

SUPPLEMENTARY INFORMATION FILE

**CANDIDATE REGULATORS OF EARLY LEAF DEVELOPMENT IN MAIZE PERTURB HORMONE
SIGNALLING AND SECONDARY CELL WALL FORMATION WHEN CONSTITUTIVELY
EXPRESSED IN RICE**

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LEGENDS

Dataset S1 (xls). Data used for regression analysis of vein number versus leaf width (cm).

Corresponding non-transgenic control samples are listed as 'regression' in each of 4 datasets (Kitaake T1 lines - leaf 6 & 8; IR64 T0 lines - leaf 6; IR64 T1 lines - leaf 6; IR64 T0 lines - penultimate leaf).

Dataset S2 (xls). Phenotyping data for IR64 T0 lines, showing measurements of vein number, and leaf width. Transgene presence as determined by PCR is indicated.

Dataset S3 (xls). Phenotyping data for Kitaake & IR64 T1 lines, showing measurements of vein number, distance between veins, cell number between veins and bundle sheath cell size. Transgene transcript level as determined by PCR is shown where measured.

Dataset S4 (xls). Primer sequences used for cloning and for RT-PCR.

Information S1. Sequences of all 60 genes transformed into rice.

Information S2. Individual datasheets for T0 rice lines summarized in Dataset S2. Each page shows the gene expression profile in maize foliar and husk leaf primordia as reported in ¹ (green circles refer to relative expression level in samples of the shoot apical meristem plus plastochron 1/2 leaf primordia, orange circles to levels in P3/4 leaf primordia and blue circles to levels in P5 leaf primordia); construct composition and ID; number and ID of independent transgenic rice lines. Cropped agarose gel images show PCR products of representative genomic PCR reactions (detecting presence of transgene). Linear regression plots illustrate number of veins versus leaf width in each line (x axis = leaf width in cm; y axis = vein number). Representative leaf samples are shown with views of the adaxial leaf surface taken using a hand-held microscope.

Information S3. Individual datasheets for T1 rice lines summarized in Dataset S3. Each page shows the gene expression profile in maize foliar and husk leaf primordia as reported in ¹ (green circles refer

to relative expression level in samples of the shoot apical meristem plus plastochron 1/2 leaf primordia, orange circles to levels in P3/4 leaf primordia and blue circles to levels in P5 leaf primordia); construct composition and ID; number and ID of independent transgenic rice lines. Where available, cropped agarose gel images show PCR products of representative genomic PCR reactions (detecting presence of transgene) and RT-PCR reactions (detecting transgene transcripts). qRT-PCR data are shown for one construct. Polynomial (Kitaake lines) or linear (IR64 lines) regression plots illustrate number of veins versus leaf width in each line. Representative leaf samples (either hand cut transverse leaf sections viewed by fluorescence microscopy or views of the adaxial leaf surface taken using a hand-held microscope) are shown in each case. Fluorescence images are visualized through autofluorescence of chlorophyll (red) and lignin (blue).

Figure S1. Phylogenetic tree of GRMZM2G074032 (*ZmIDD16*). Maximum-likelihood tree of the GRMZM2G074032 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis and rice proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S2. Phylogenetic tree of GRMZM2G027068 (*ZmbHLH106*). Maximum-likelihood tree of the GRMZM2G027068 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S3. Phylogenetic tree of GRMZM2G140694 (*ZmHCA2*). Maximum-likelihood tree of one of the sub-groups of the GRMZM2G140694 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis HCA2, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S4. Phylogenetic tree of GRMZM2G480386 (YUCCA). Maximum-likelihood tree of the GRMZM2G480386 orthogroup (as defined by Orthofinder ²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Rice YUCCA8 plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S5. Phylogenetic tree of GRMZM2G011463 (*ZmSAUR60*). Maximum-likelihood tree of the GRMZM2G011463 orthogroup (as defined by Orthofinder ²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Rice and Arabidopsis proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S6. Phylogenetic tree of GRMZM2G098988 (*ZmSACL3*). Maximum-likelihood tree of the GRMZM2G098988 orthogroup (as defined by Orthofinder ²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis SACL3, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S7. Phylogenetic tree of GRMZM2G163724 (LRR kinase). Maximum-likelihood tree of the GRMZM2G163724 orthogroup (as defined by Orthofinder ²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis RDK1, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S8. Phylogenetic tree of GRMZM2G111045 (R2R3 MYB). Maximum-likelihood tree of the GRMZM2G111045 orthogroup (as defined by Orthofinder ²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis LMI2, plus the maize

protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S9. Phylogenetic tree of GRMZM2G114893 (Unknown). Maximum-likelihood tree of the GRMZM2G114893 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. The maize protein encoded by the gene analysed in this study is highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S10. Phylogenetic tree of GRMZM2G377217 (*ZmWRKY12*). Maximum-likelihood tree of one of the sub-groups of the GRMZM2G377217 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis proteins plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S11. Details of activation tagged line M0094935. **a)** Schematic of rice chromosome 3 showing insertion site of T-DNA (red arrow) upstream of Os03g50920 in line M0094935. **b)** Gel images of RT-PCR reactions showing transcript levels of 03g50920 relative to actin in 21 day old seedling shoots of wild-type (WT) and the insertion line (M0094935).

REFERENCES

1. Wang, P., Kelly, S., Fouracre, J. P. & Langdale, J. A. Genome-wide transcript analysis of early maize leaf development reveals gene cohorts associated with the differentiation of C4 Kranz anatomy. *Plant J.* **75**, 656–670 (2013).
2. Emms, D. M. & Kelly, S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol.* **16**, 157 (2015).

Information File S1. Sequences of all 60 genes transformed into rice.

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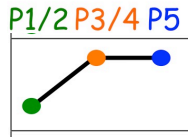
Information S2. Individual datasheets for T0 rice lines summarized in Dataset S2. Each page shows the gene expression profile in maize foliar and husk leaf primordia as reported in ¹ (green circles refer to relative expression level in samples of the shoot apical meristem plus plastochron 1/2 leaf primordia, orange circles to levels in P3/4 leaf primordia and blue circles to levels in P5 leaf primordia); construct composition and ID; number and ID of independent transgenic rice lines. Cropped agarose gel images show PCR products of representative genomic PCR reactions (detecting presence of transgene). Linear regression plots illustrate number of veins versus leaf width in each line (x axis = leaf width in cm; y axis = vein number). Representative leaf samples are shown with views of the adaxial leaf surface taken using a hand-held microscope.

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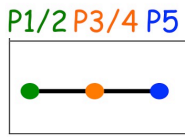
ZnF DoF

Gene ID: JL44

Foliar expression



Husk expression

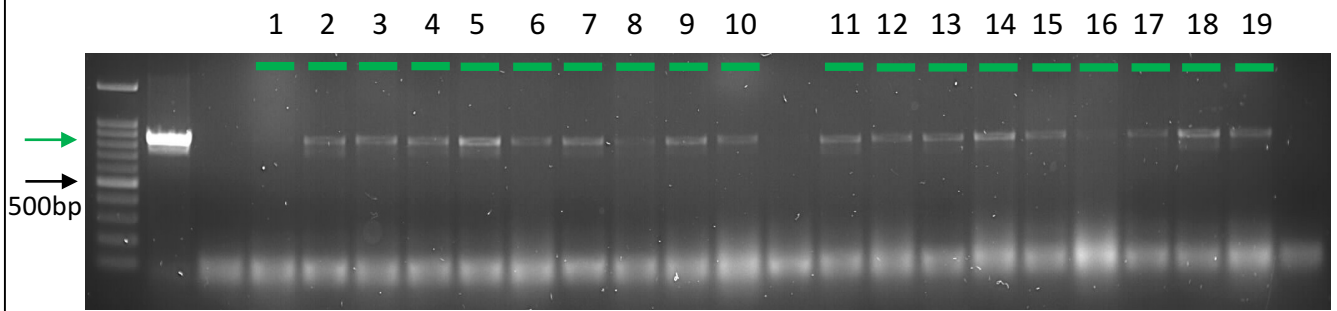


Construct: ZmUBI_{pro}:cDNA (ID# IRS1085)

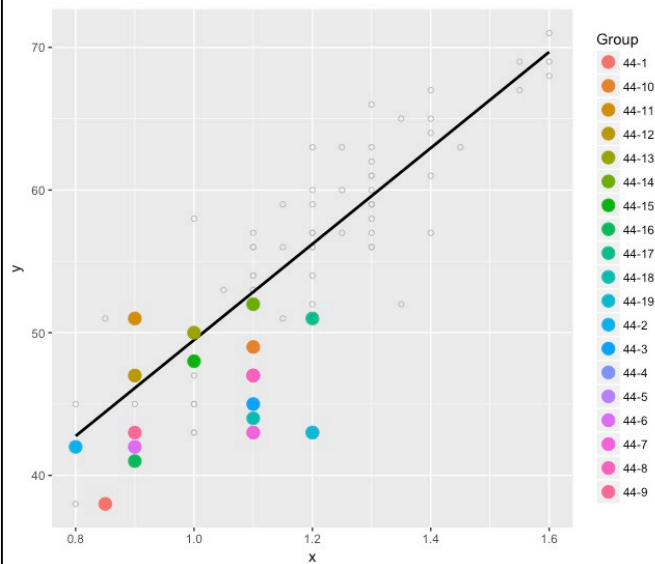
Nineteen independent T0 lines:

IR64-IRS1085-001 to IR64-IRS1085-019

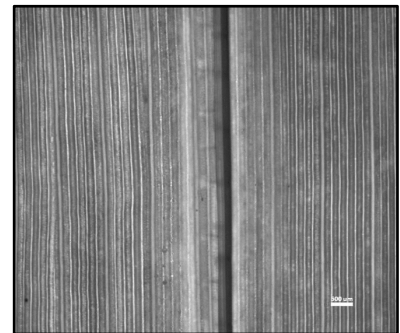
Genomic PCR



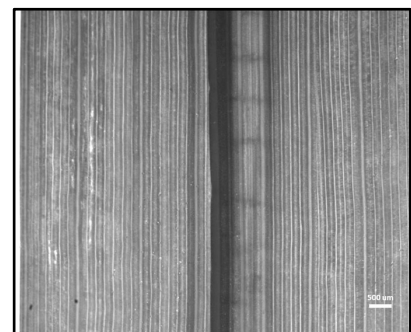
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 15 leaf



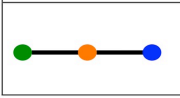
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Myb

Gene ID: JL45

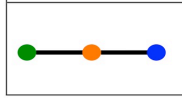
Foliar expression

P1/2 P3/4 P5



Husk expression

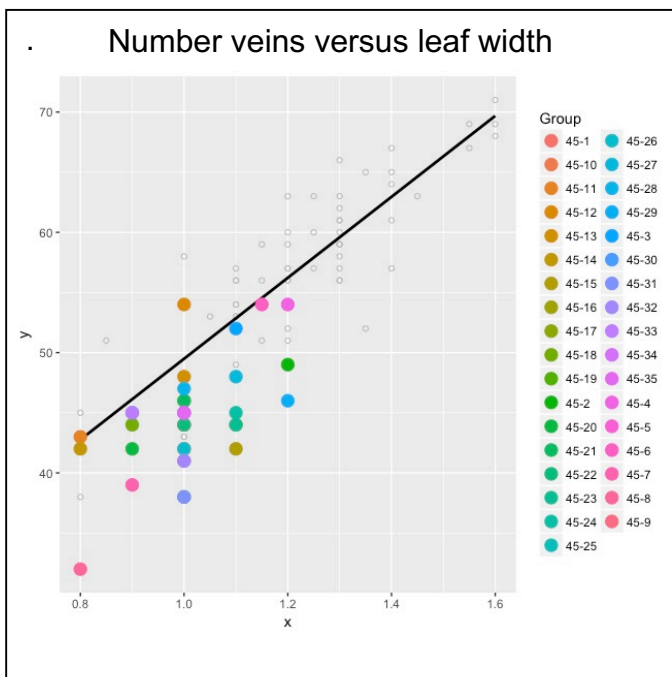
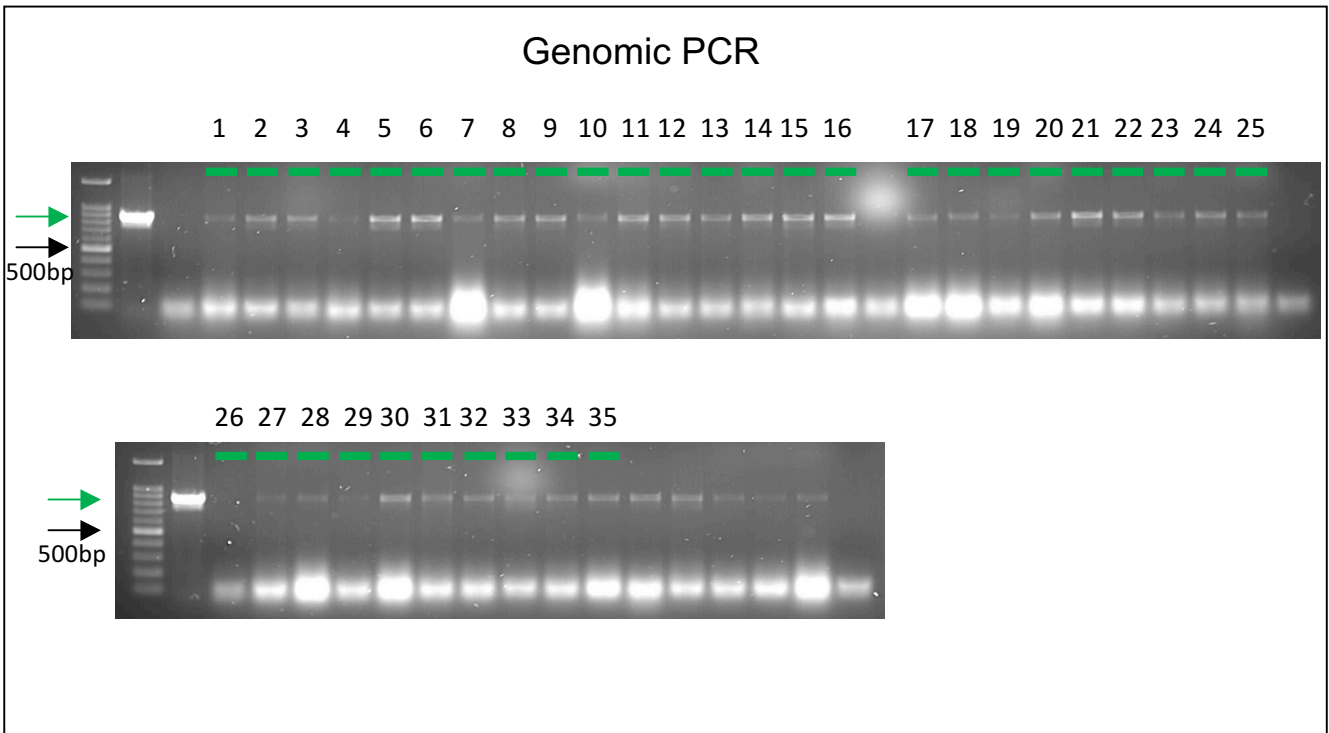
P1/2 P3/4 P5



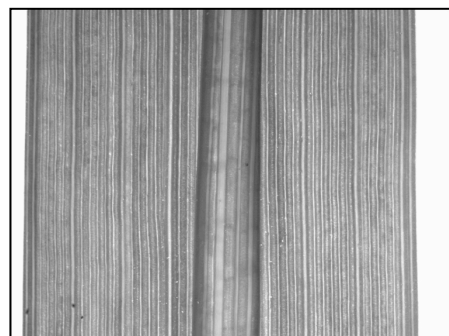
Construct: ZmUBI_{pro}:cDNA (ID# IRS1086)

Thirty five independent T0 lines:

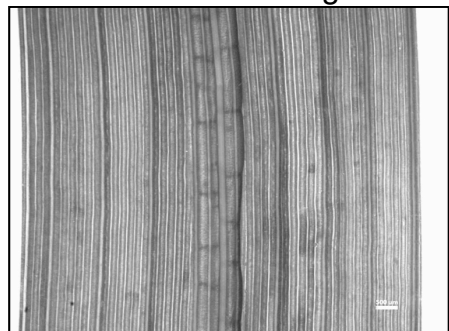
IR64-IRS1086-001 to IR64-IRS1086-035



Representative surface image of segregating WT leaf



Representative surface image of line 35 leaf

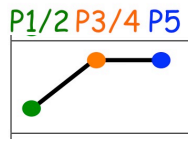


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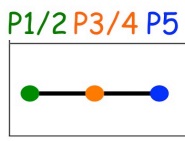
AP2 domain

Gene ID: JL53

Foliar expression



Husk expression

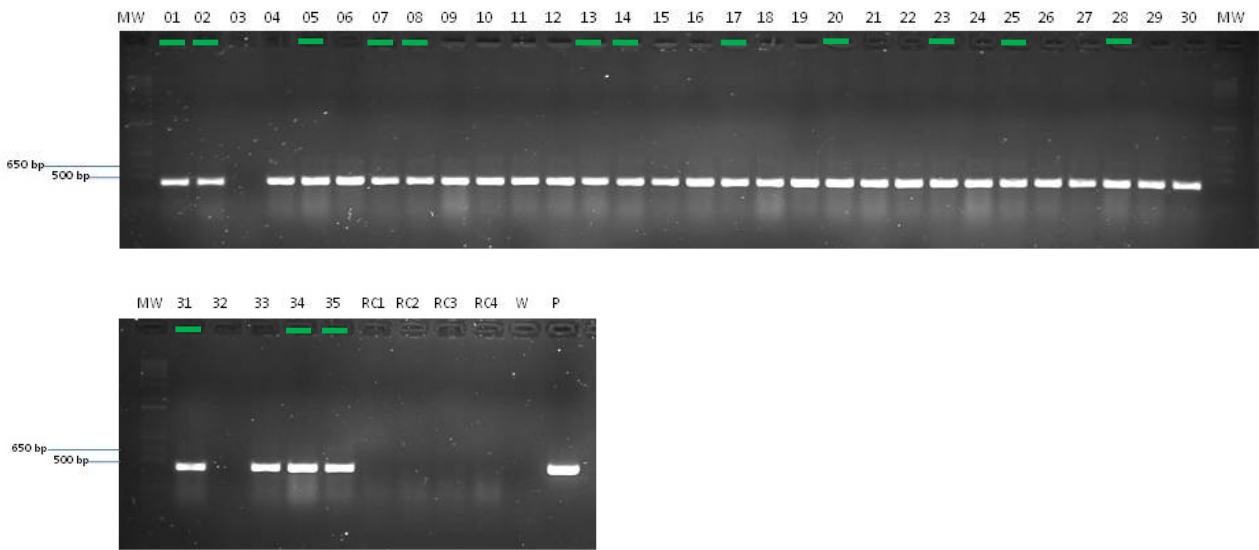


Construct: ZmUBI_{pro}:cDNA (ID# IRS952)

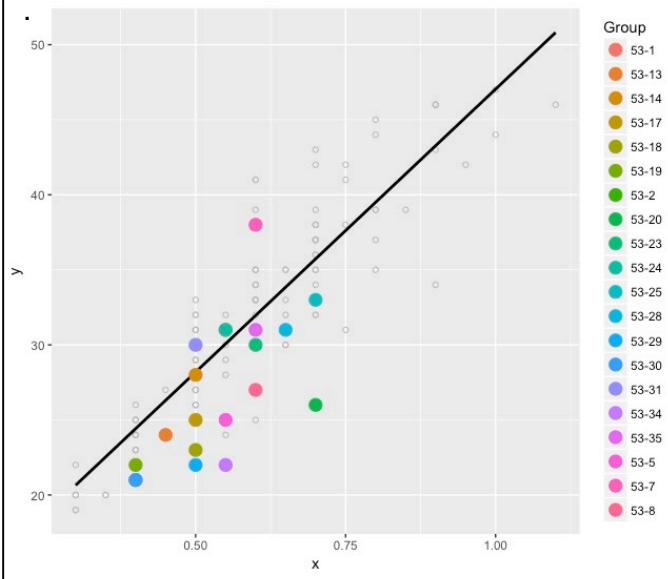
Twenty independent T0 lines:

IR64-IRS952-001,002,005,007,008,013,014,017-020,023-025,028-031,034,035

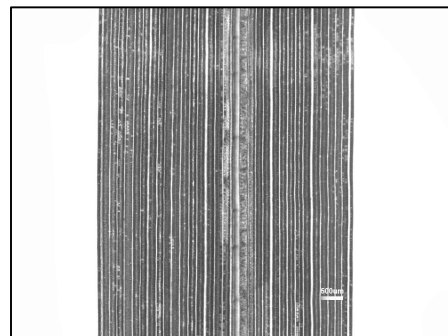
Genomic PCR



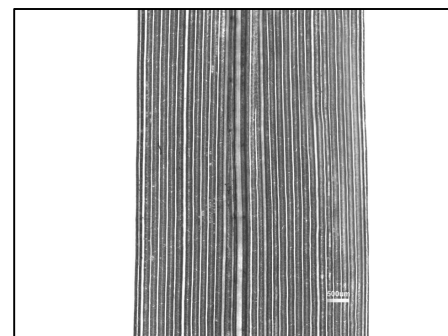
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 14 leaf

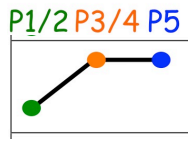


GRMZM2G077219

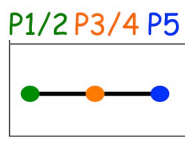
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Gene ID: JL54

Foliar expression



Husk expression

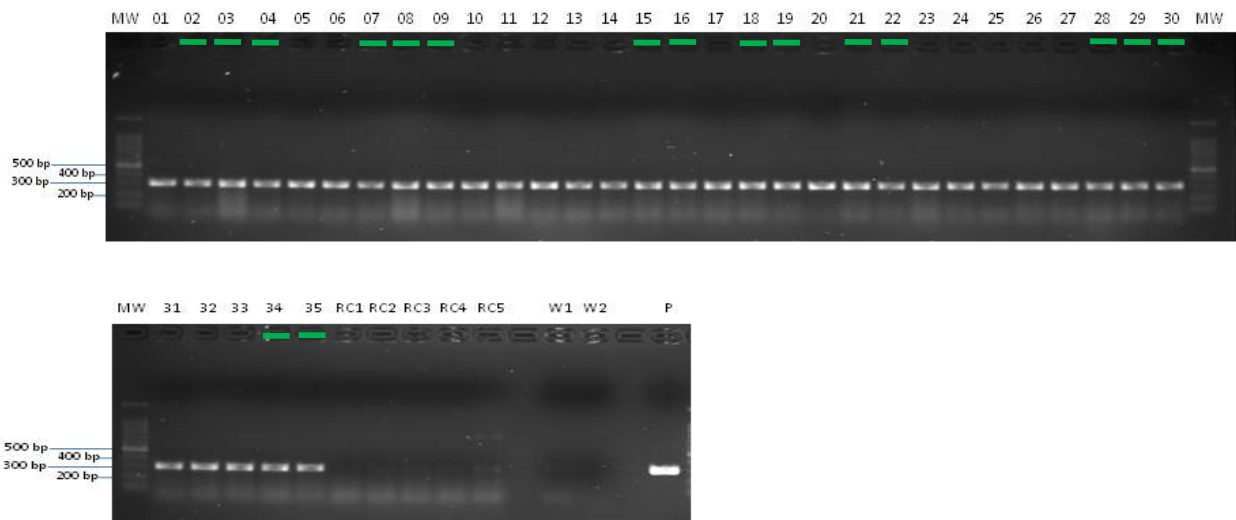


Construct: ZmUBI_{pro}:cDNA (ID# 1010)

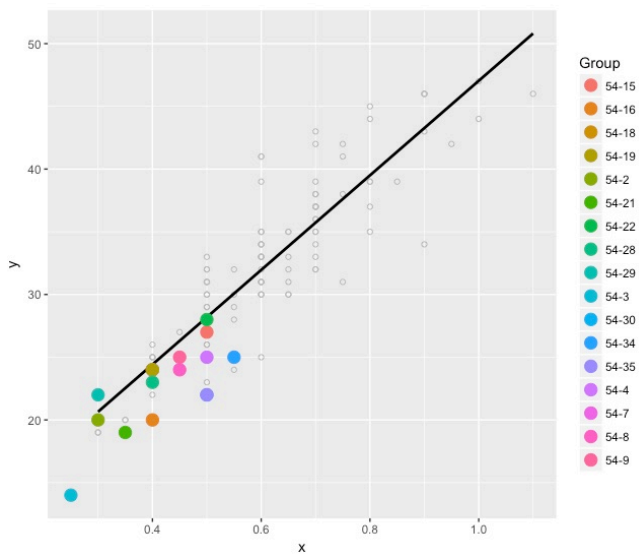
Seventeen independent T0 lines:

IR64-IRS1010-002-004,007-009,015,016,018,019, 021,022, 028-030, 034,035

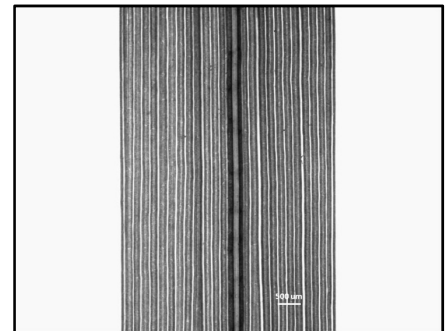
Genomic PCR



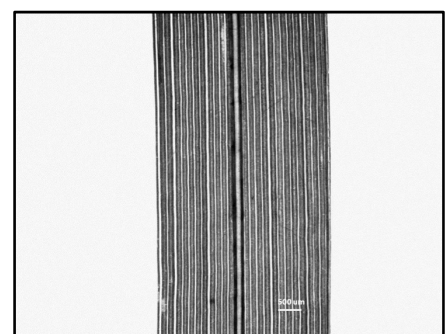
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 19 leaf

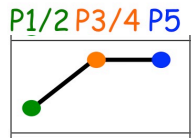


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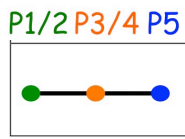
bHLH

Gene ID: JL55

Foliar expression



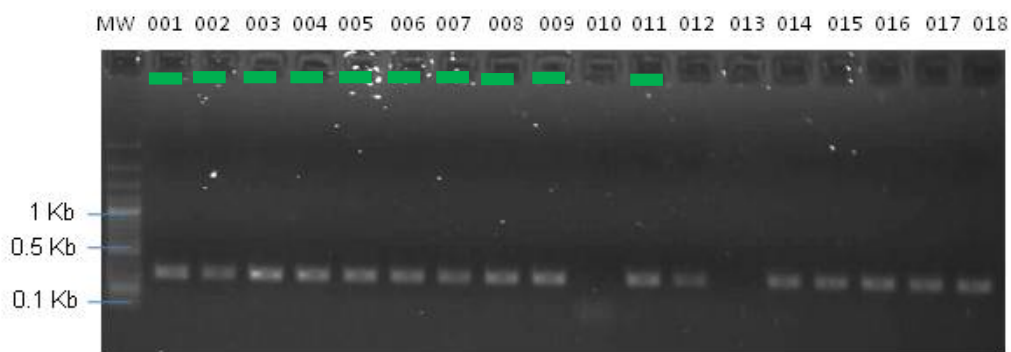
Husk expression



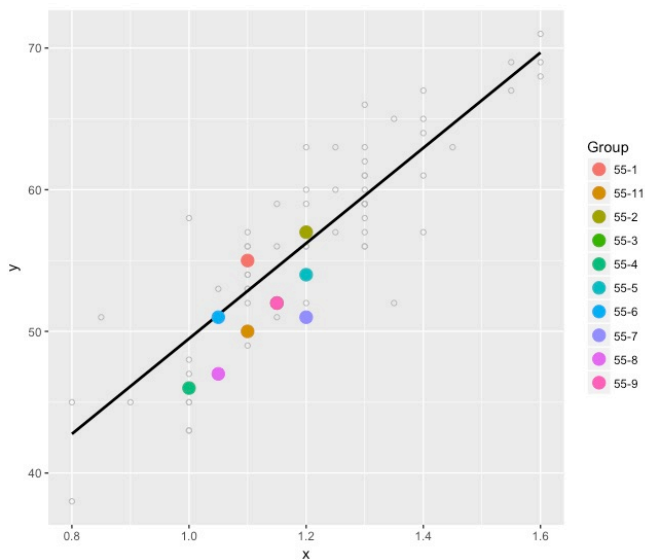
Construct: ZmUBI_{pro}:cDNA (ID# 878)

Ten independent T0 lines:
IR64-IRS878-001-009, 011

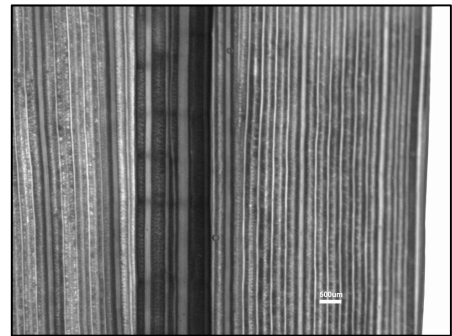
Genomic PCR



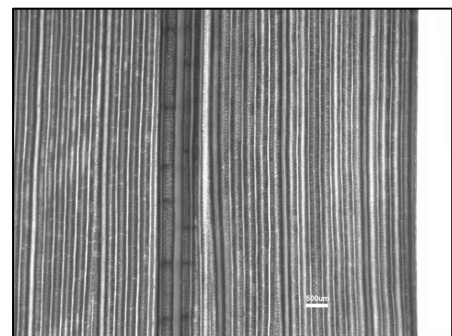
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 1 leaf



GRMZM2G171365

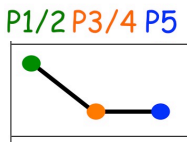
MADS box

Gene ID: JL56

Foliar expression



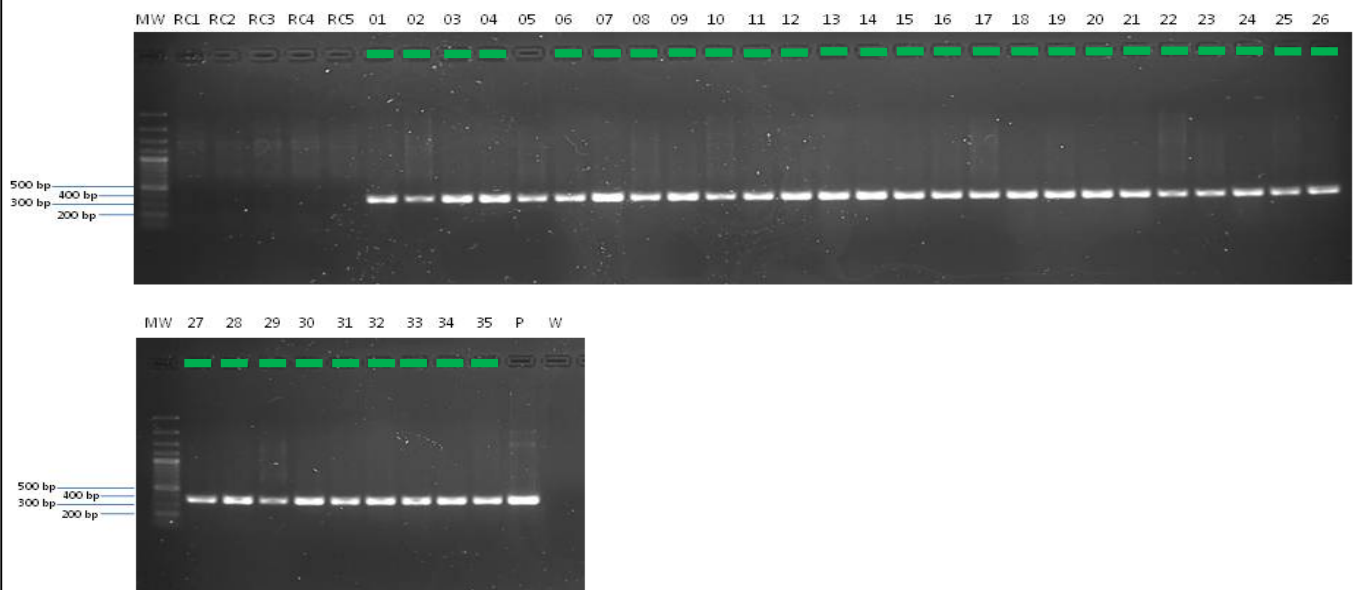
Husk expression



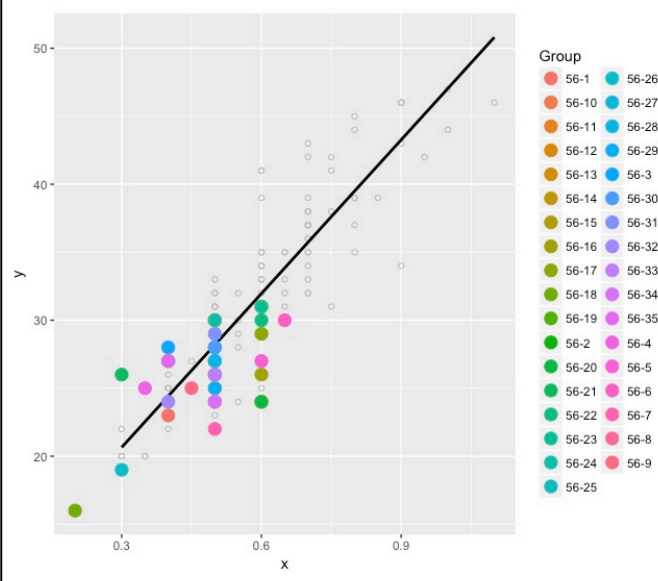
Construct: ZmUBI_{pro}:cDNA (ID# 921)

Thirty four independent T0 lines:
IR64-IRS921-001-004,006-035

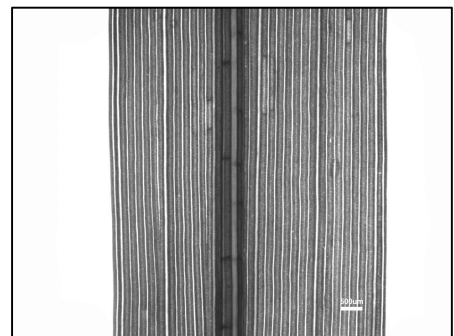
Genomic PCR



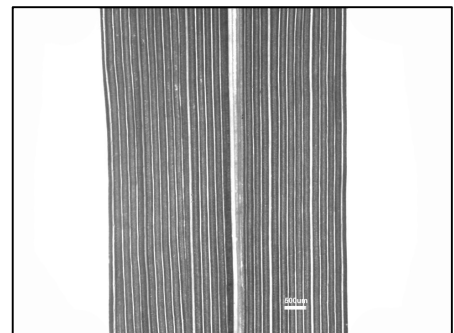
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 30 leaf



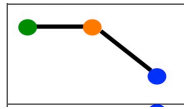
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LFY-like

