Genome-Wide Association Mapping of Preharvest Sprouting Traits in PNW Wheat

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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

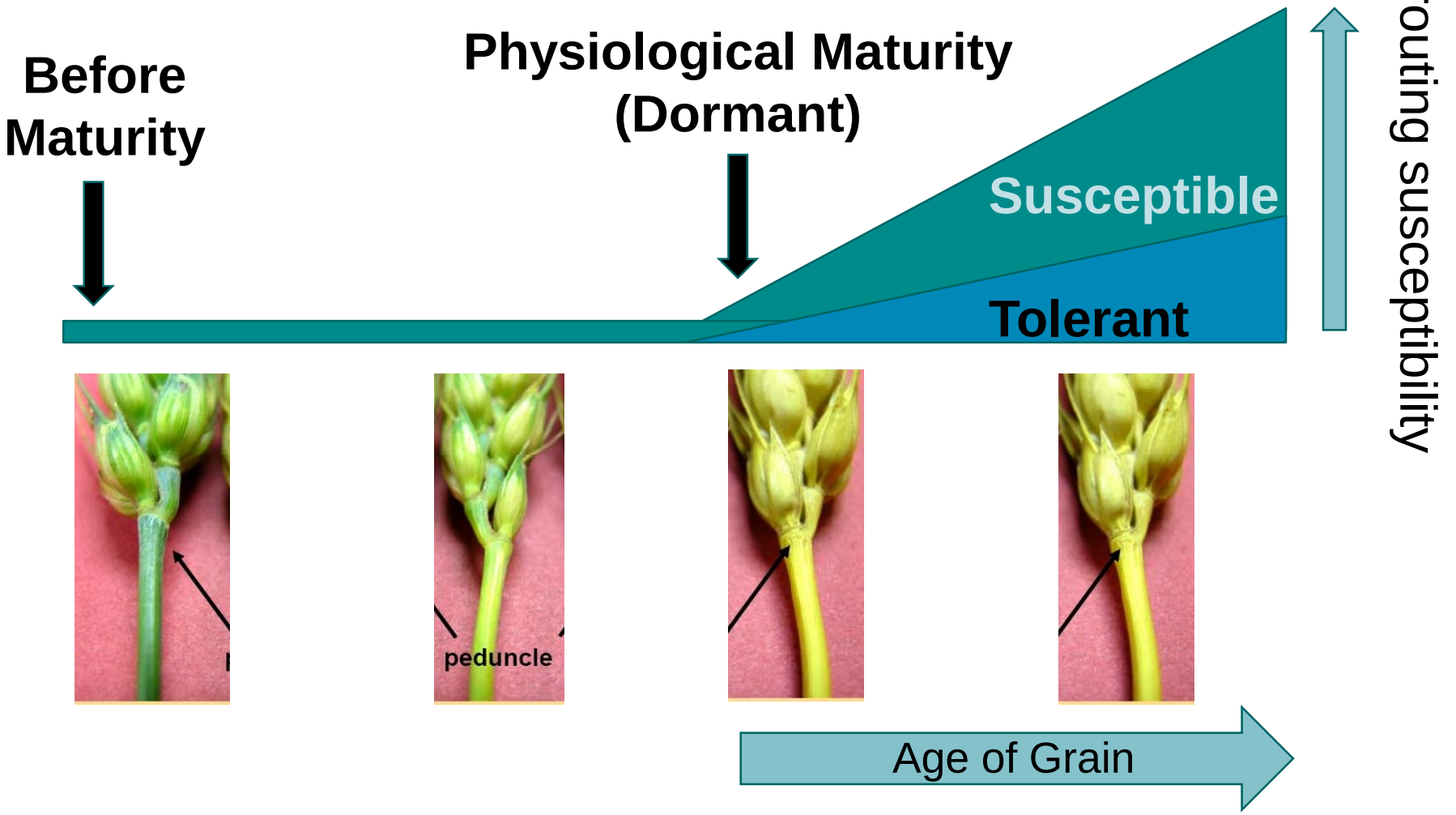
Dormant

After-ripening

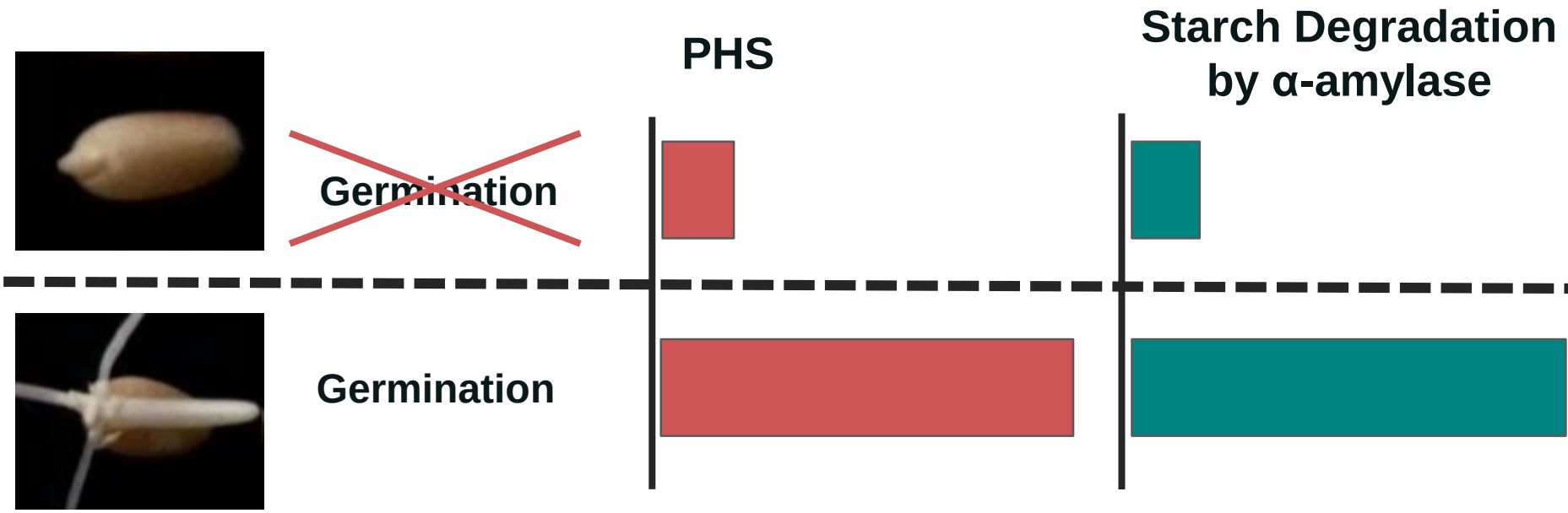
Cold Imbibition

Non-Dormant

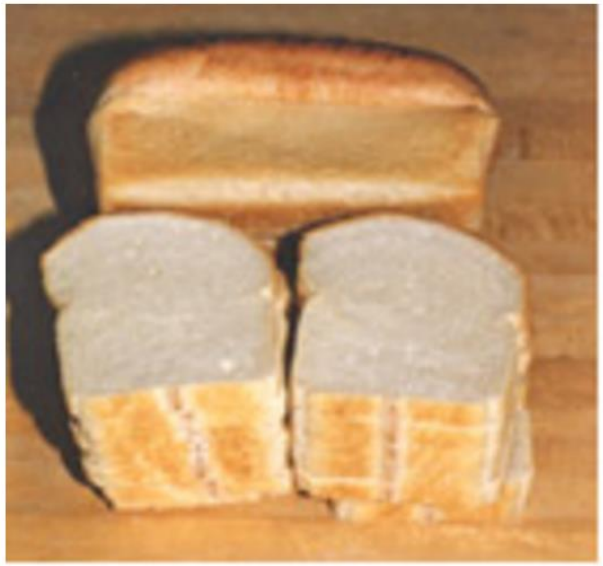
Susceptibility to preharvest sprouting depends on maturity date



What does this mean to the farmers?



What does this mean for end-use?

