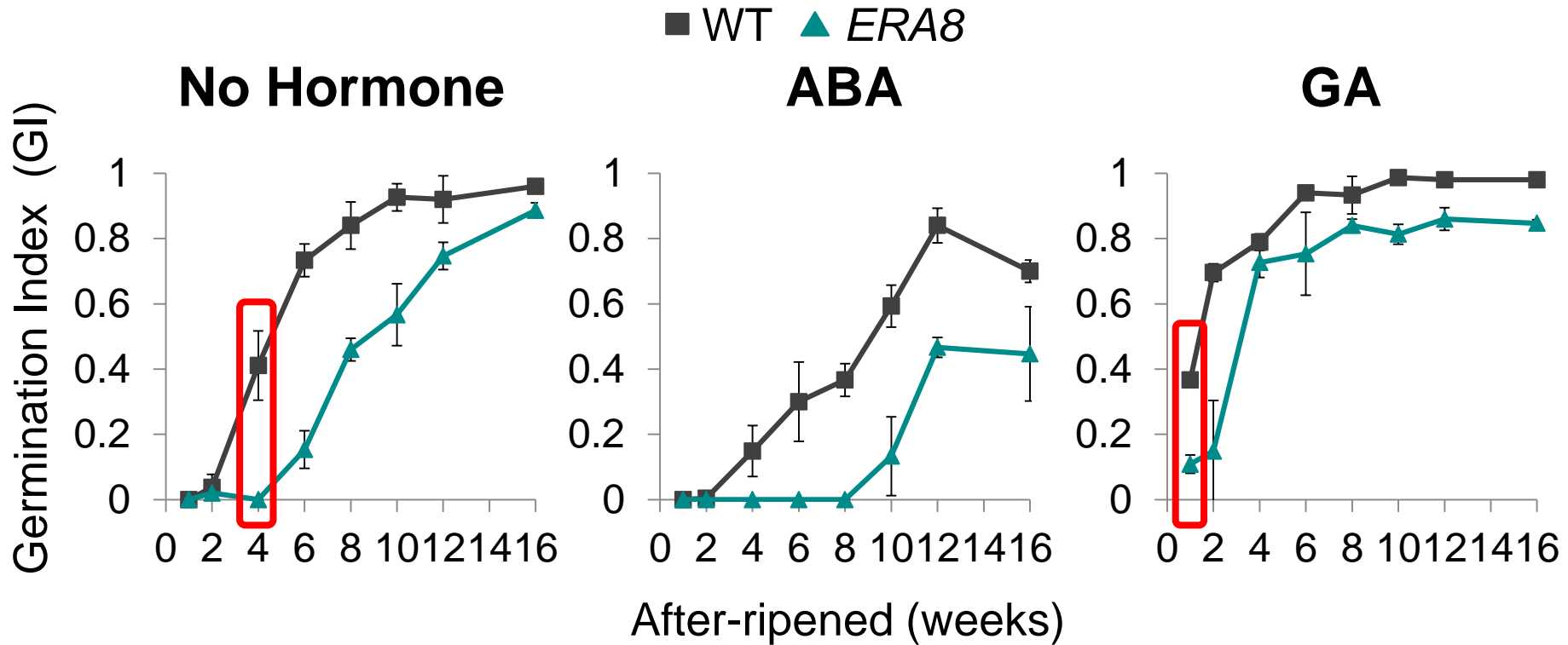
- *ERA8* is more sensitive to ABA than WT
- *ERA8* initially is insensitive to GA (rescue of germination)