Gene ID: JL57

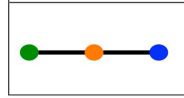
Foliar expression

P1/2 P3/4 P5



Husk expression

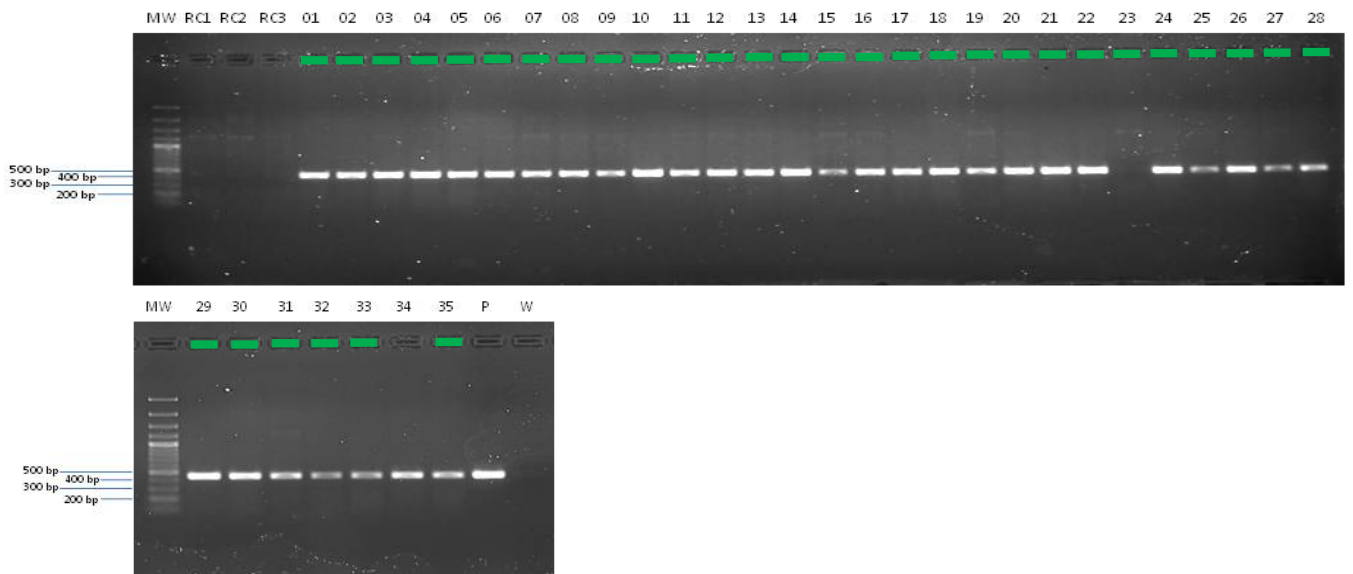
P1/2 P3/4 P5



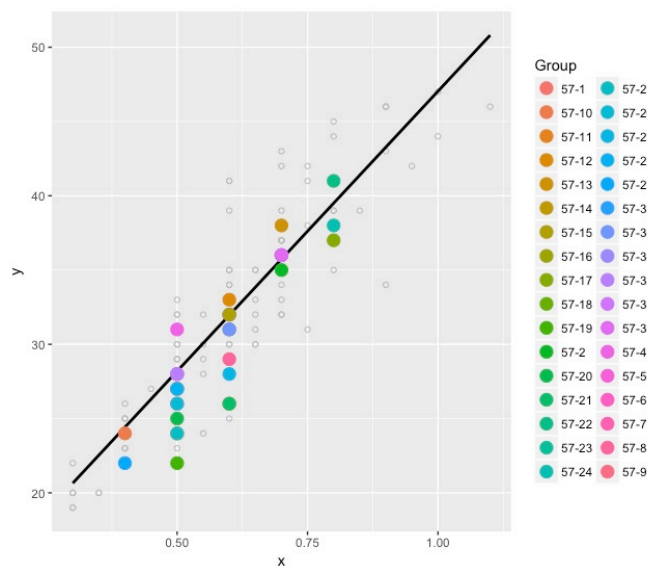
Construct: ZmUBI_{pro}:cDNA (ID# 922)

Thirty four independent T0 lines:
IR64-IRS922-001-033, 035

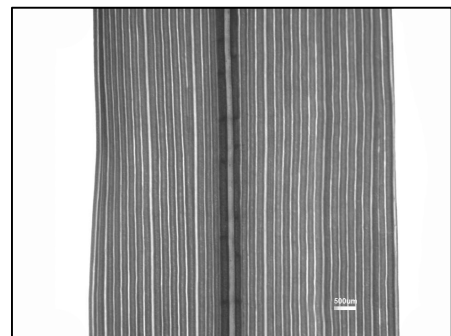
Genomic PCR



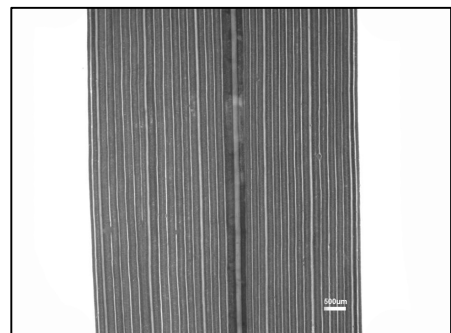
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 12 leaf

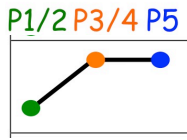


GRMZM2G139324

unknown

Gene ID: JL59

Foliar expression

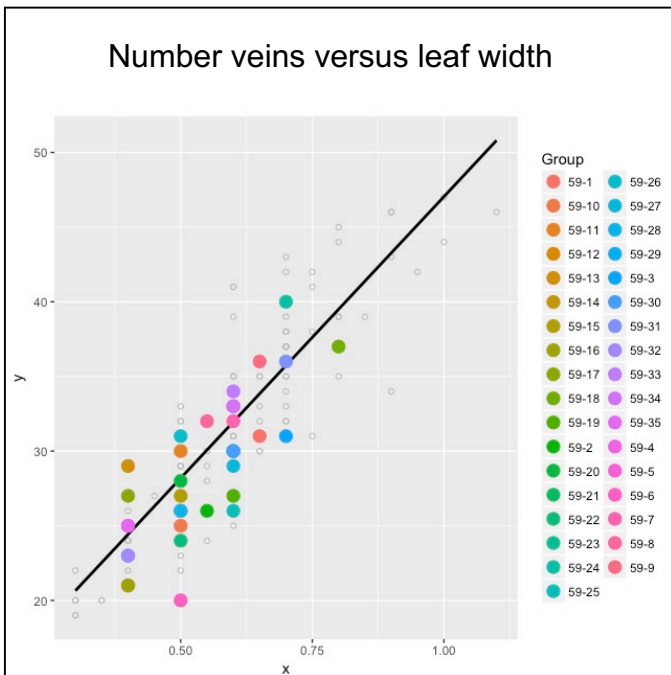
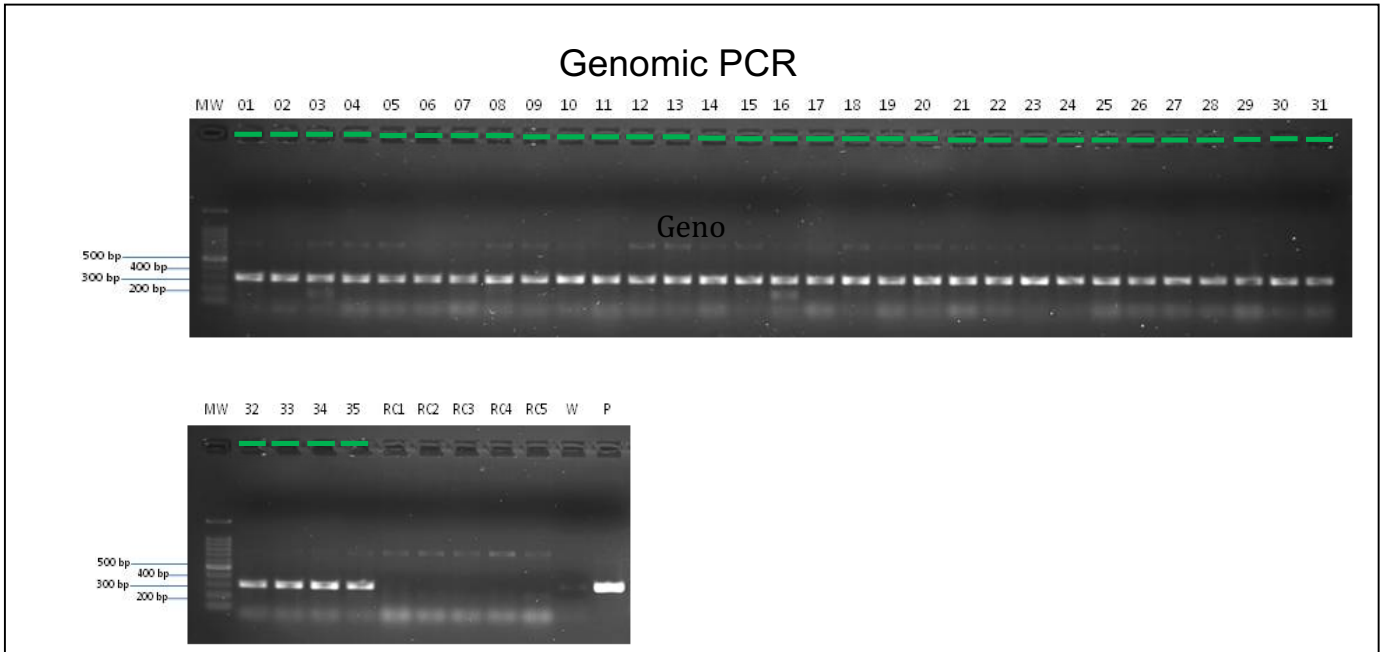


Husk expression

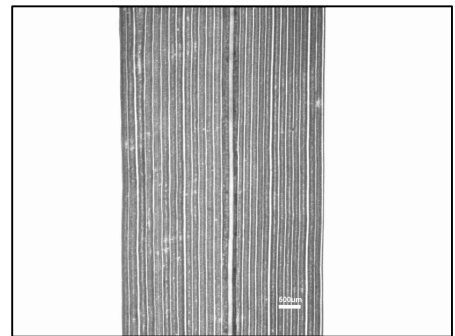


Construct: ZmUBI_{pro}:cDNA (ID# 1004)

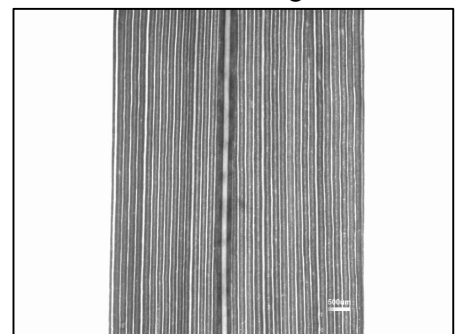
Thirty five independent T0 lines:
IR64-IRS1004-001-035



Representative surface image of segregating WT leaf



Representative surface image of line 5 leaf

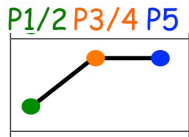


GRMZM2G061314

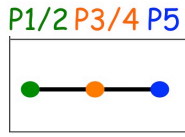
LRR binding protein

Gene ID: JL60

Foliar expression

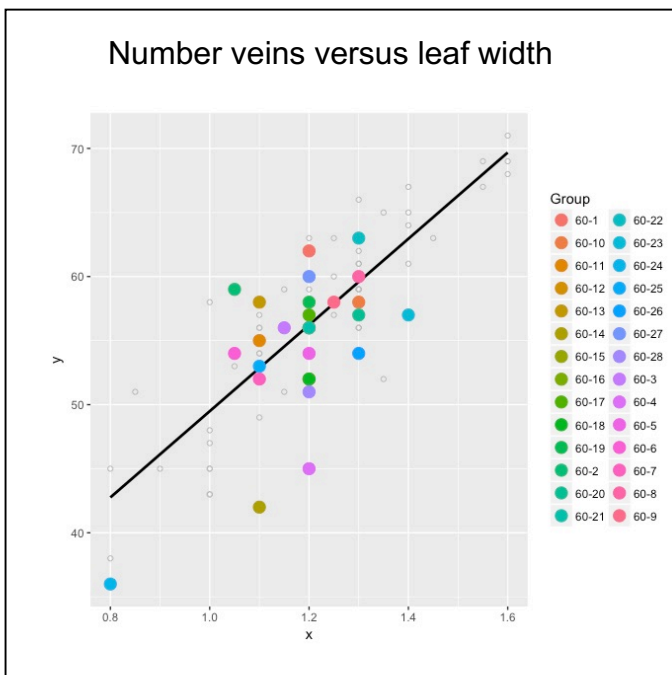
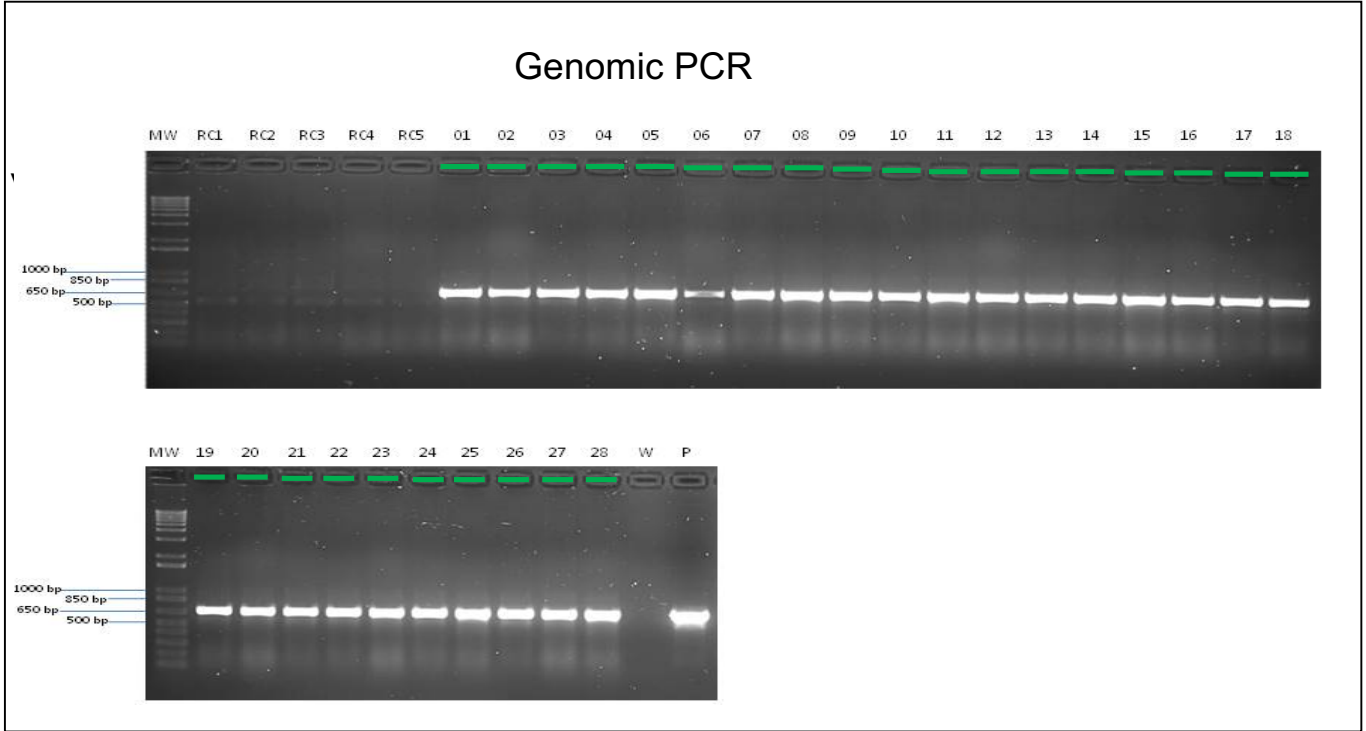


Husk expression

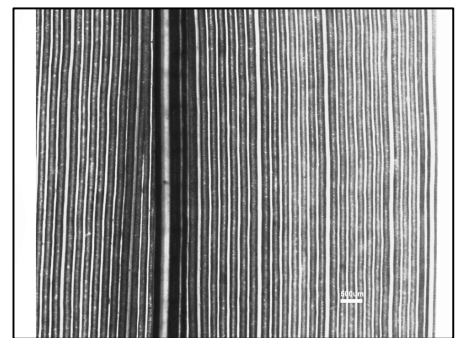


Construct: ZmUBI_{pro}:cDNA (ID# 945)

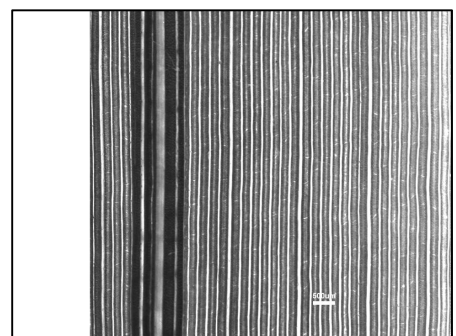
Twenty eight independent T0 lines:
IR64-IRS945-001-028



Representative surface image of segregating WT leaf



Representative surface image of line 16 leaf

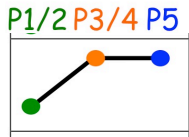


GRMZM2G151955

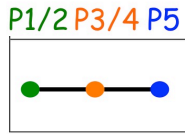
LRR RLK

Gene ID: JL61

Foliar expression



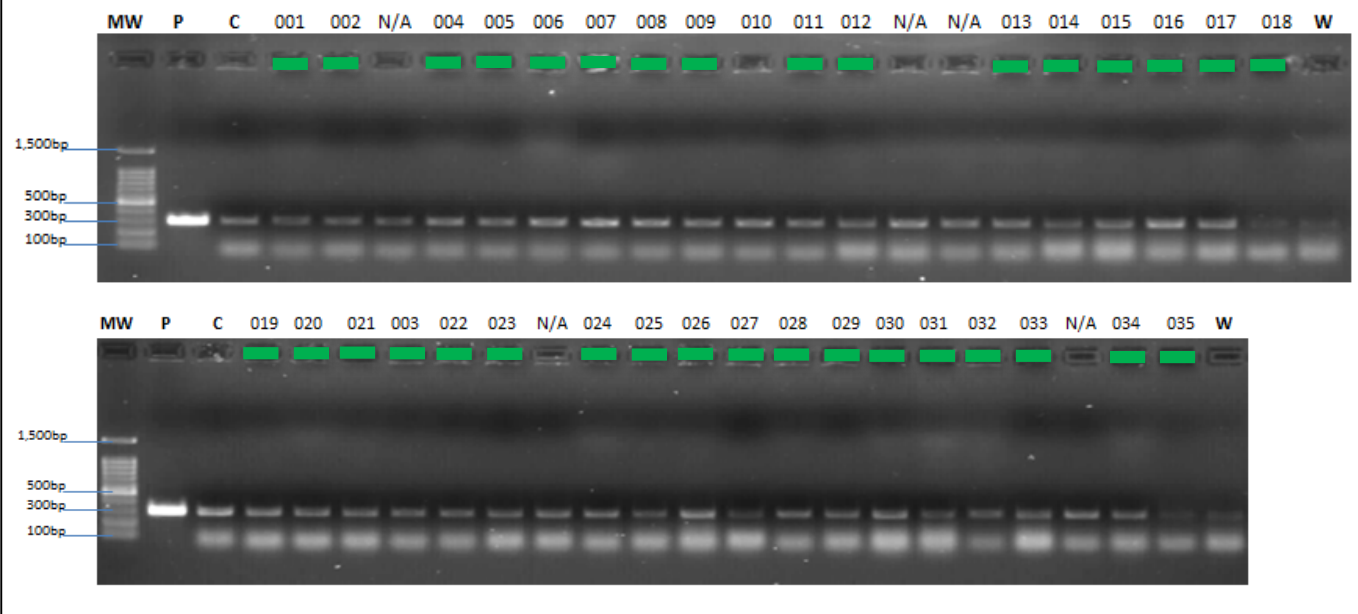
Husk expression



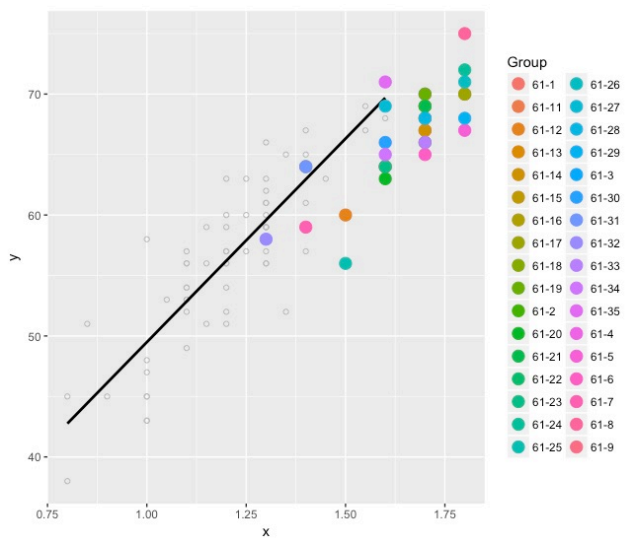
Construct: ZmUBI_{pro}:cDNA (ID# 1078)

Thirty four independent T0 lines:
IR64-IRS1078-001-009, 011-035

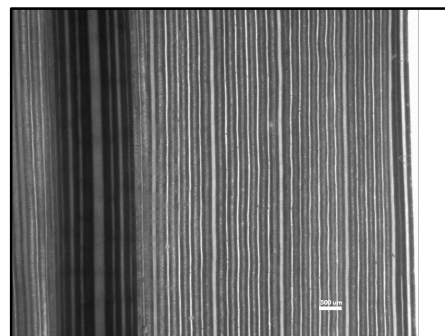
Genomic PCR



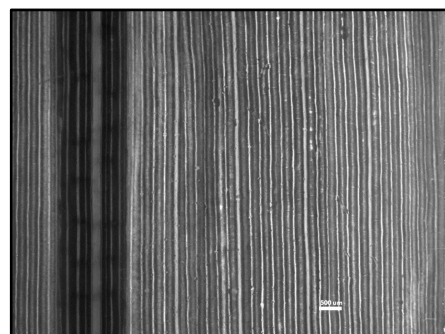
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 3 leaf

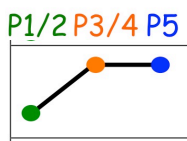


GRMZM2G159953

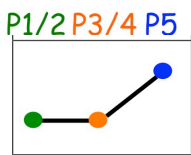
Lectin family RLK

Gene ID: JL62

Foliar expression



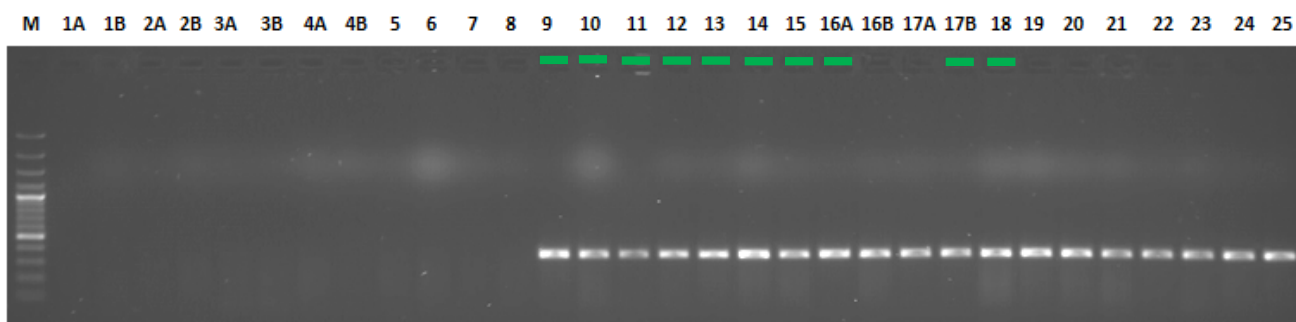
Husk expression



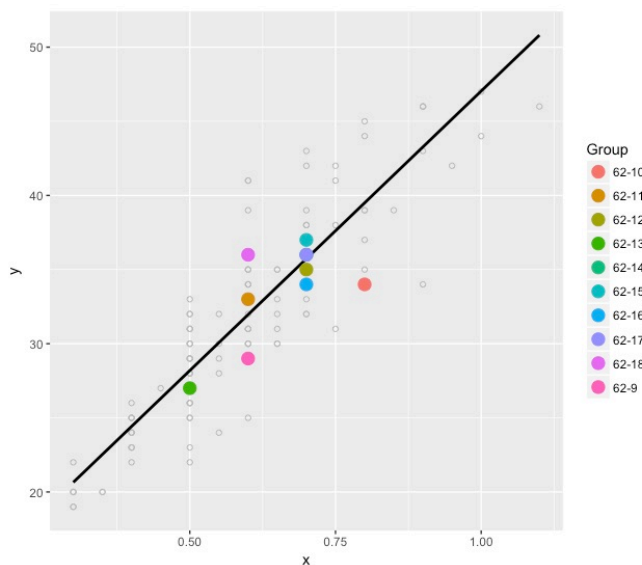
Construct: ZmUBI_{pro}:cDNA (ID# 913)

Ten independent T0 lines:
IR64-IRS913-009-016A, 017B, 018

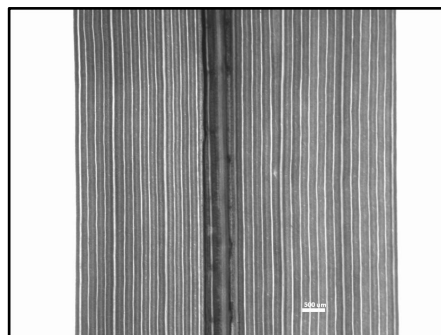
Genomic PCR



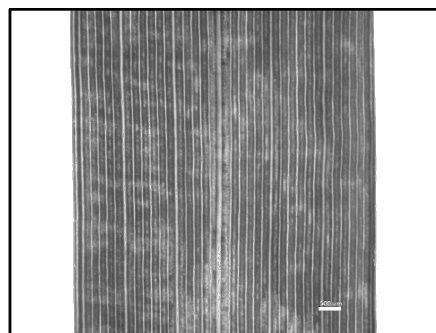
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 12 leaf

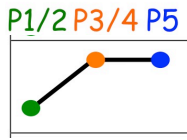


GRMZM2G039934

TDR/PXY LRR RLK

Gene ID: JL63

Foliar expression



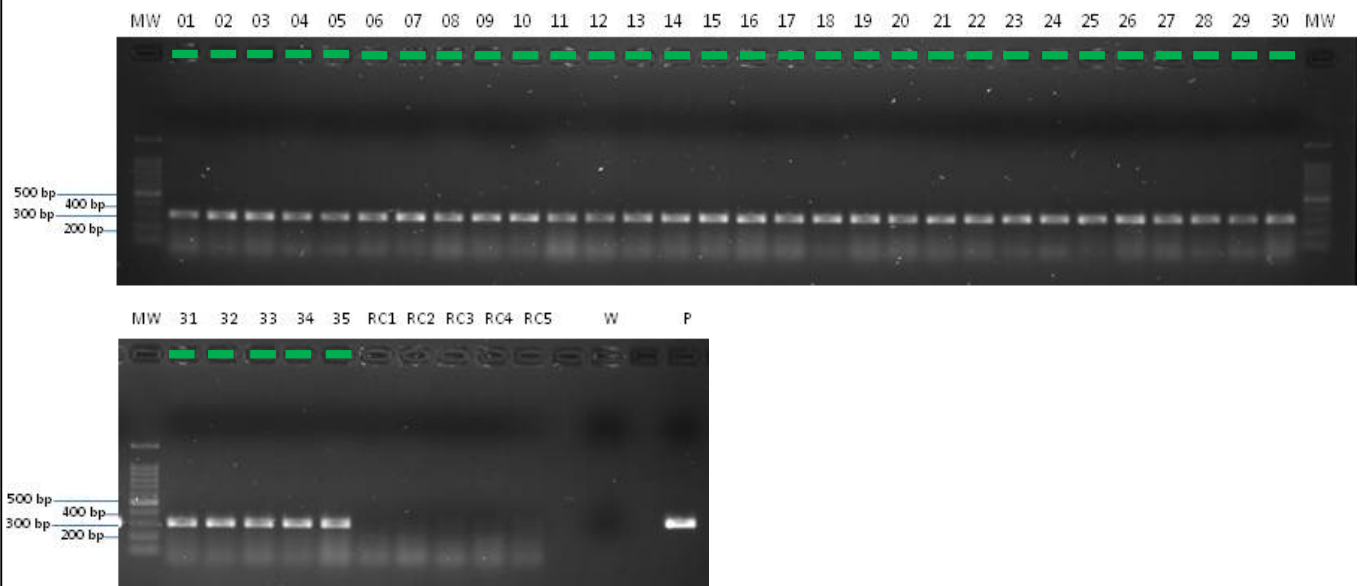
Husk expression



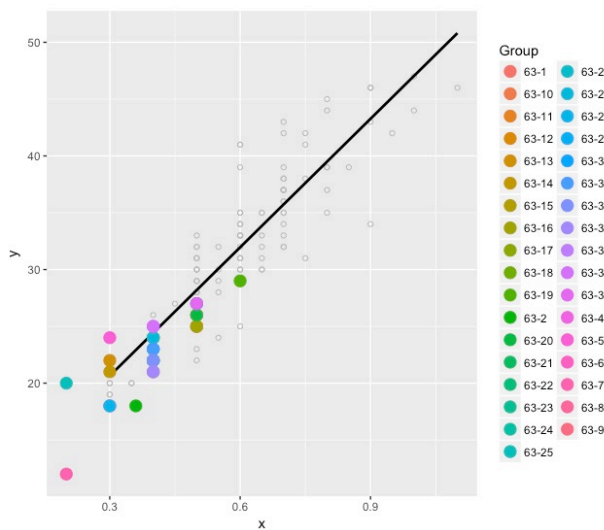
Construct: ZmUBI_{pro}:cDNA (ID# 1011)

Thirty five independent T0 lines:
IR64-IRS1011-001-035

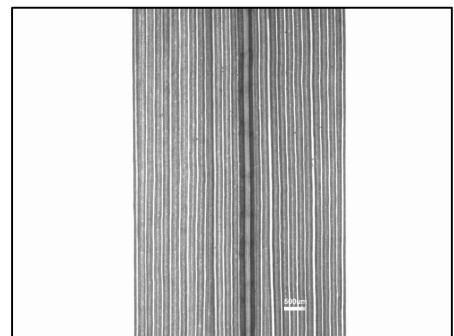
Genomic PCR



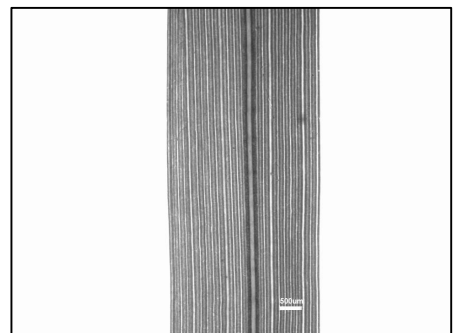
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 23 leaf

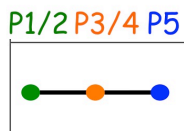


GRMZM2G046316

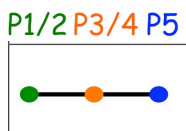
LRR-RLK

Gene ID: JL64

Foliar expression

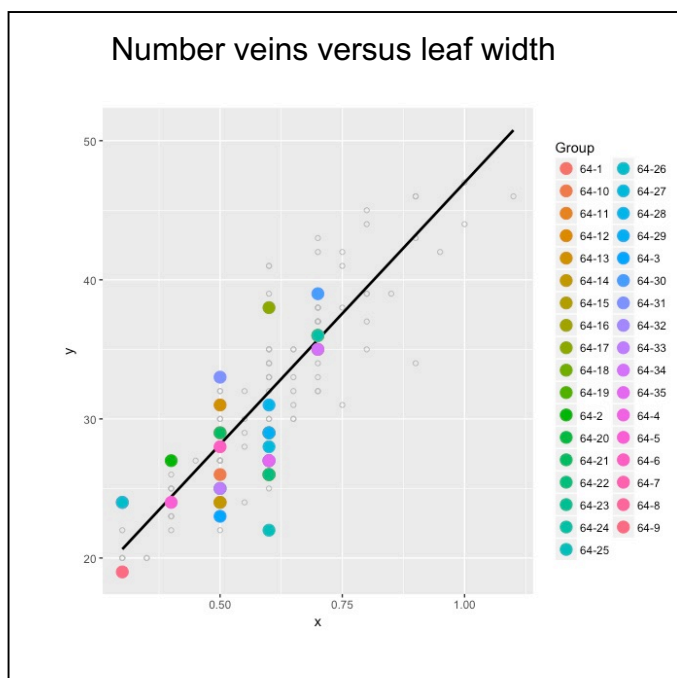
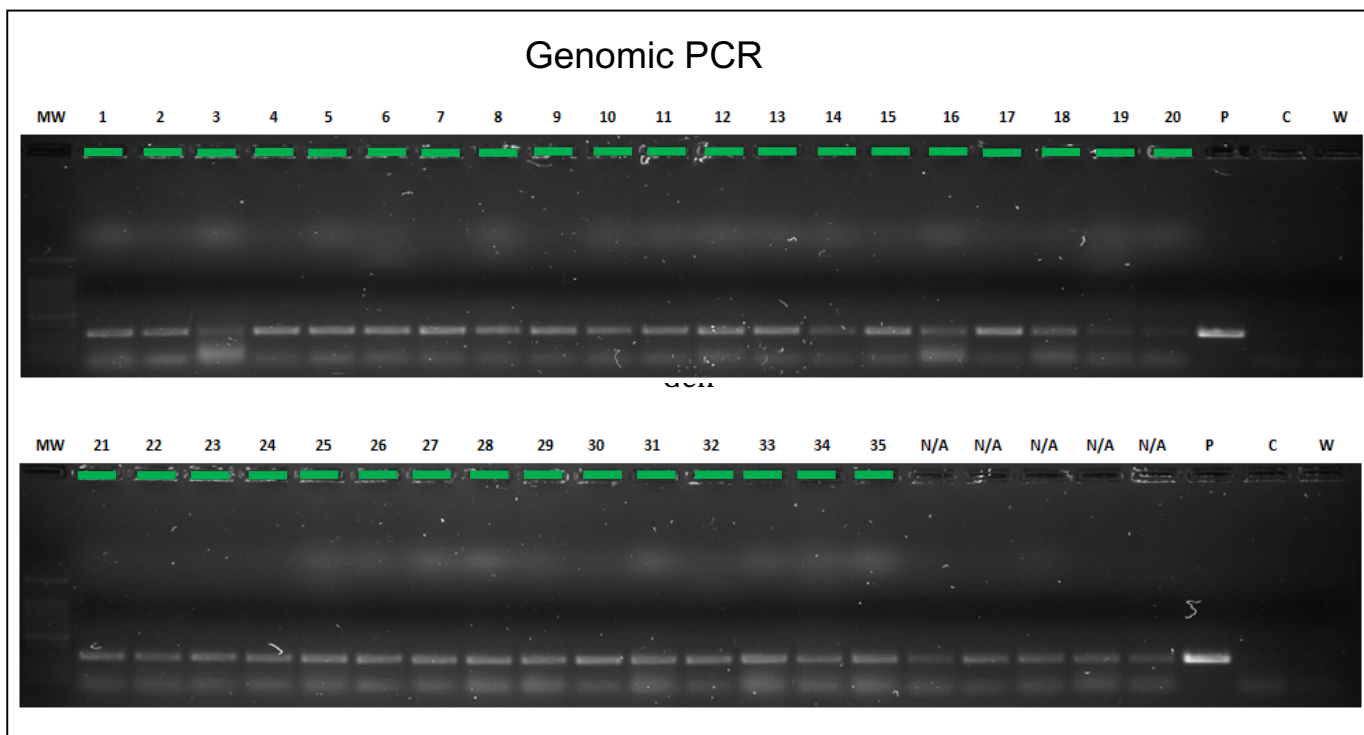


Husk expression

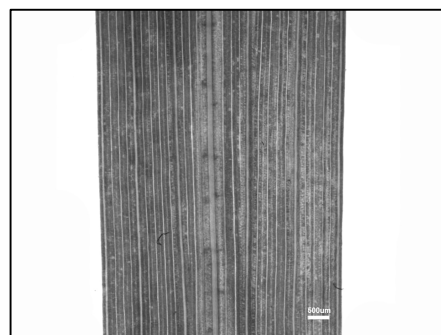


Construct: ZmUBI_{pro}:cDNA (ID#1106)