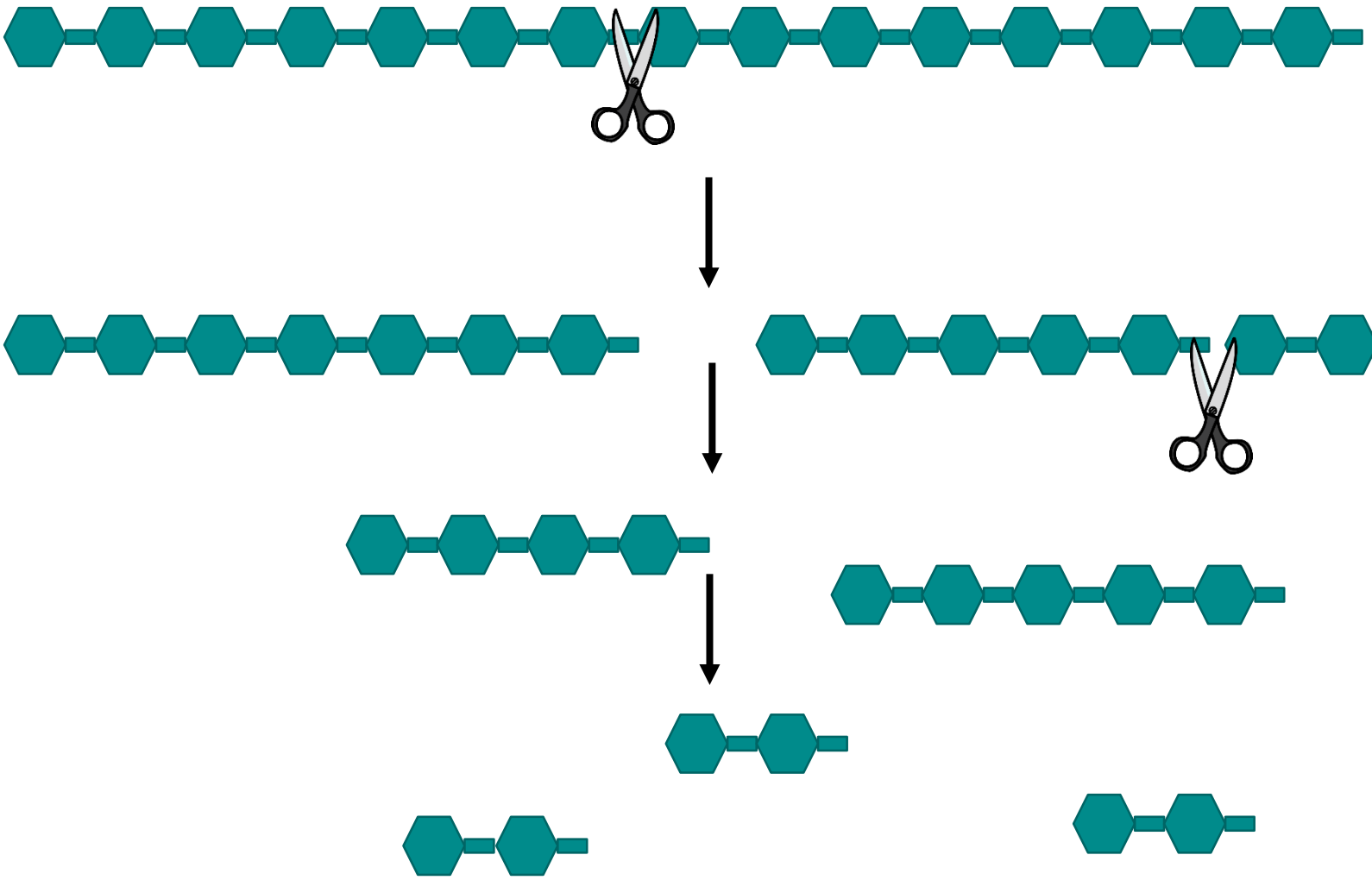


Sound

Sprouted

Severely Sprouted

As alpha-amylase cuts, the starch chains get smaller and provide less structural integrity.



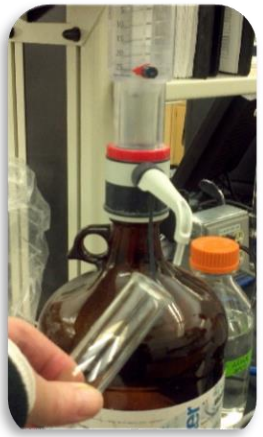
Gravy **gels** if starch strands are long

Gravy is **watery** if starch strands are short

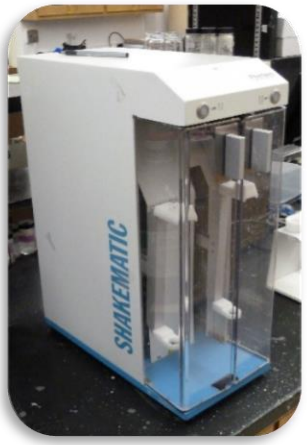
Hagberg-Perten Falling Number

1. Grind

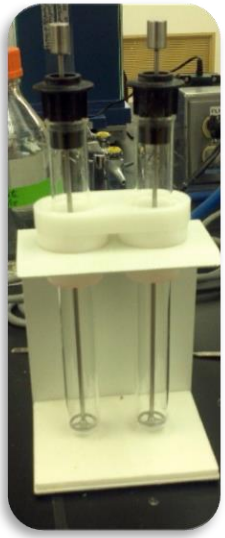
2. Weigh & adjust for moisture



3. Add water



4. Mix



5. Add stirrers

6. Stir and heat for 60 sec

7. Drops & Measures Time. Correct for altitude of 2500ft.

Hagberg-Perten Falling Number

Low FN is associated with low end use quality

1. Grind

2. Weigh & adjust for moisture

3. Add water

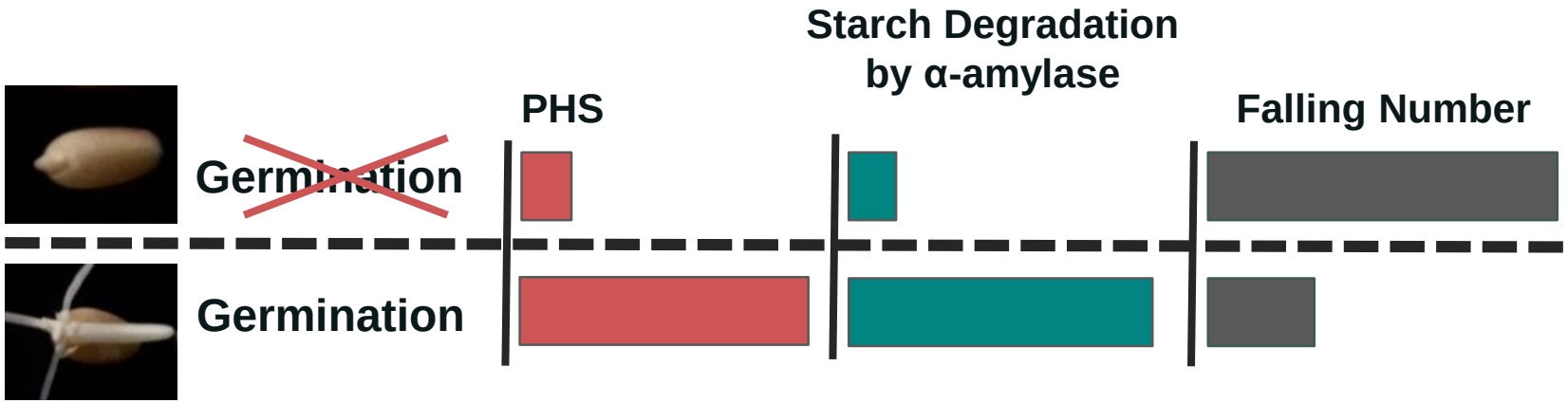
4. Mix

5. Add stirrers

6. Stir and heat for 60 sec

7. Drops & Measures Time. Correct for altitude of 2500ft.

What does this mean to the farmers?