# *ERA8* after-ripens more slowly than WT



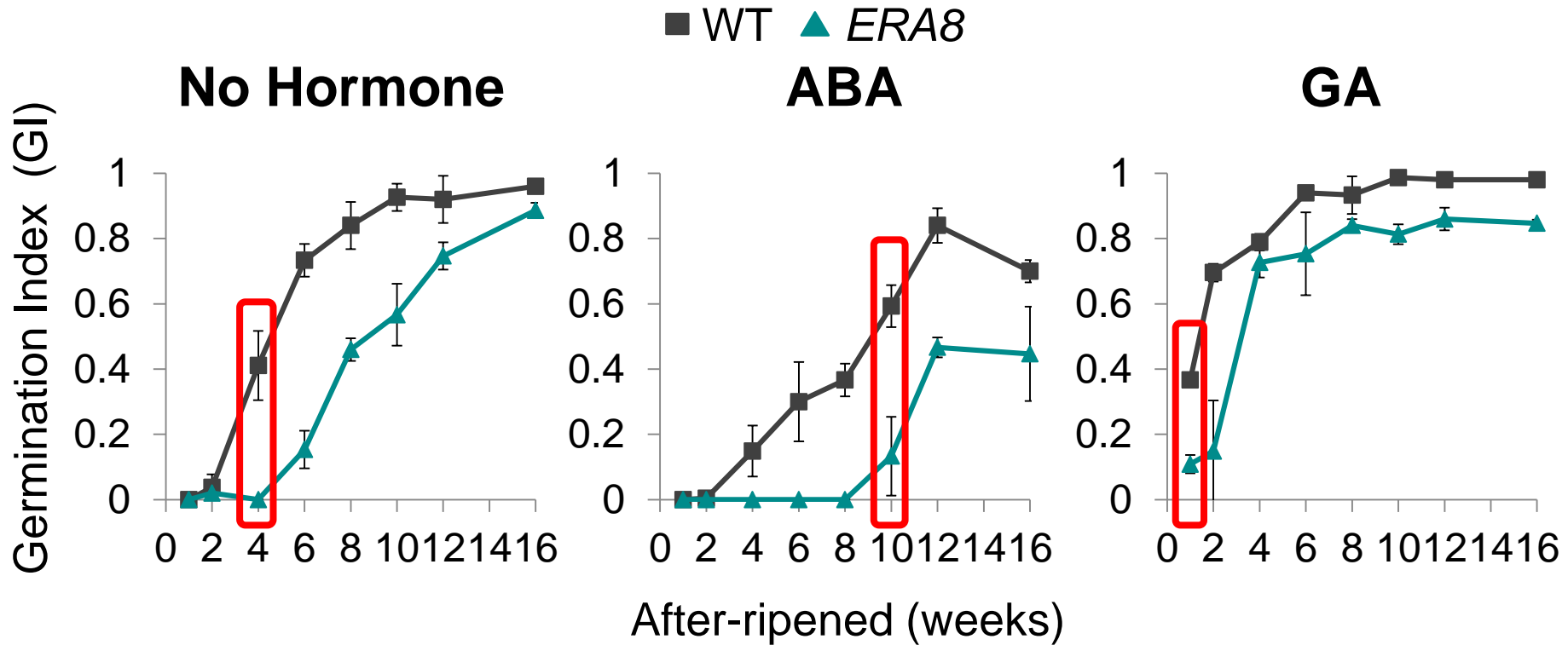
- *ERA8* is more sensitive to ABA than WT
- *ERA8* initially is insensitive to GA (rescue of germination)

# *ERA8* after-ripens more slowly than WT



- *ERA8* is more sensitive to ABA than WT
- *ERA8* initially is insensitive to GA (rescue of germination)

# *ERA8* after-ripens more slowly than WT



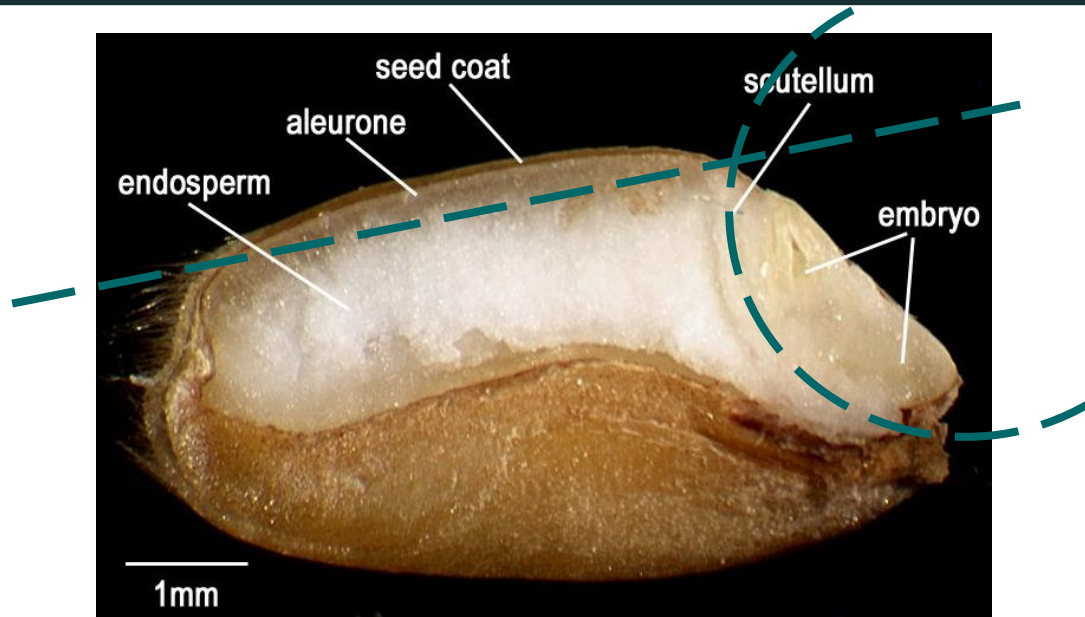
- *ERA8* is more sensitive to ABA than WT
- *ERA8* initially is insensitive to GA (rescue of germination)