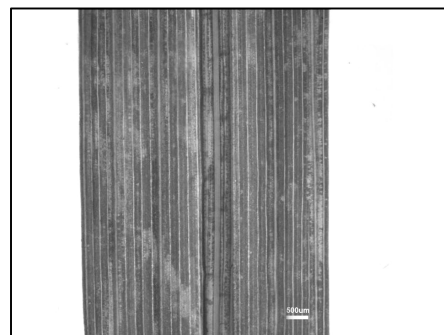
Thirty five independent T0 lines:
IR64-IRS1106-001-035



Representative surface image of segregating WT leaf



Representative surface image of line 23 leaf



GRMZM2G034155

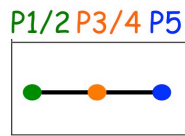
LRR-RLK

Gene ID: JL65

Foliar expression

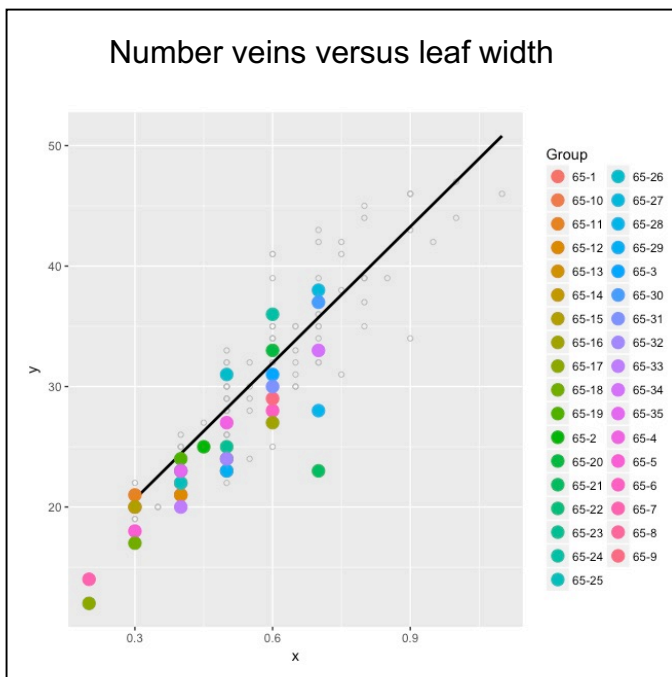
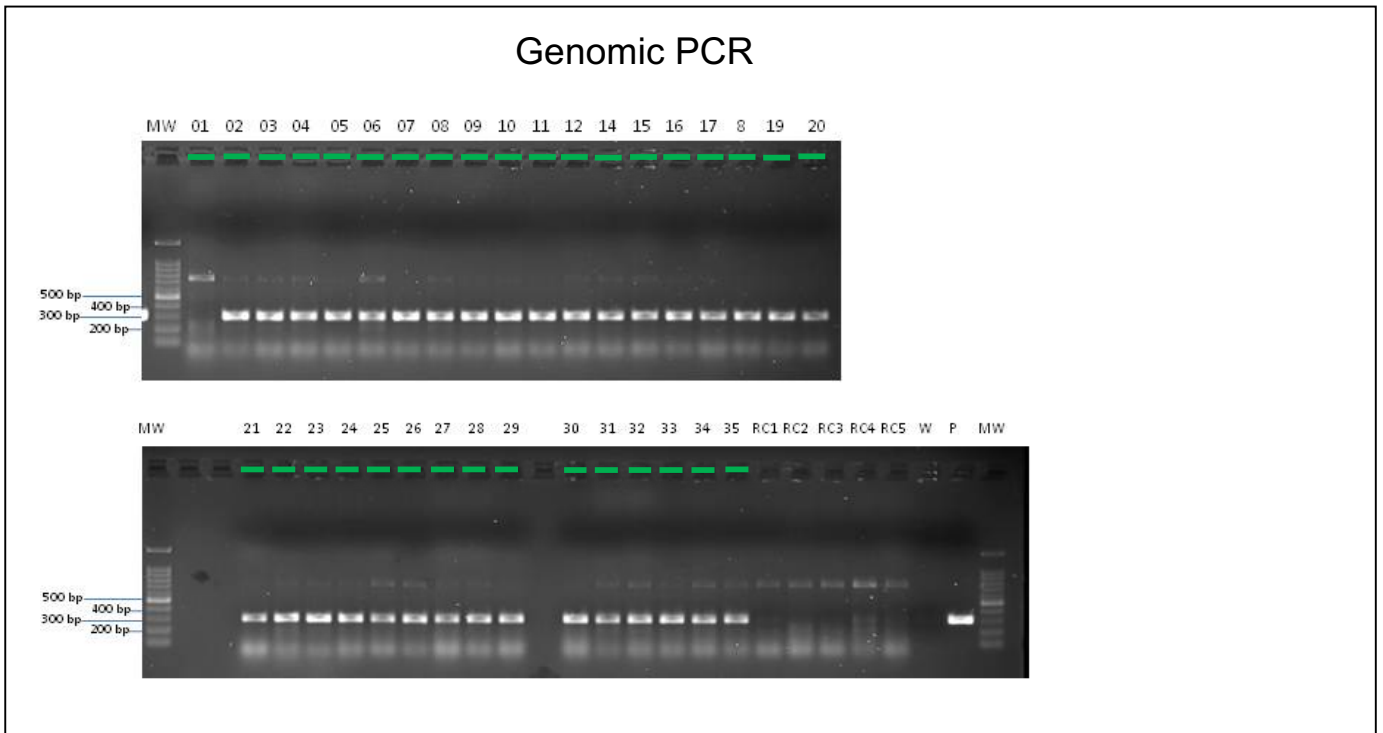


Husk expression

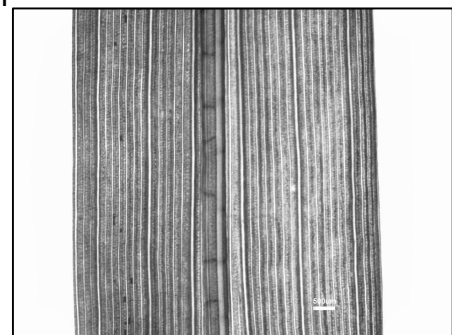


Construct: ZmUBI_{pro}:cDNA (ID# 1005)

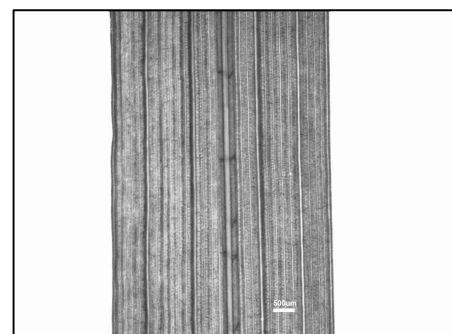
Thirty five independent T0 lines:
IR64-IRS1005-001-035



Representative surface image of segregating WT leaf



Representative surface image of line 4 leaf



GRMZM2G114276

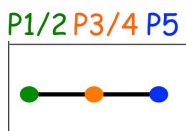
LRR-RLK

Gene ID: JL66

Foliar expression



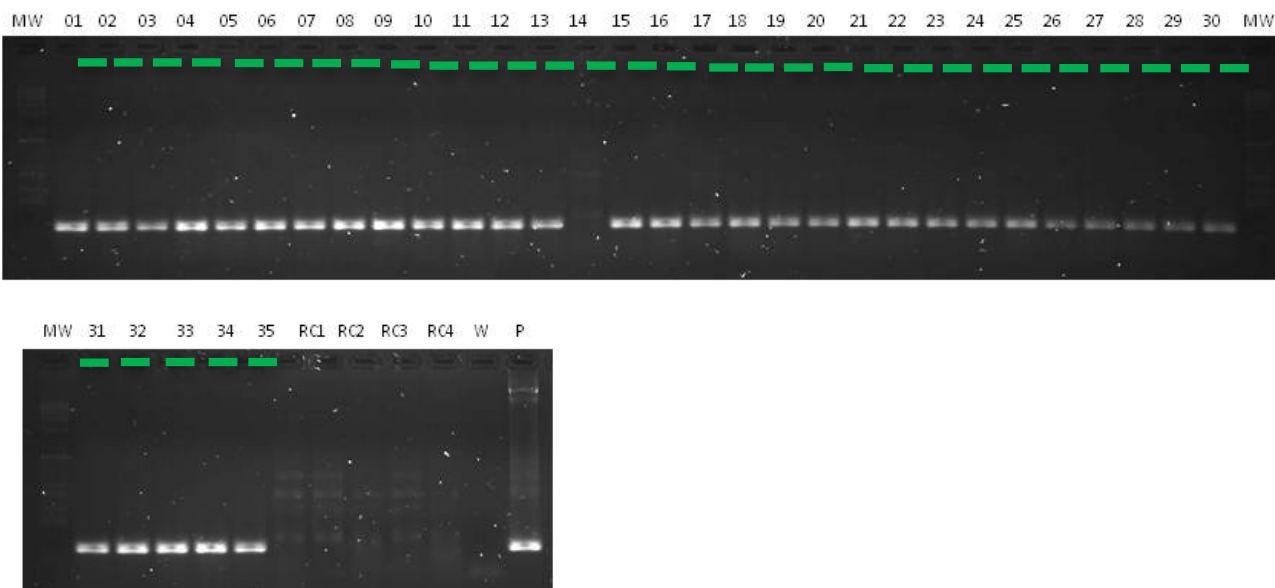
Husk expression



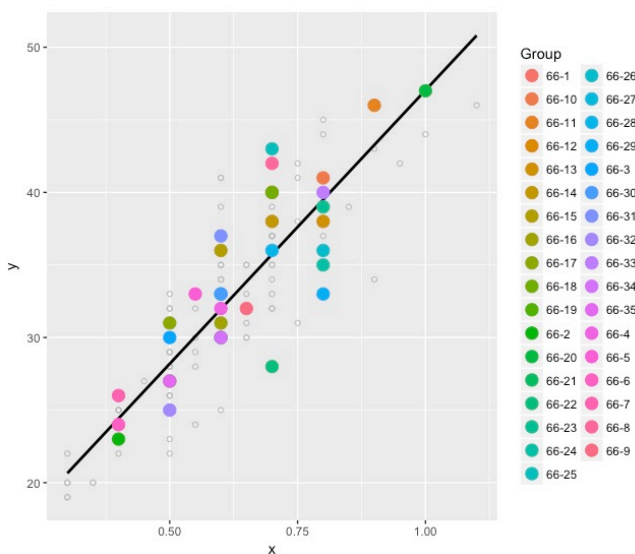
Construct: ZmUBI_{pro}:cDNA (ID# 959)

Thirty five independent T0 lines:
IR64-IRS959-001-035

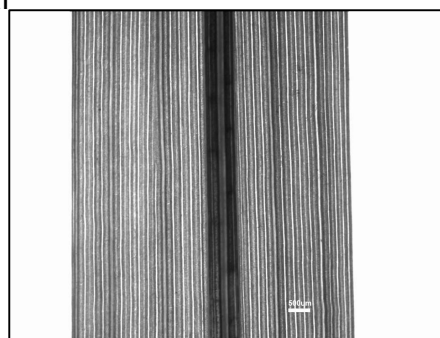
Genomic PCR



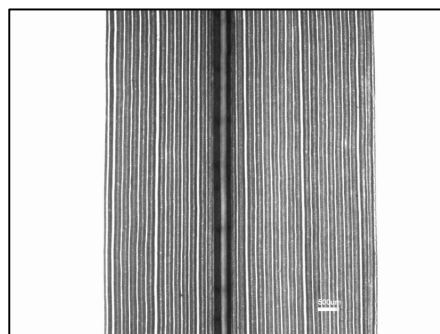
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 9 leaf

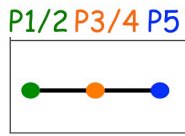


GRMZM2G059117

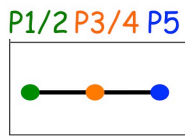
LRR-RLK

Gene ID: JL67

Foliar expression



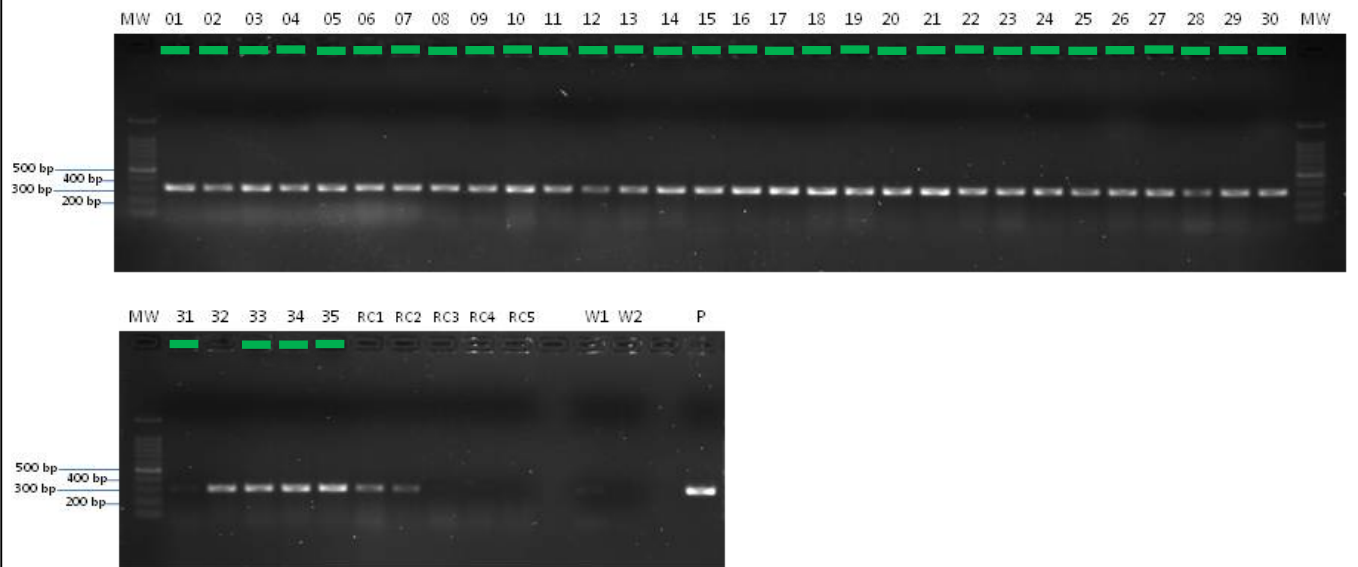
Husk expression



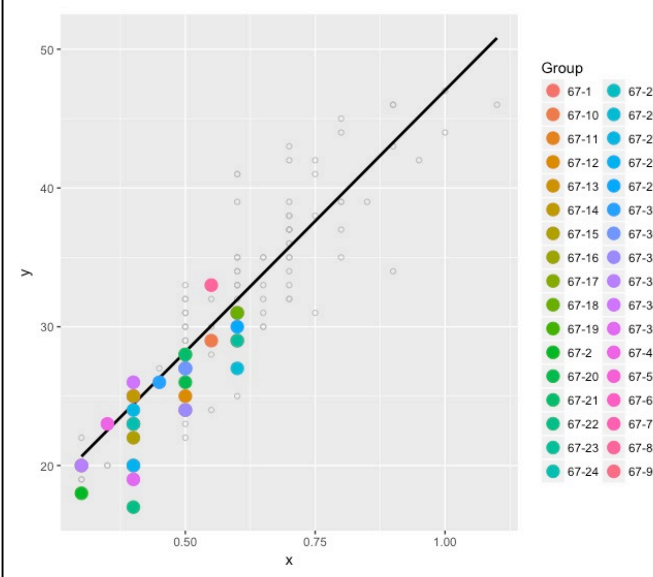
Construct: ZmUBI_{pro}:cDNA (ID# 1006)

Thirty four independent T0 lines:
IR64-IRS1006-001-031, 033-035

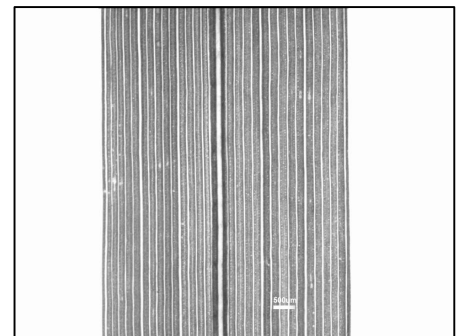
Genomic PCR



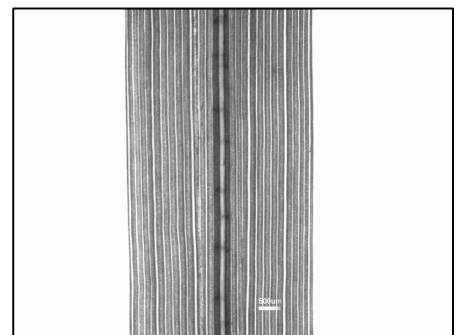
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 3 leaf



GRMZM2G089819

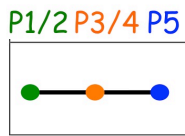
Brassinosteroid LRR-RLK

Gene ID: JL68

Foliar expression

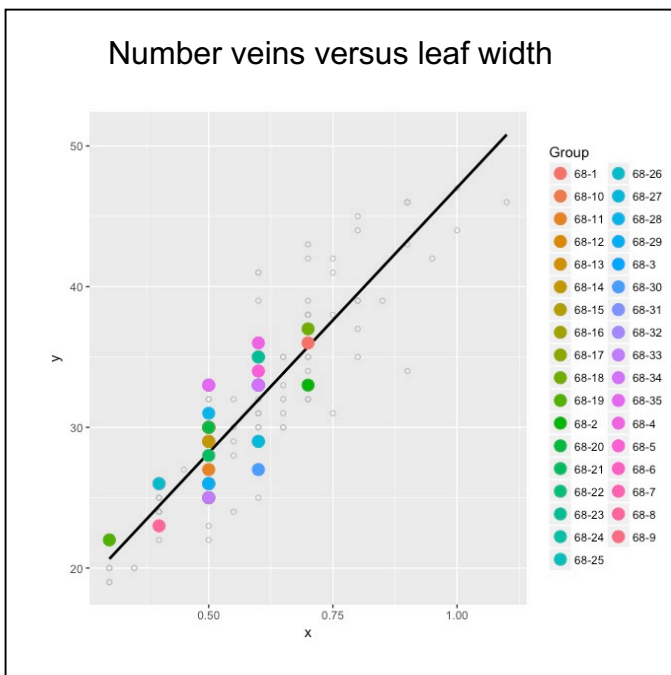
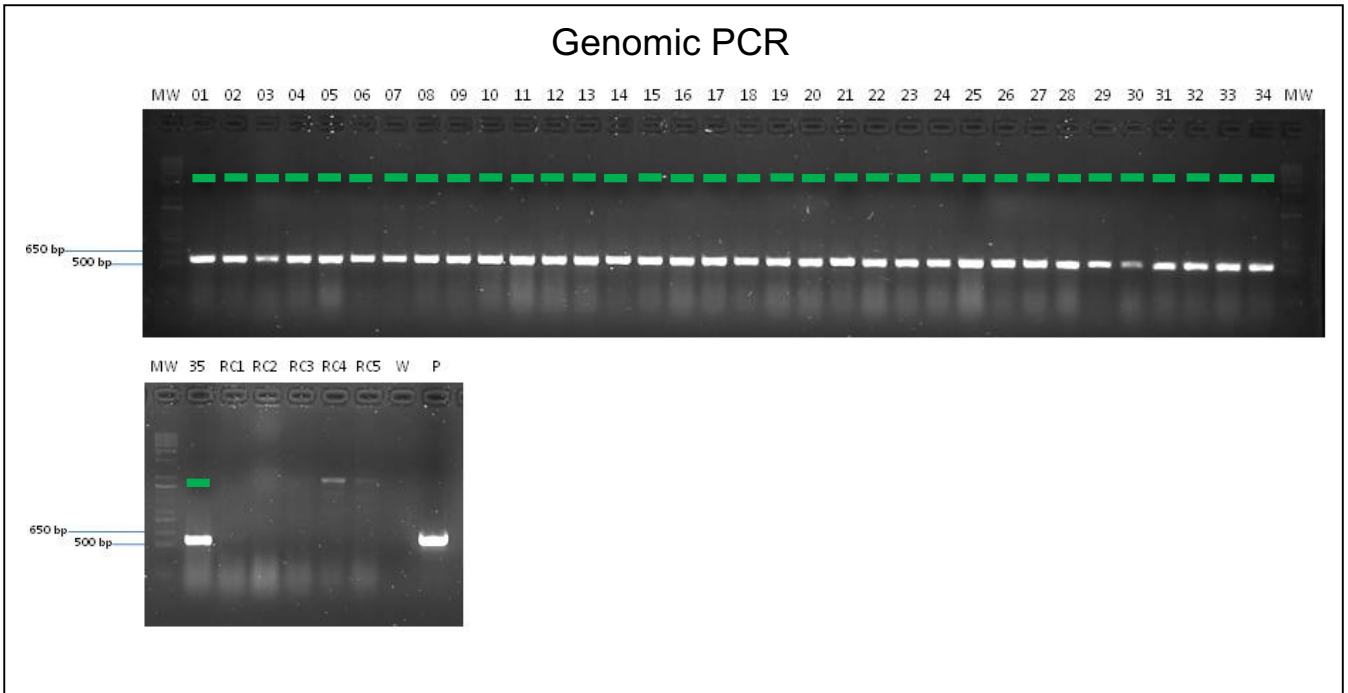


Husk expression

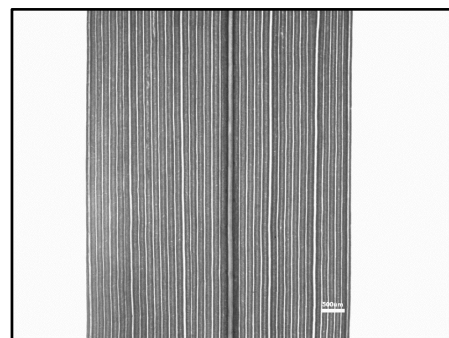


Construct: ZmUBI_{pro}:cDNA (ID# 953)

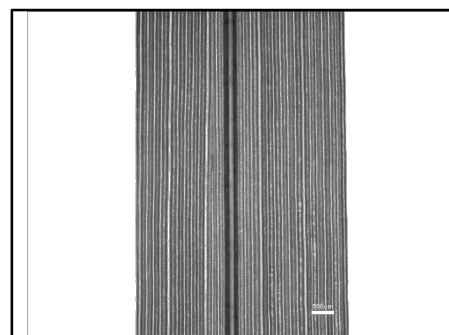
Thirty five independent T0 lines:
IR64-IRS953-001-035



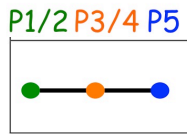
Representative surface image of segregating WT leaf



Representative surface image of line 14 leaf



Foliar expression

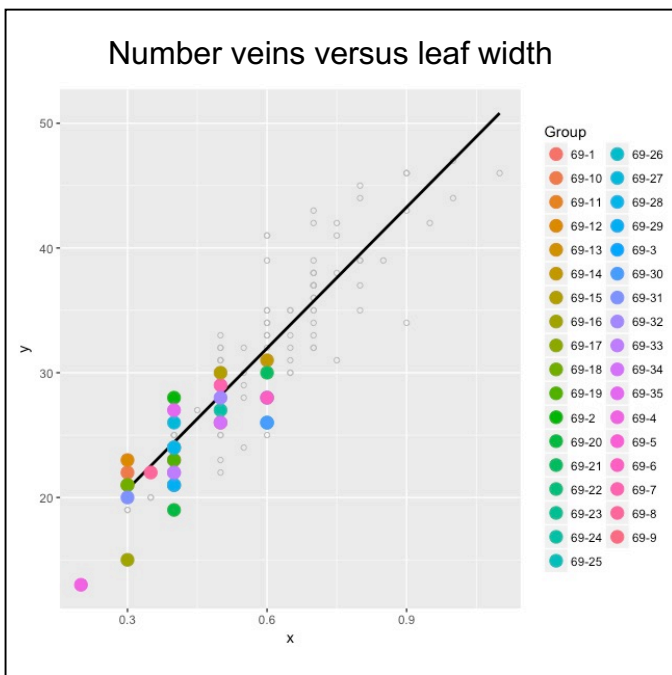
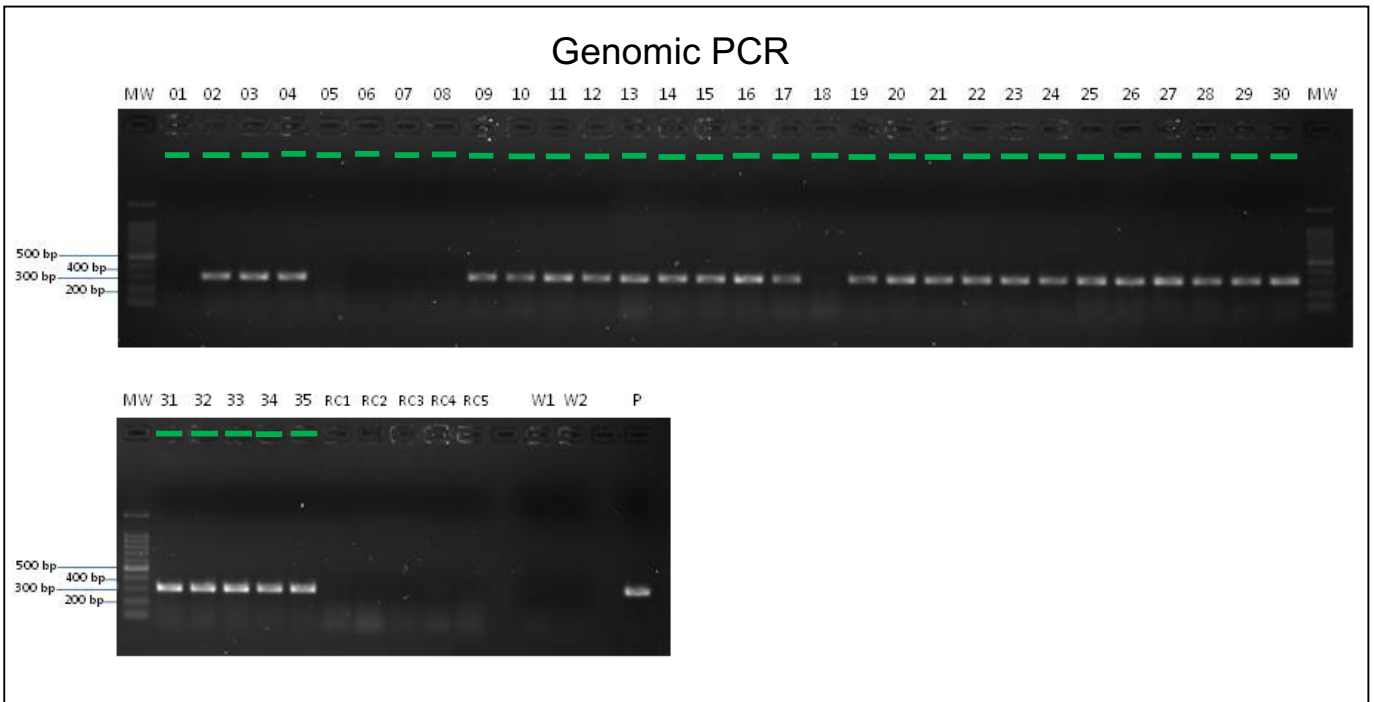


Husk expression

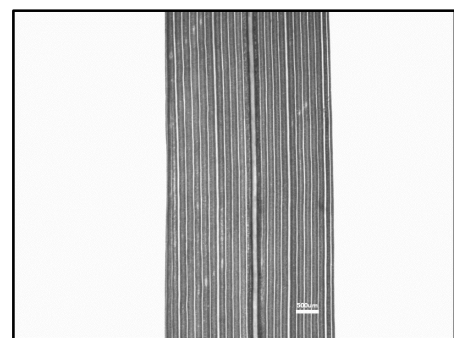


Construct: ZmUBI_{pro}:cDNA (ID# 1007)

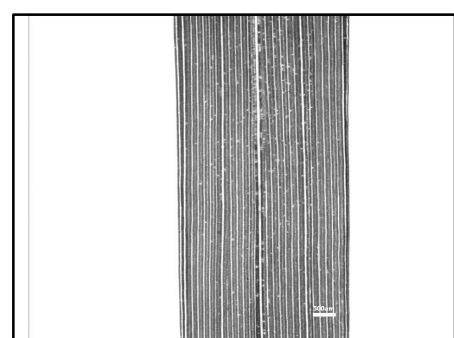
Thirty five independent T0 lines:
IR64-IRS1007-001-035



Representative surface image of segregating WT leaf



Representative surface image of line 27 leaf



GRMZM2G087243

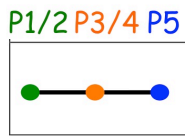
Armadillo like

Gene ID: JL70

Foliar expression



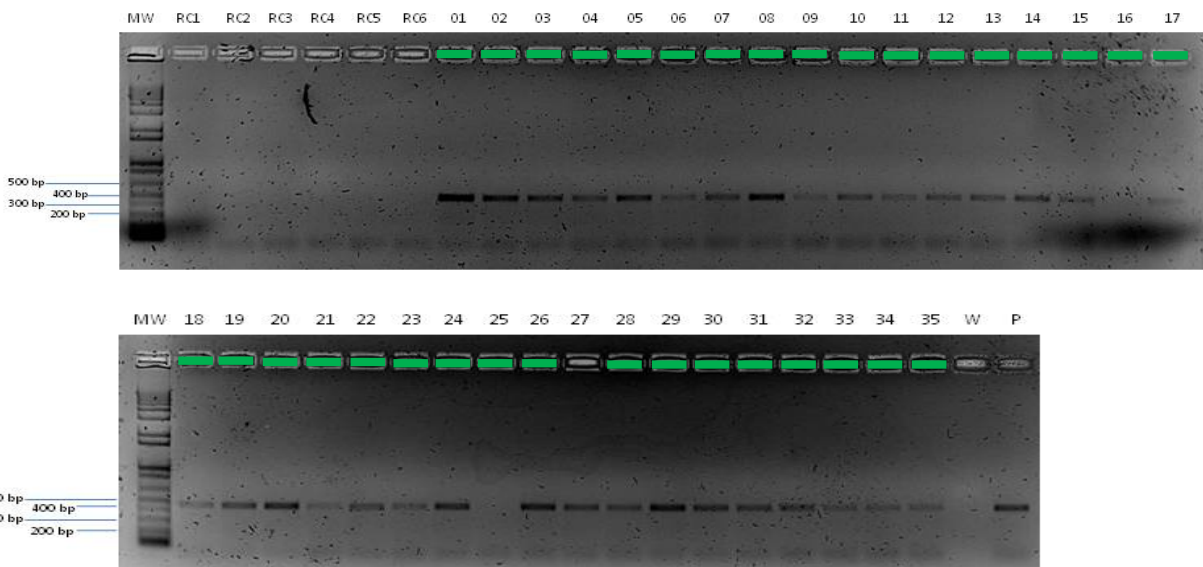
Husk expression



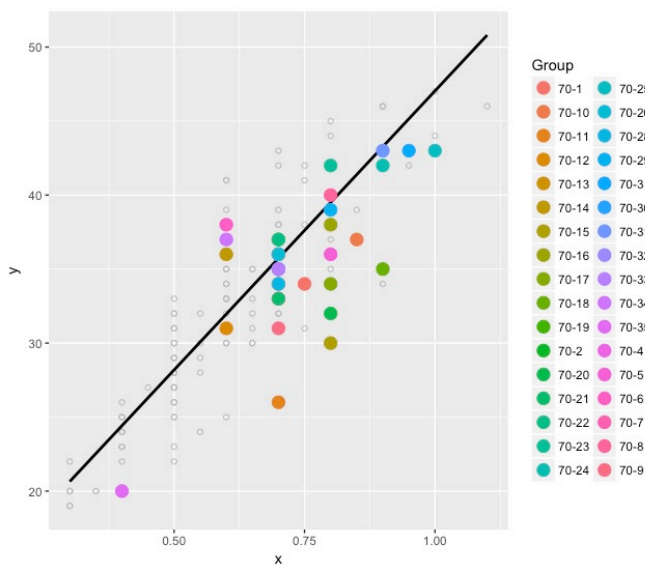
Construct: ZmUBI_{pro}:cDNA (ID# 929)

Thirty four independent T0 lines:
IR64-IRS929-001-026, 028-035

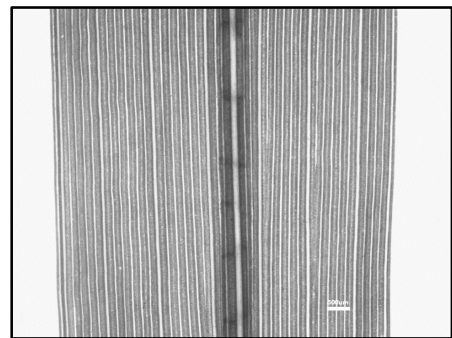
Genomic PCR



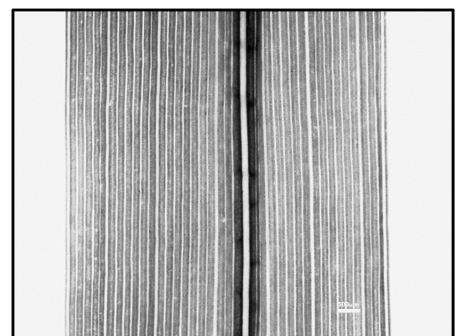
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 2 leaf

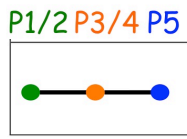


GRMZM2G133716

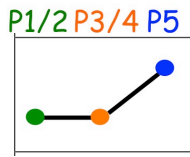
Forkhead domain

Gene ID: JL71

Foliar expression



Husk expression

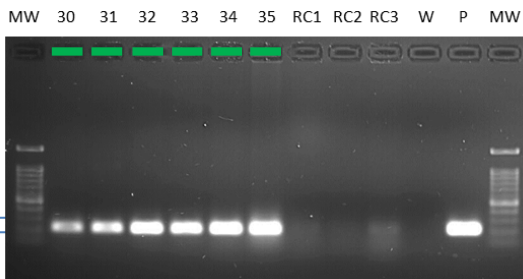
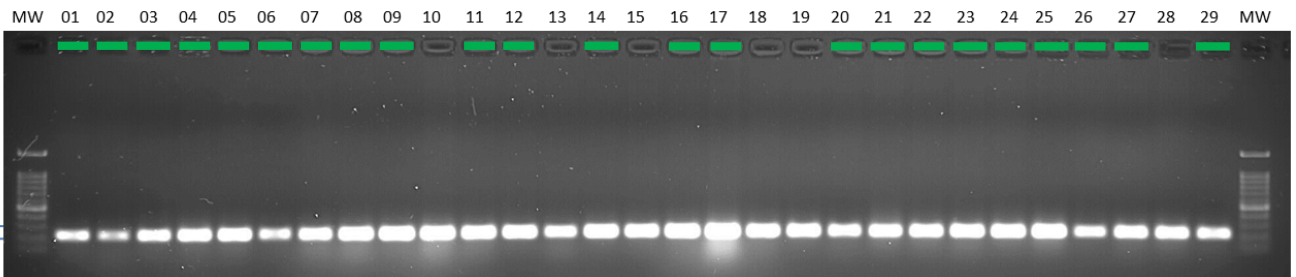


Construct: ZmUBI_{pro}:cDNA (ID# 986)