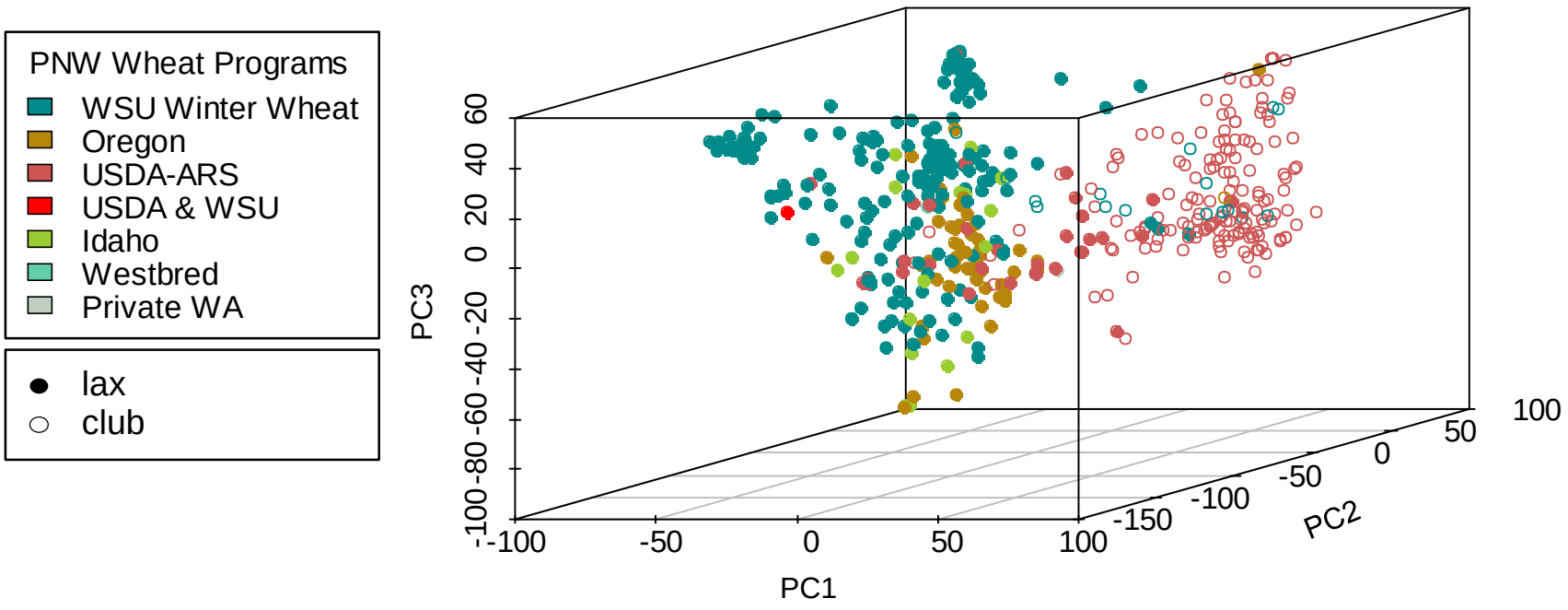


Improving PHS Tolerance

Genome-Wide Association Study

Mutation Breeding

Genetic Principal Component Analysis based on SNP genotyping of 469 lines



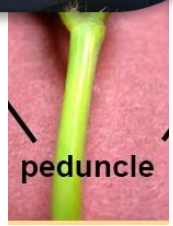
The panel is derived from at least six white winter wheat breeding programs.

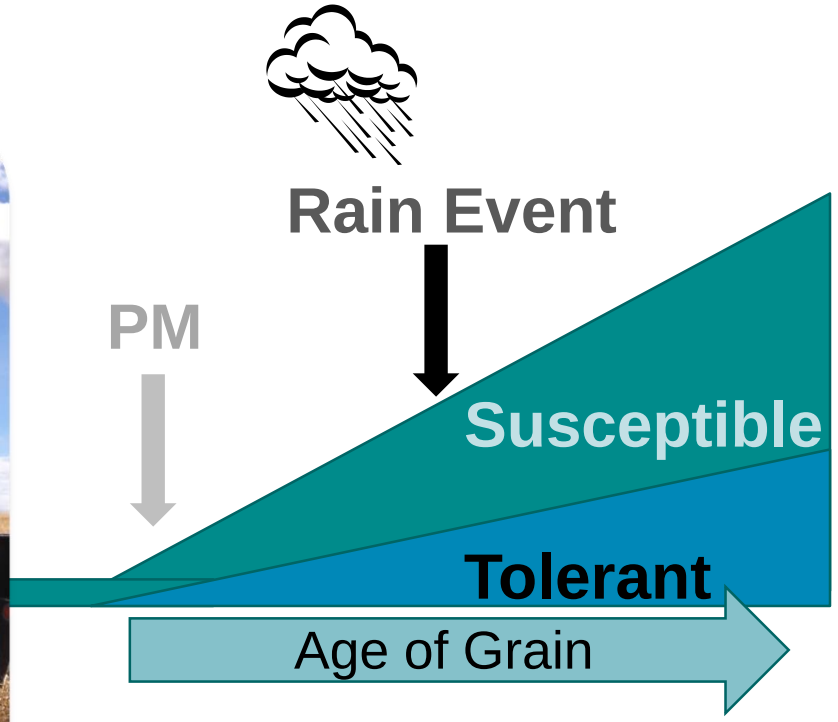
Jernigan and Godoy et al., 2018 | Martinez et al., 2016

Hypothesis

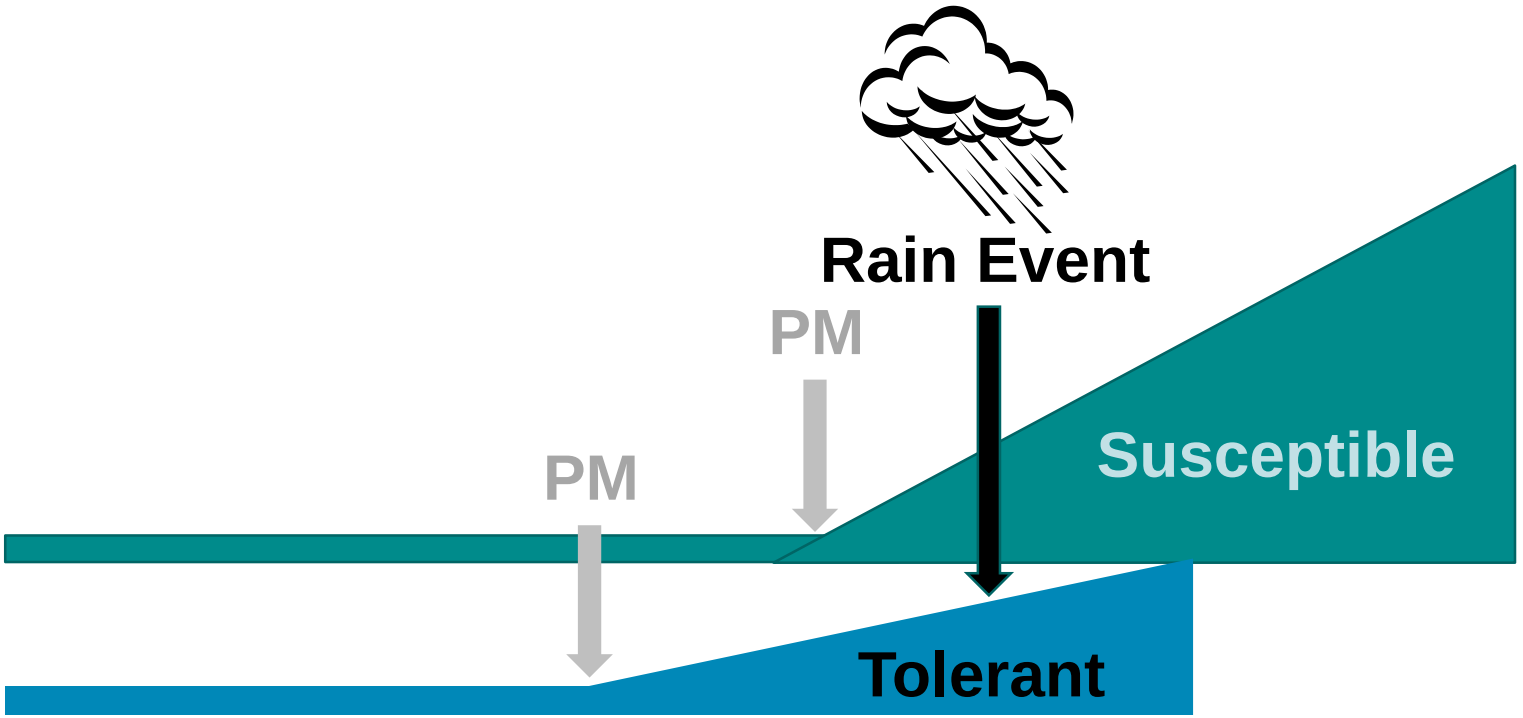
If sprouting is the main cause of low FN, then similar loci should be detected based on Falling Numbers and on the appearance on visible sprouting in spike wetting tests through association mapping.

