1 PREHARVEST SPROUTING

HOW TO TEST

Tolerant 0 — Susceptible 9

Physiological Maturity (PM)
5d — Greenhouse Mist — 4d — Sprouting score

perten.com

THE PROBLEM

Tolerant Line — Combine Harvest

Susceptible Line — Sprouts — Decrease in quality
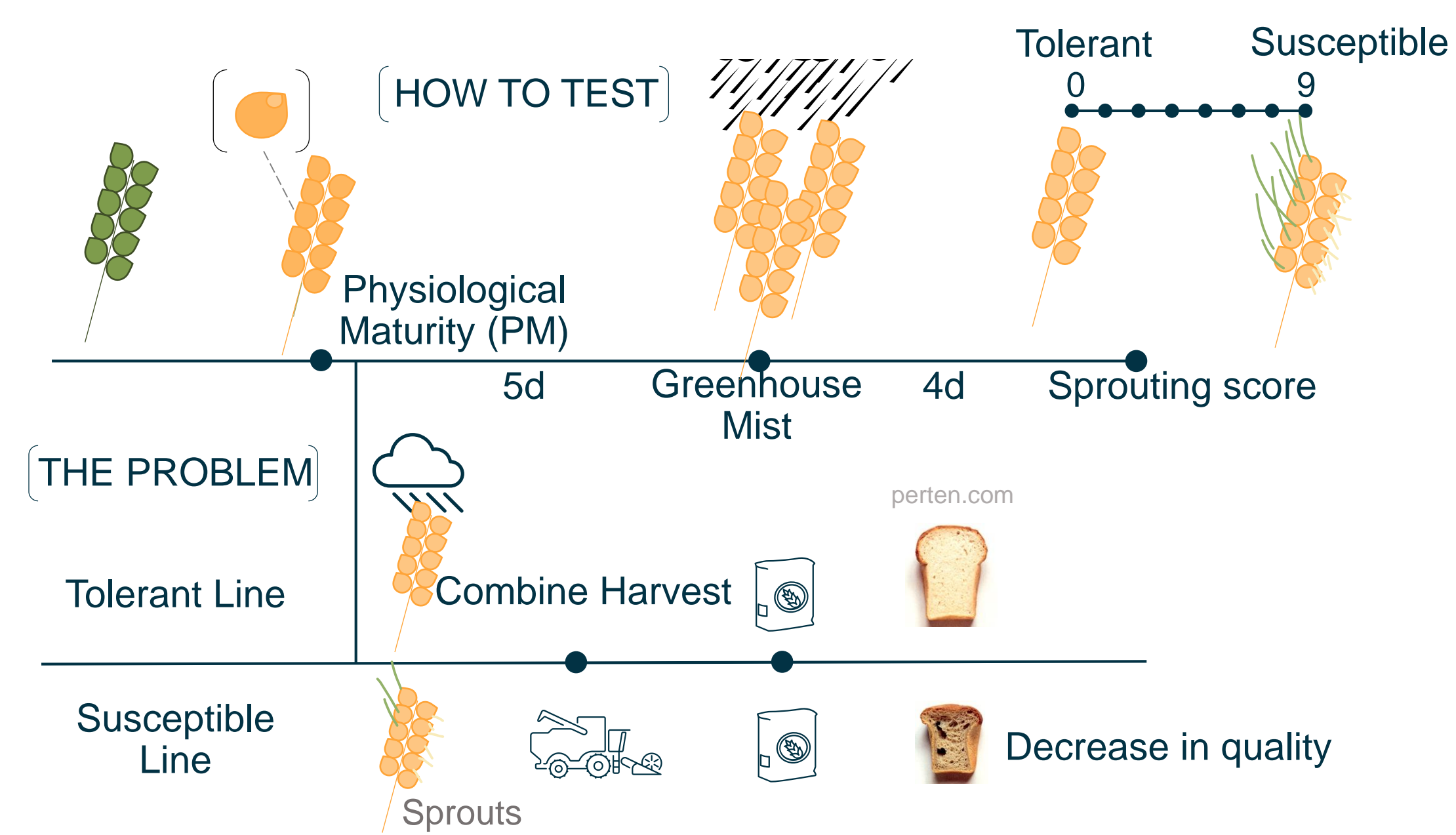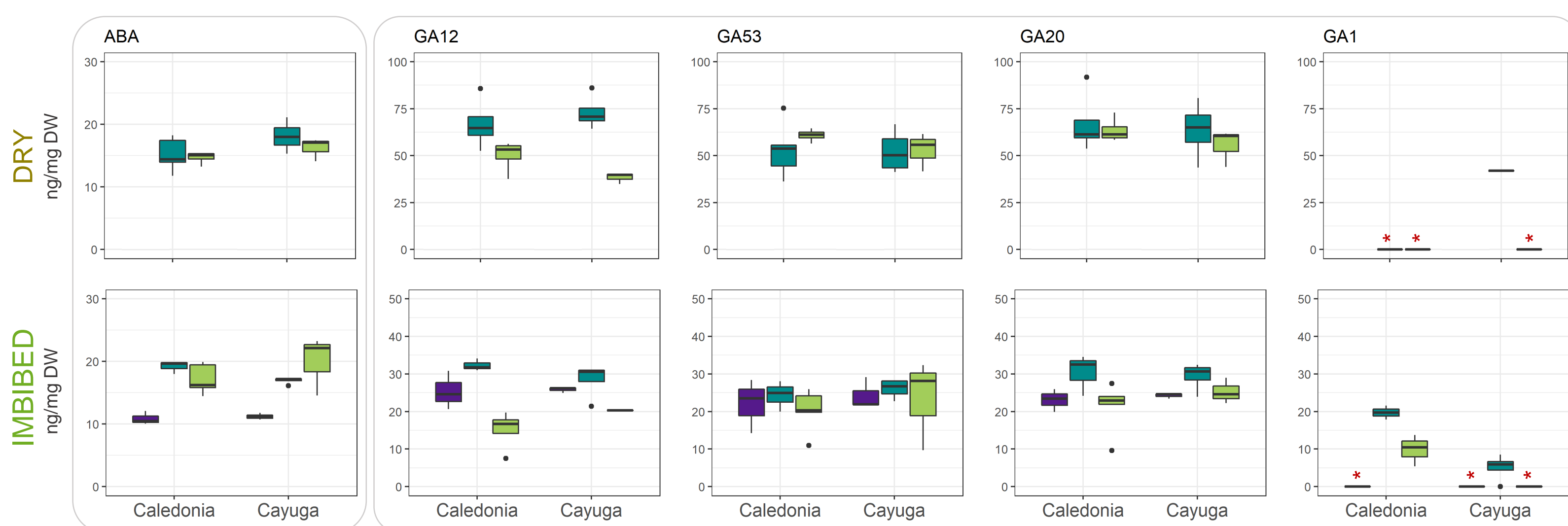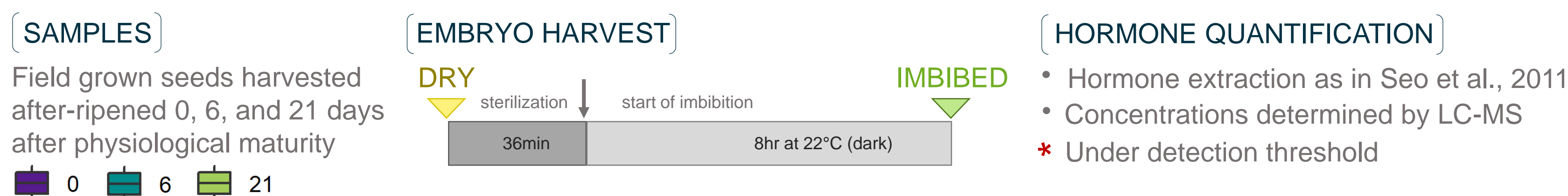
A PHS tolerant gene is associated with a 3.1 Mb region on chromosome 2B. Previous studies have reported 14/19 of the genes in this region were expressed in seed tissue.