1. Is the *ERA8* phenotype due to overaccumulation of ABA or a change in ABA signaling?

2. Is the *ERA8* phenotype associated with changes in other hormones?

# Hormone Content Measurements

After-ripened	Plated	Imbibed	Dissected	Hormones
1 wk	 No Hormone	→ 8 h	<ul style="list-style-type: none"> <li>•Embryo</li> <li>•Aleurone Cross Section</li> </ul>	ABA
4 wk		→ 18 h		IAA
10 wk				JA
				JA-Ile



# Does *ERA8* have higher IAA levels?

# Does *ERA8* have higher IAA levels?

- In wheat, more dormant cultivars are more sensitive to IAA inhibition of germination (Ramaih et al.,2003)

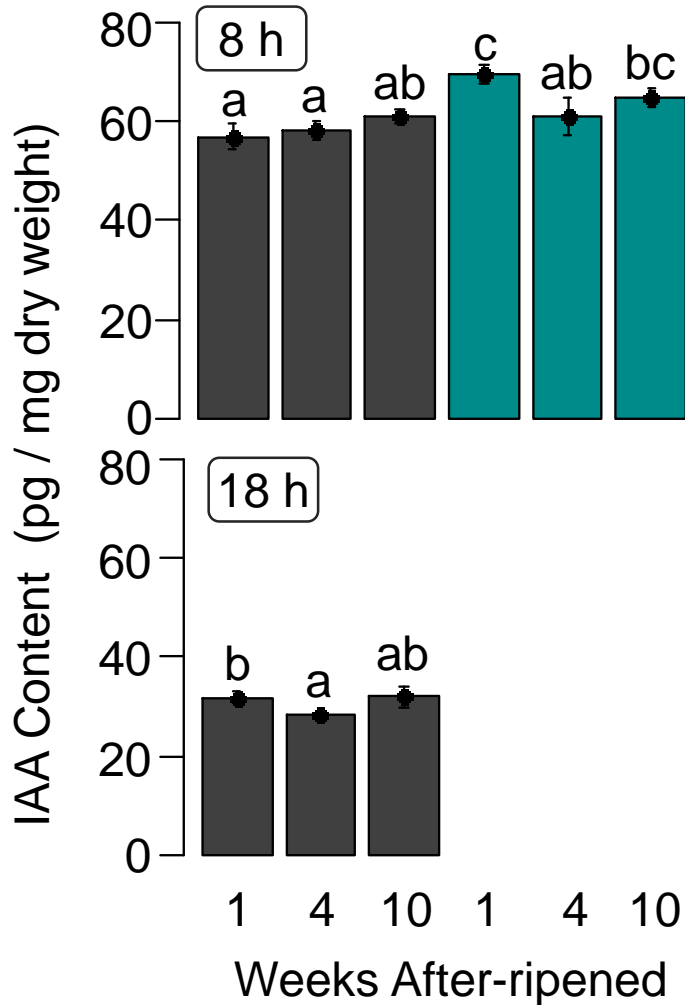


# Does *ERA8* have higher IAA levels?

- In wheat, more dormant cultivars are more sensitive to IAA inhibition of germination (Ramaih et al.,2003)
- Exogenously applied IAA inhibits germination (Morris et al., 1988)

