The First Step to Tackling the FN Problem: Identifying PHS Tolerant Genes/QTL in PNW Germplasm

Shantel A. Martinez
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PNW Quality Council

Affiliations
Current: Plant Breeding & Genetics, Cornell University
Previous: Crop & Soil Science, Washington State University
Authors
Jayfred Godoy
Meng Huang
Zhiwu Zhang
Arron H Carter
Kimberly Garland Campbell
Camille M Steber

Acknowledgments
Rehana Parveen
Tracy J Harris
Preharvest Sprouting

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

Wheat Seed Dormancy

The inability to germinate even under favorable environmental conditions

Dormant

Non-Dormant

PHS Tolerant

PHS Susceptible

After-ripening Cold Imbibition
Susceptibility to preharvest sprouting depends on maturity date.

- Before Maturity
- Physiological Maturity (Dormant)

Sprouting susceptibility

Age of Grain

- Tolerant
- Susceptible
PHS is a result of a-amylase activity breaking down starch chains.
Hagberg-Perten Falling Number: The Industry Standard to Measure PHS

300 seconds

Low FN is associated with low end use quality
You are Not Alone - 2018 Preharvest Sprouting

England

I guess this is what you'd call pre-harvest sprouting #Harvest18

Kansas

I'm always happy to have rain, but not the view I want during #WheatHarvest18 #kswx

New York

A lot of hopes and dreams were crushed this week. Mon. I finished my harvest for PHS trials, then it rained for 2.5 days. Today we all went out to harvest the barley trials, SPROUTED. Went to harvest my mapping pop for planting seed, SPROUTED. Welcome to NY summers?

Canada

#wheatharvest18 started here at fermeschauvinfarms.com in StoneyPoint. Decent yields for no rain … #OntAg #AgMoreThanEver #goodineverygrain

Nebraska

North Dakota

(2017)
Improving Preharvest Sprouting in the PNW: Understanding the Genetic Tolerance that Exists in the Current Breeding Programs

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

Jernigan and Godoy et al., 2018 | Martinez et al., 2016
Falling Numbers Test: Samples are Harvested at “Harvest Maturity”
Greenhouse Spike Wetting Test:
Samples are Harvested at Physiological Maturity

5 days AR

PM

Rain Event

Susceptible

Tolerant

Age of Grain

Misted 6 sec / min

Scored every 24 hrs for 7 days

Greenhouse Spike Wetting Test:
Samples are Harvested at Physiological Maturity

5 days AR

PM

Rain Event

Susceptible

Tolerant

Age of Grain

Misted 6 sec / min

Scored every 24 hrs for 7 days
Visible Sprout Scored

PHS Tolerant

PHS Susceptible

1 2 3 4 5 6 7 8 9 10

Roots
Germination
Coleoptile
Seedling Growth
1st Leaf

McMaster & Derera et al., 1976
**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.
The FN Trait Was Tested Over 5 Environments, 3 Different Events

Pul: Pullman
CF: Central Ferry
The environment has a large affect on the FN response

<table>
<thead>
<tr>
<th></th>
<th>Pul13</th>
<th>CF14</th>
<th>Pul15</th>
<th>CF15</th>
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<td>0.29**</td>
<td>0.29**</td>
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<tr>
<td>CF16</td>
<td>0.33**</td>
<td><strong>0.46</strong></td>
<td>0.30**</td>
<td>0.34**</td>
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</tbody>
</table>

**: p ≤ 0.001  * : p < 0.05
Visible Sprouting Was Tested Over 5 Environments, Same GH Rain Event

Day 3

Day 4

Day 5

Day 6

Day 7

Germination

Seedling Growth
Visible Sprout Correlations Across Environments Were as Good as Other SWT Studies

<table>
<thead>
<tr>
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<td>0.29**</td>
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<tr>
<td>CF15</td>
<td>0.38**</td>
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<td>Pul15</td>
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<td>0.16*</td>
<td>0.46**</td>
<td>0.36**</td>
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Kulwal et al., 2012; Jaiswal et al., 2012; Ogbonnaya et al., 2008; Zhou et al., 2017
The Correlations Between FN and Visible Sprout are Not Highly Negative