Fine-mapping and characterizing the *QPhs.cnl-2B.1* found in the Northeast soft white wheat, Cayuga

2 GWAS

CORNELL MASTER NURSERY
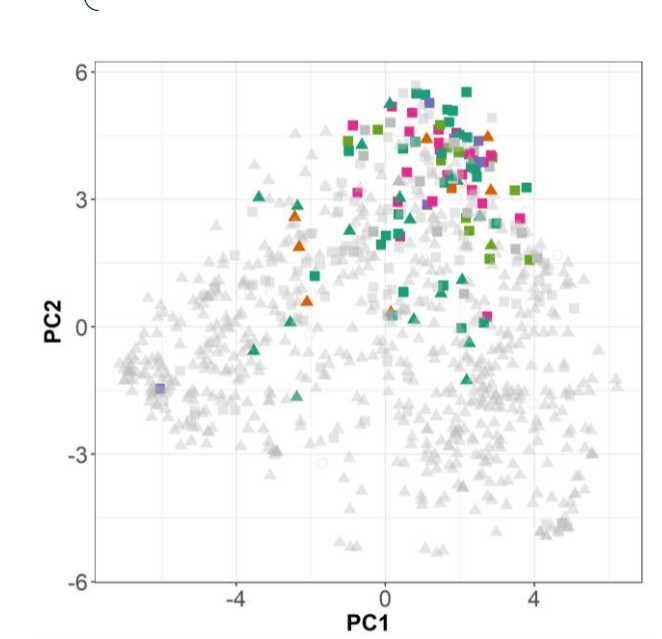
The PHS tolerant MTA *QPhs.cnl-2B.1*, contributed by the cultivar Cayuga, was highly significant at 174.8 Mb in the Cornell elite master nursery.

The other major PHS tolerant QTL contributed by Cayuga is on chromosome 6D, *QPhs.cnl-6D.1* , as shown in Munkvold et al., 2009

GBS-derived KASP markers have been created for both QTL

Pyramiding multiple Cayuga alleles results in increased PHS tolerance, however a single QTL present drastically reduces sprouting compared to Caledonia

QPhs.cnl-2B.1

Pedigrees with Cayuga
QPhs.cnl-2B.1 and *QPhs.cnl-6D.1*

Caledonia

Cayuga

CALEDONIA IN PEDIGREE | 2B-S / 6D-T | 2B-T / 6D-S | 2B-T / 6D-T

n = 1317

MSU, Cornell, OH, Eastern, Midwest, Private

3 RECOMBINATION EVENTS

CORNELL MASTER LINES

Which recombination event is the source of the Group II's large variation in PHS score?

Tolerant 'T' markers have moderate to susceptible phenotype, not what you would expect if 163-165Mb region was needed for PHS tolerance

Cayuga — Caledonia
Superior

PHS BLUP Scores

CAYUGA X CALEDONIA BC1

More general variation in the BC1 population is likely due to PHS tolerant QTL on other chromosomes

PHS BLUP Scores

4 QTL MAPPING

CAYUGA X CALEDONIA

QPhs.cnl-2B.1 — 298.6 Mb — **Chrm 2B**
QPhs.cnl-2B.3 63 Mb — *QPhs.cnl-2B.1* — unknown Mb

Munkvold et al. 2009
Somyong et al. 2014
GWAS
QTL Analysis

MTA

AX-94487047

430 BC1F5:8
PHS BLUP Score; 7 environments
SSR, Axiom, KASP markers
'cim' of qtl package in R

barc55 — wmc474
wmc453

QPhs.cnl-2B.1 is linked to the 175.6 - 181.6 Mb region

40 HC Genes 6 Mb region

172.6 | 175.6 | 178.7 | 180.5 | 181.6 | 184.3 | 184.6

wheat-expression.com
36 gene expression studies
118 seed tissue samples
65 different wheat varieties

GWAS MTA — *gid1a* — *TaAFP-B*

dpa
35+
20-30
14-15
2-12

104 Seed Samples

Many genes show gene expression in wheat seeds, except 27/104 genes (light grey) have 0.0 tpm gene expression across all 118 samples.