# Does *ERA8* have higher IAA levels?

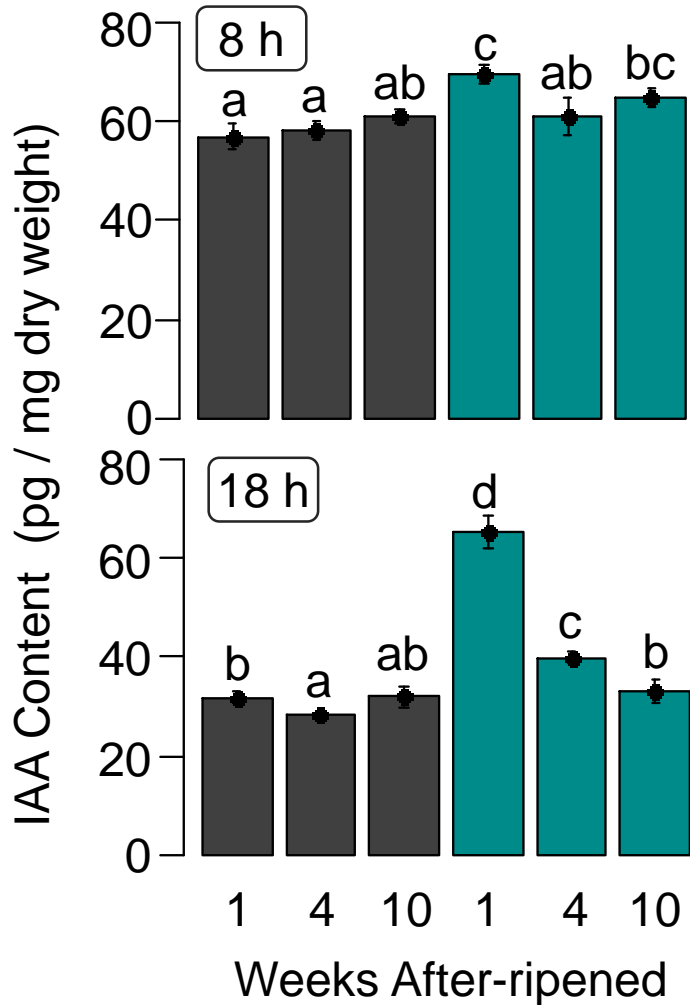
Aleurone    ■ WT    ■ *ERA8*



- In wheat, more dormant cultivars are more sensitive to IAA inhibition of germination (Ramaih et al., 2003)
- Exogenously applied IAA inhibits germination (Morris et al., 1988)