FN: the degree of dormancy depends upon maturity of individual varieties



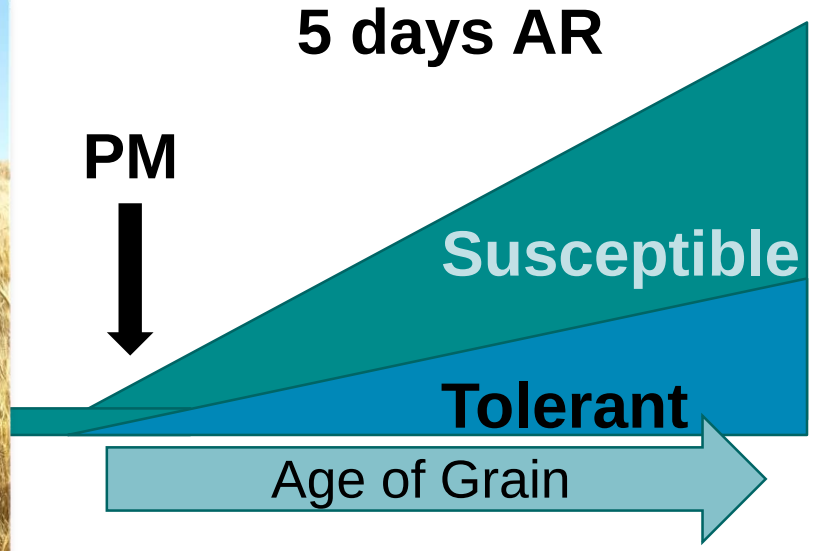


Effect of rain on sprouting depends on its timing relative to grain maturity



If we base our conclusions about PHS on a single rain event, then an early-maturing tolerant line may seem “worse” than a late maturity sprouting susceptible line.

Spike wetting test samples were harvested at physiological maturity (PM)