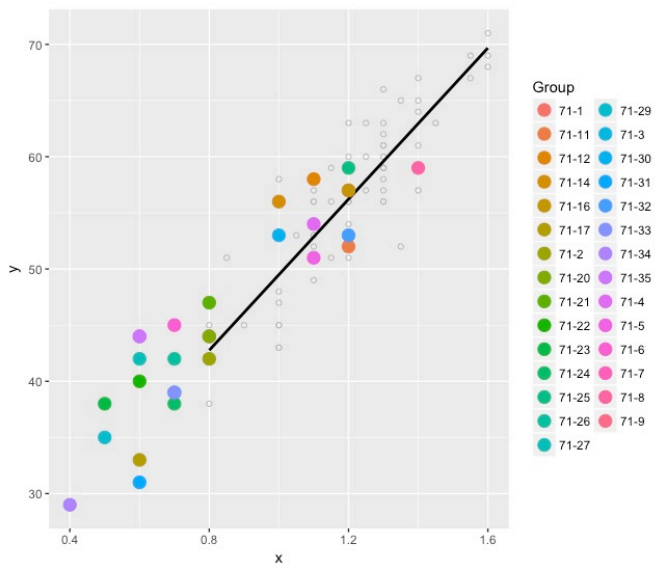
Twenty nine independent T0 lines:

IR64-IRS986-001-009,011,012,014,016,017,020-027,029,030,031-035

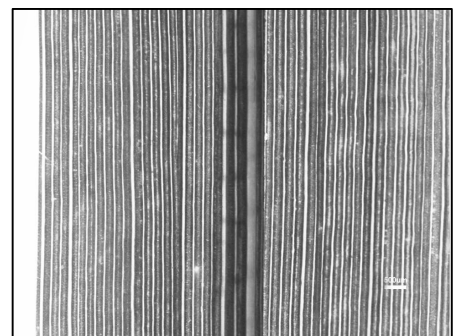
Genomic PCR



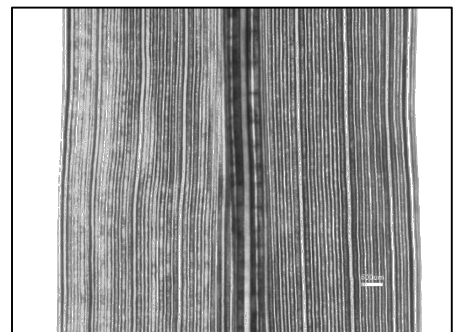
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 21 leaf



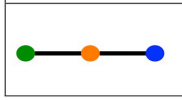
GRMZM2G469304

Ternary complex factor

Gene ID: JL72

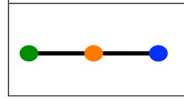
Foliar expression

P1/2 P3/4 P5



Husk expression

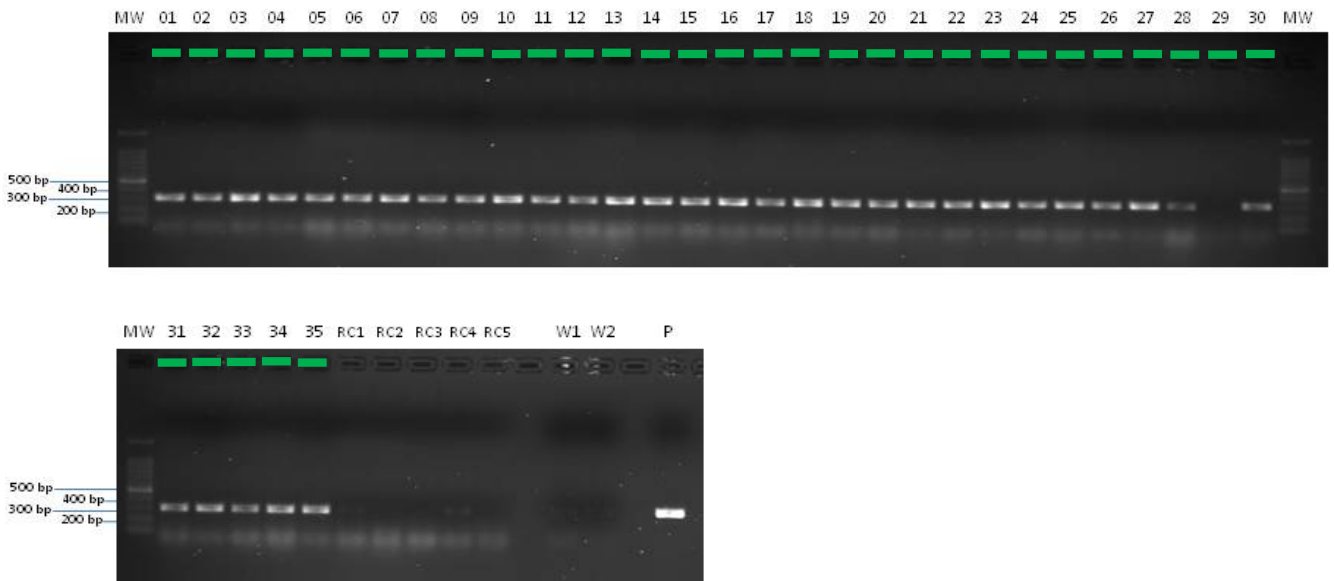
P1/2 P3/4 P5



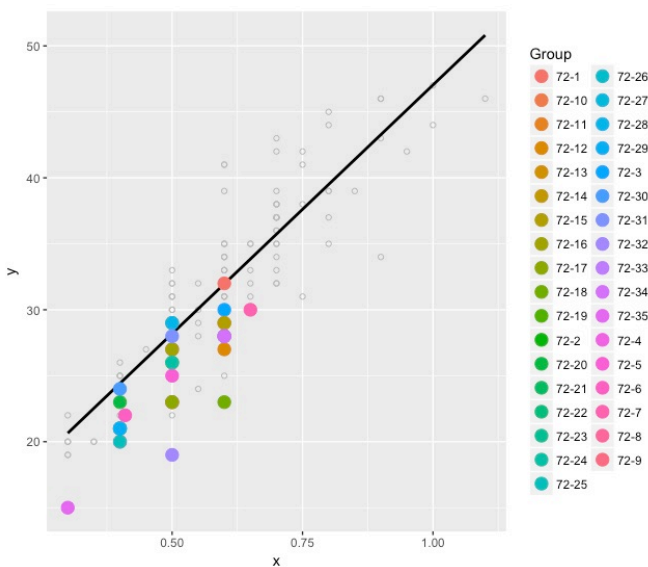
Construct: ZmUBI_{pro}:cDNA (ID# 1008)

Thirty five independent T0 lines:
IR64-IRS1008-001-035

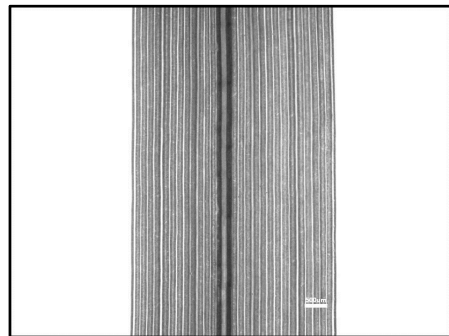
Genomic PCR



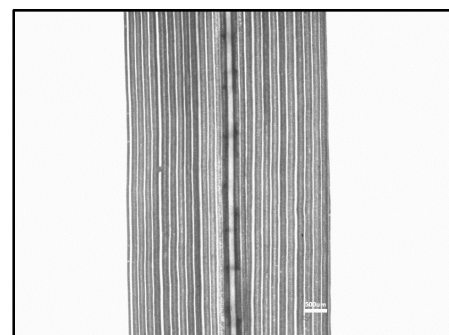
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 5 leaf



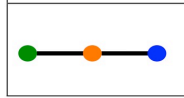
GRMZM5G850129

Growth regulatory factor

Gene ID: JL75

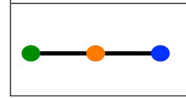
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

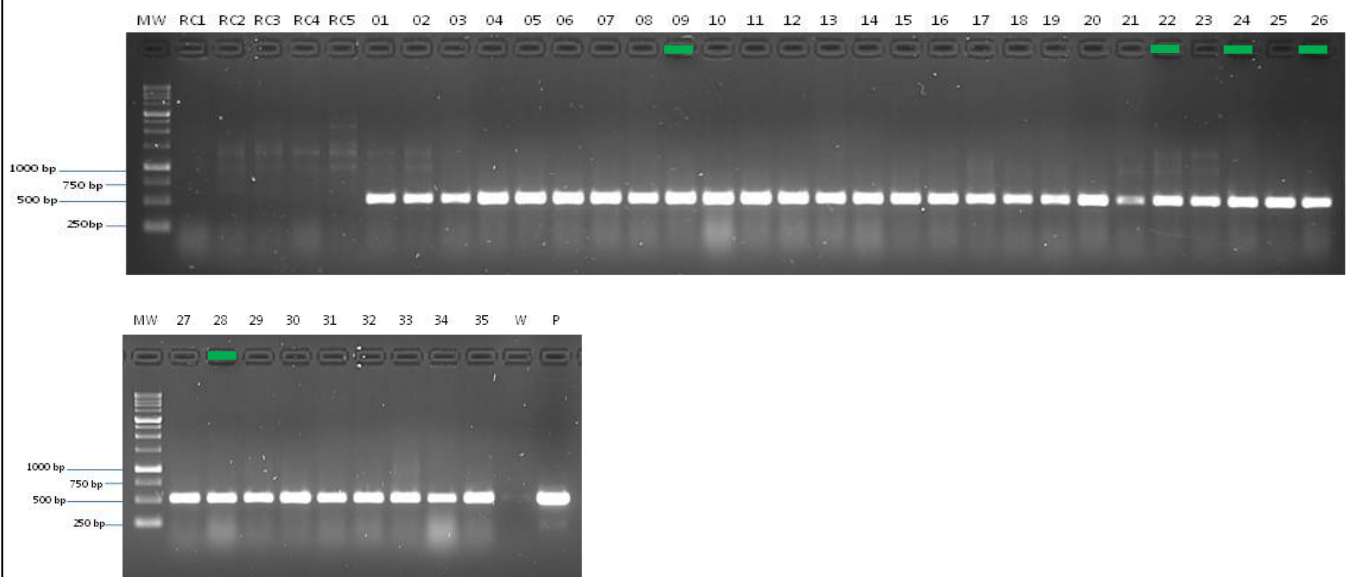


Construct: ZmUBI_{pro}:cDNA (ID# 948)

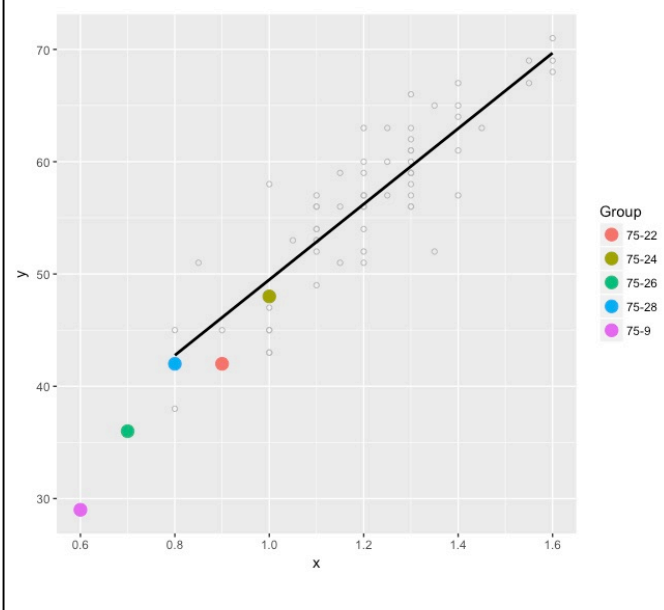
Five independent T0 lines:

IR64-IRS948-009,022,024,026,028

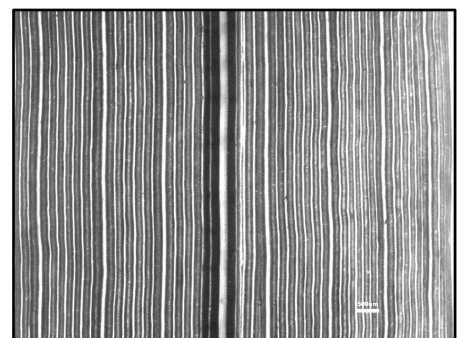
Genomic PCR



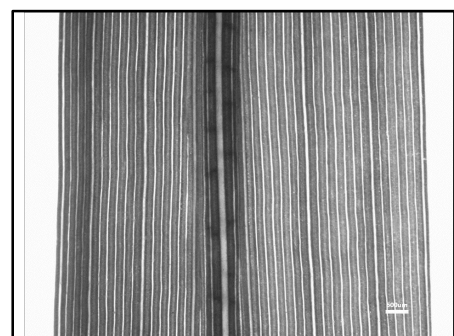
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 22 leaf



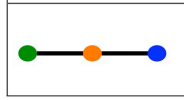
GRMZM2G061734

SBP domain TF

Gene ID: JL76

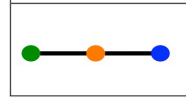
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

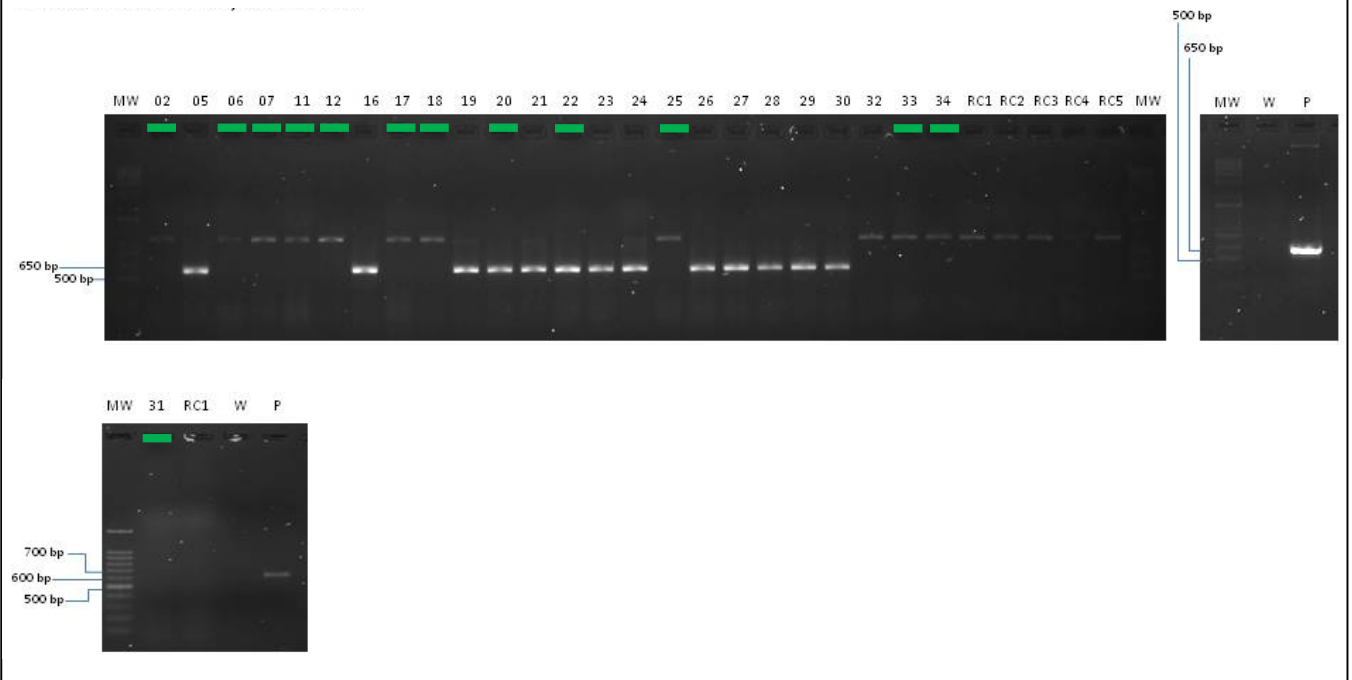


Construct: ZmUBI_{pro}:cDNA (ID# 960)

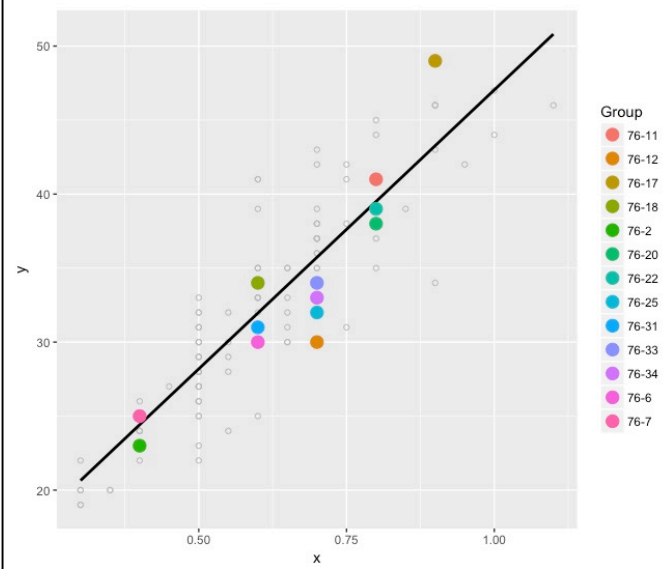
Thirteen independent T0 lines:

IR64-IRS960-002,006,007,011,012,017,018,020,022,025,031,033,034

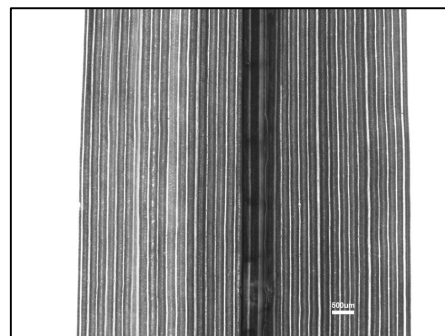
Genomic PCR



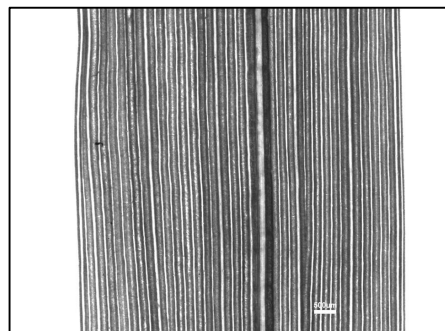
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 34 leaf



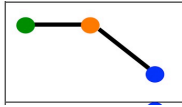
GRMZM2G318592

ZnF C2H2

Gene ID: JL77

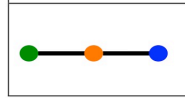
Foliar expression

P1/2 P3/4 P5



Husk expression

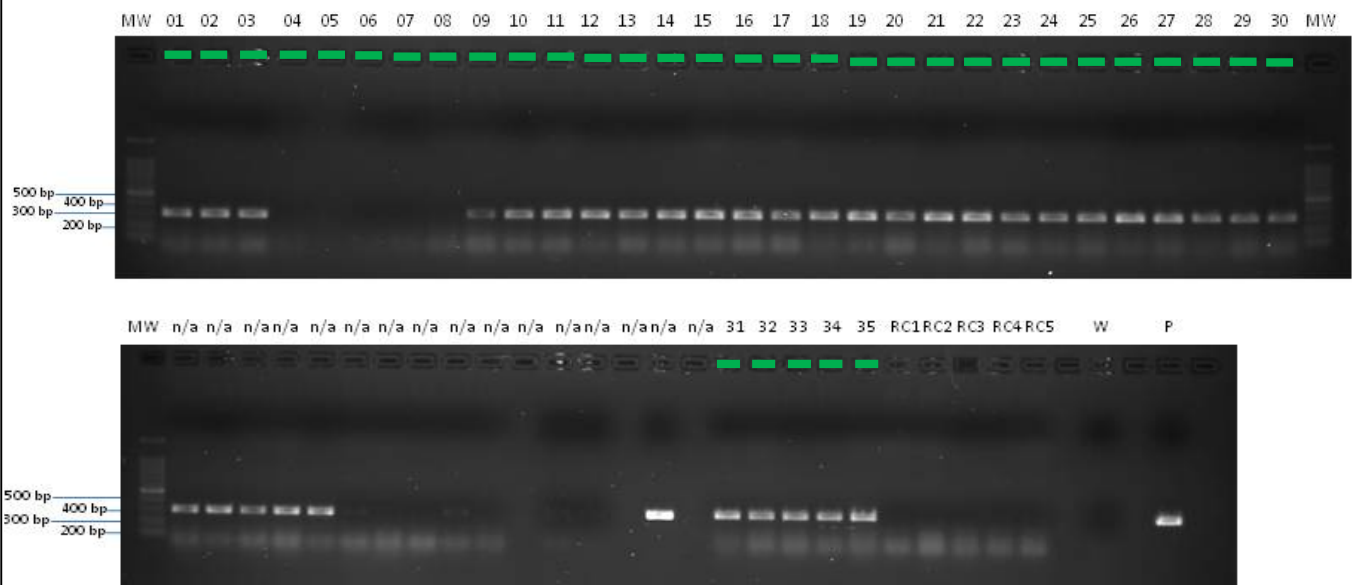
P1/2 P3/4 P5



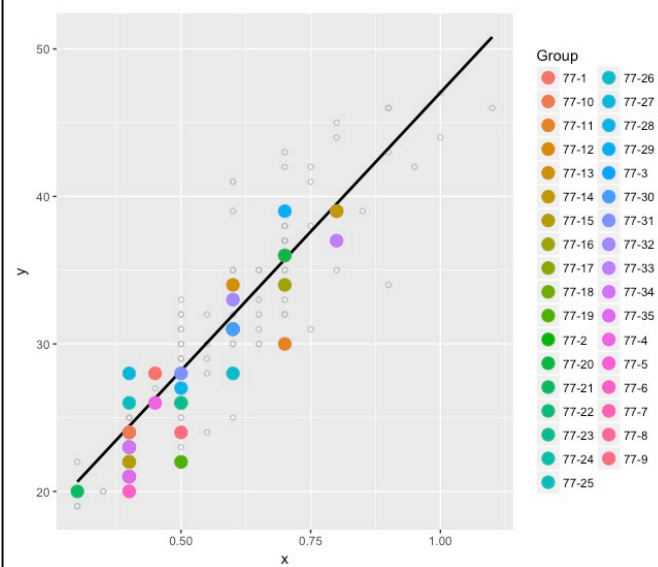
Construct: ZmUBI_{pro}:cDNA (ID# 1009)

Thirty five independent T0 lines:
IR64-IRS1009-001-035

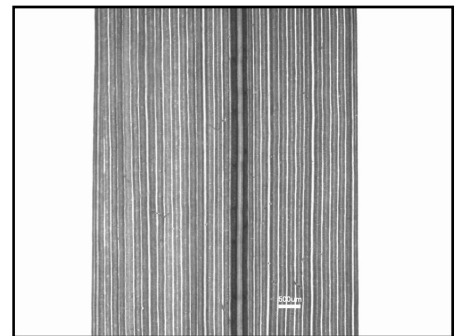
Genomic PCR



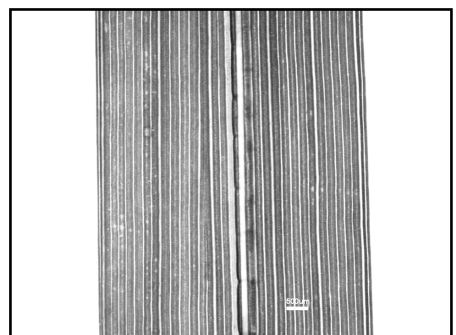
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 3 leaf



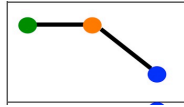
GRMZM2G095899

bHLH

Gene ID: JL78

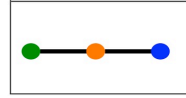
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

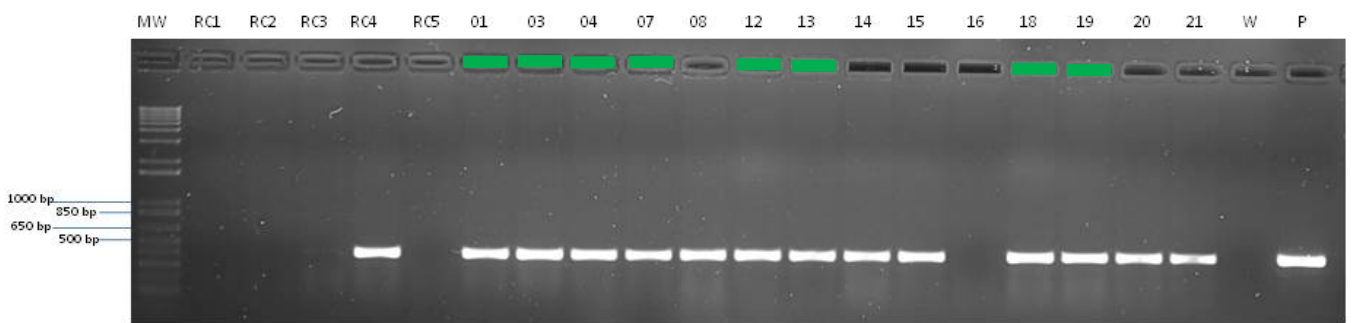


Construct: ZmUBI_{pro}:cDNA (ID# 942)

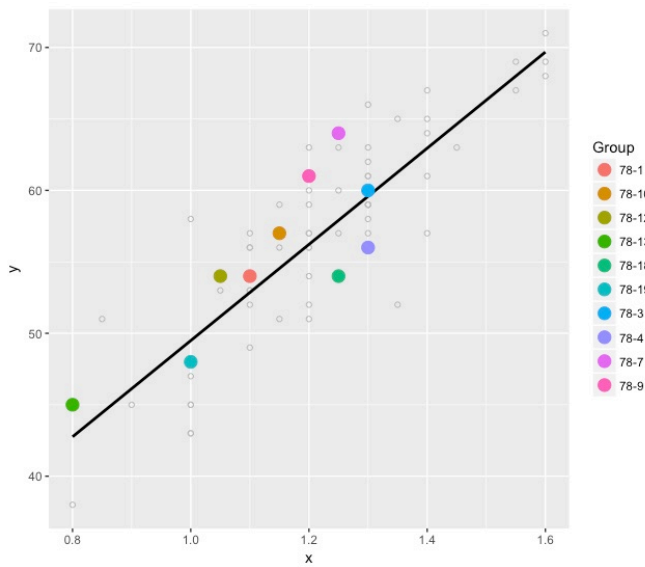
Ten independent T0 lines:

IR64-IRS942-001,003,004,007,009,010,012,013,018,019

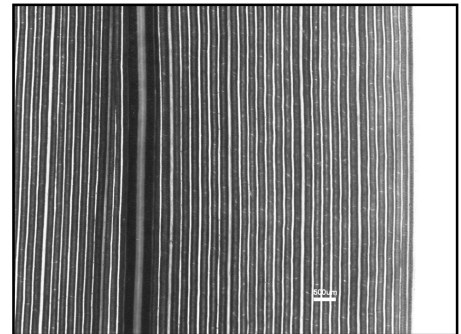
Genomic PCR



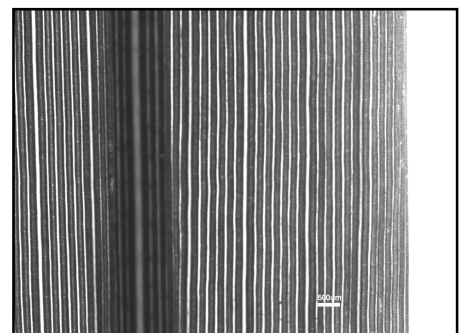
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 10 leaf



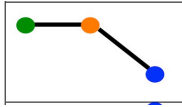
GRMZM2G015666

bHLH

Gene ID: JL79

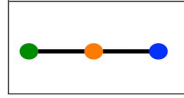
Foliar expression

P1/2 P3/4 P5



Husk expression

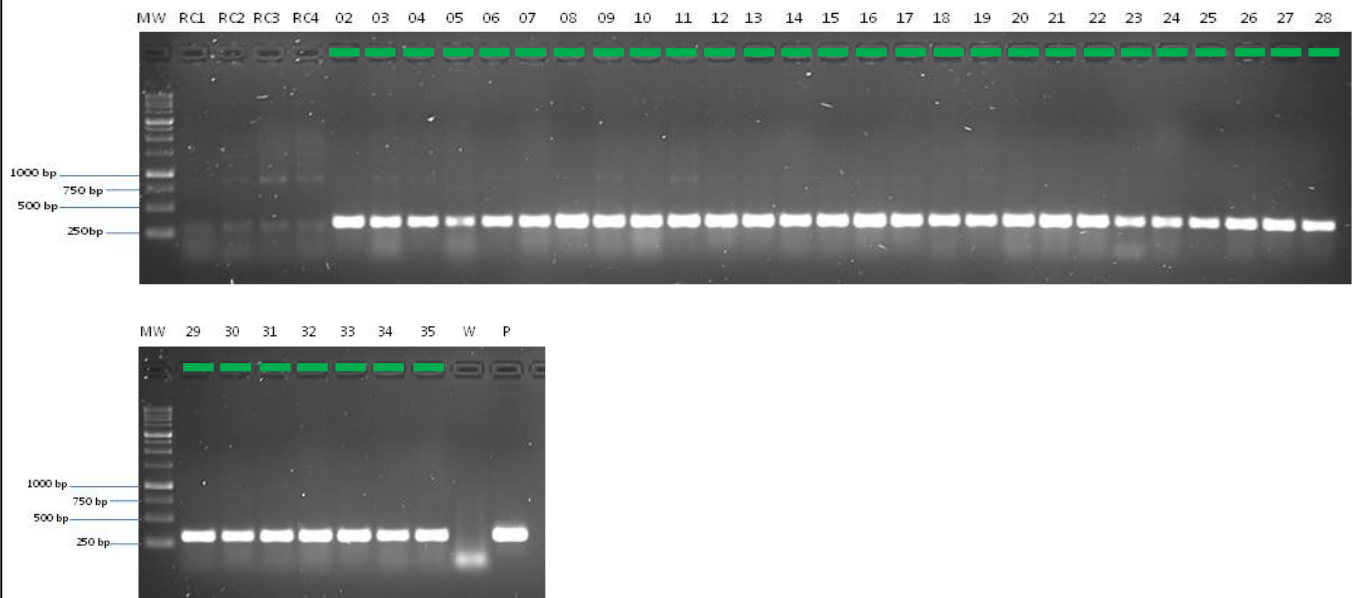
P1/2 P3/4 P5



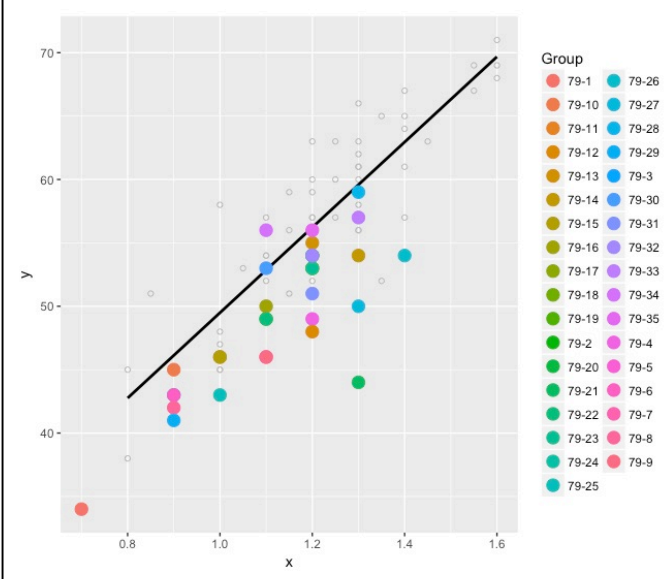
Construct: ZmUBI_{pro}:cDNA (ID# 946)

Thirty five independent T0 lines:
IR64-IRS946-001-035

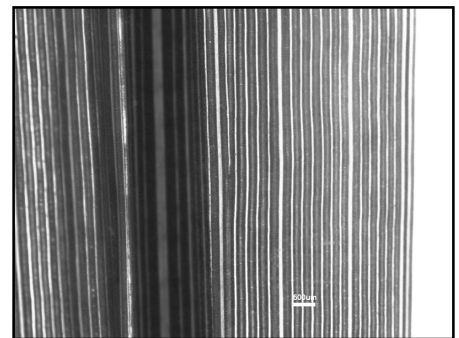
Genomic PCR



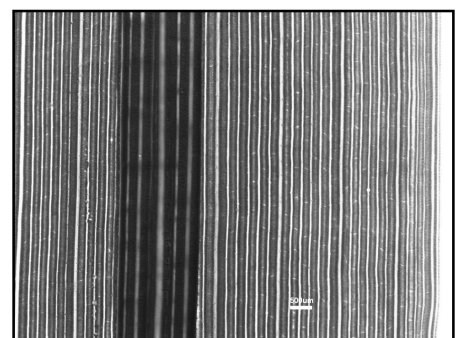
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 2 leaf



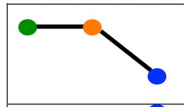
GRMZM2G126018

SBP domain TF

Gene ID: JL80

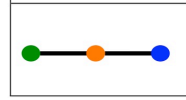
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

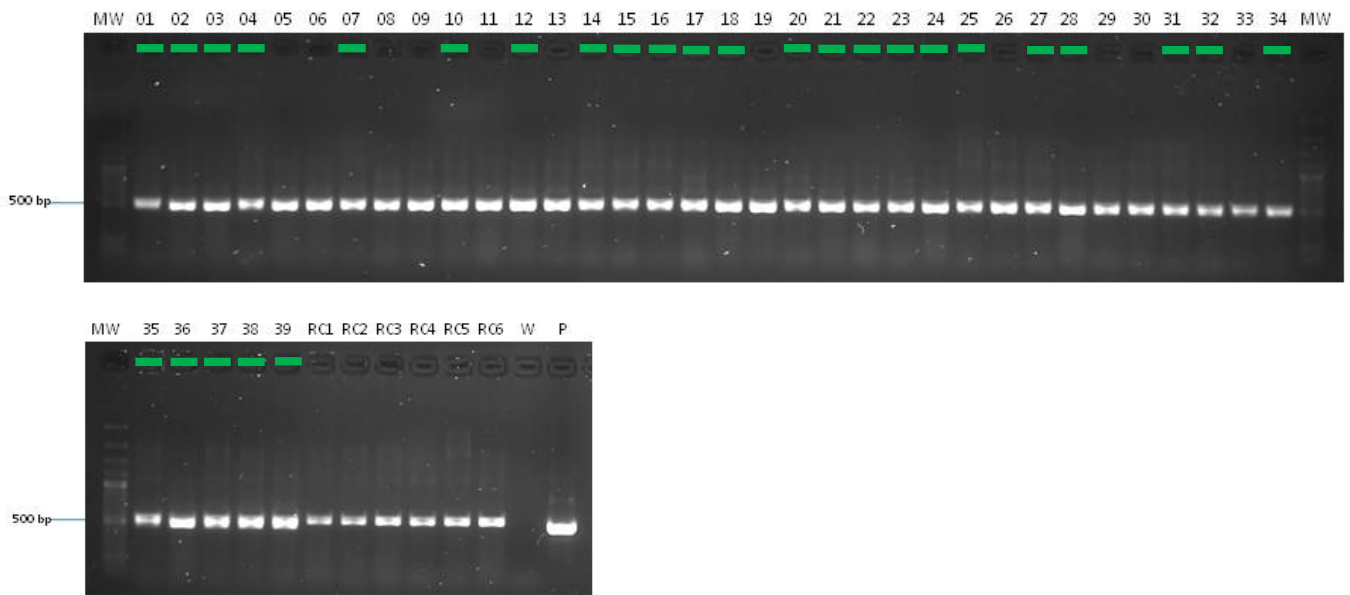


Construct: ZmUBI_{pro}:cDNA (ID# 954)