# Does *ERA8* have higher IAA levels?

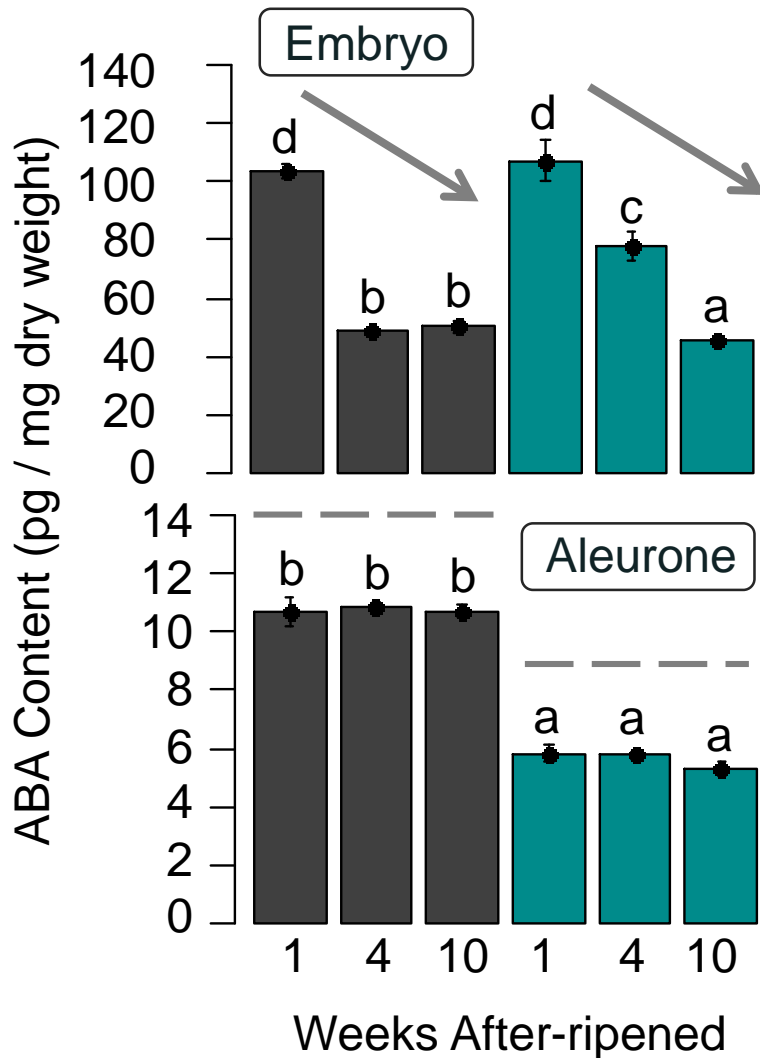
Aleurone    ■ WT    ■ *ERA8*



- In wheat, more dormant cultivars are more sensitive to IAA inhibition of germination (Ramaih et al., 2003)
- Exogenously applied IAA inhibits germination (Morris et al., 1988)
- At 1 week of after-ripening, *ERA8* has higher IAA levels than WT
- IAA levels decrease with after-ripening in *ERA8*.

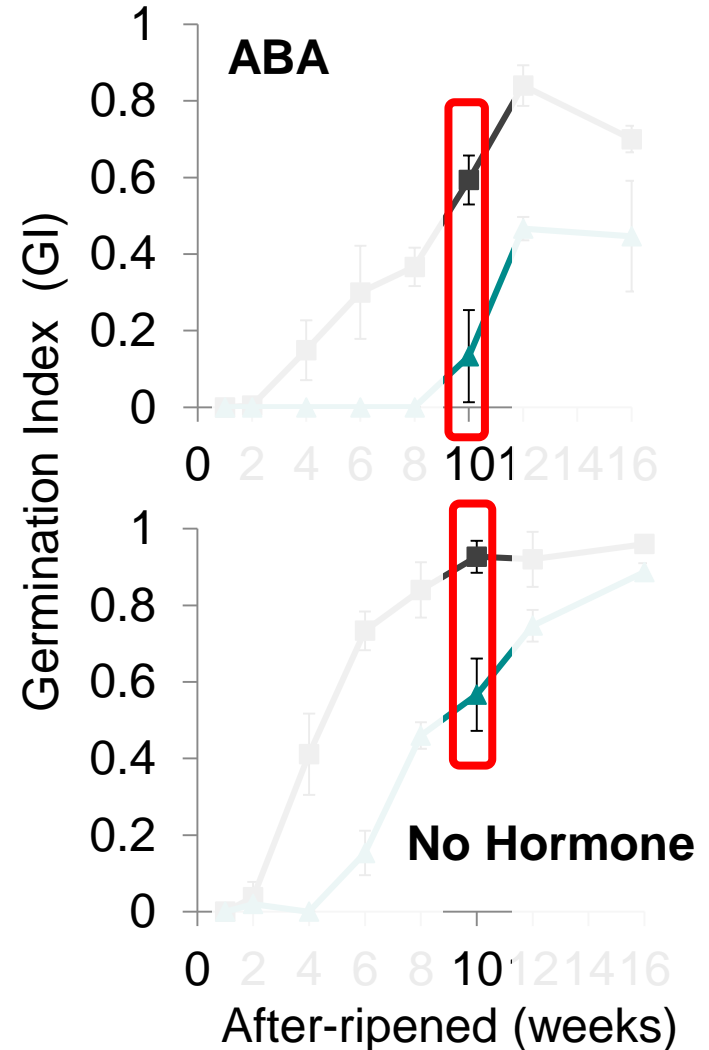
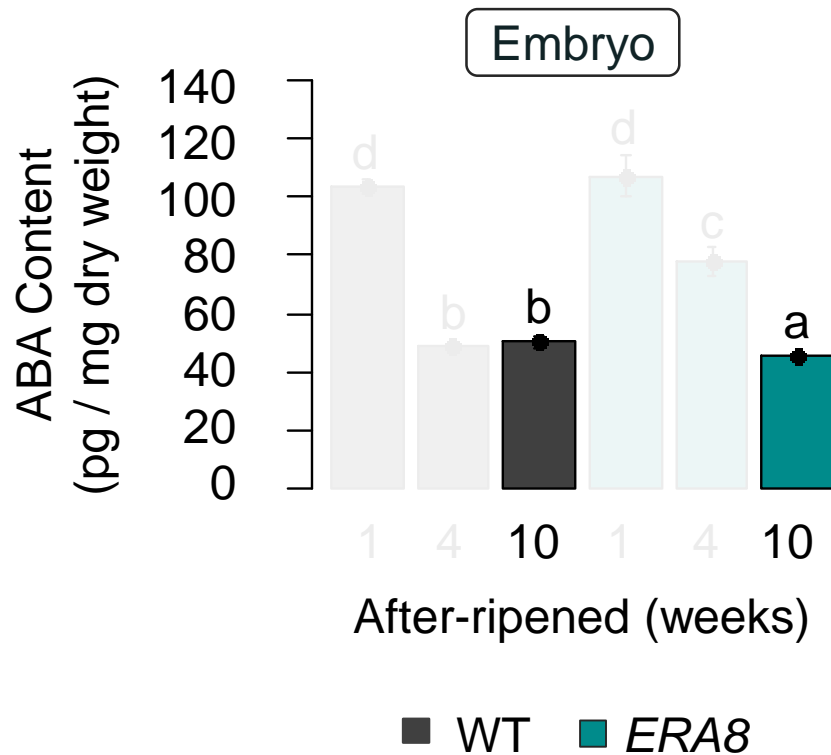
# Does *ERA8* have higher ABA levels?