Greenhouse Spike Wetting Test

Misted 6 sec / min
Scored every 24 hrs for 7 days

Visible Sprout Scored

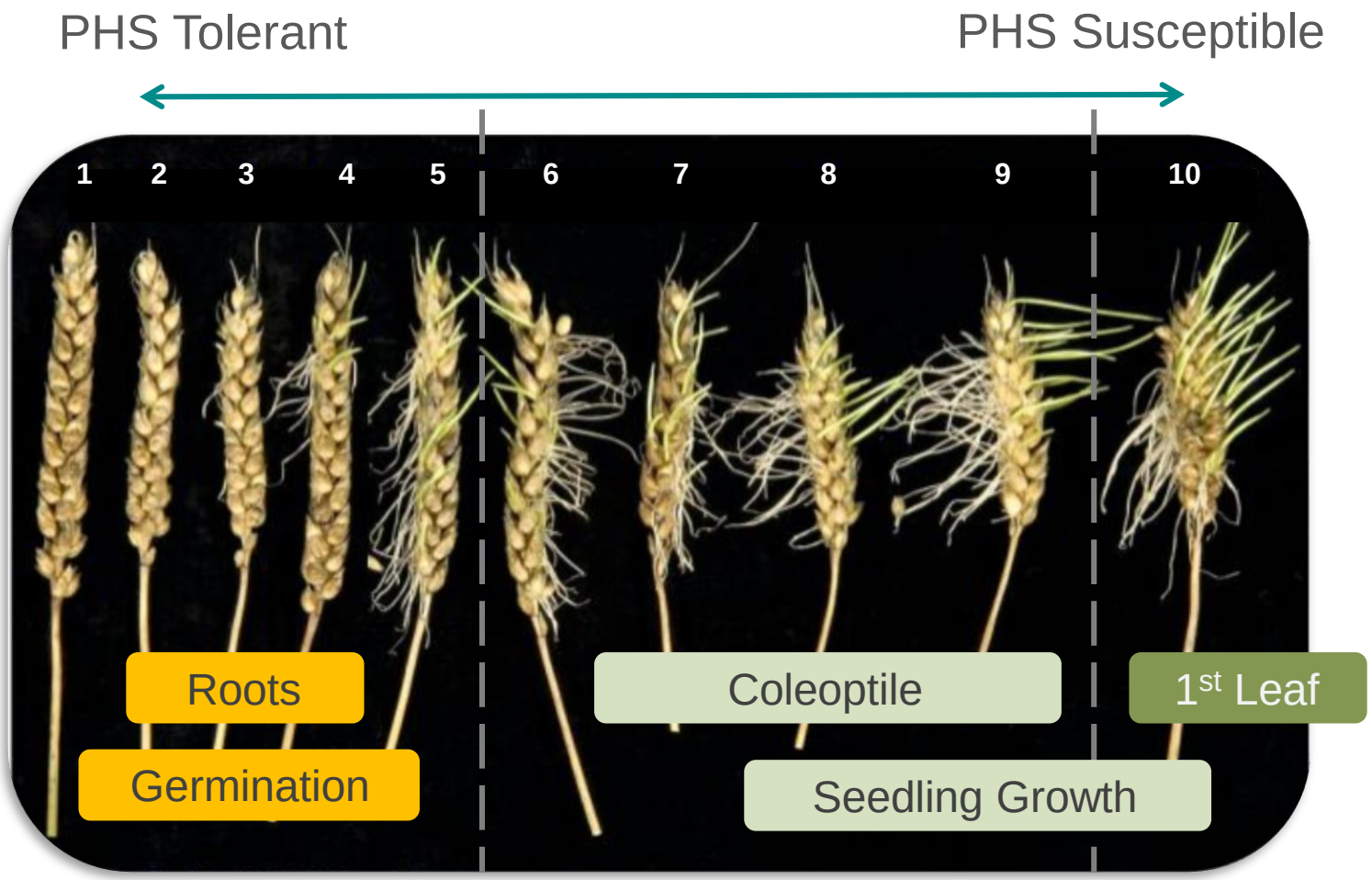


Photo: Kent Loeffler | McMaster & Derera *et al*., 1976

Association Mapping Population

- 469 white winter wheat PNW breeding lines & released cultivars

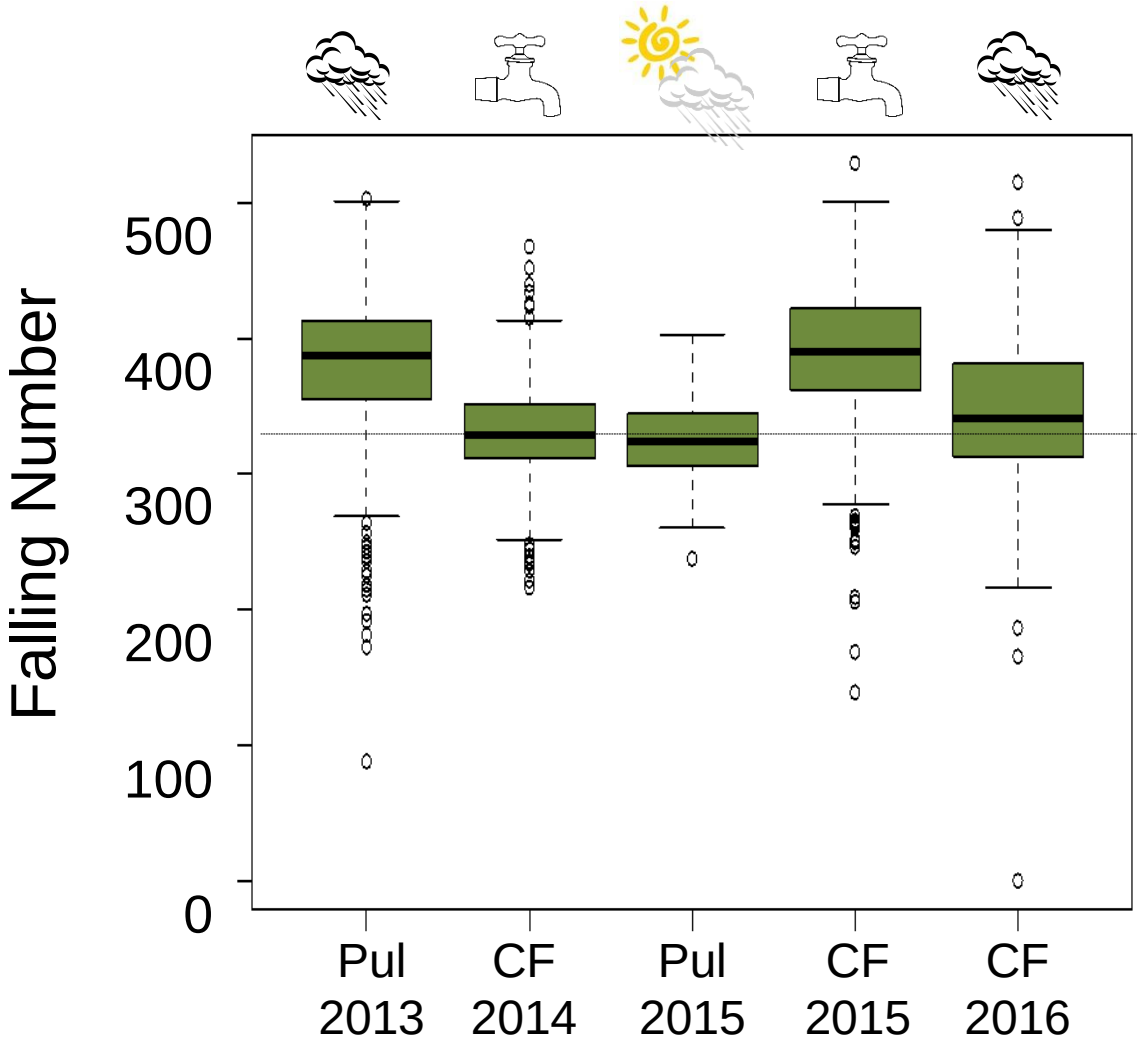
Phenotype

- Spike wetting tests
- Falling Numbers

Environments

- Grown in Pullman (Pul) and Central Ferry (CF), WA 2013-2015

FN Phenotypic Distributions



Pul: Pullman

CF: Central Ferry

Martinez et al., 2018

FN Environment Correlations




	Pul13	CF14	Pul15	CF15
CF14	0.29**			
Pul15	0.23**	**0.42****		
CF15	0.23**	0.29**	0.29**	
CF16	0.33**	**0.46****	0.30**	0.34**

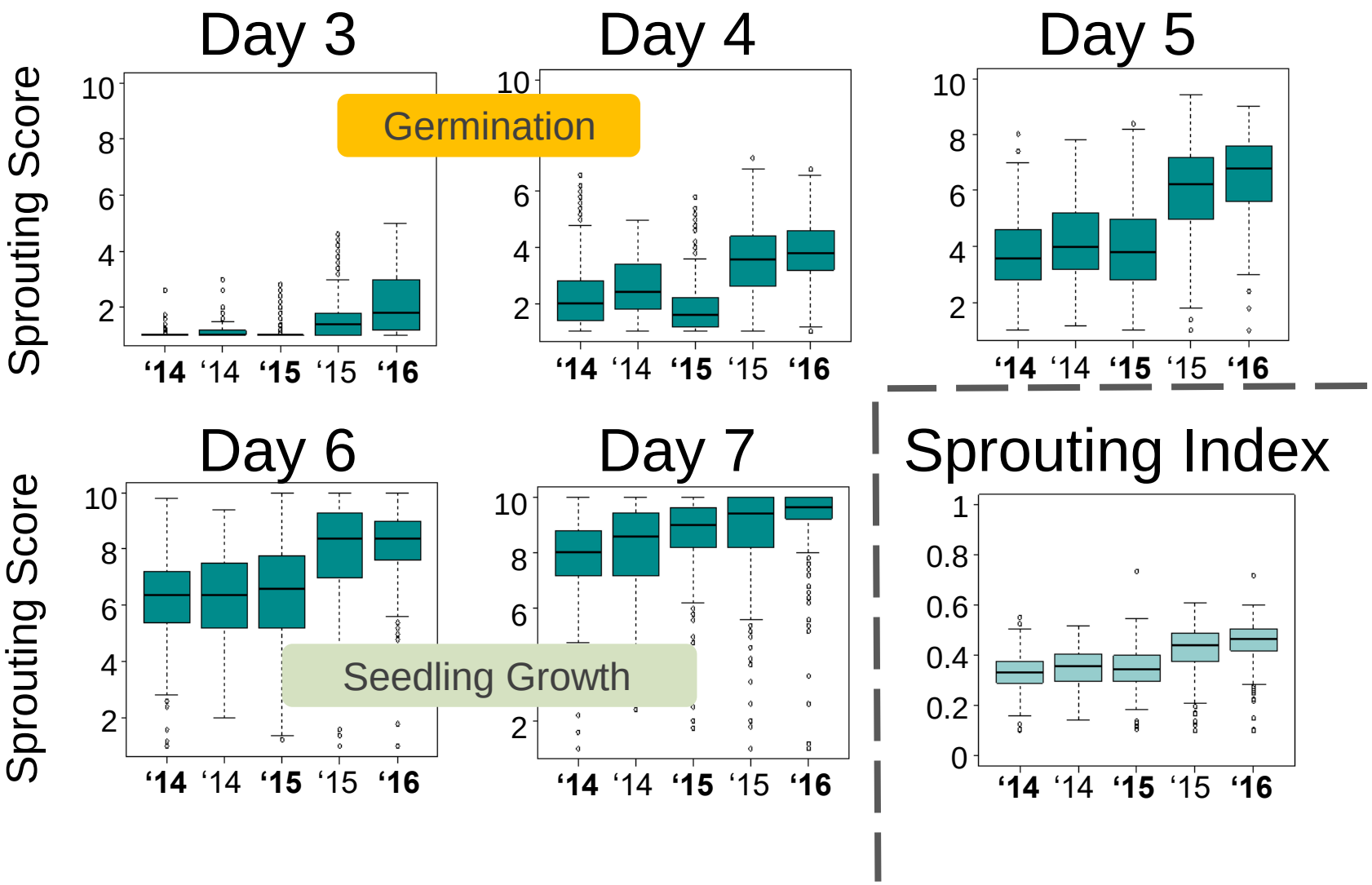



**: $p \leq 0.001$ * : $p < 0.05$

Martinez et al., 2018

Visible Sprout Phenotypic Distributions

Pullman Central Ferry



Visible Sprout Environment Correlations

Day 6 | Seedling Growth

	CF14	Pul16	Pul14	CF15
Pul16	0.39**			
Pul14	0.39**	0.29**		
CF15	0.38**	0.30**	**0.40****	
Pul15	0.34**	0.16*	**0.46****	0.36**

**: $p \leq 0.001$ * : $p \leq 0.05$

In fact, the correlations between our environments were as good as those in other spike-wetting test studies: Kulwal et al., 2012; Jaiswal et al., 2012; Ogbonnaya et al., 2008; Zhou et al., 2017