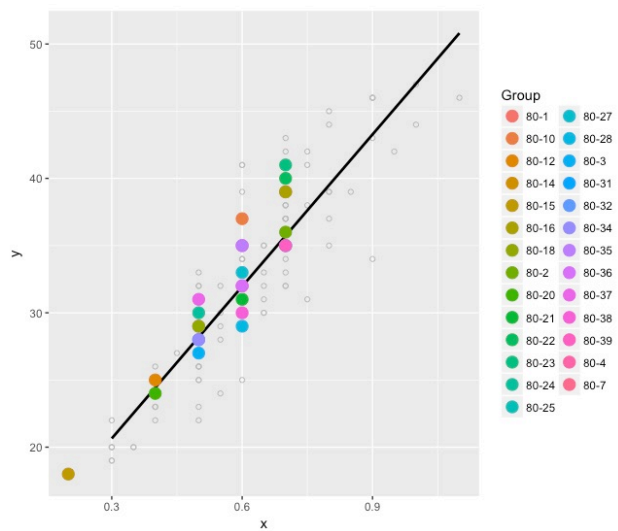
Twenty seven independent T0 lines:

IR64-IRS954-001-004,007,010,012,014-018,020-025,027,028,031,032,034-039

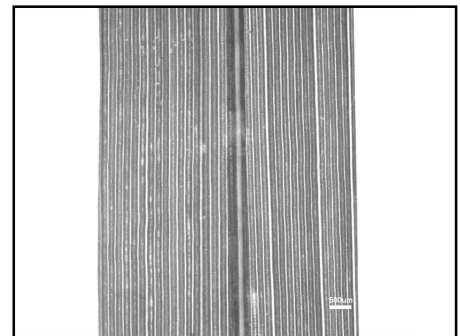
Genomic PCR



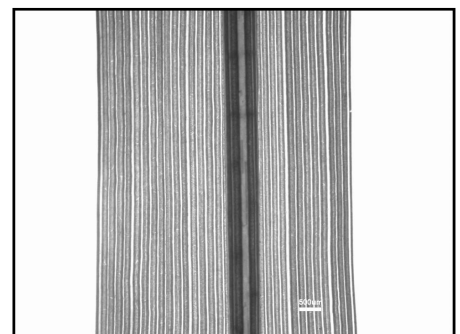
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 21 leaf

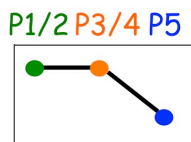


GRMZM2G312419

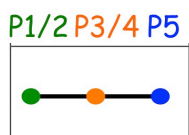
R2R3 Myb

Gene ID: JL81

Foliar expression



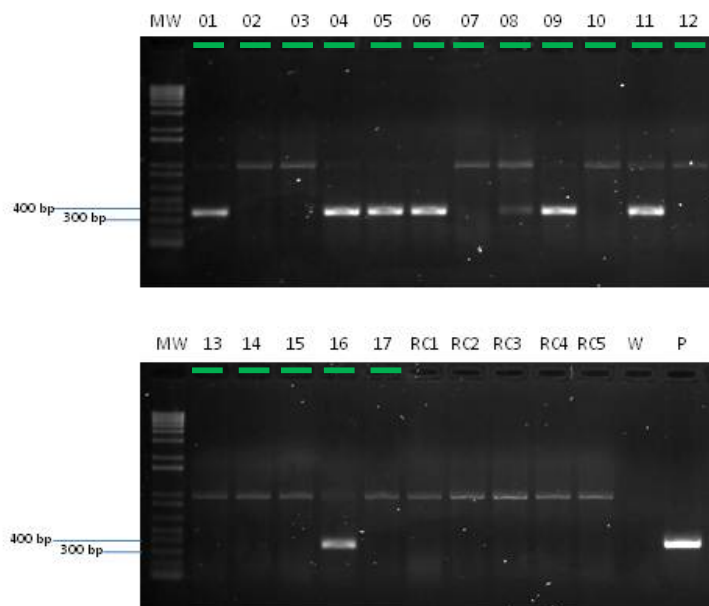
Husk expression



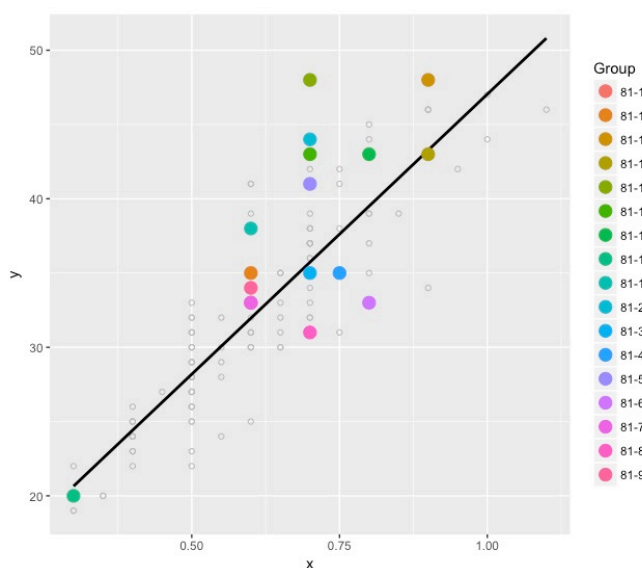
Construct: ZmUBI_{pro}:cDNA (ID# 961)

Seventeen independent T0 lines:
IR64-IRS961-001-017

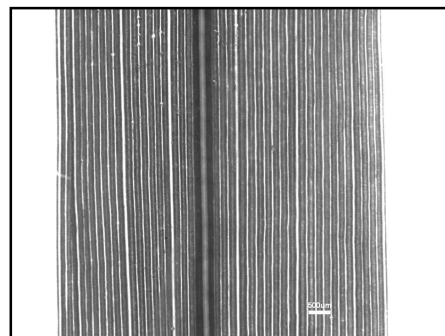
Genomic PCR



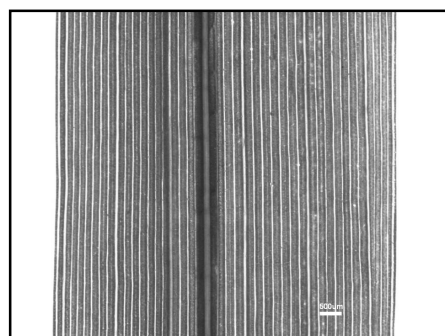
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 5 leaf



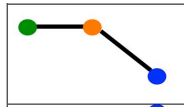
GRMZM2G478876

Serine threonine kinase

Gene ID: JL82

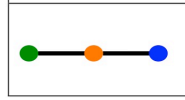
Foliar expression

P1/2 P3/4 P5



Husk expression

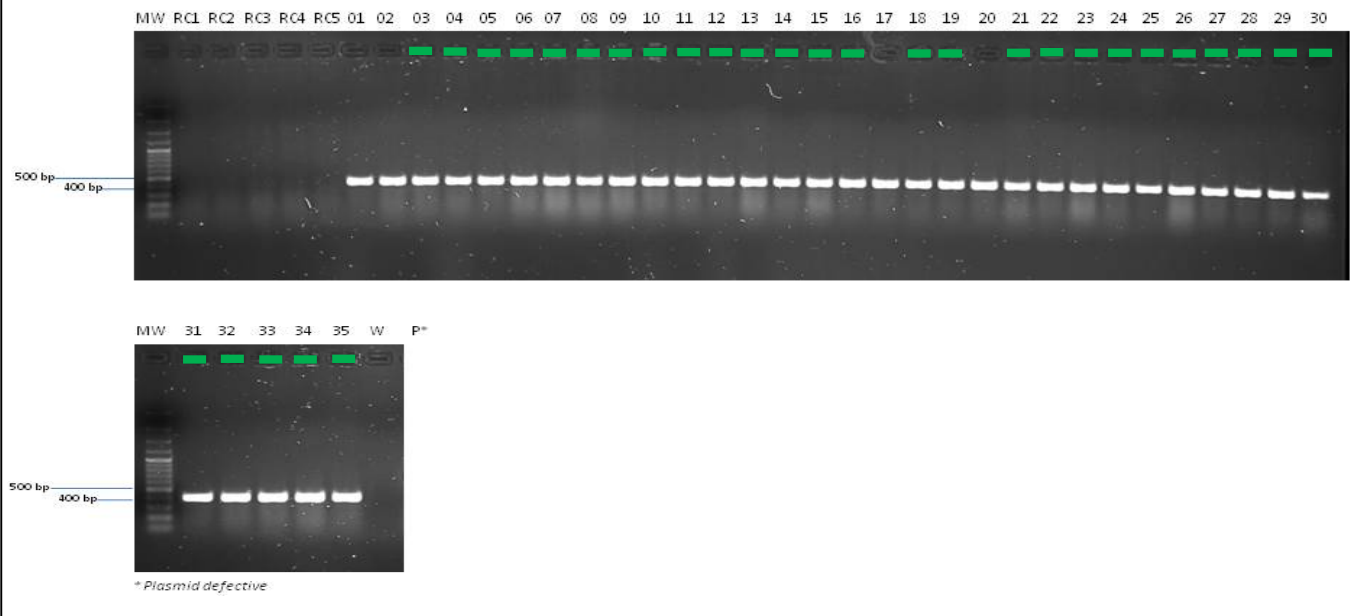
P1/2 P3/4 P5



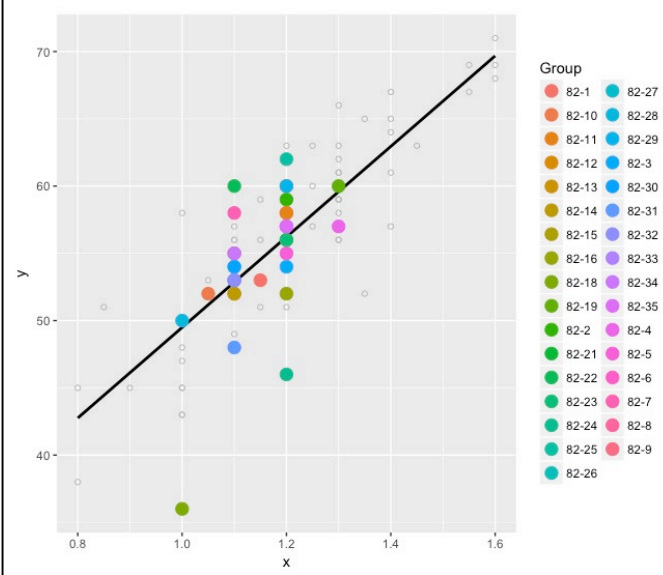
Construct: ZmUBI_{pro}:cDNA (ID# 940)

Thirty one independent T0 lines:
IR64-IRS940-003-016,018,019,021-035

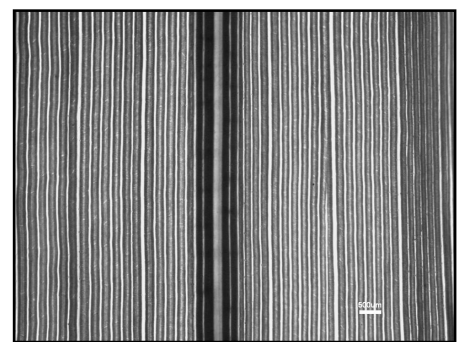
Genomic PCR



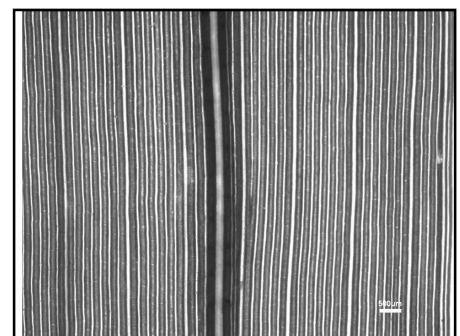
Number veins versus leaf width



Representative surface image of segregating WT leaf



Representative surface image of line 21 leaf



Information S3. Individual datasheets for T1 rice lines summarized in Dataset S3. Each page shows the gene expression profile in maize foliar and husk leaf primordia as reported in ¹⁹ (green circles refer to relative expression level in samples of the shoot apical meristem plus plastochron 1/2 leaf primordia, orange circles to levels in P3/4 leaf primordia and blue circles to levels in P5 leaf primordia); construct composition and ID; number and ID of independent transgenic rice lines. Where available, cropped agarose gel images show PCR products of representative genomic PCR reactions (detecting presence of transgene) and RT-PCR reactions (detecting transgene transcripts). qRT-PCR data are shown for one construct. Polynomial (Kitaake lines) or linear (IR64 lines) regression plots illustrate number of veins versus leaf width in each line. Representative leaf samples (either hand cut transverse leaf sections viewed by fluorescence microscopy or views of the adaxial leaf surface taken using a hand-held microscope) are shown in each case. Fluorescence images are visualized through autofluorescence of chlorophyll (red) and lignin (blue).

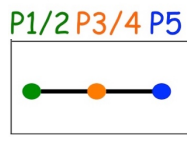
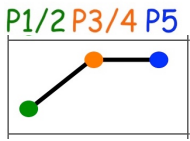
GRMZM2G028046

MYB-interacting protein (ZmMIL1)

Gene ID: JL2

Foliar expression

Husk expression

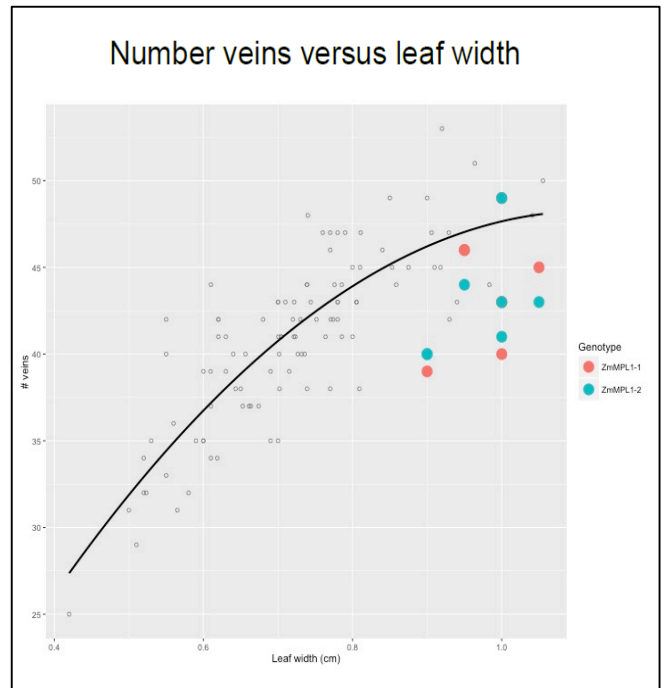
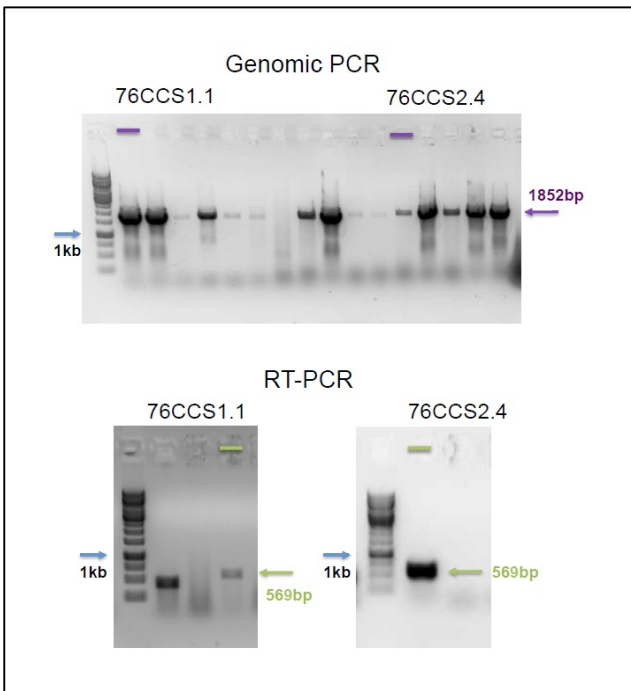


Construct: ZmUBI_{pro}:cDNA (ID# 76CC)

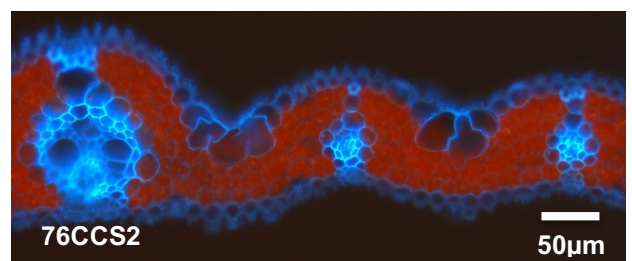
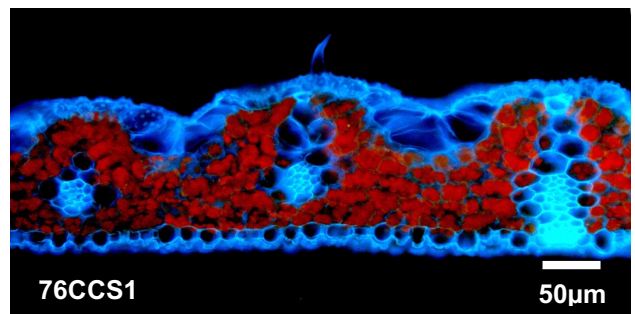
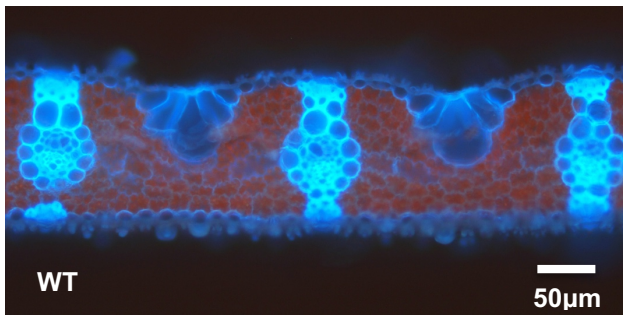
Two independent T1 lines:

ZmMIL1-1 (76CCS1 – 8 individuals)

ZmMIL1-2 (76CCS2 – 7 individuals)



Representative leaf cross sections:



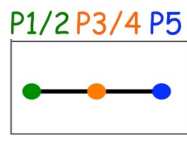
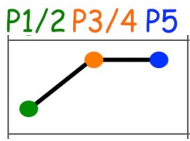
GRMZM2G136494

MYB-interacting protein (ZmMIL2)

Gene ID: JL3

Foliar expression

Husk expression



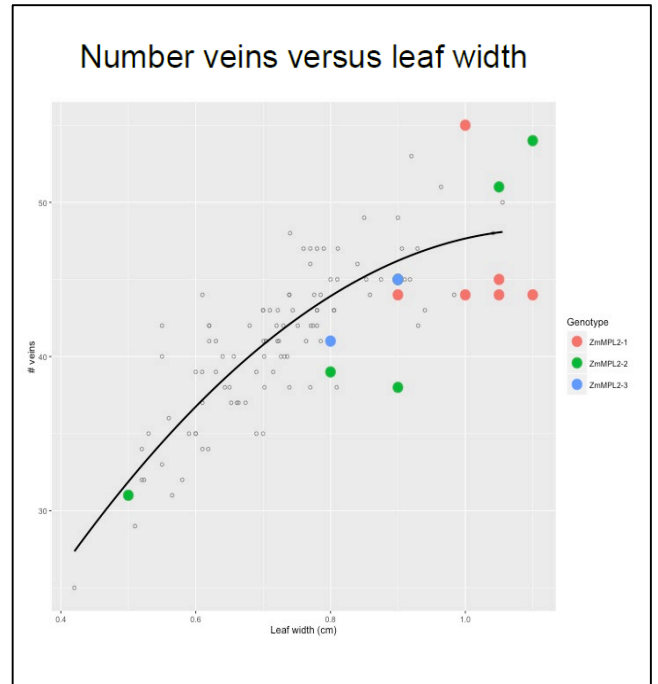
Construct: ZmUBI_{pro}:cDNA (ID# 84CC)

Three independent T1 lines:

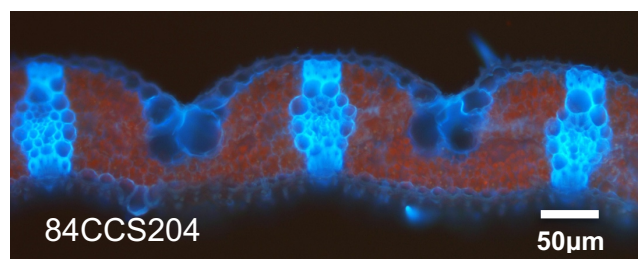
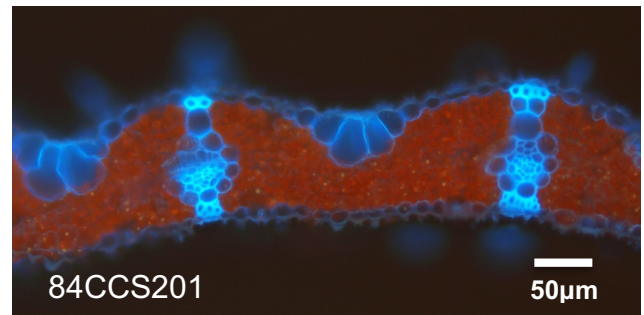
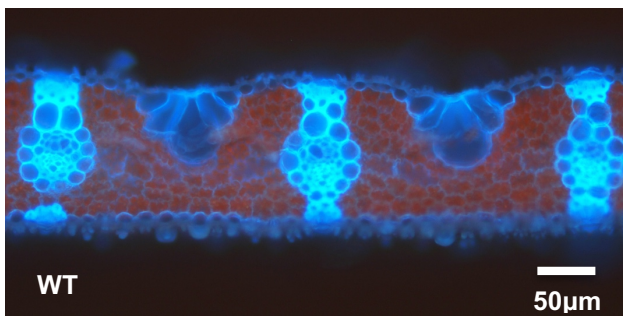
ZmMIL2-1 (84CCSR1 – 7 individuals)

ZmMIL2-2 (84CCS201 – 6 individuals)

ZmMIL2-3 (84CCS204 – 2 individuals)



Representative leaf cross sections:



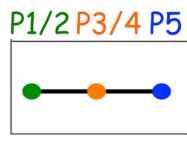
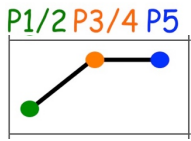
GRMZM2G045883

bHLH (ZmSPL1)

Gene ID: JL4

Foliar expression

Husk expression

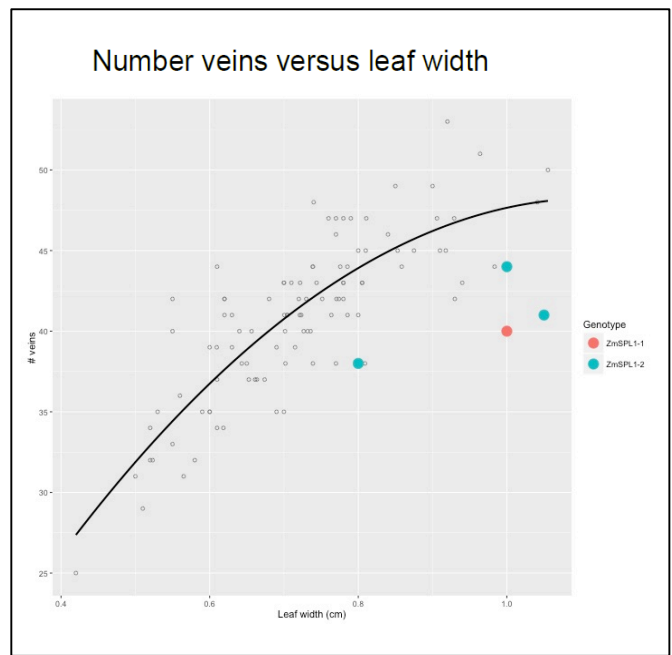
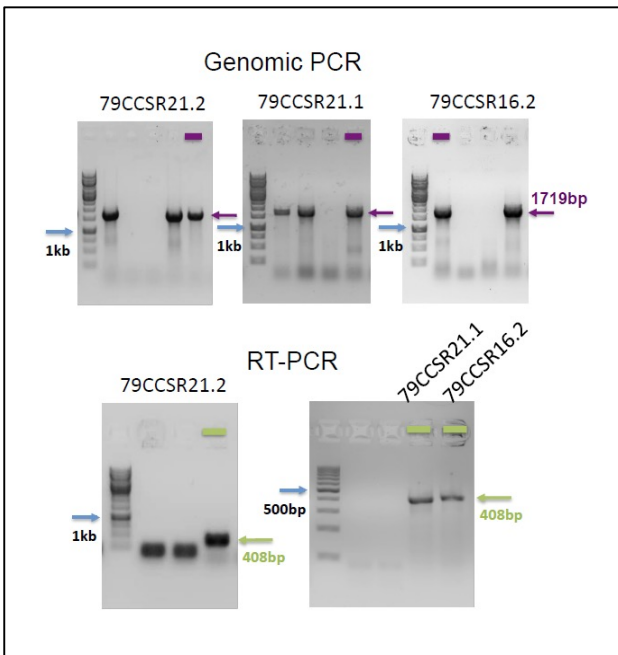


Construct: ZmUBI_{pro}:cDNA (ID# 79CC)

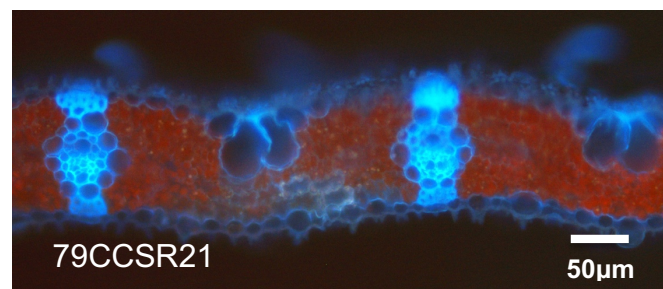
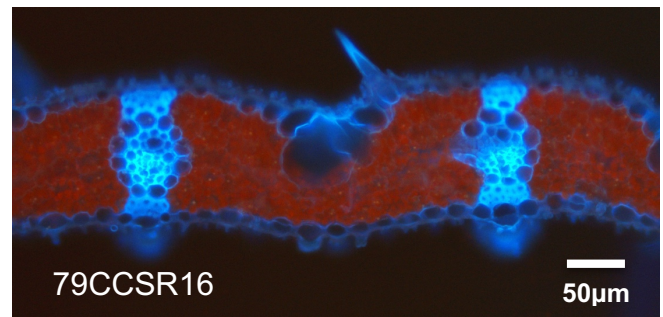
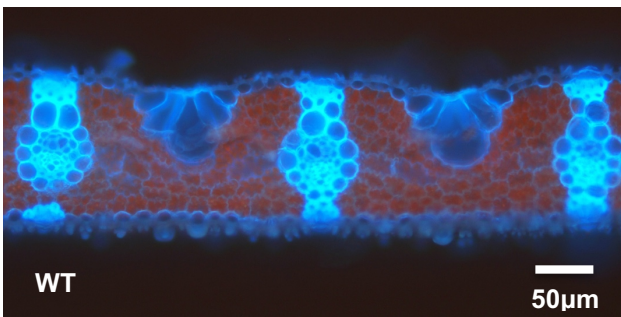
Two independent T1 lines:

ZmSPL1-1 (79CCSR16 – 1 individual)

ZmSPL1-2 (79CCSR21 – 3 individuals)



Representative leaf cross sections:



GRMZM2G131516

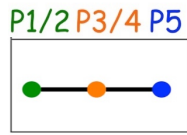
GRAS (ZmSCR1)

Gene ID: JL8

Foliar expression

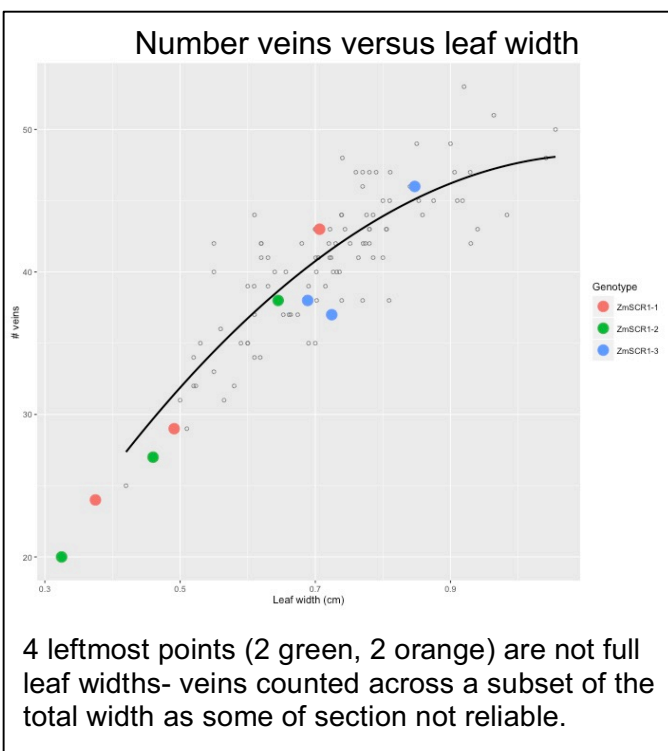
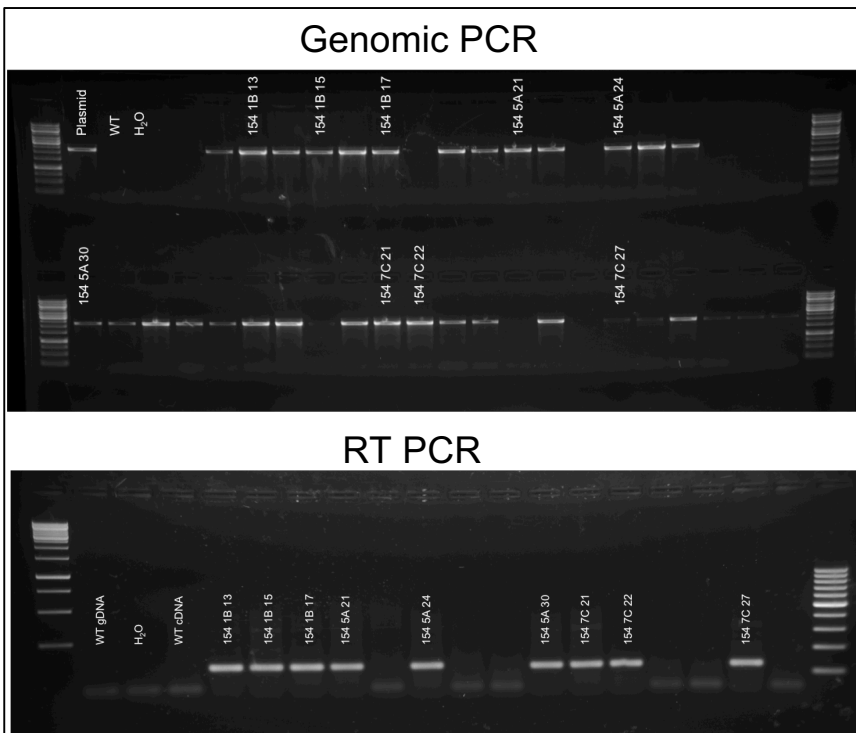


Husk expression

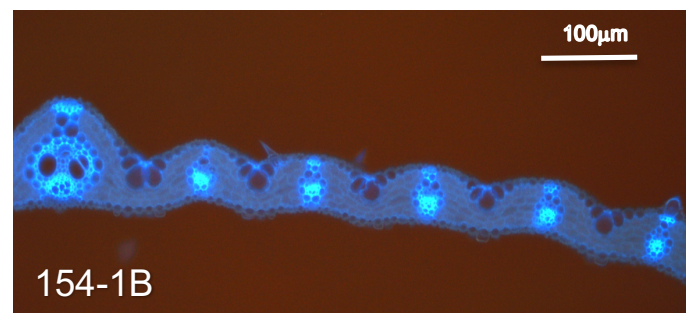
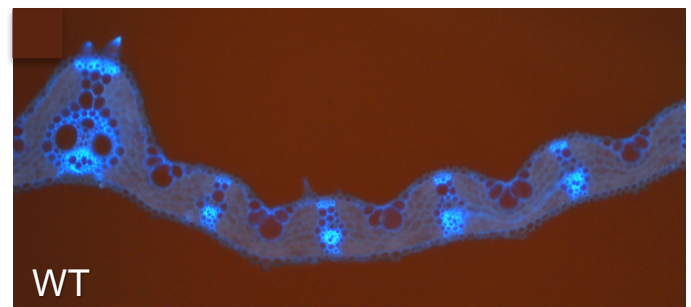


Construct: ZmUBI_{pro}:cDNA
(rice codon optimised) (ID# MS154)

Three independent T1 lines:
ZmSCR1-1 (1B – 3 individuals)
ZmSCR1-2 (5A – 3 individuals)
ZmSCR1-3 (7C – 3 individuals)



Representative leaf cross sections:



AC215201.3_FG008

bHLH

Gene ID: JL13

Foliar expression

Husk expression

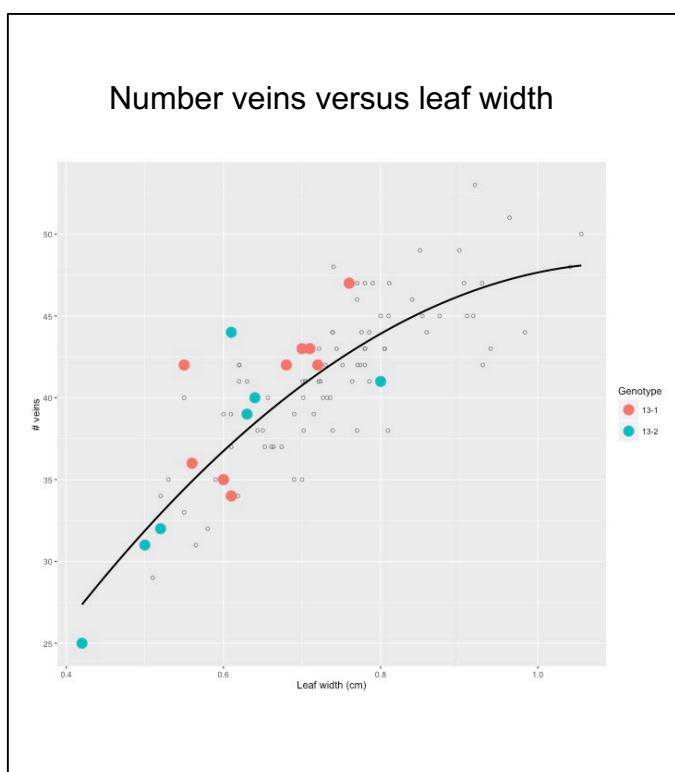
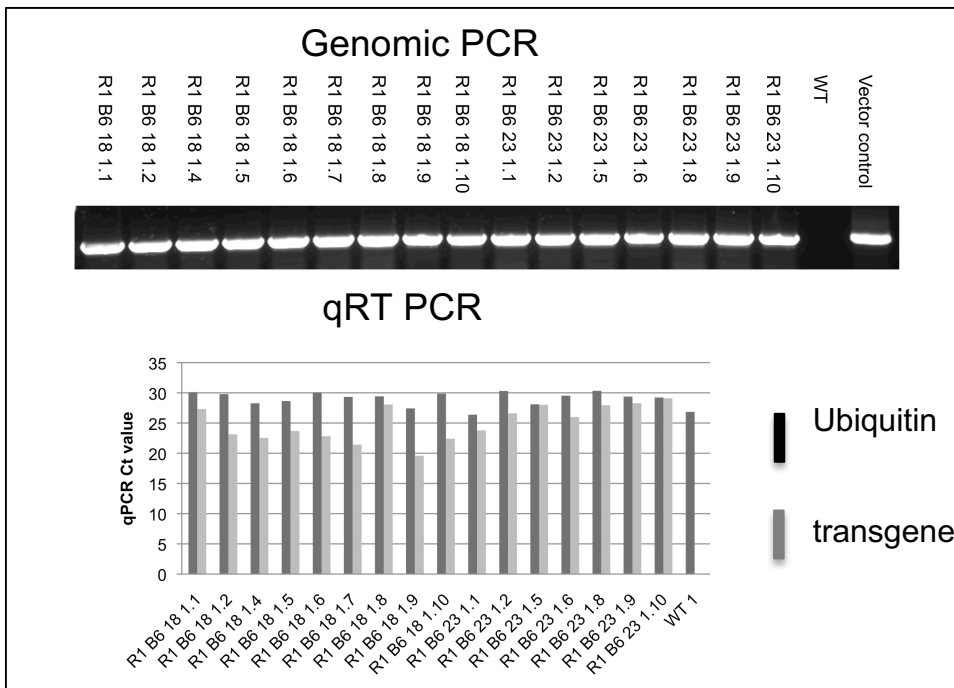