■ WT ■ *ERA8*

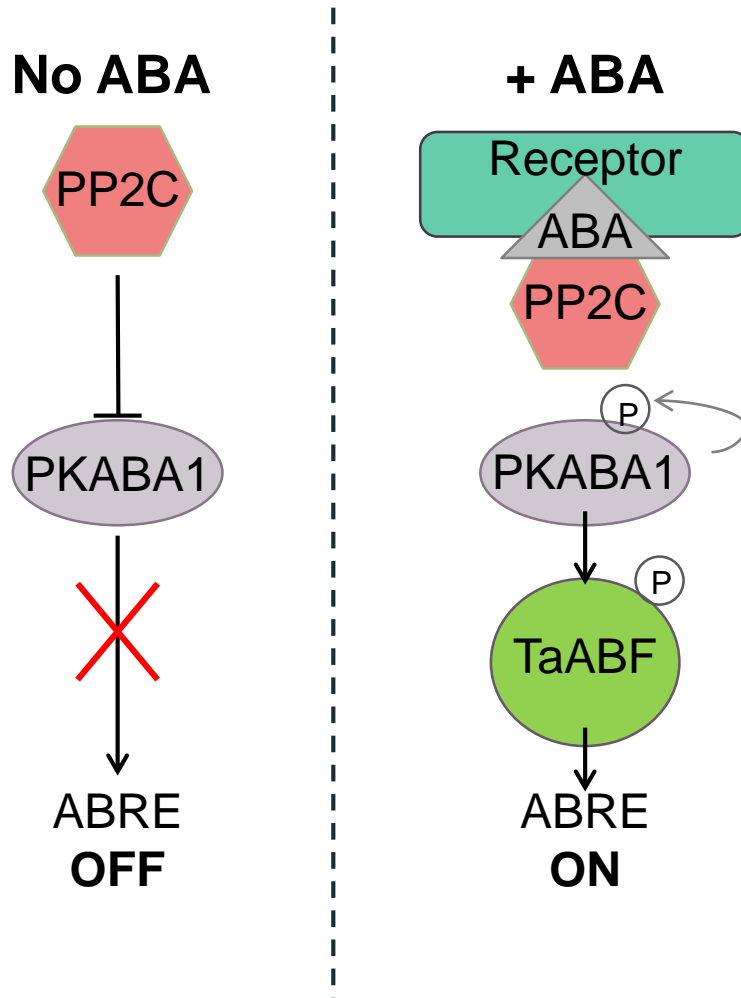


- Initially WT and *ERA8* are not different in the embryo
- ABA is decreasing with AR of **BOTH** *ERA8* and WT
- ABA levels in *ERA8* were actually lower in the aleurone than WT.
- ABA content in the aleurone does not parallel the embryo

# Hormone Content vs Germination Profile



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



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

## 3 possibilities

- ABA overaccumulation
- ABA transport
- ABA sensitive

# Conclusions

- Increased ABA sensitivity in *ERA8* is associated with higher seed dormancy and PHS tolerance.