Martinez et al., 2018

FN (PM+2wk) versus Visible Sprout (PM) Correlations

Germination | Seedling Growth

	3 days	4 days	5 days	6 days	7 days	SI
Pul13	-0.16**	-0.24**	-0.17**	-0.18**	-0.20**	-0.21**
CF14	-0.07	-0.09*	-0.06	-0.09	-0.10*	-0.10*
Pul15	-0.07	-0.13*	-0.12*	-0.12*	-0.17**	-0.15**
CF15	-0.09	-0.04	0.00	0.01	0.00	-0.01
CF16	-0.17**	-0.19**	-0.18**	-0.17**	-0.17**	-0.19**

**: $p \leq 0.001$ * : $p \leq 0.05$

-0.80** -0.83**

Rasul et al., 2009; Jiménez et al., 2016

Martinez et al., 2018

Genetic Repeatability Increases as Covariates and Replications are Taken into Account

Trait	Simple $R^{2\,a}$	Covariate $R^{2\,b}$	Line mean basis $R^{2\,c}$
FN	0.197	0.500	0.667
3days	0.109	0.145	0.459
4days	0.163	0.240	0.612
5days	0.151	0.214	0.577
6days	0.229	0.276	0.656
7days	0.218	0.230	0.599
SI	0.228	0.315	0.697