Construct: ZmUBI_{pro}:cDNA (ID# R1B6)

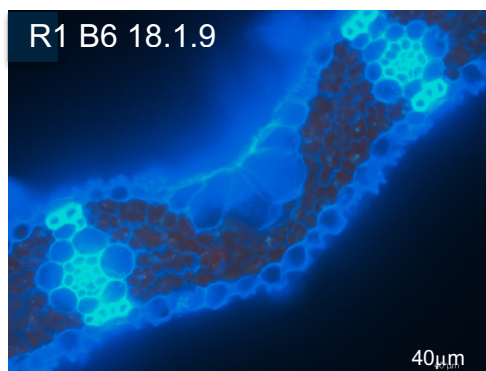
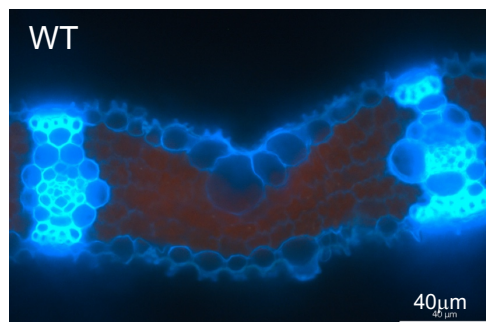
Two independent T1 lines:

13-1 (R1B618.1 – 10 individuals)

13-2 (R1B623.1 – 10 individuals)



Representative leaf cross sections:

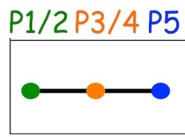


GRMZM2G016477

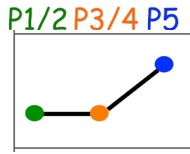
LRR kinase

Gene ID: JL15

Foliar expression



Husk expression



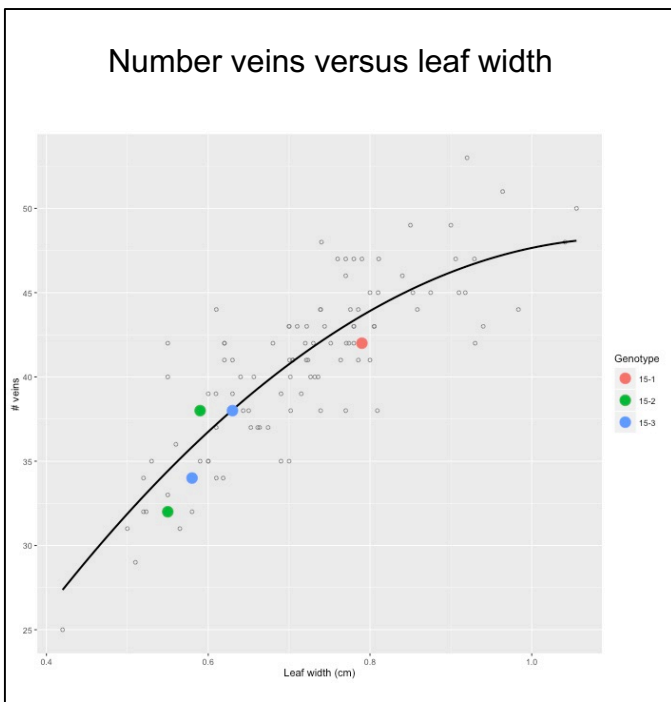
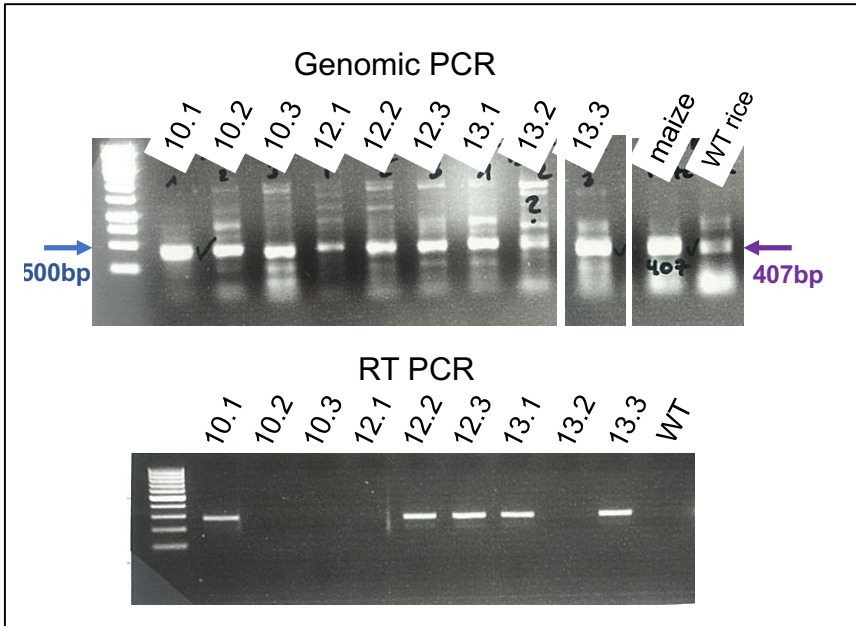
Construct: ZmUBI_{pro}:cDNA (ID# MS80)

Three independent T2 lines:

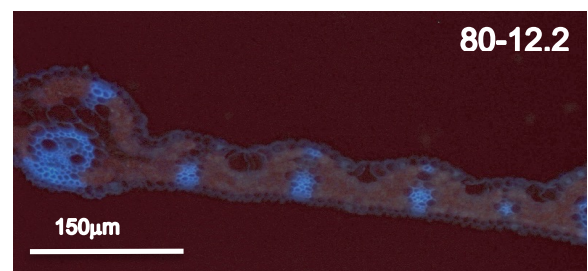
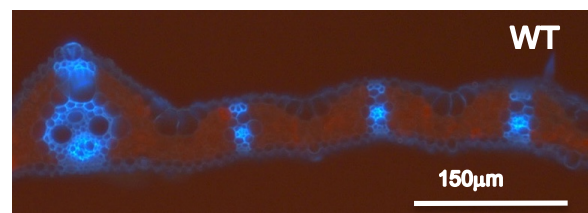
15-1 (MS80-10 – 1 individual)

15-2 (MS80-12 – 2 individuals)

15-3 (MS80-13 – 2 individuals)



Representative leaf cross sections:



GRMZM2G119359

Growth Regulating Factor

Gene ID: JL26

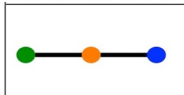
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

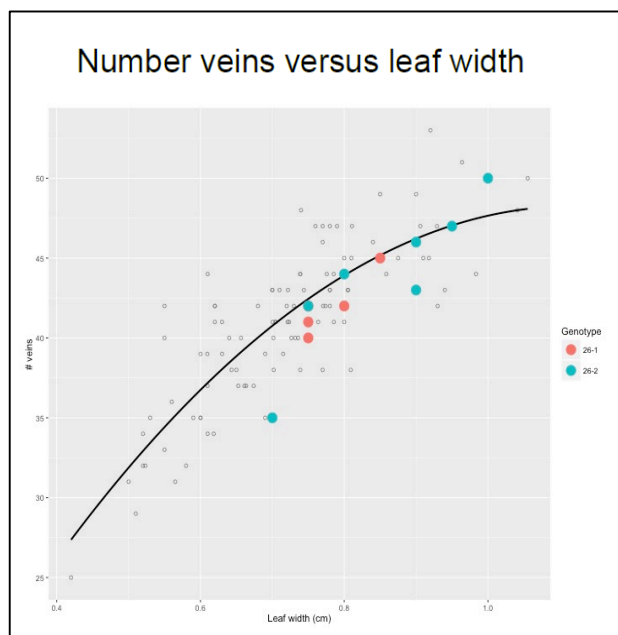


Construct: ZmUBI_{pro}:cDNA (ID# 70CC)

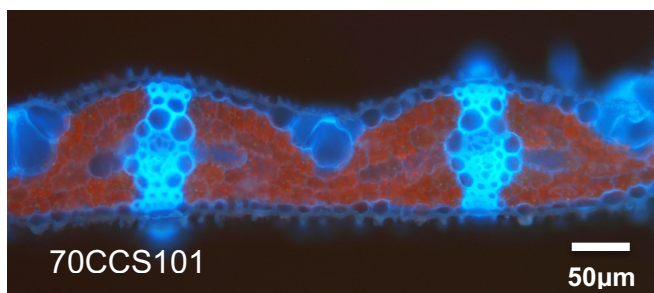
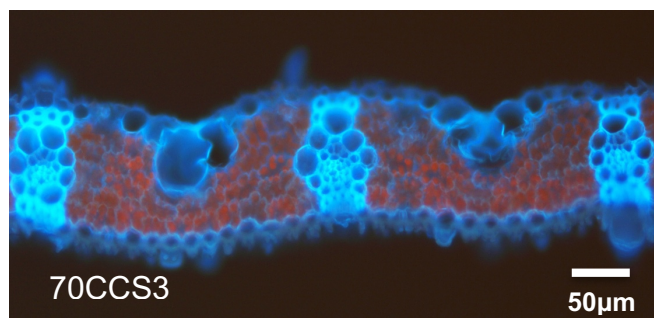
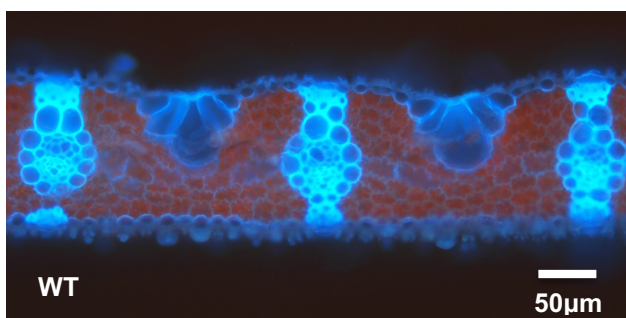
Two independent T2 lines:

26-1 (70CCS3 – 5 individuals)

26-2 (70CCS101 – 7 individuals)



Representative leaf cross sections:

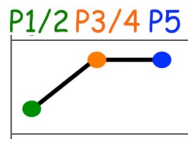


GRMZM5G893117

Growth Regulating Factor

Gene ID: JL27

Foliar expression



Husk expression



Construct: ZmUBI_{pro}:cDNA (ID# 71CC)
ZmUBI_{pro}:gDNA (ID# 71GC)

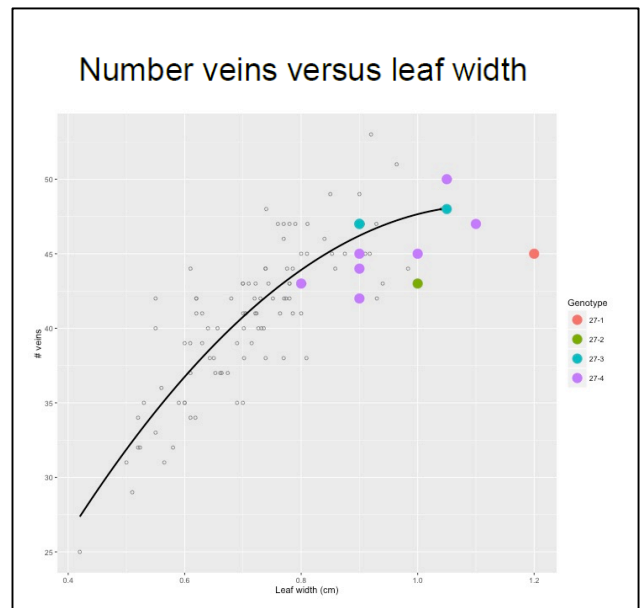
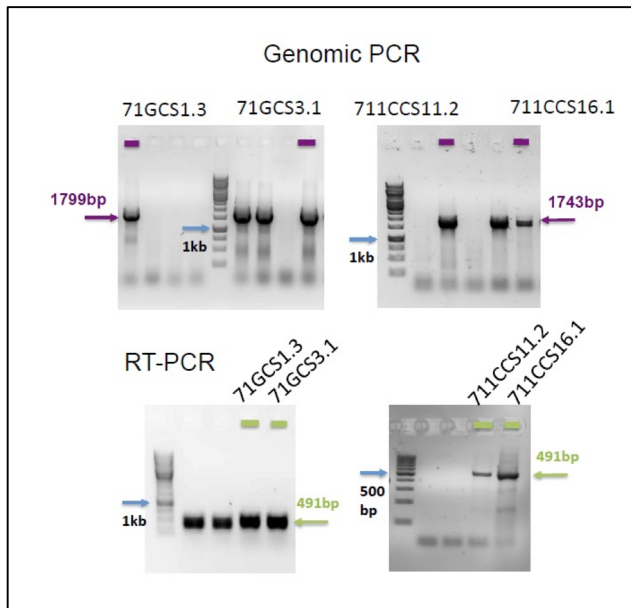
Four independent T1 lines:

27-1 (71CCS11 – 1 individual)

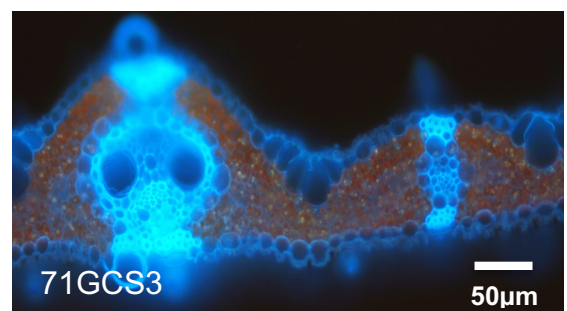
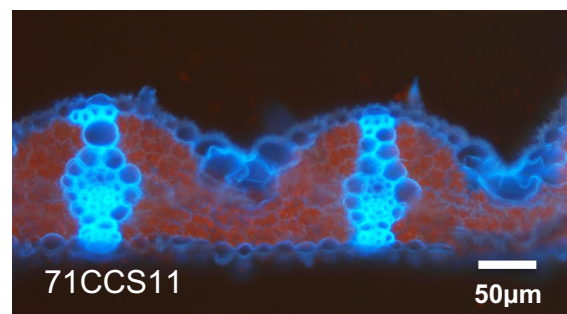
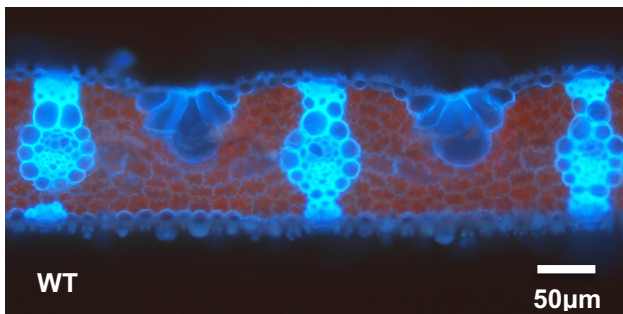
27-2 (71CCS16 – 1 individual)

27-3 (71GCS1 – 3 individuals)

27-4 (71GCS3 – 7 individuals)



Representative leaf cross sections:



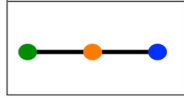
GRMZM2G178182

bHLH

Gene ID: JL29

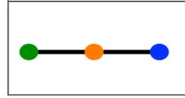
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

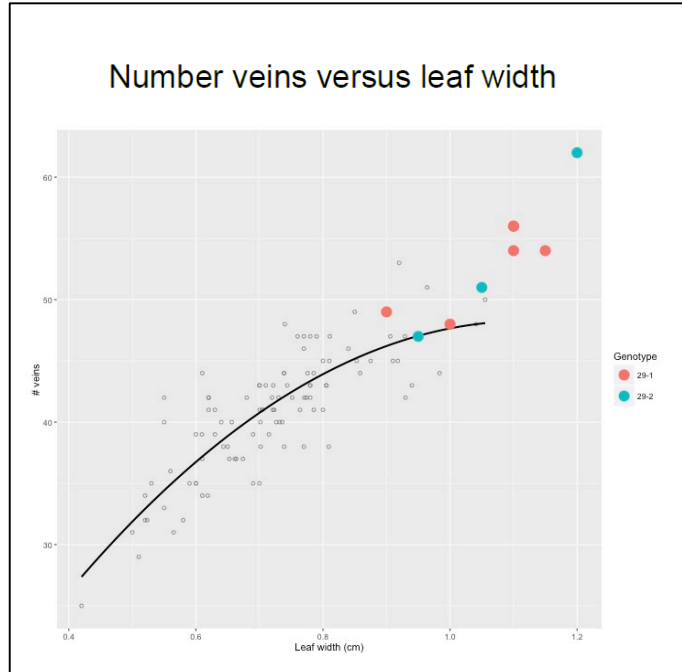
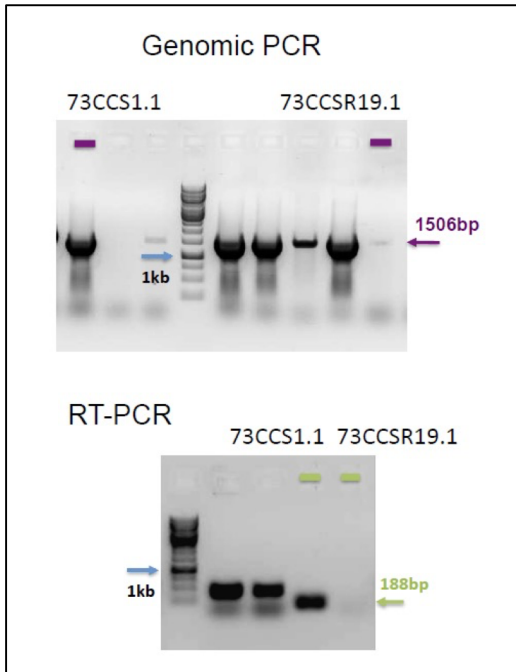


Construct: ZmUBI_{pro}:cDNA (ID# 73CC)

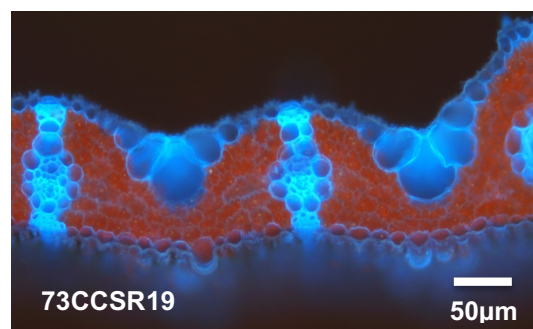
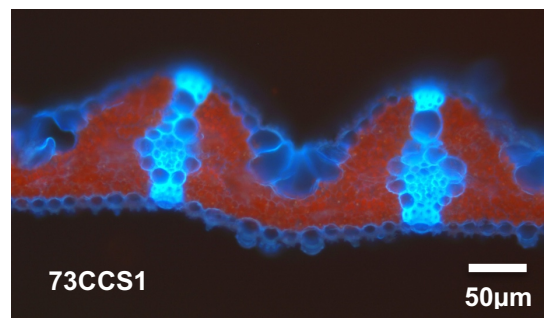
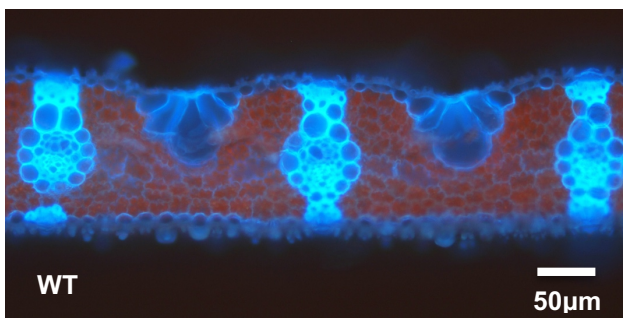
Two independent T1 lines:

29-1 (73CCS1 – 6 individuals)

29-2 (73CCSR19 – 3 individuals)



Representative leaf cross sections:



GRMZM2G140669

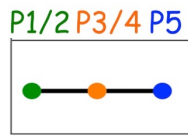
GATA ZnF

Gene ID: JL30

Foliar expression



Husk expression

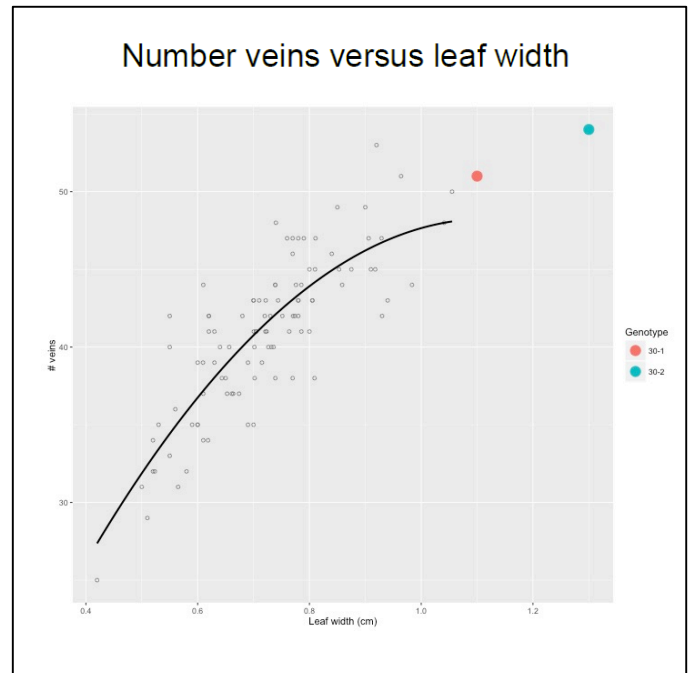


Construct: ZmUBI_{pro}:cDNA (ID# 74CC)

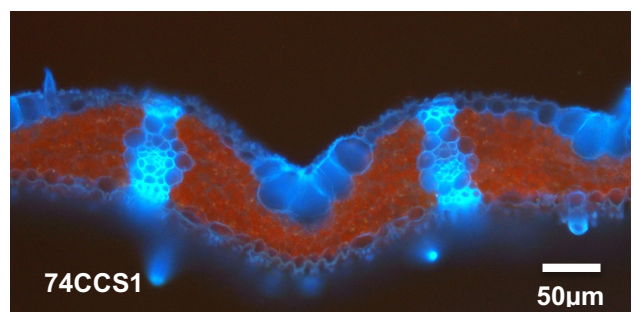
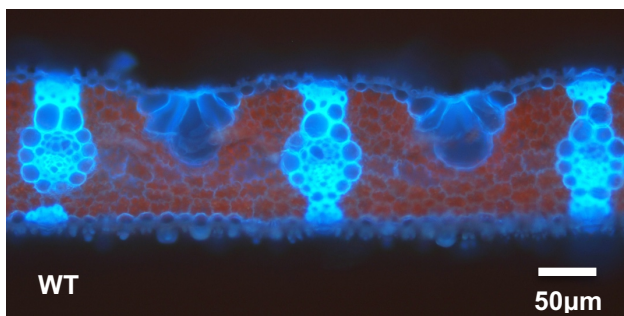
Two independent T1 lines:

30-1 (74CCS1 – 1 individual)

30-2 (74CCS10 – 1 individual)



Representative leaf cross sections:



GRMZM2G472945

TUBBY-like

Gene ID: JL31

Foliar expression



Husk expression

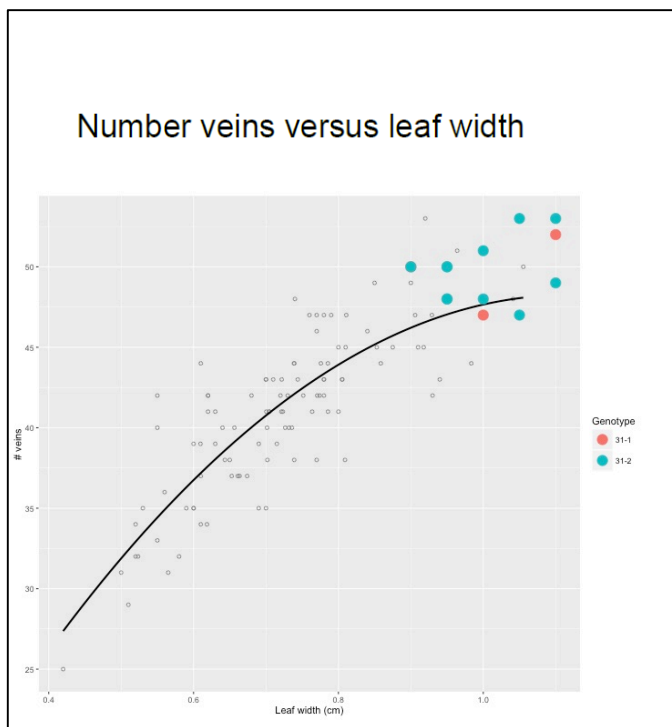
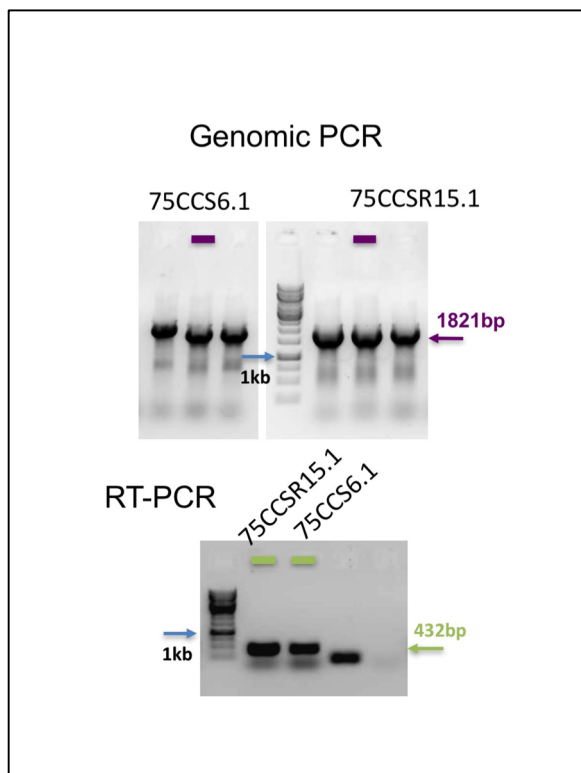


Construct: ZmUBI_{pro}:cDNA (ID# 75CC)

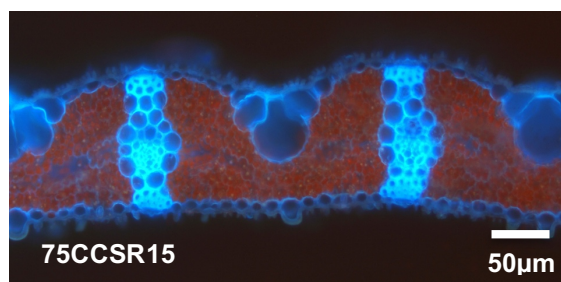
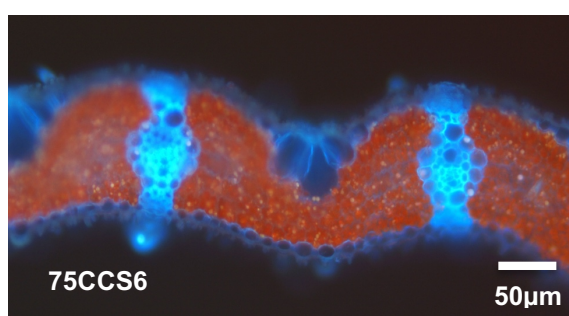
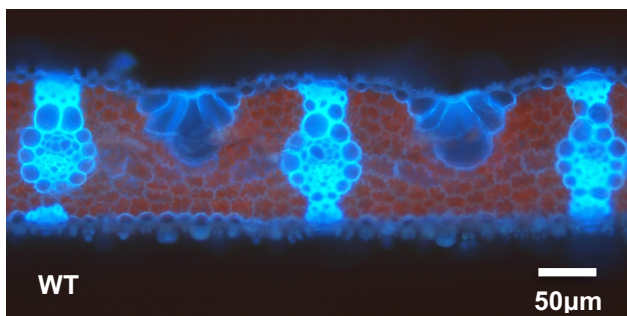
Two independent T1 lines:

31-1 (75CCS6 – 3 individuals)

31-1 (75CCSR15 – 11 individuals)



Representative leaf cross sections:

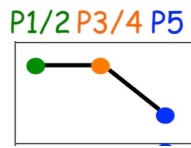


GRMZM2G146688

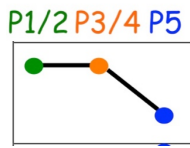
AP2

Gene ID: JL32

Foliar expression



Husk expression



Construct: ZmUBI_{pro}:cDNA (ID# 77CC)
ZmUBI_{pro}:gDNA (ID# 77GC)

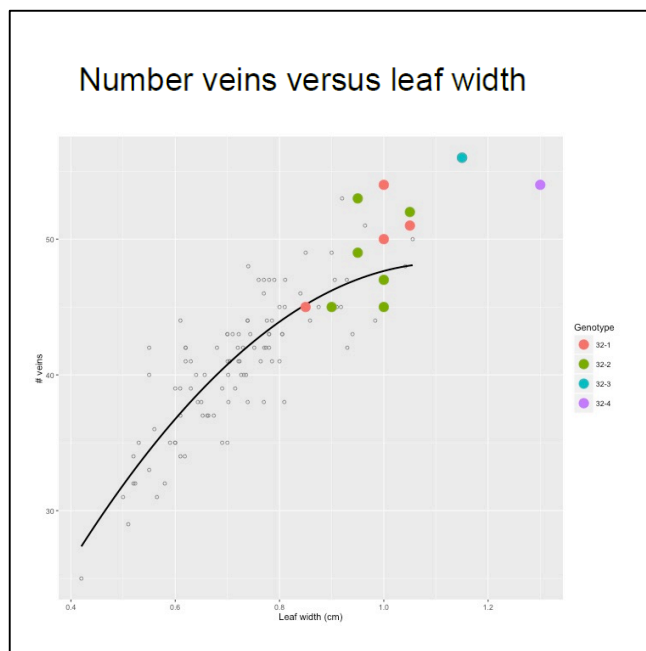
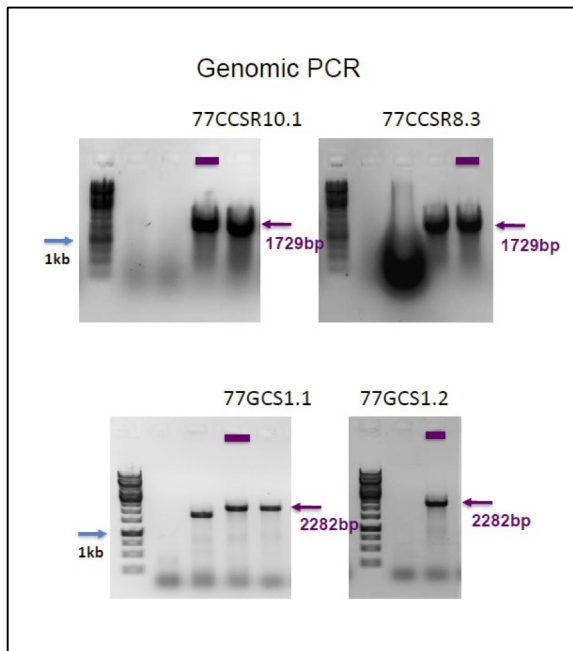
Four independent T1 lines: This gene has been transformed with both CDS (77CC) and genomic sequence (77GC), but no gene expression was detected in either attempt.