# Conclusions

- Increased ABA sensitivity in *ERA8* is associated with higher seed dormancy and PHS tolerance.
- *ERA8* is associated with elevated levels of IAA, an inhibitor of wheat germination.



# Conclusions

- Increased ABA sensitivity in *ERA8* is associated with higher seed dormancy and PHS tolerance.
- *ERA8* is associated with elevated levels of IAA, an inhibitor of wheat germination.
- In wheat, loss of seed dormancy is associated with decreasing ABA levels.

# Conclusions

- Increased ABA sensitivity in *ERA8* is associated with higher seed dormancy and PHS tolerance.
- *ERA8* is associated with elevated levels of IAA, an inhibitor of wheat germination.
- In wheat, loss of seed dormancy is associated with decreasing ABA levels.
- The *ERA8* mutation is not associated with a failure in ABA turnover with after-ripening.

# Conclusions

- Increased ABA sensitivity in *ERA8* is associated with higher seed dormancy and PHS tolerance.
- *ERA8* is associated with elevated levels of IAA, an inhibitor of wheat germination.
- In wheat, loss of seed dormancy is associated with decreasing ABA levels.
- The *ERA8* mutation is not associated with a failure in ABA turnover with after-ripening.
- The *ERA8* phenotype likely results from increased ABA signaling, such as a gain-of-function mutation in a positive regulator of ABA response.

# Acknowledgments

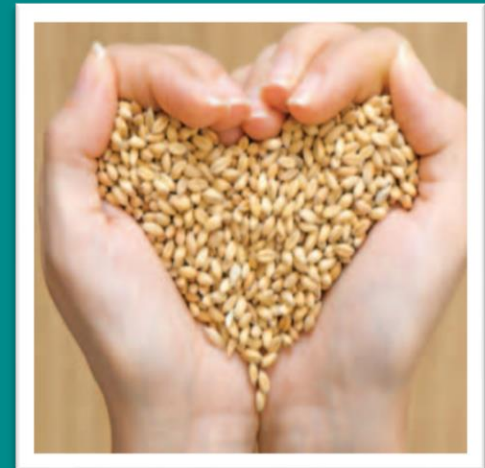
Cornell University:  
Mark Sorrells

RIKEN:  
Mitsunori Seo  
Yumiko Takebayashi

Washington State University:  
Camille M Steber  
Kimberly Garland Campbell  
Keiko M Tuttle  
Tracy J Harris  
Lucia Strader  
Elizabeth Schramm  
Sven Nelson



Arron H Carter  
Scot H Hulbert  
Campbell & Steber Lab  
USDA Field Crew



# References & Questions

**Klingler**, J. P., Batelli, G. & Zhu, J.-K. ABA receptors: the START of a new paradigm in phytohormone signalling. *J. Exp. Bot.* 61, 3199–3210 (2010).

**McMaster**, G. J. & **Derera**, N. F. Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Research Communications* 4, 251–254 (1976).

**Morris**, C. F., Paulsen, G. M., Mueller, D. D. & Faubion, J. M. Identification of L-Tryptophan as an endogenous inhibitor of embryo germination in white wheat. *Plant Physiology* 88, 435–440 (1988).

**Schramm**, E. C., Nelson, S. K., Kidwell, K. K. & Steber, C. M. Increased ABA sensitivity results in higher seed dormancy in soft white spring wheat cultivar 'Zak'. *Theor Appl Genet* 126, 791–803 (2013).

**Ramaih**, S., Guedira, M. & Paulsen, G. M. Relationship of indoleacetic acid and tryptophan to dormancy and preharvest sprouting of wheat. *Functional Plant Biology* 30, 939–945 (2003).

**Johnson**, R.R., Wagner, R.L., Verhey, S.D., & Walker-Simmons, M.K. The Abscisic Acid-Responsive Kinase PKABA1 Interacts with a Seed-Specific Abscisic Acid Response Element-Binding Factor, TaABF, and Phosphorylates TaABF Peptide Sequences. *Plant Physiol.* 130, 837–846 (2002).