5 CANDIDATE GENES

Ramírez-González et al., 2019 (doi 10.1126/science.aar6089)

Gene ID / Marker	Pos	Functional Description	Ortholog [a] / LOD	Seed Expr. [b]
wmc474	172,687,312	Marker	LOD 2.5	-
31 GENES	-	-	-	-
AX-94487047	175,640,531	Marker	LOD 14.8	-
TraesCS2B01G198400	175,895,190	30S ribosomal protein S3	-	Yes
-	176,172,350	Critical Recombination within Cayuga crosses		
TraesCS2B01G198500	176,172,676	PHD finger alfin-like protein	TraesCS2D01G179400	Yes
TraesCS2B01G198600	176,299,996	alpha/beta-Hydrolases superfamily protein	-	Yes
TraesCS2B01G198700	176,406,772	Nuclear transcription factor Y subunit	TraesCS2D01G179600	Yes
TraesCS2B01G198800	176,412,003	6,7-dimethyl-8-ribityllumazine synthase	-	Yes
TraesCS2B01G198900	176,414,909	DUF2996 family protein	TraesCS2D01G179700	Yes
TraesCS2B01G199000	176,708,590	Acidic leucine-rich nuclear phosphoprotein 32-related protein	-	Yes
TraesCS2B01G199100	177,218,818	Ankyrin repeat family protein	-	No
TraesCS2B01G199200	177,545,817	Embryogenesis transmembrane protein	TraesCS2D01G180100	No
TraesCS2B01G199300	177,583,467	sugar transporter, putative (DUF1195)	TraesCS2D01G180200	No
TraesCS2B01G199400	177,597,685	Pectinesterase	TraesCS2D01G180300	Yes
TraesCS2B01G199500	177,657,471	Agenet domain-containing protein / bromo-adjacent domain-containing protein, putative	TraesCS2A01G173300	Yes
TraesCS2B01G199600	178,161,075	Heavy metal transport/detoxification superfamily protein	TraesCS2D01G180500	Yes
TraesCS2B01G199700	178,168,111	Gibberellin A receptor GID1A	TraesCS2D01G180600	Yes
TraesCS2B01G199800	178,338,316	Nuclear transcription factor Y subunit B	TraesCS2D01G180700	Yes
TraesCS2B01G199000	178,711,584	ATP-dependent RNA helicase-like protein	TraesCS2D01G180800	Yes
TraesCS2B01G200000	178,723,855	Werner Syndrome-like exonuclease	-	No
TraesCS2B01G200100	178,730,819	Sulfotransferase	TraesCS2D01G181000	Yes
TraesCS2B01G200200	178,759,101	lysine ketoglutarate reductase trans-splicing-like protein (DUF707)	TraesCS2A01G173700	No
GBS-178.7	178,761,042	Marker	LOD 13.9	-

[a] Orthologs were listed in the Persophone browser linked to the IWGSC RefSeq v1.0 URGI JBrowse
[b] Gene expression in seeds were determined using the wheat eFP browser

6 HORMONE PROFILE

SAMPLES
Field grown seeds harvested after-ripened 0, 6, and 21 days after physiological maturity

0 — 6 — 21

EMBRYO HARVEST
DRY — sterilization — start of imbibition — IMBIBED
36min — 8hr at 22°C (dark)

HORMONE QUANTIFICATION
• Hormone extraction as in Seo et al., 2011
• Concentrations determined by LC-MS
* Under detection threshold

ABA | GA12 | GA53 | GA20 | GA1

DRY ng/mg DW
IMBIBED ng/mg DW

Caledonia — Cayuga

GA1 was inconsistent in extraction efficiency. In spite of GA1 being more detectable in imbibed seed, GA precursors are lower in imbibed seed.