x = accessions
y = trait

y ~ x model y ~ x + covariates model

$$R^2 = V_g / (V_g + (V_e / n))$$

Genome-wide Association Study of FN and Visible Sprout

15,229 polymorphic markers | 21 chromosomes | 469 accessions

2 *QFN.wsu* 9 *QFN.wsu* 34 *QPHS.wsu*



CF16	Pul15	CF15
3+2	3	0
Pul13		**CF14**
3		0

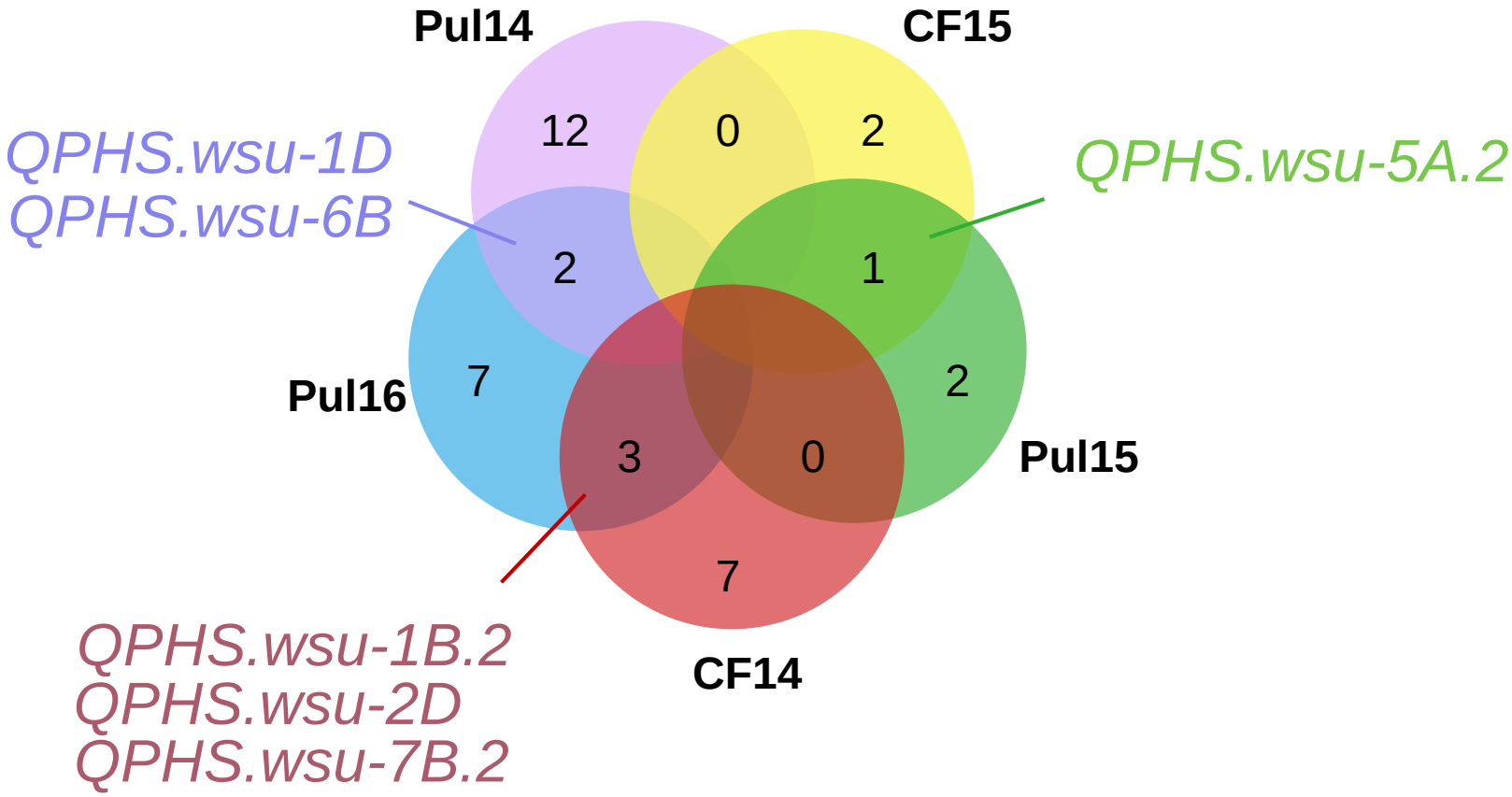
Germination | Seedling Growth

Day 3 Day 4	Day 5	Day 6 Day 7	SI
12	6	13	3

There were no *QFN.wsu* and *QPHS.wsu* that co-localized with one another

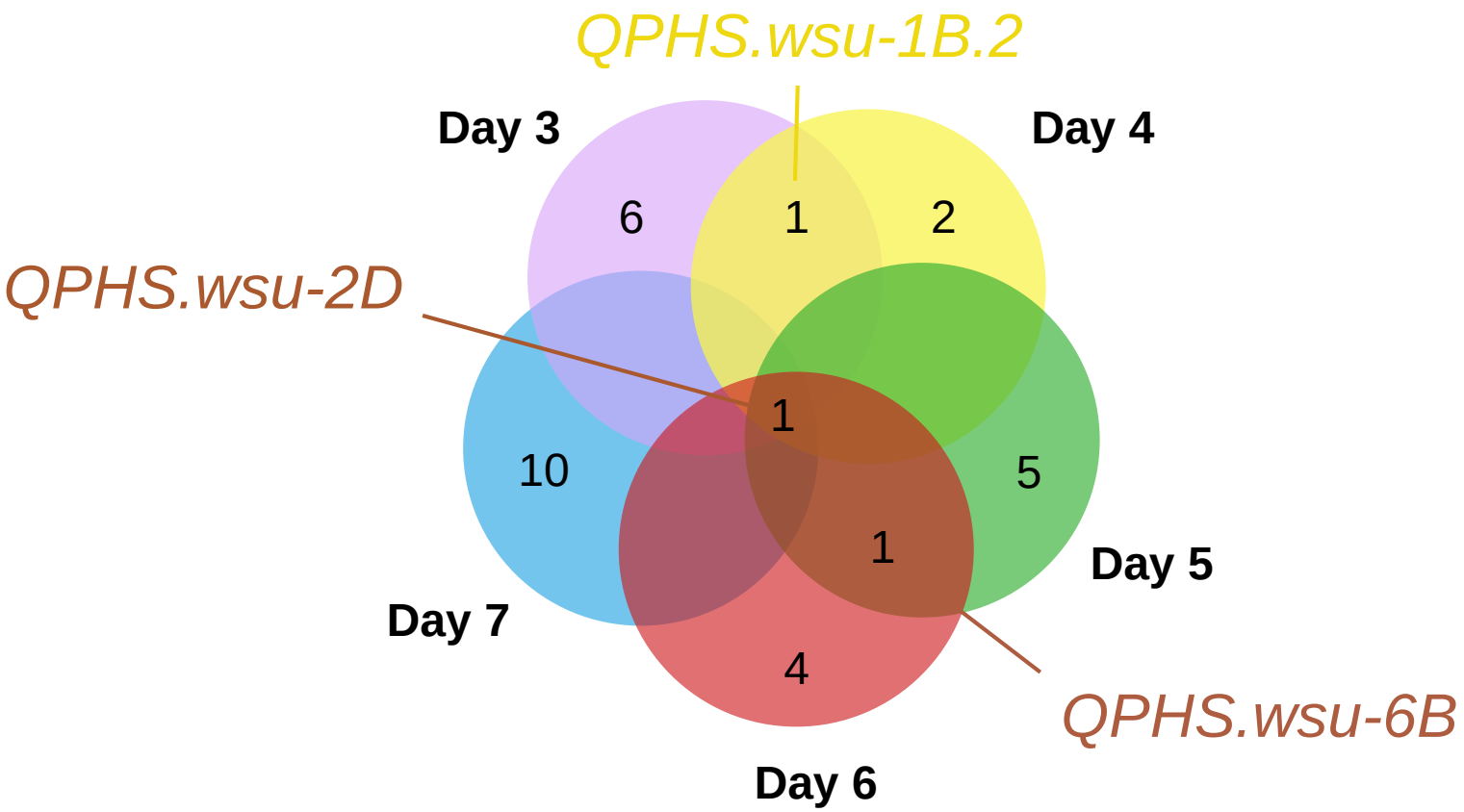
Martinez et al., 2018

Visible Sprouting QTN *QPHS.wsu*



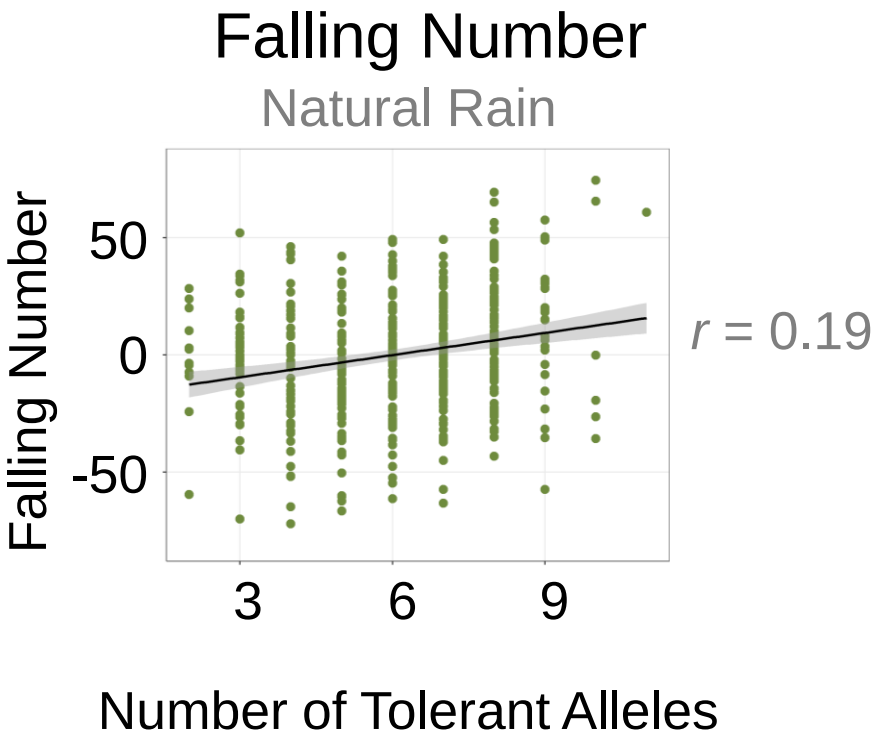
Martinez et al., 2018

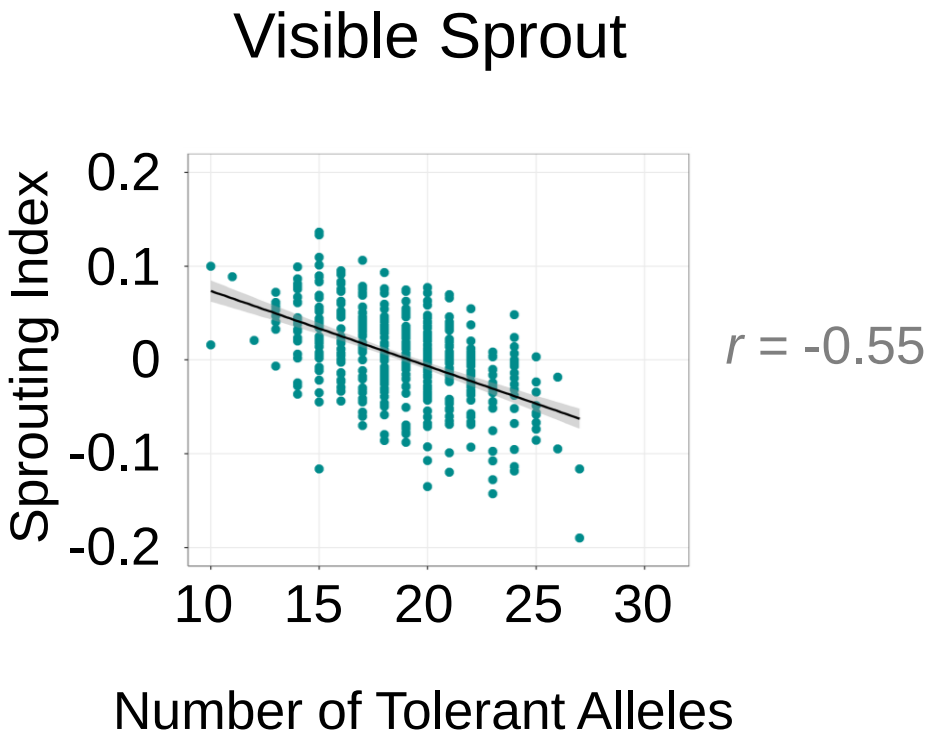
Visible Sprouting QTN *QPHS.wsu*



Martinez et al., 2018

Quantitative Effects of PHS-related QTN





This is been published many times, but its important to know the effect of many QTN on the trait so the breeders can potentially pyramid these PHS tolerant QTN with one another to obtain the desired amount of tolerance