<table>
<thead>
<tr>
<th></th>
<th>Germination</th>
<th>Seedling Growth</th>
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<tr>
<td></td>
<td>3 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Pul13</td>
<td>-0.16**</td>
<td>-0.24**</td>
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<tr>
<td>CF14</td>
<td>-0.07</td>
<td>-0.09*</td>
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<tr>
<td>Pul15</td>
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<tr>
<td>CF15</td>
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<tr>
<td>CF16</td>
<td>-0.17**</td>
<td>-0.19**</td>
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**: p ≤ 0.001  * : p ≤ 0.05

-0.80**  -0.83**

Rasul et al., 2009; Jiménez et al., 2016
When Implementing Spike-Wetting Tests in a Breeding Program: The Germplasm / Environment Could Affect the Variance

Cornell University & Washington State University, unpublished
Genome-wide Association Study of FN and Visible Sprout

15,229 polymorphic markers | 21 chromosomes | 469 accessions

9 QFN.wsu

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34 QPHS.wsu

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<tbody>
<tr>
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<td>Day 5</td>
<td>Day 6</td>
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<td>Day 7</td>
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<tr>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
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.github.com/shantel-martinez/FNWorkshop2019
Will Only Looking at the Low FN Values Give Different QTN?

![Box plot showing Falling Number values for Pul 2013, CF 2014, Pul 2015, CF 2015, and CF 2016. The horizontal line at 400 FN Cap indicates the FN cap value.]
# Genome-wide Association Study of FN and Visible Sprout

15,229 polymorphic markers | 21 chromosomes | 469 accessions

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<th>34 QPHS.wsu</th>
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<tbody>
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<td>CF15</td>
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<td>0</td>
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<tr>
<td>Pul13</td>
<td>CF14</td>
<td>Day 3</td>
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<tr>
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<td>Day 5 6</td>
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<td>Day 6 13</td>
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<td>Day 7 3</td>
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<tr>
<td></td>
<td></td>
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### Germination vs. Seedling Growth

- **Germination**: Day 3, Day 4, Day 5, Day 6, Day 7
- **Seedling Growth**: SI

*Images of weather icons and growth stages are shown.*

*Source: [github.com/shantel-martinez/FNWorkshop2019]*
There were no `QFN.wsu` and `QPHS.wsu` that co-localized with one another.
Visible Sprouting QTL QPHS.wsu Across Environments

Diagram showing the overlap of QTLs across different environments:
- **QPHS.wsu-1D**
- **QPHS.wsu-6B**
- **QPHS.wsu-1B.2**
- **QPHS.wsu-2D**
- **QPHS.wsu-7B.2**

Environments:
- Pul14
- CF15
- Pul16
- Pul15
- CF14

Numbers indicating the number of overlapping QTLs across environments.
PHS and Dormancy Genes RefSeqv1.0
Positions

IWGSC, 2018
Visible Sprouting QTL Located Near Known PHS Genes

- Sprouting Assay
- Dormancy Assay
- Falling Numbers
- Grain Color
- Quality
- LMA

Martinez et al. QTN

2B

QPhs.cnl-2B.1
QPhs.wsu-2B
TaSdr-B1

3A

QPHS.wsu-3A.1
QPHS.wsu-3A.2
MFT

4A

QPHS.wsu-4A.1
QPHS.wsu-4A.2
MKK3
QPHS.wsu-4A.3
QPHS.wsu-4A.4
QFN.wsu-4A

13, 16, 16, 18, 18, 19, 20, 21,
31, 32, 34, 34, 36, 36, 37,
44, 45, 45, 49, 49, 51, 52
Strongest PHS QTL, $QPHS.wsu-2D$, Close to the *Compactum* ($C$) Locus

Photos by T. DeMacon | Johnson et al., 2008
2 of the 11 QFN.wsu appear to be unique

10 of the 34 QPHS.wsu appear to be unique

The others were found near other known PHS-related loci
Breeding for PHS Conclusion

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

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

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

How do we make these markers/QTL useful in breeding programs

How do we turn this into a tool the breeders can use now

github.com/shantel-martinez/FNWorkshop2019