From the few samples that were detected with GA1, PHS tolerant Cayuga had lower levels than PHS susceptible Caledonia.
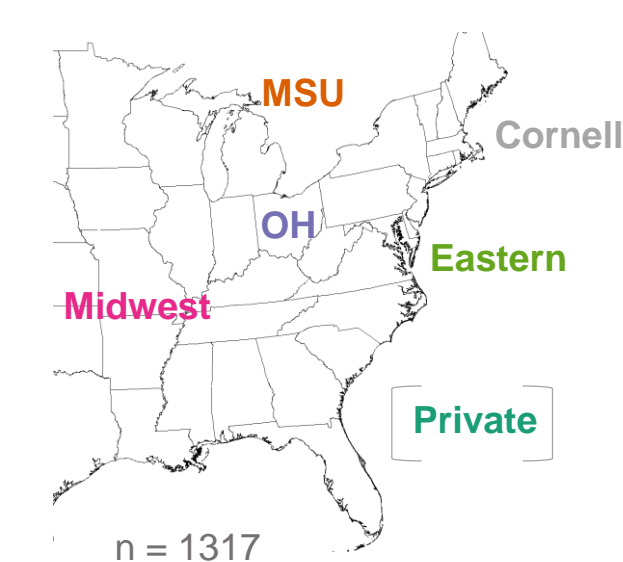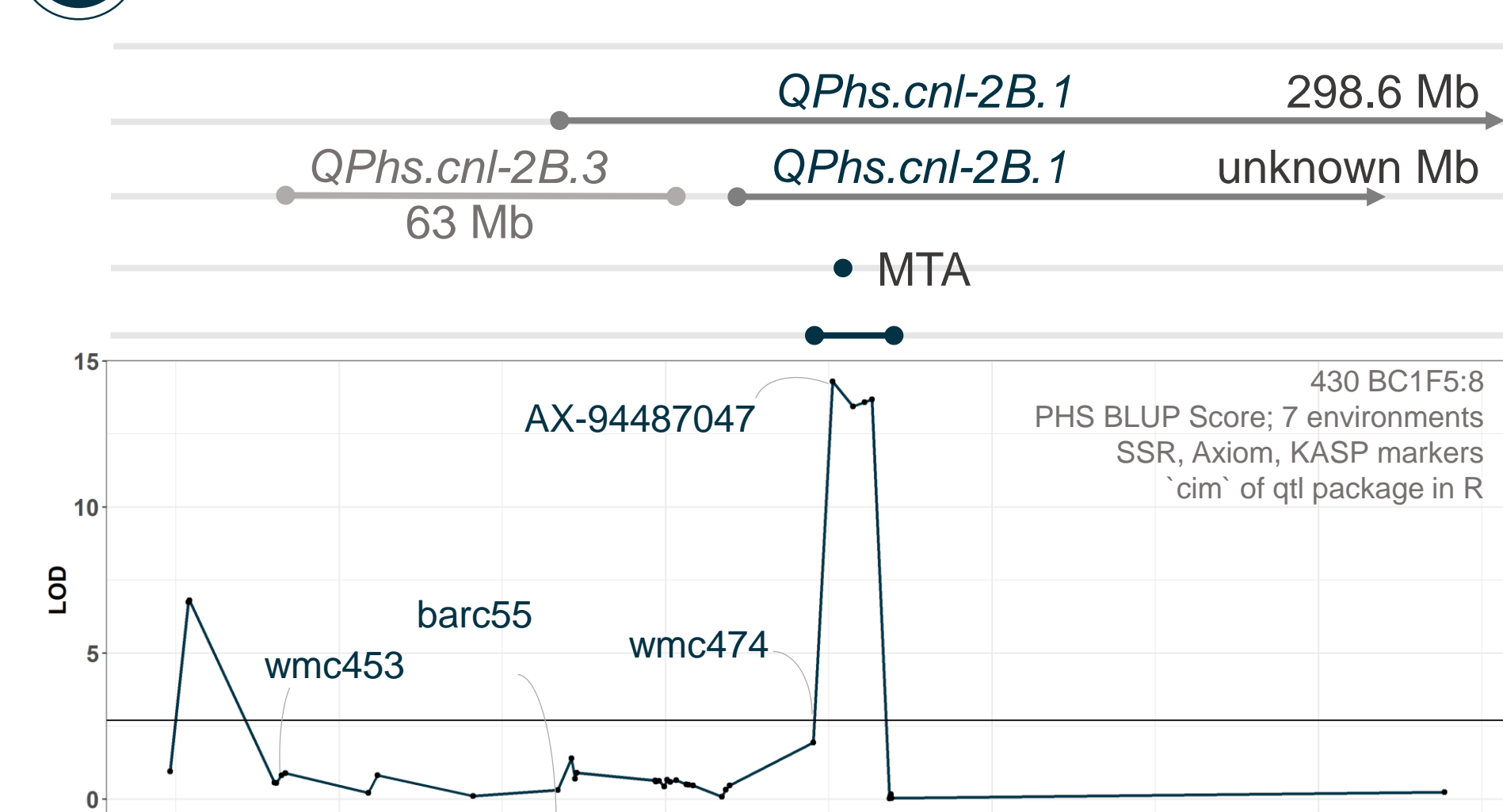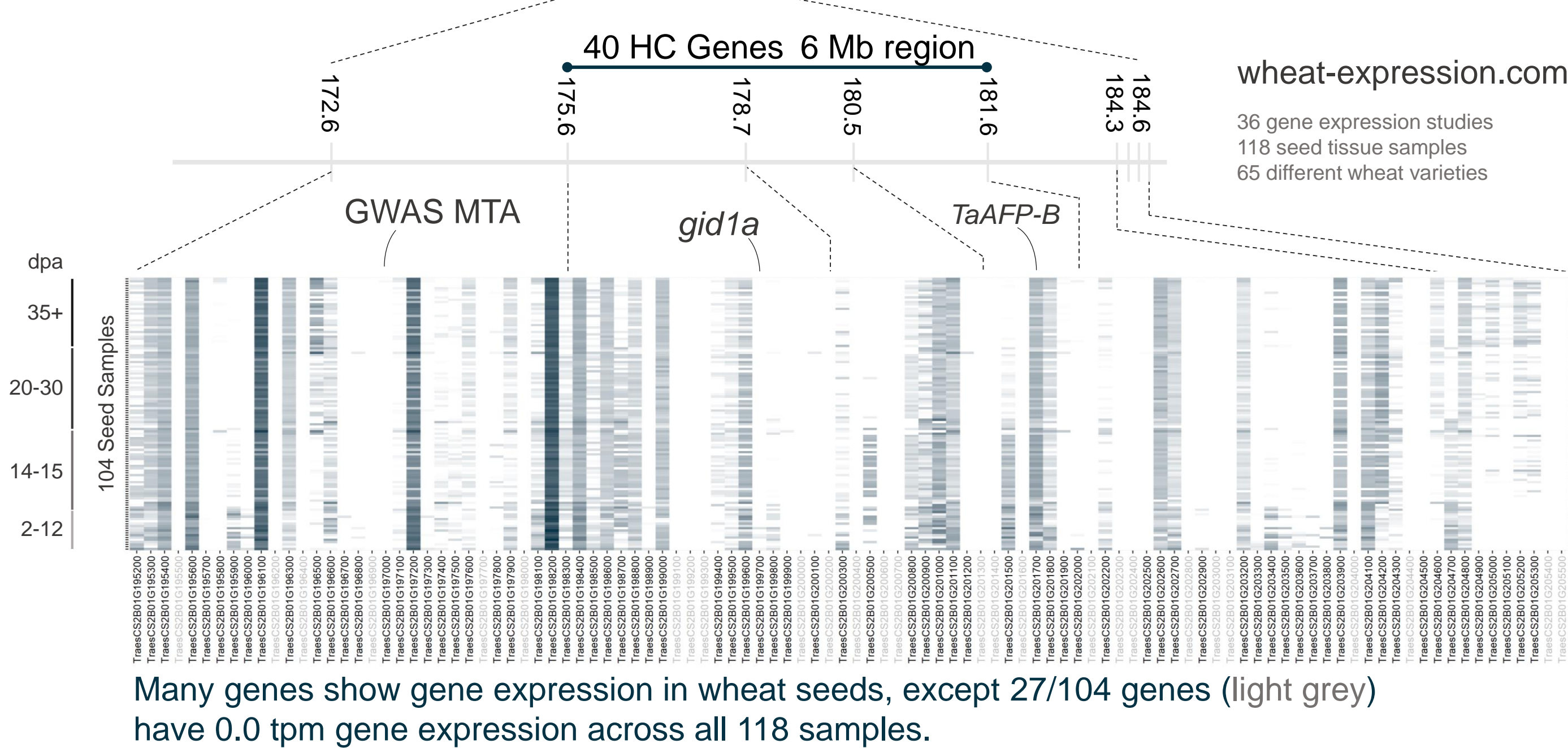
ABA and GA precursor hormone levels appear to not differ between Caledonia and Cayuga, implying the gene mechanism of *QPhs.cnl-2B.1* may not be involved with these metabolites.