32-1 (77CCSR10 – 5 individuals)

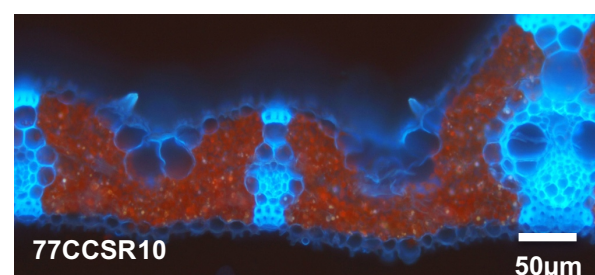
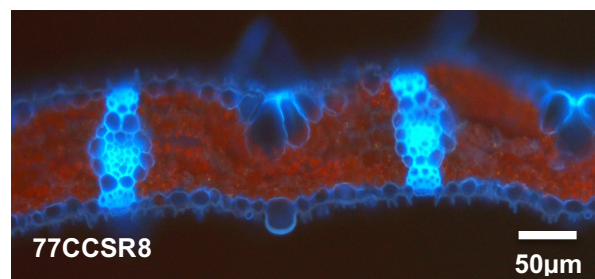
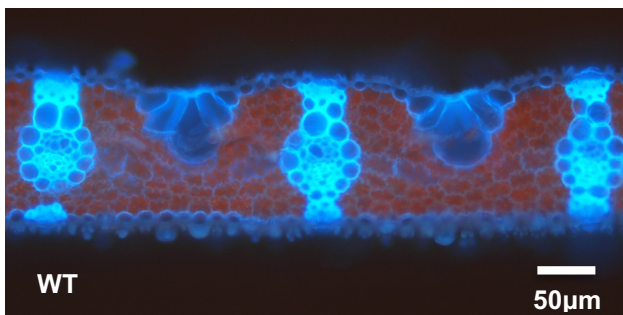
32-2 (77CCSR8 – 6 individuals)

32-3 (77GCS1 – 1 individual)

32-4 (77GCS18 – 1 individual)



Representative leaf cross sections:



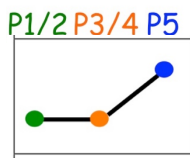
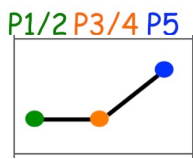
GRMZM2G023051

Unknown

Gene ID: JL35

Foliar expression

Husk expression

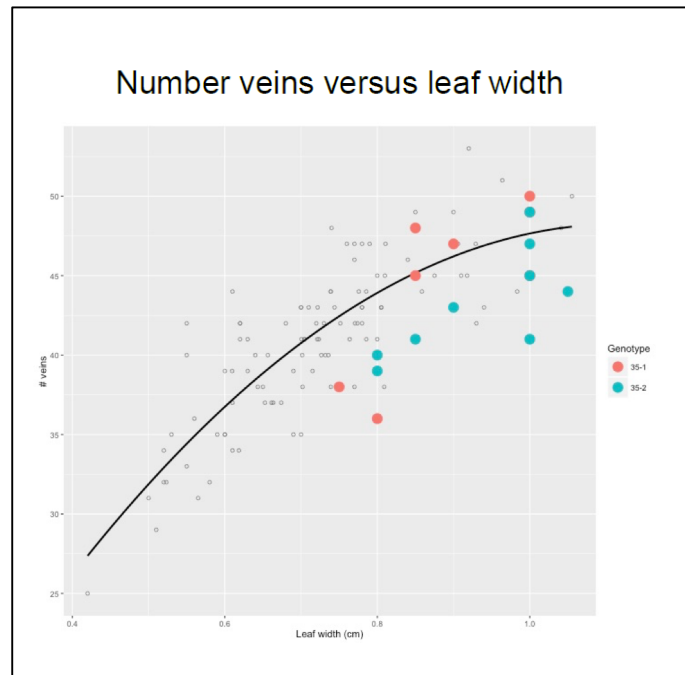
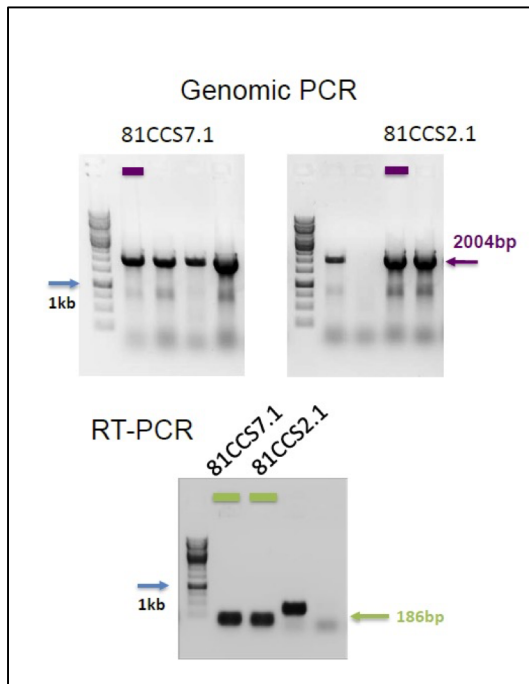


Construct: ZmUBI_{pro}:cDNA (ID# 81CC)

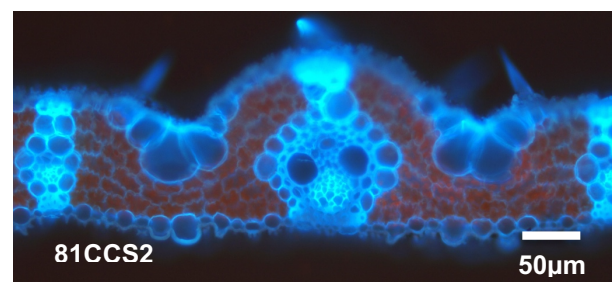
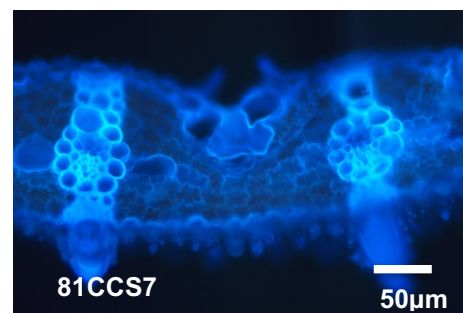
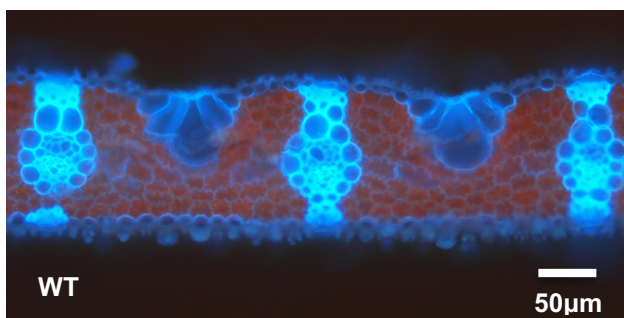
Two independent T1 lines:

35-1 (81CCS7 – 8 individuals)

35-2 (81CCS2 – 9 individuals)



Representative leaf cross sections:



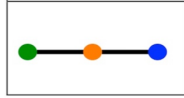
GRMZM2G109480

Unknown

Gene ID: JL36

Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

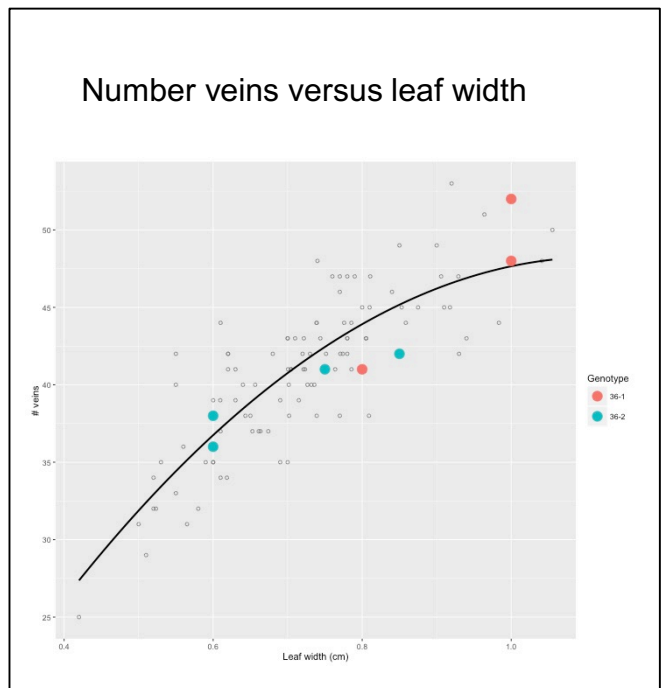
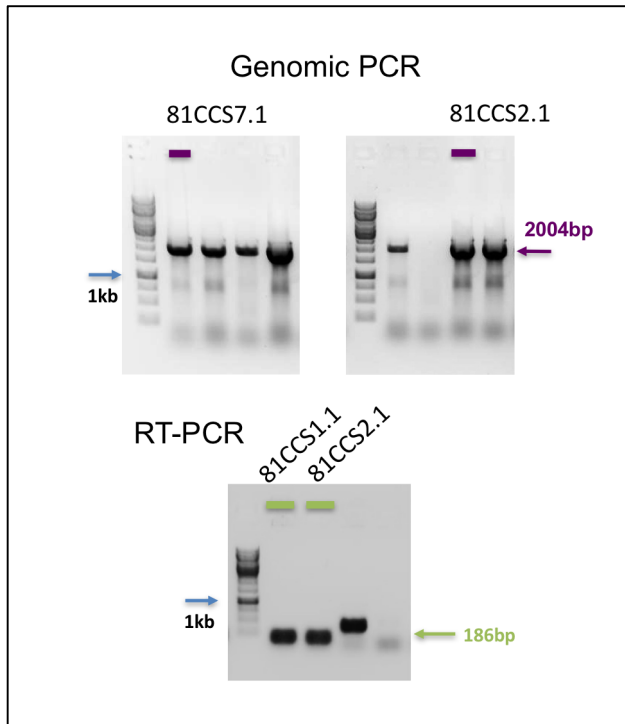


Construct: ZmUBI_{pro}:cDNA (ID# 82CC)

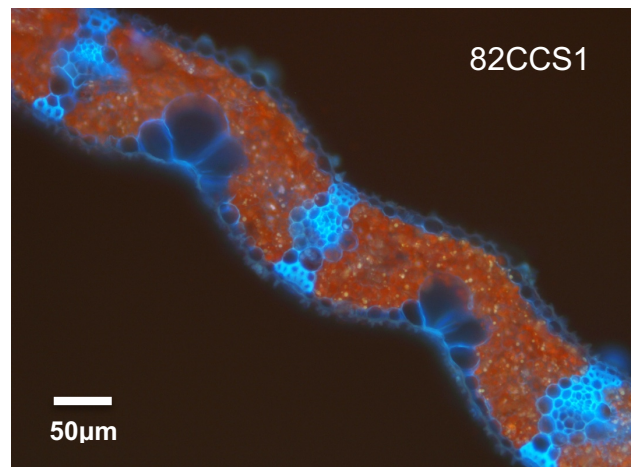
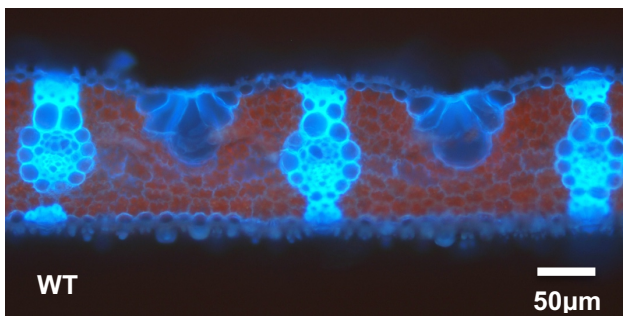
Two independent T1 lines:

36-1 (82CCS1 – 3 individuals)

36-2 (82CCS2 – 4 individuals)



Representative leaf cross sections:

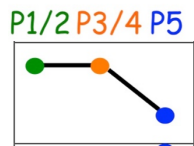


GRMZM2G097275

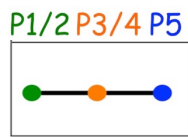
SBP

Gene ID: JL47

Foliar expression



Husk expression

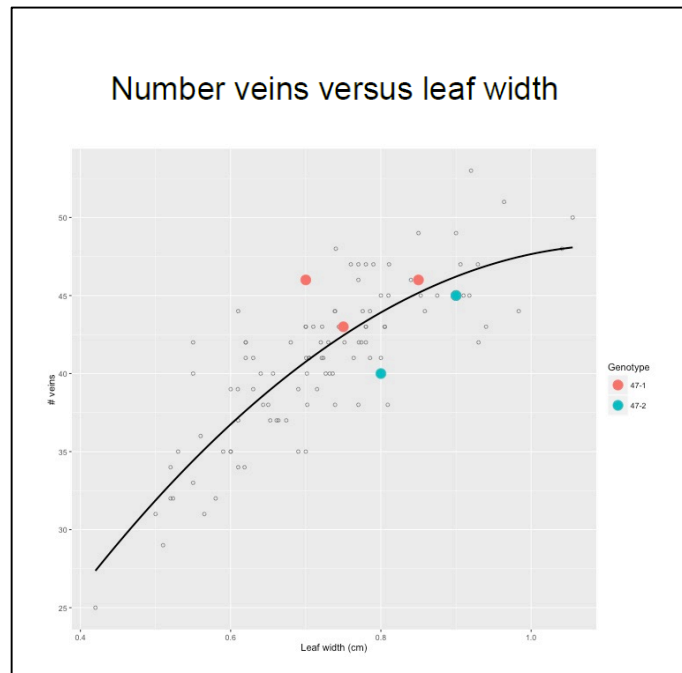
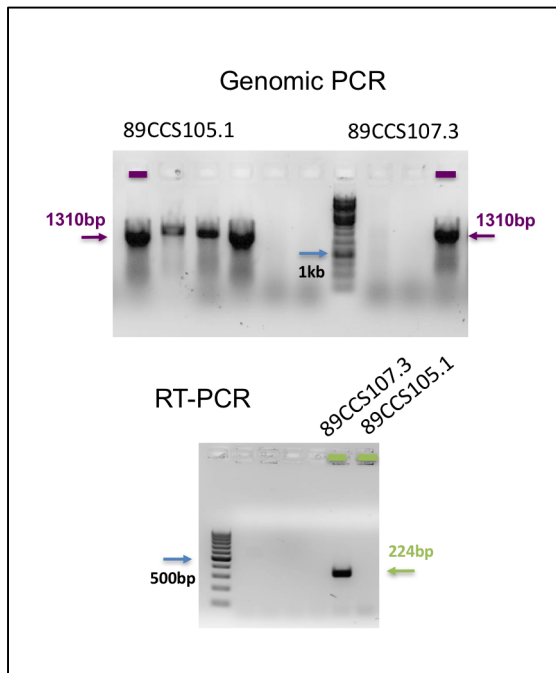


Construct: ZmUBI_{pro}:cDNA (ID# 89CC)

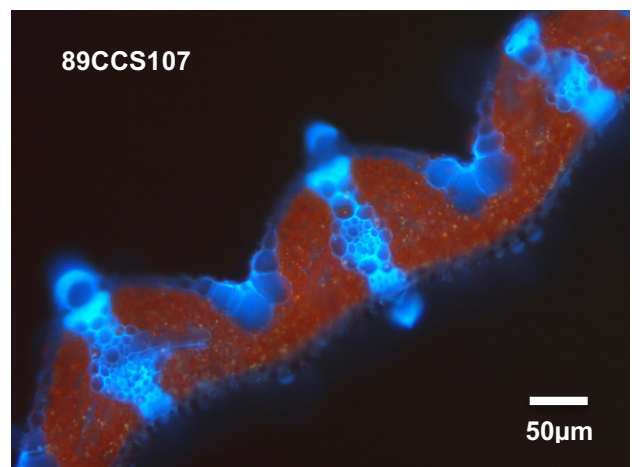
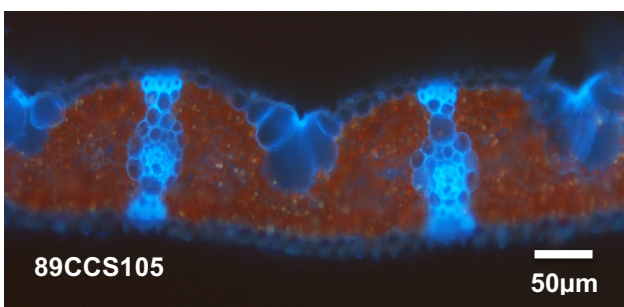
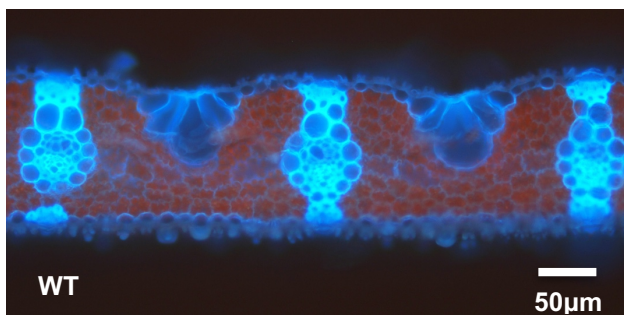
Two independent T1 lines:

47-1 (89CCS105 – 4 individuals)

47-2 (89CCS107 – 2 individuals)



Representative leaf cross sections:



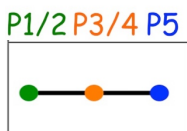
GRMZM2G148467

SBP

Gene ID: JL48

Foliar expression

Husk expression

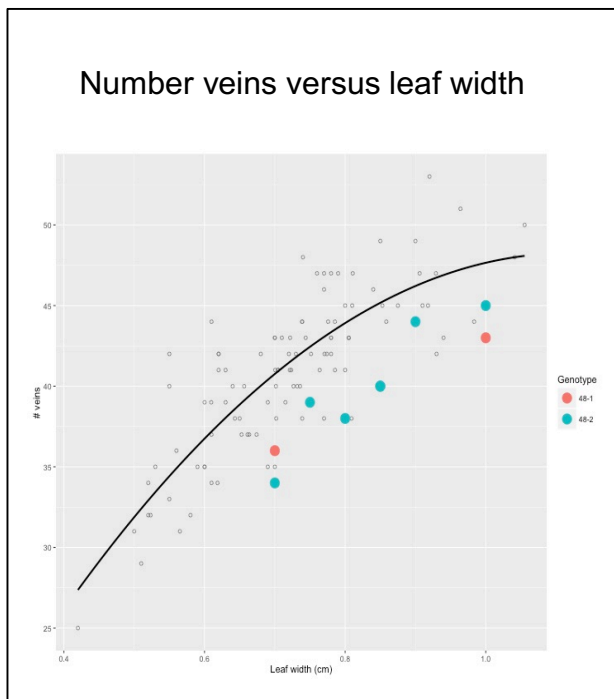
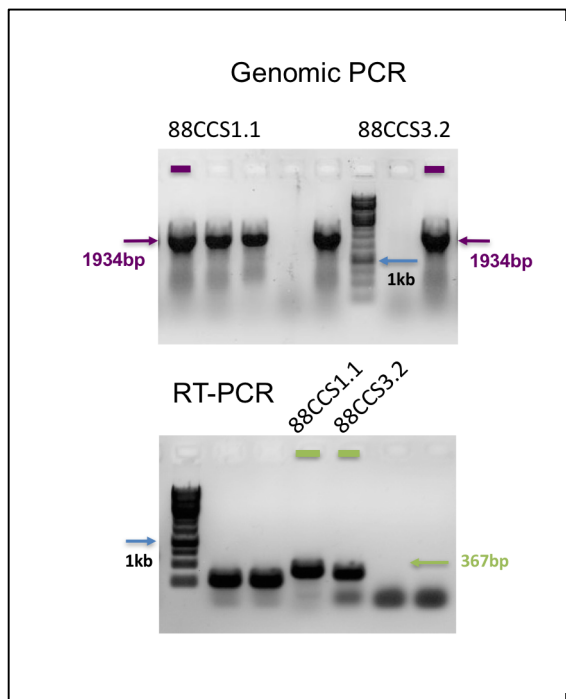


Construct: ZmUBI_{pro}:cDNA (ID# 88CC)

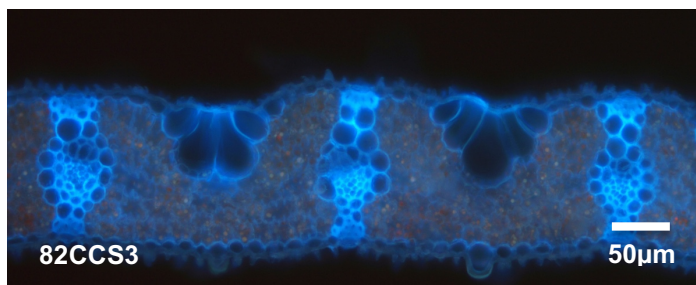
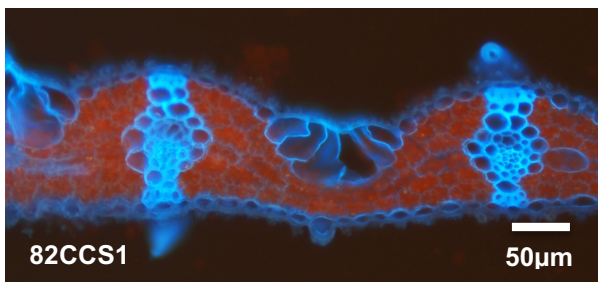
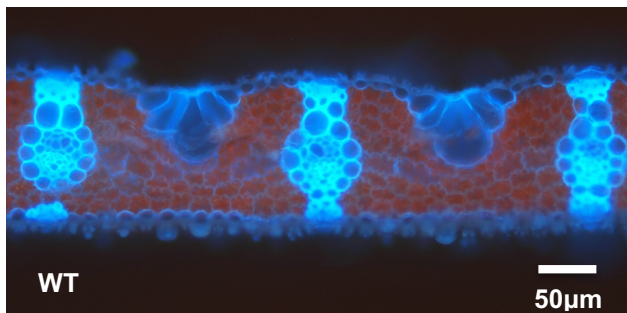
Two independent T1 lines:

48-1 (88CCS1 – 2 individuals)

48-2 (82CCS3 – 7 individuals)



Representative leaf cross sections:



GRMZM2G028643

LRR kinase

Gene ID: JL49

Foliar expression



Husk expression

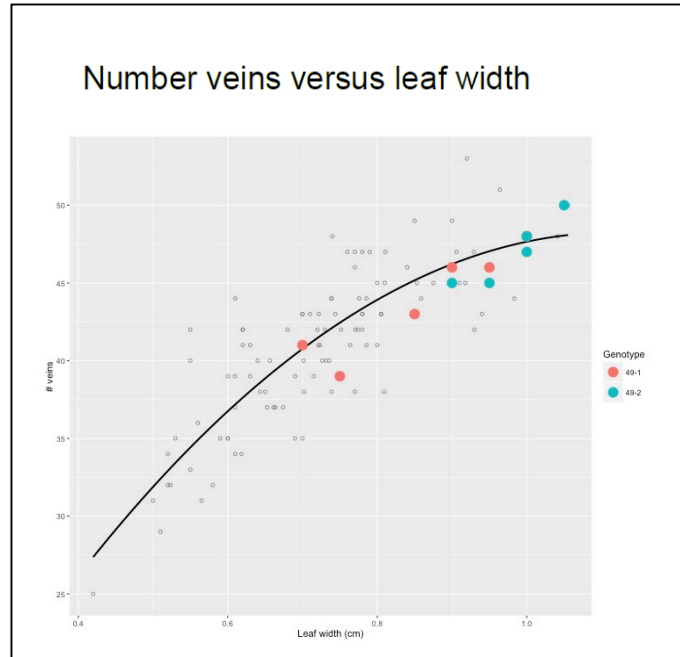
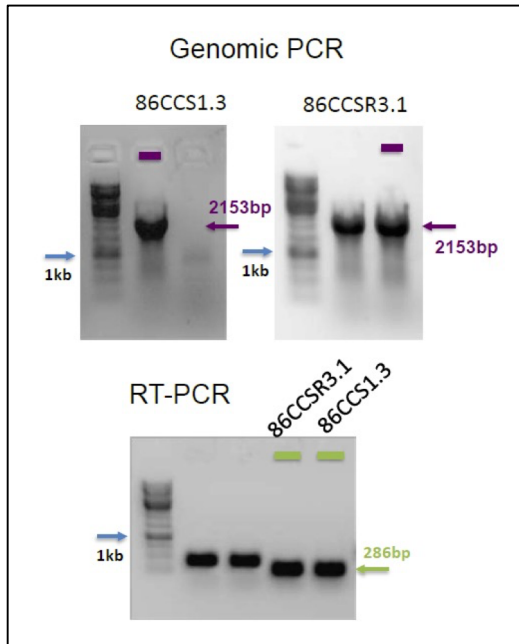


Construct: ZmUBI_{pro}:cDNA (ID# 86CC)

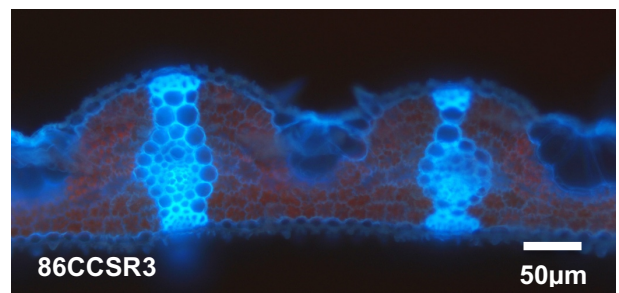
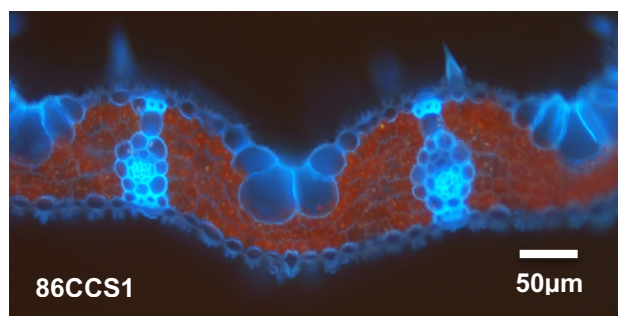
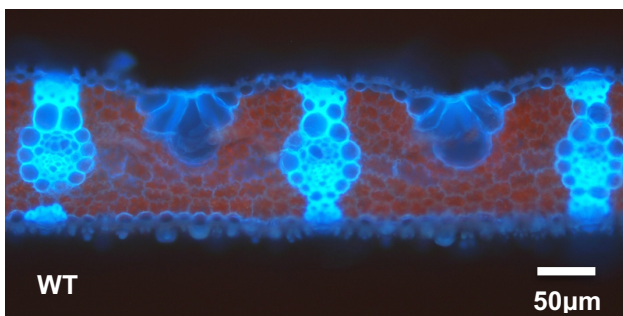
Two independent T1 lines:

49-1 (86CCS1 – 6 individuals)

49-2 (86CCSR3 – 5 individuals)



Representative leaf cross sections:



GRMZM2G178102

Class III-HD ZIP

Gene ID: JL51

Foliar expression



Husk expression

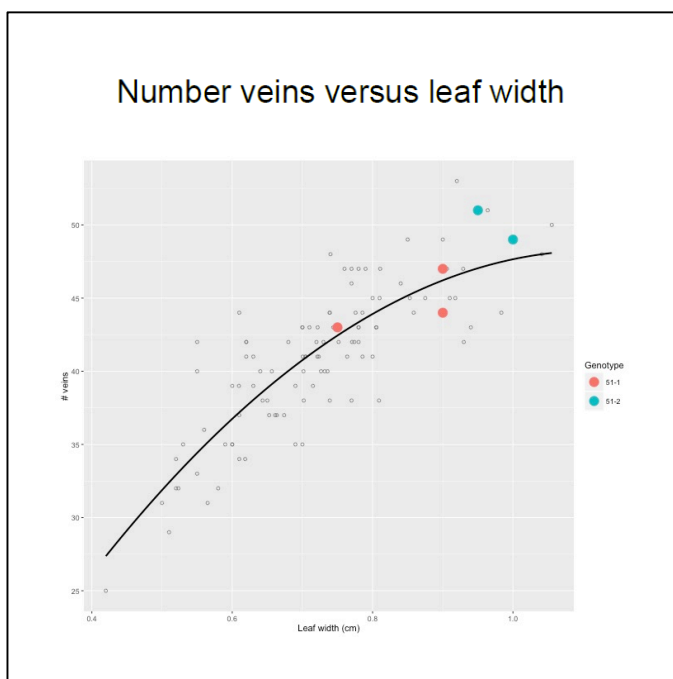
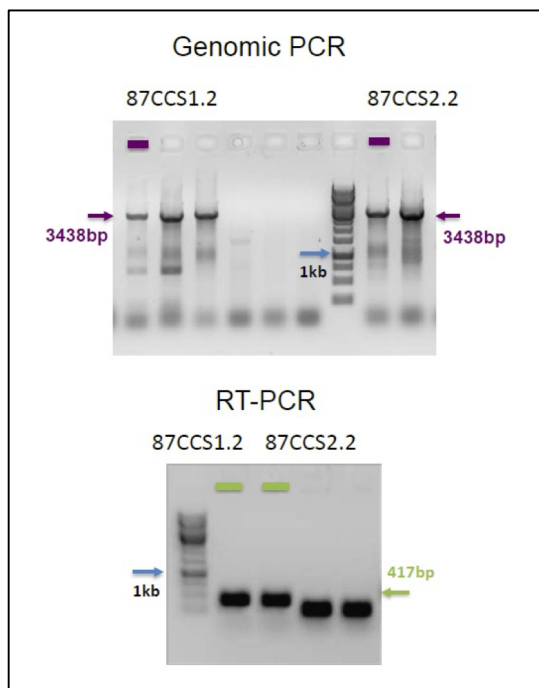


Construct: ZmUBI_{pro}:cDNA (ID# 87CC)

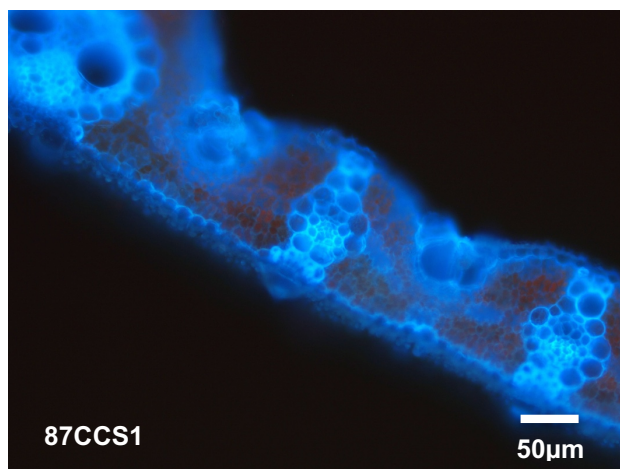
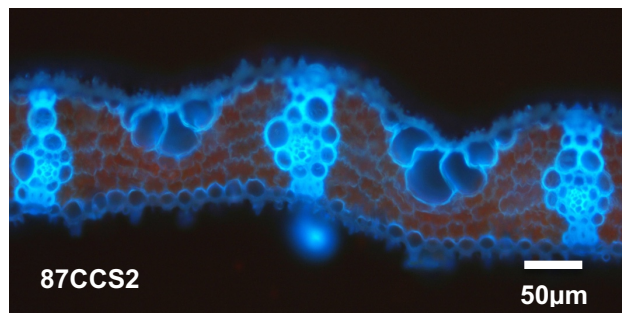
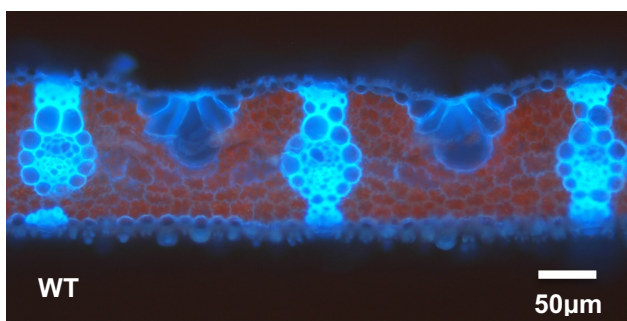
Two independent T1 lines:

51-1 (87CCS1 – 3 individuals)

51-2 (87CCS2 – 2 individuals)



Representative leaf cross sections:

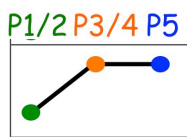


GRMZM2G061314

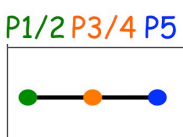
LRR binding protein

Gene ID: JL60

Foliar expression

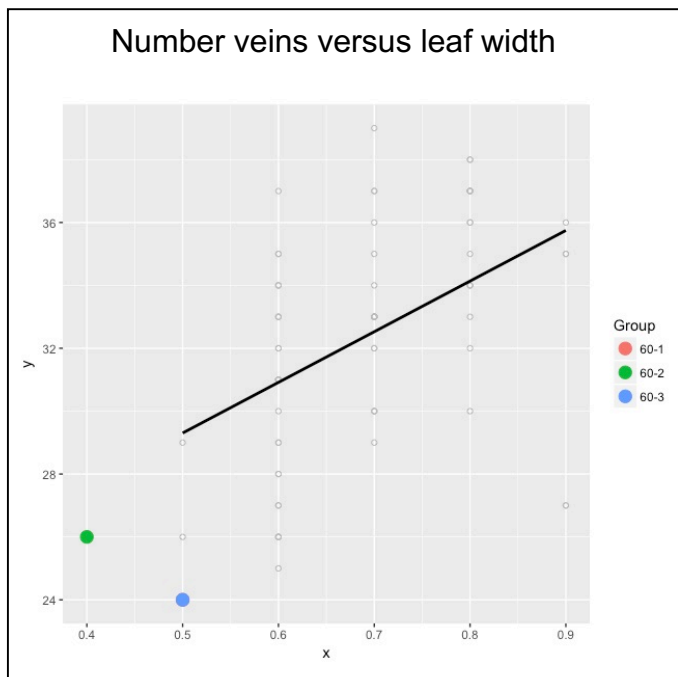
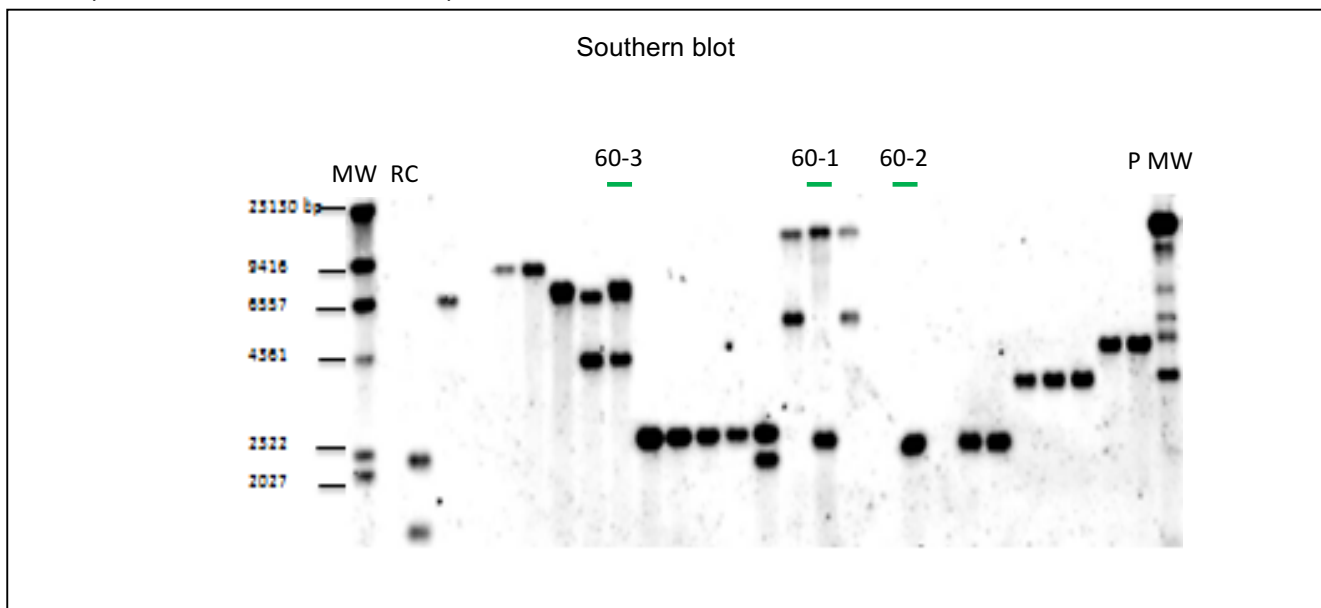


Husk expression

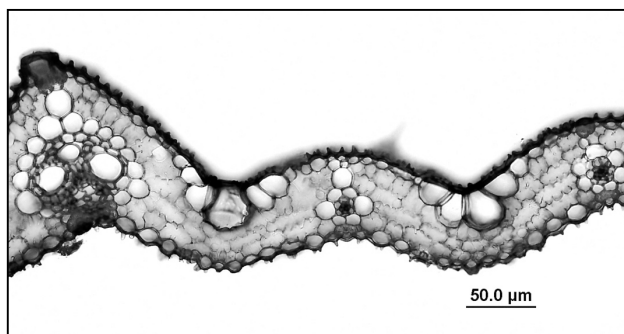


Construct: ZmUBI_{pro}:cDNA (ID# 945)

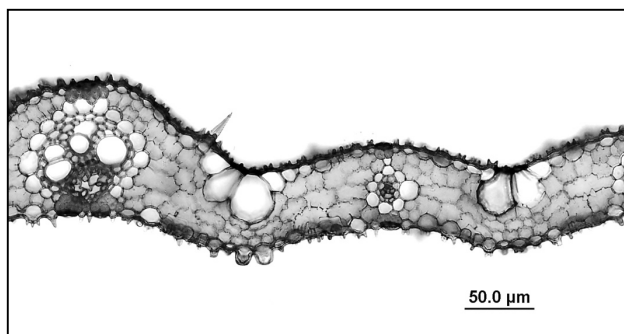
Three independent T1 lines:
60-1 (IRS945-19 – 1 individual)
60-2 (IRS945-20 – 1 individual)
60-3 (IRS945-11 – 1 individual)