Martinez et al., 2018

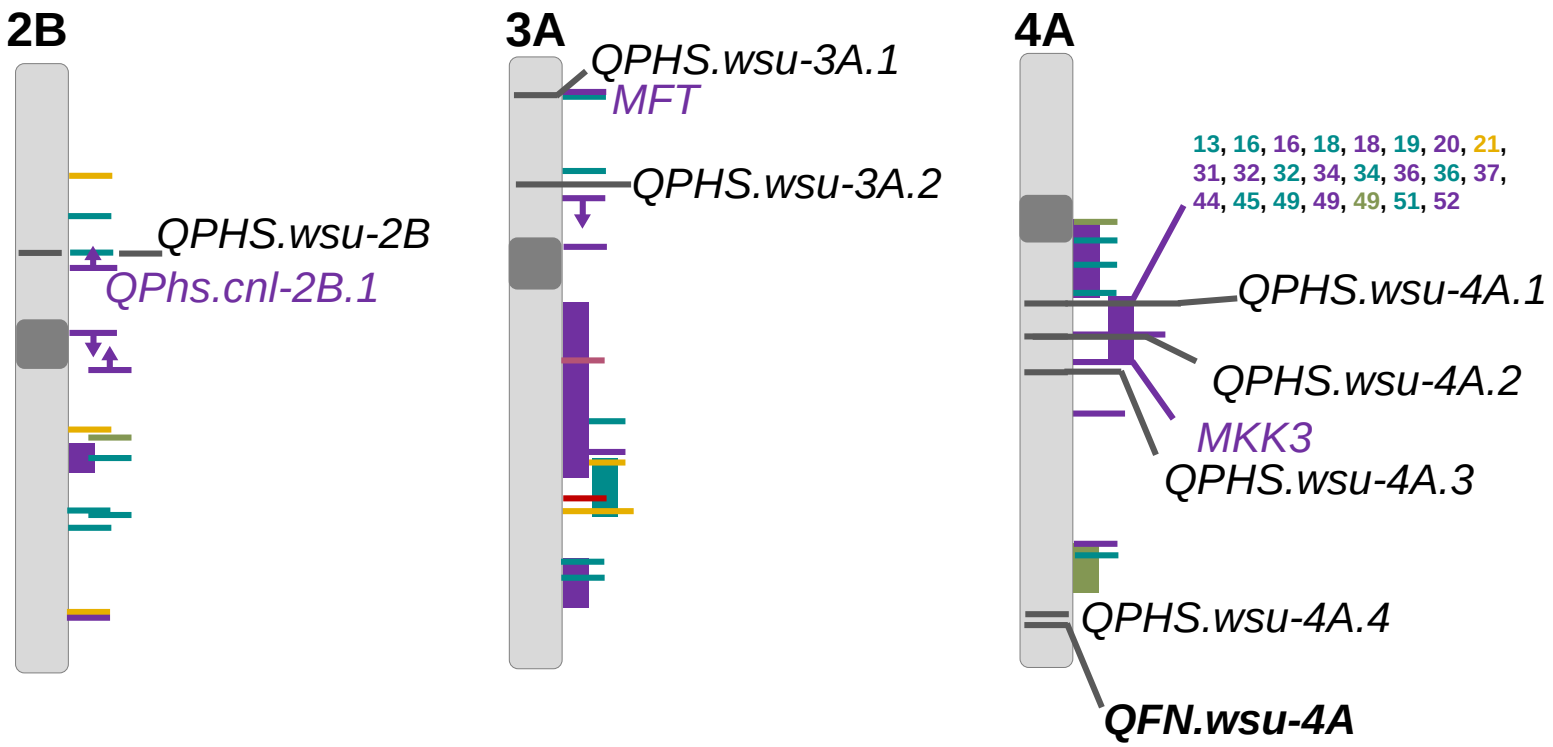
2 of the 11 *QFN.wsu* were unique

10 of the 34 *QPHS.wsu* were unique

The others were found near other known PHS-related loci

Dormancy (PHS) QTL and Known Genes

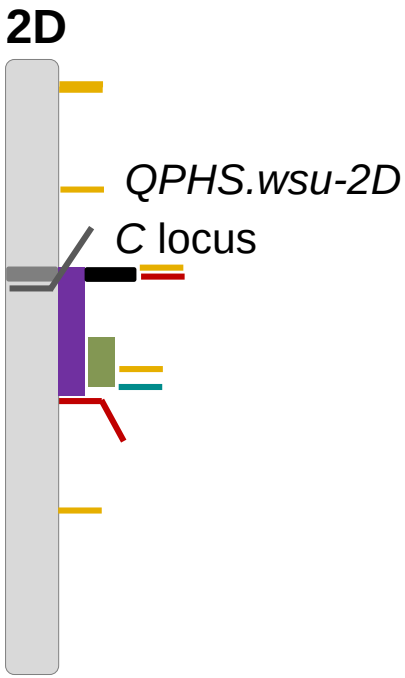
Sprouting Assay Falling Numbers Grain Color Martinez et al. QTN
Dormancy Assay Quality LMA



Martinez et al., 2018

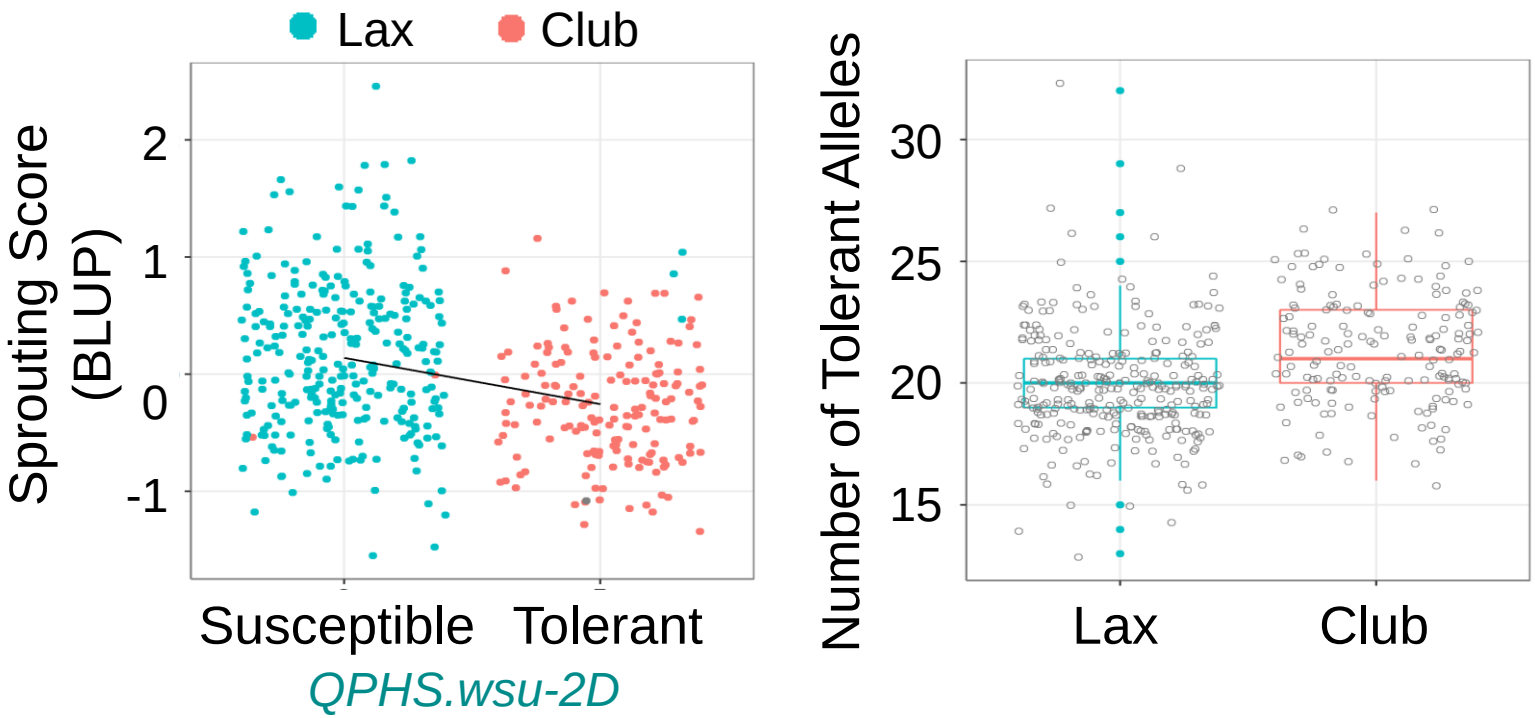
Compactum (*C*) Locus on 2D

Sprouting Assay Falling Numbers Grain Color Martinez et al. QTN
Dormancy Assay Quality LMA



Photos by T. DeMacon | Johnson et al., 2008 | Martinez et al., 2018

Strongest PHS QTL: *QPHS.wsu-2D*



Taking a further look into the phenotype of the club lines with the c locus and the lax lines without the c locus: We see here that across all visible sprouting env, 98% of the club lines have the 2D PHS tolerant allele. However when you look at all the PHS QTN, not just 2D QTN, the club lines have more tolerant alleles on average compared to the lax lines.

This could mean the i) 2D QTN is mapping the c locus but we have 2% of lines that conflict with that theory and previous work on a much smaller sample set suggests that the c locus results in PHS susceptibility, ii) the PHS tolerant 2D QTN could be linked to the c locus but not the same gene, or iii) we could just be seeing such a high tolerance to PHS because of historical breeding efforts for PHS in the club wheat breeding program

PHS GWAS Conclusions

GWAS for FN and visible sprout detected different QTN, although both co-localized with known PHS-related loci

The club *C* locus was linked to the strongest *QPHS.wsu-2D* QTL

FN is a measure of a-amylase activity. The lack of correlation between FN and visible sprout may mean that a-amylase is regulated differently with respect to the timing of germination in different varieties.

Collaborators

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Questions