7 HOMEOLOGOUS GENES

Whole genome homeologous genes calls defined in Santantonio et al., 2019

QPhs.cnl-2B.1 — *QPhs.cnl-2D.1*

Mb Pos
172.6 WMC474
174.5 GBS
174.8 GBS
178.7 GBS
184.3 GBS

95 HC Genes

37 HC genes are homeologous to 2D genes

66 HC genes have homeologous genes on 2A and/or 2D

Mb Pos
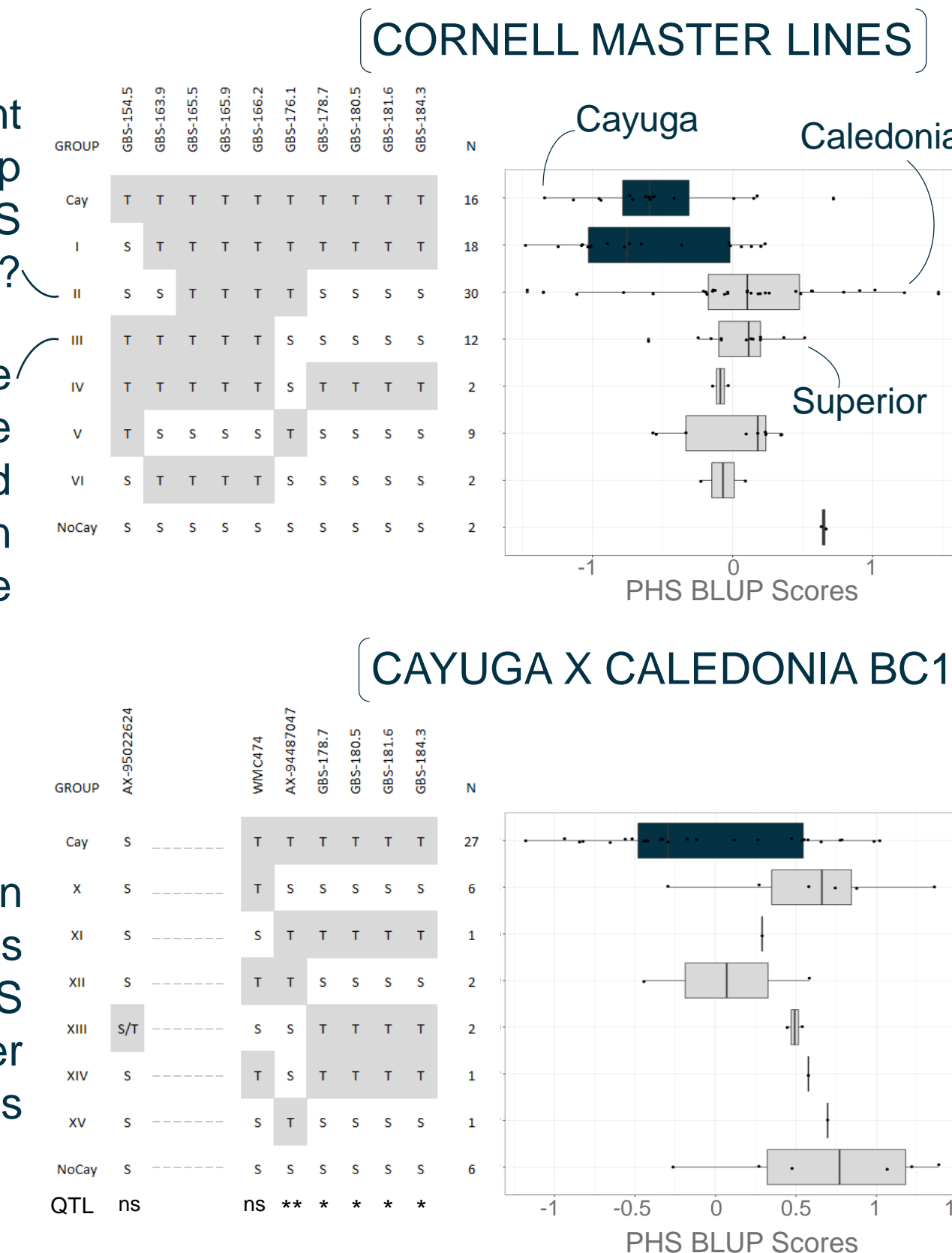12.5 wPT-6003
27 wPT-9997
43.3 barc1123
119
131

616 HC Genes

Munkvold et al. (2009) discovered another PHS tolerant QTL contributed by Cayuga on Chrm 2D, mapped between 12.5 - 43.3 Mb.

All of the homeologous 2B genes in our region map between 119 - 131 Mb region on 2D

FUTURE WORK

Confirm seed gene expression on Cayuga and Caledonia

Identify additional BC2 F2:3 recombinants with only the 2B Cayuga QTL affecting the phenotype, not other chrm QTL

Sequence the candidate genes in Cayuga to identify sequence variation and design genome specific gRNA

Knock out candidate genes controlling *QPhs.cnl-2B.1* in Cayuga and screen for a PHS susceptible phenotype

REFERENCES

Munkvold et al. 2009 doi: 10.1007/s00122-009-1123-1
Seo et al., 2011 doi: 10.1007/978-1-61779-231-1_7
Santantonio et al., 2019 doi: 10.1534/genetics.118.301851
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*AFRI EWD Project Director   Proposal #2017-07109

To connect, take a photo with your phone
Or go straight to the website: shantel-martinez.github.io
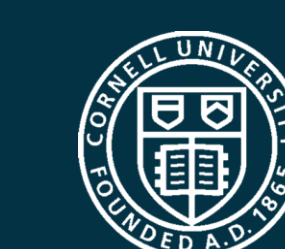
Cornell University

USDA United States Department of Agriculture   National Institute of Food and Agriculture

in shantel-a-martinez

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