Representative cross section of segregating WT leaf



Representative cross section of line 60-3 leaf

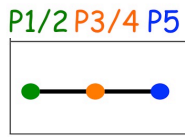


GRMZM2G089819

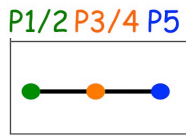
Brassinosteroid LRR RLK

Gene ID: JL68

Foliar expression



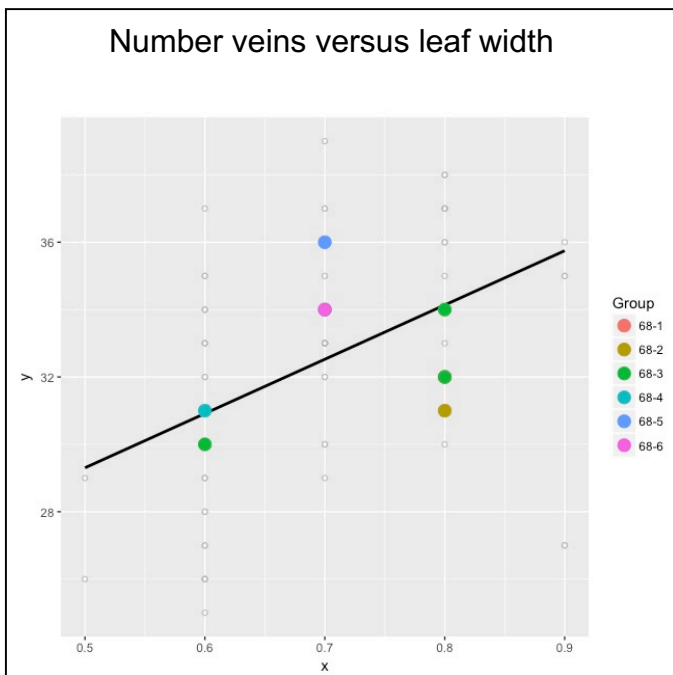
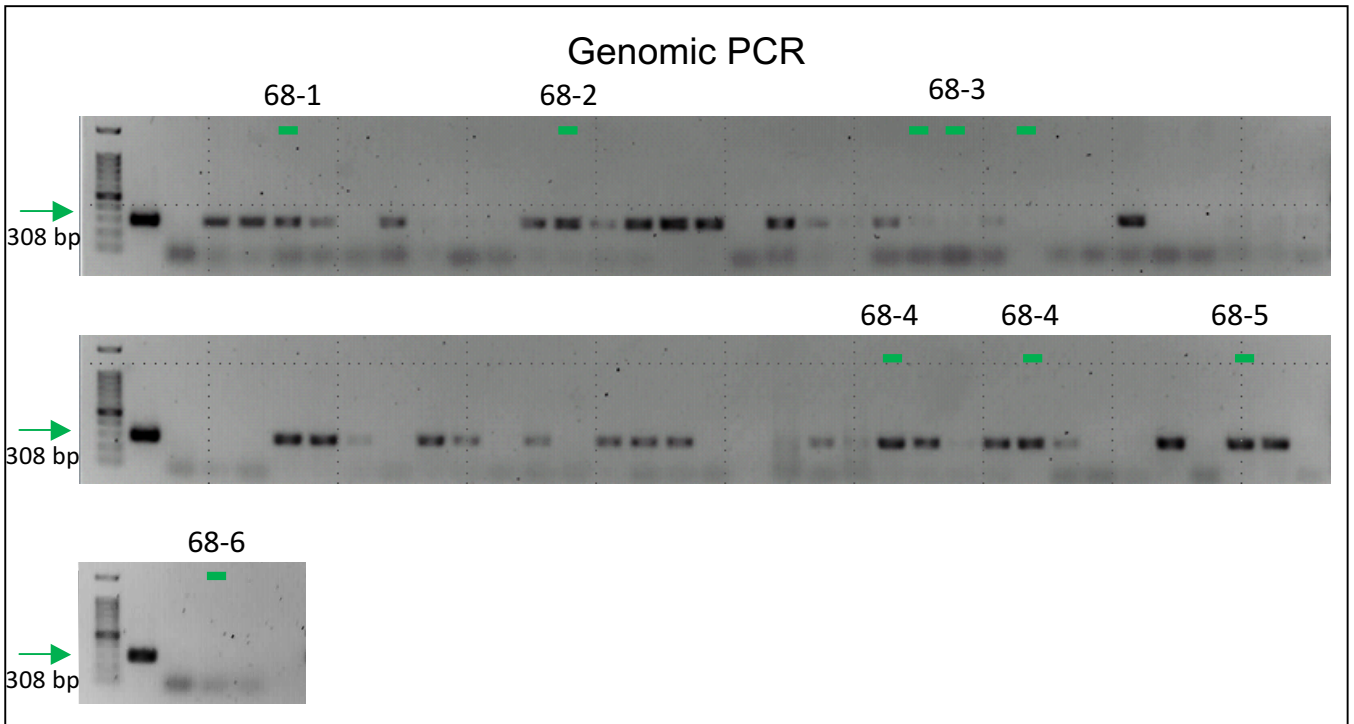
Husk expression



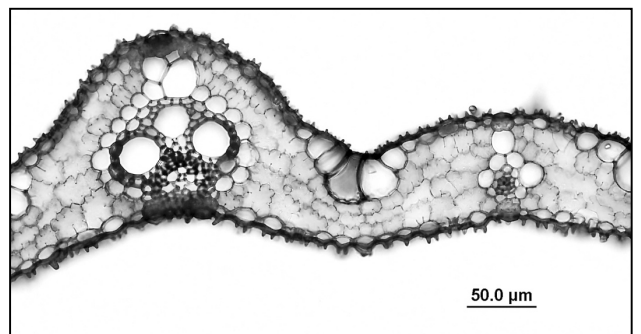
Construct: ZmUBI_{pro}:cDNA (ID# 953)

Six independent T1 lines:

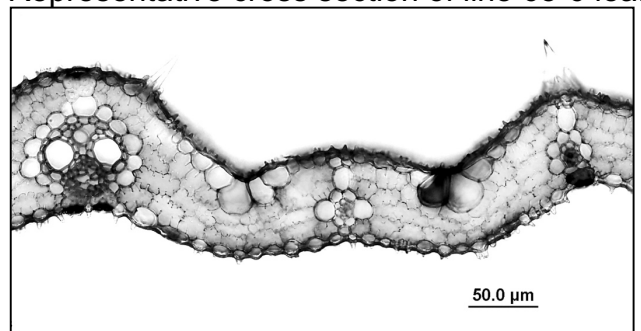
- | | |
|---------------------------------|---------------------------------|
| 68-1 (IRS953-1 – 1 individual) | 68-4 (IRS953-6 – 2 individuals) |
| 68-2 (IRS953-2 – 1 individual) | 68-5 (IRS953-7 – 1 individual) |
| 68-3 (IRS953-3 – 3 individuals) | 68-6 (IRS953-10 – 1 individual) |



Representative cross section of segregating WT leaf



Representative cross section of line 68-6 leaf



GRMZM5G850129

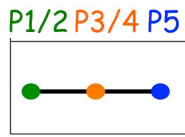
Growth Regulatory Factor

Gene ID: JL75

Foliar expression



Husk expression



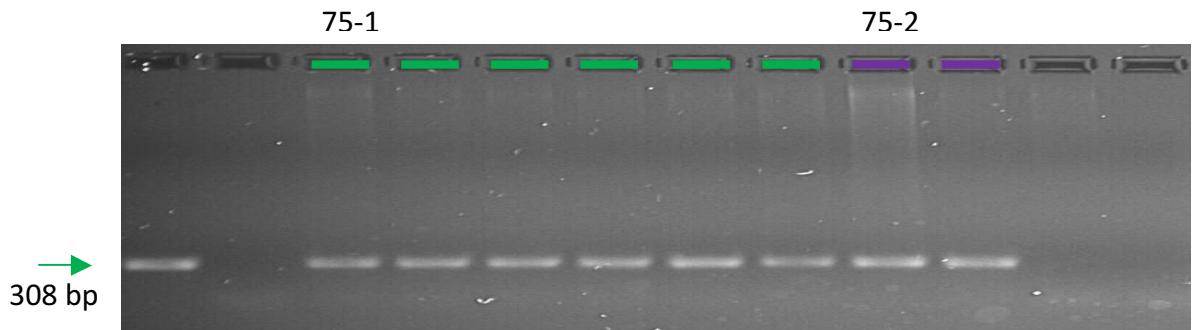
Construct: ZmUBI_{pro}:cDNA (ID# 948)

Two independent T1 lines:

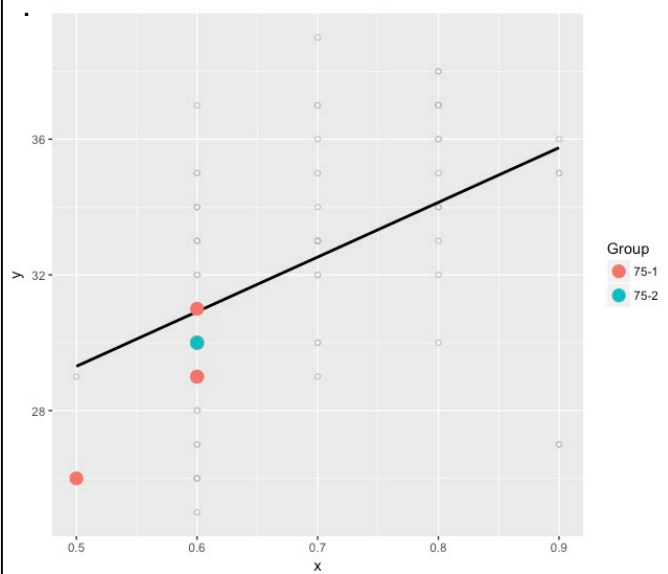
75-1 (IRS948-19 – 6 individuals)

75-2 (IRS948-23 – 2 individuals)

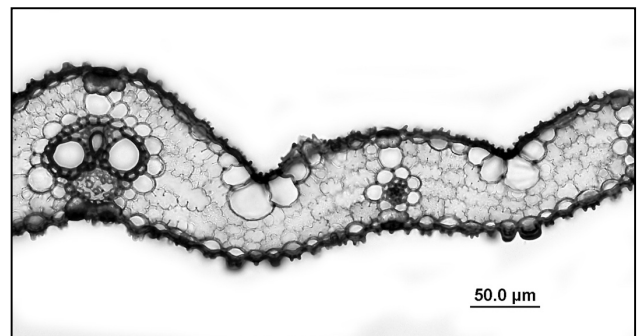
Genomic PCR



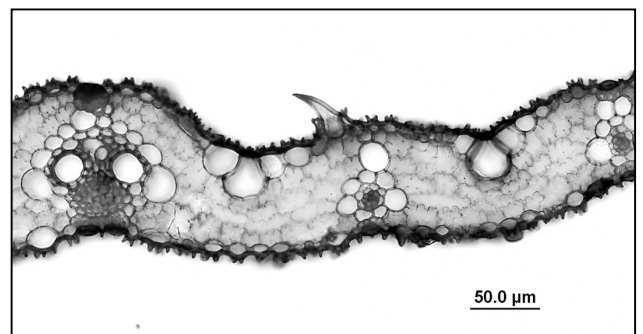
Number veins versus leaf width



Representative cross section of segregating WT leaf



Representative cross section of line 75-2 leaf



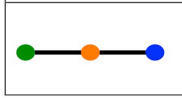
GRMZM2G061734

SBP domain

Gene ID: JL76

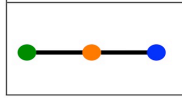
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5



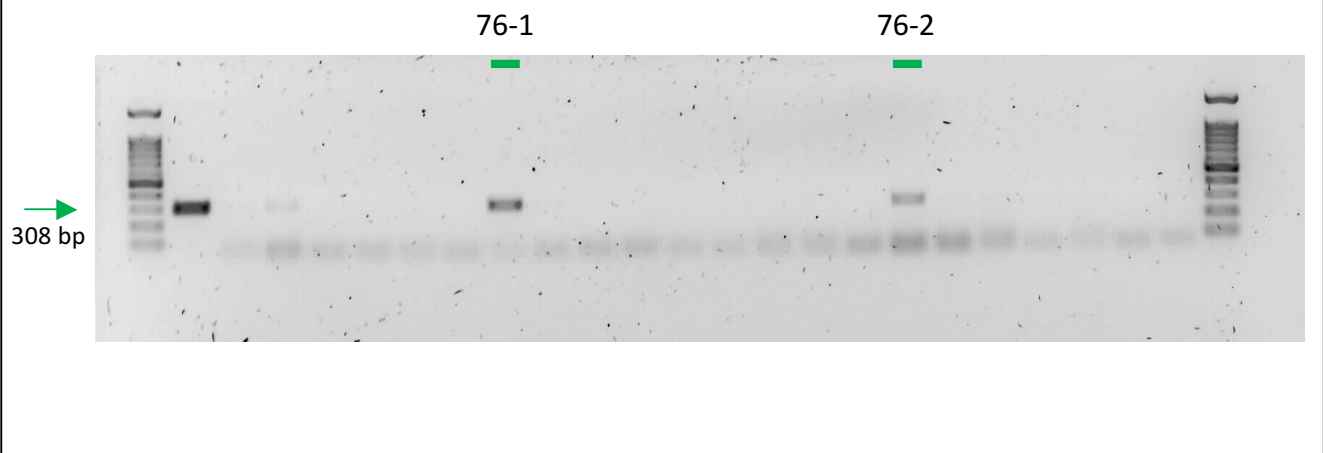
Construct: ZmUBI_{pro}:cDNA (ID# 960)

Two independent T1 lines:

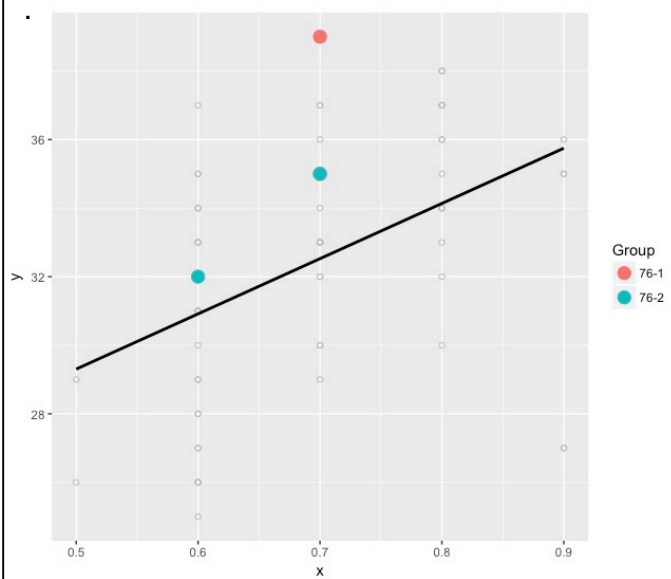
76-1 (IRS960-20 – 2 individuals)

76-2 (IRS960-22 – 2 individuals)

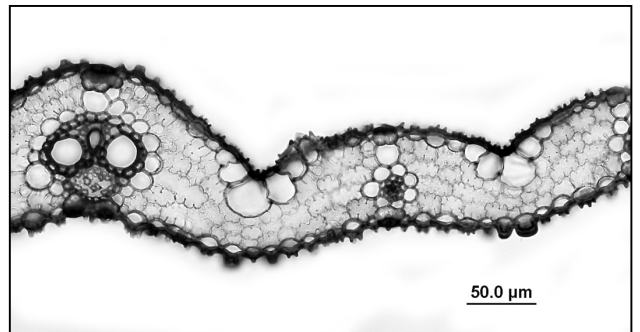
Genomic PCR



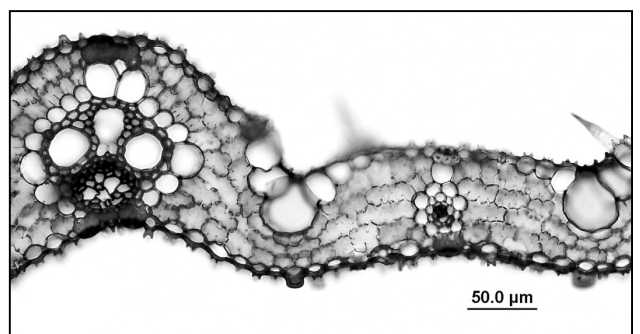
Number veins versus leaf width



Representative cross section of segregating WT leaf



Representative cross section of line 76-2 leaf

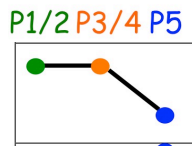


GRMZM2G126018

SBP domain

Gene ID: JL80

Foliar expression



Husk expression



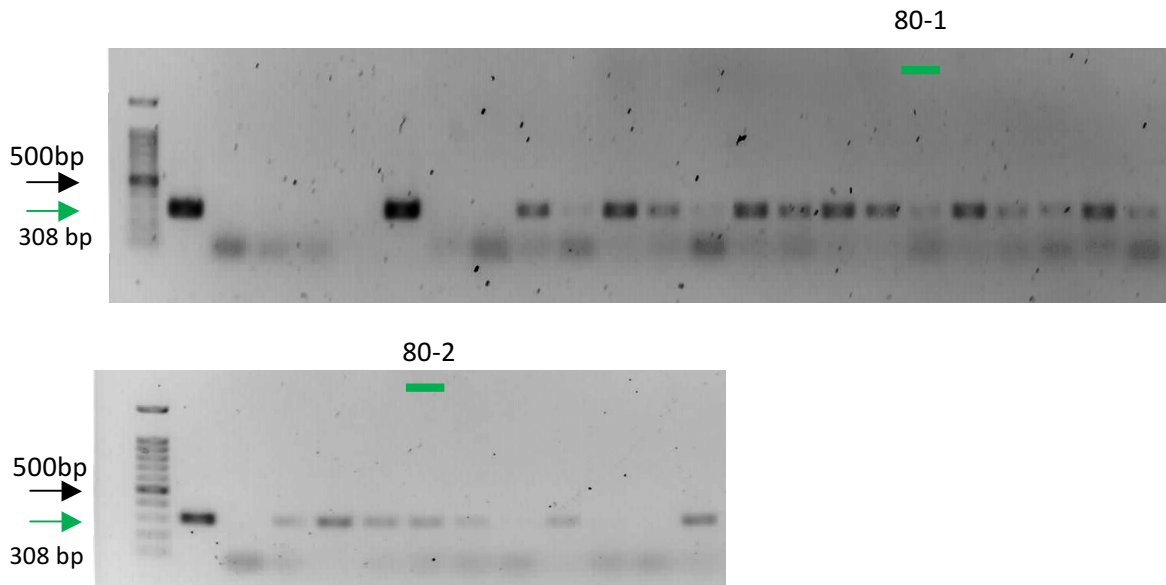
Construct: ZmUBI_{pro}:cDNA (ID# 954)

Two independent T1 lines:

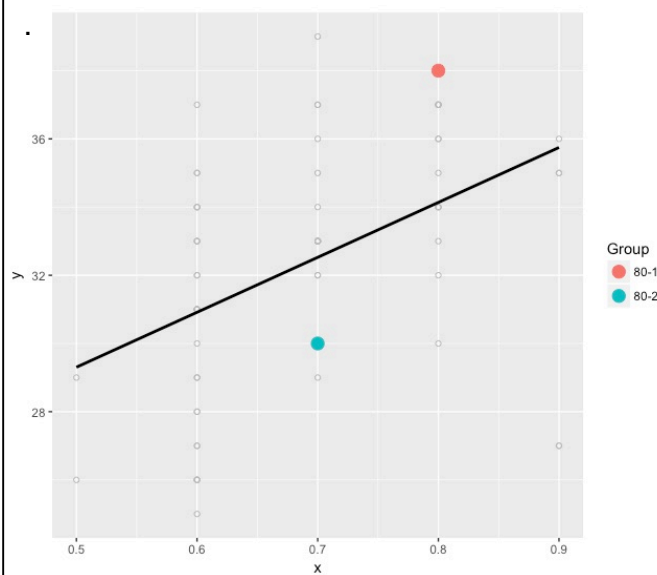
80-1 (IRS954-3 – 1 individual)

80-2 (IRS954-10 – 1 individual)

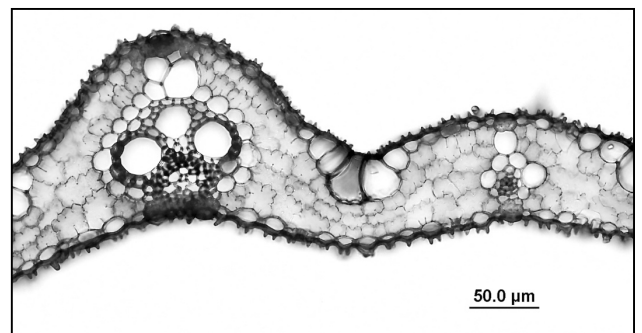
Genomic PCR



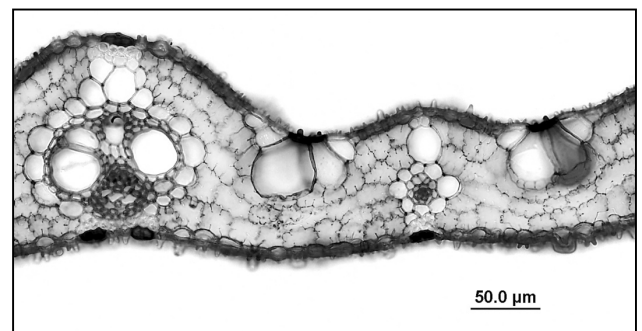
Number veins versus leaf width



Representative cross section of segregating WT leaf



Representative cross section of line 80-2 leaf

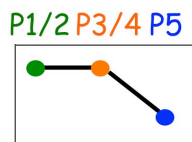


GRMZM2G312419

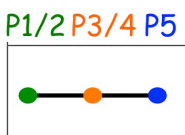
R2R3 Myb

Gene ID: JL81

Foliar expression



Husk expression



Construct: ZmUBI_{pro}:cDNA (ID# 961)

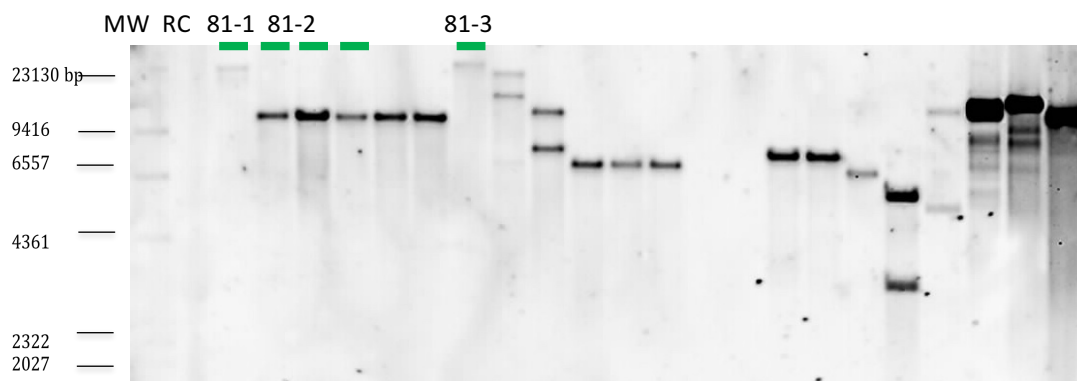
Three independent T1 lines:

81-1 (IRS961-1 – 1 individual)

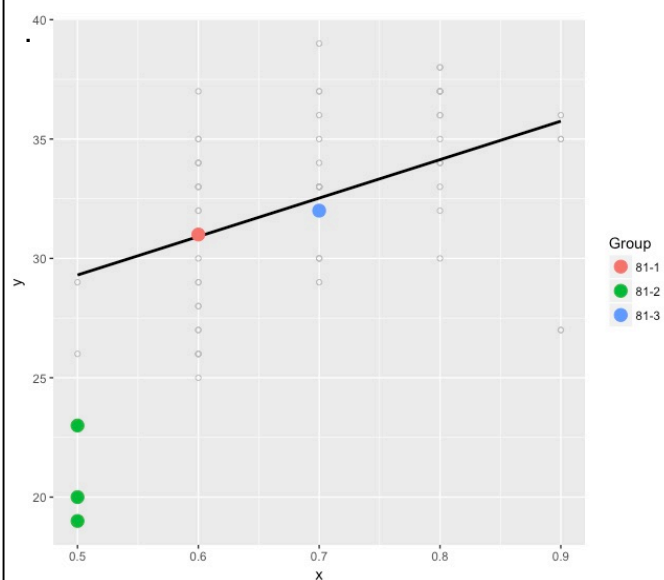
81-2 (IRS961-6 – 3 individuals)

81-3 (IRS961-9 – 1 individual)

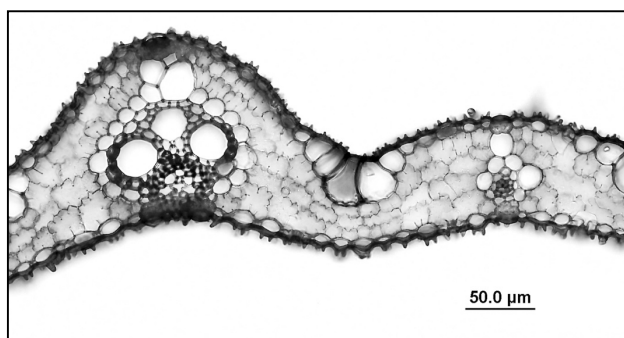
Southern blot



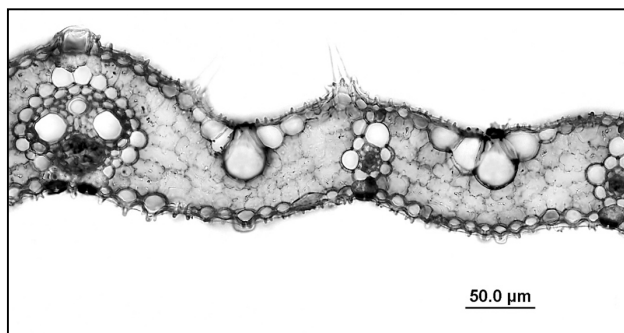
Number veins versus leaf width



Representative cross section of segregating WT leaf



Representative cross section of line 81-3 leaf



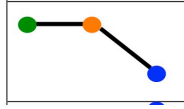
GRMZM2G478876

Serine threonine kinase

Gene ID: JL82

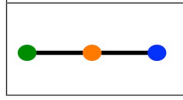
Foliar expression

P1/2 P3/4 P5



Husk expression

P1/2 P3/4 P5

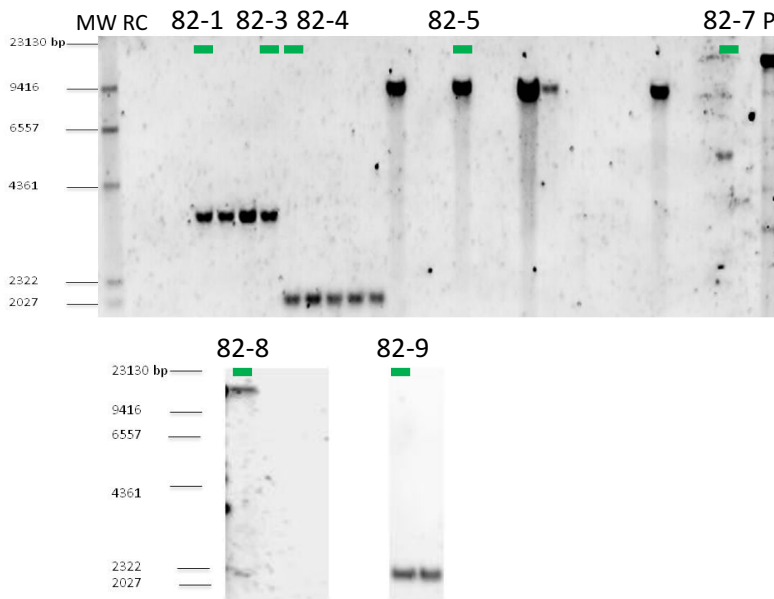


Construct: ZmUBI_{pro}:cDNA (ID# 940)

Nine independent T1 lines:

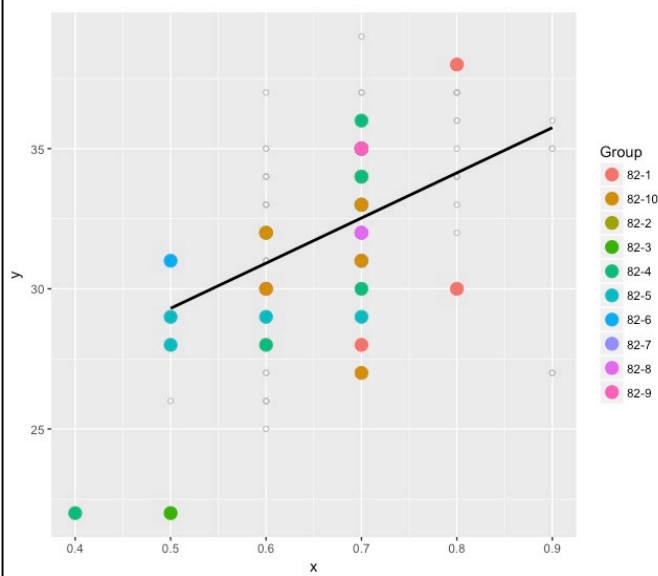
82-1 (IRS940-4–6 individuals) 82-4 (IRS940-10–7 individuals) 82-7 (IRS940-17–3 individuals)
82-2 (IRS940-5– 1 individual) 82-5 (IRS940-11–6 individuals) 82-8 (IRS940-19– 2 individuals)
82-3 (IRS940-7– 1 individual) 82-6 (IRS940-15 –2 individuals) 82-9 (IRS940-35– 6 individuals)

Southern blot

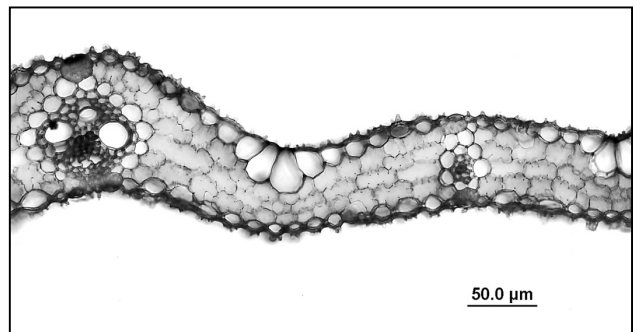


MW: DNA Molecular weight marker II, P- plasmid, RC- non-transgenic rice control

Number veins versus leaf width



Representative cross section of segregating WT leaf



Representative cross section of line 82-8 leaf

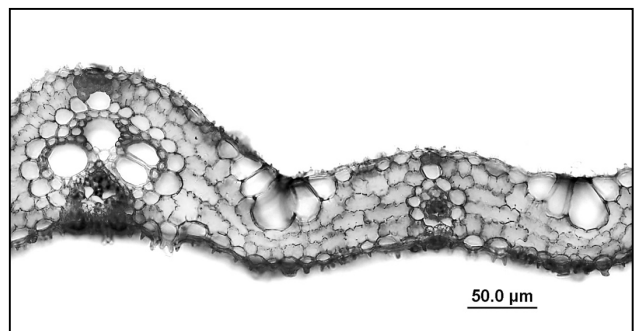


Figure S1. Phylogenetic tree of GRMZM2G074032 (*ZmIDD16*). Maximum-likelihood tree of the GRMZM2G074032 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis and rice proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S2. Phylogenetic tree of GRMZM2G027068 (*ZmHLH106*). Maximum-likelihood tree of the GRMZM2G027068 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S3. Phylogenetic tree of GRMZM2G140694 (*ZmHCA2*). Maximum-likelihood tree of one of the sub-groups of the GRMZM2G140694 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis HCA2, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S4. Phylogenetic tree of GRMZM2G480386 (*YUCCA*). Maximum-likelihood tree of the GRMZM2G480386 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Rice YUCCA8 plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S5. Phylogenetic tree of GRMZM2G011463 (*ZmSAUR60*). Maximum-likelihood tree of the GRMZM2G011463 orthogroup (as defined by Orthofinder²). Numbers at each node

are support values based on the consensus tree of 10 bootstrap replicates. Rice and Arabidopsis proteins, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S6. Phylogenetic tree of GRMZM2G098988 (*ZmSACL3*). Maximum-likelihood tree of the GRMZM2G098988 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis SACL3, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S7. Phylogenetic tree of GRMZM2G163724 (LRR kinase). Maximum-likelihood tree of the GRMZM2G163724 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis RDK1, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

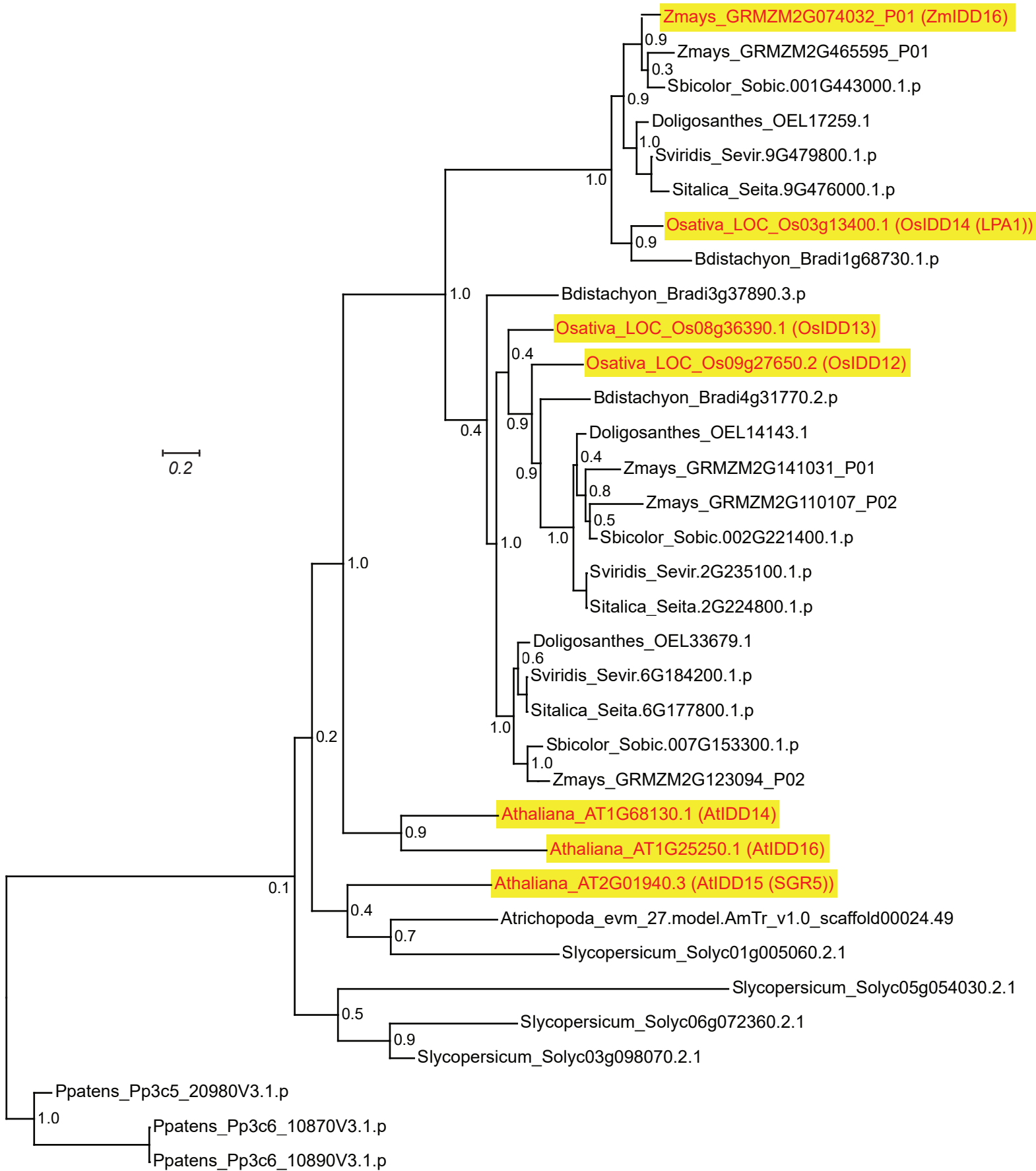
Figure S8. Phylogenetic tree of GRMZM2G111045 (R2R3 MYB). Maximum-likelihood tree of the GRMZM2G111045 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis LMI2, plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S9. Phylogenetic tree of GRMZM2G114893 (Unknown). Maximum-likelihood tree of the GRMZM2G114893 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. The maize protein encoded by the gene analysed in this study is highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

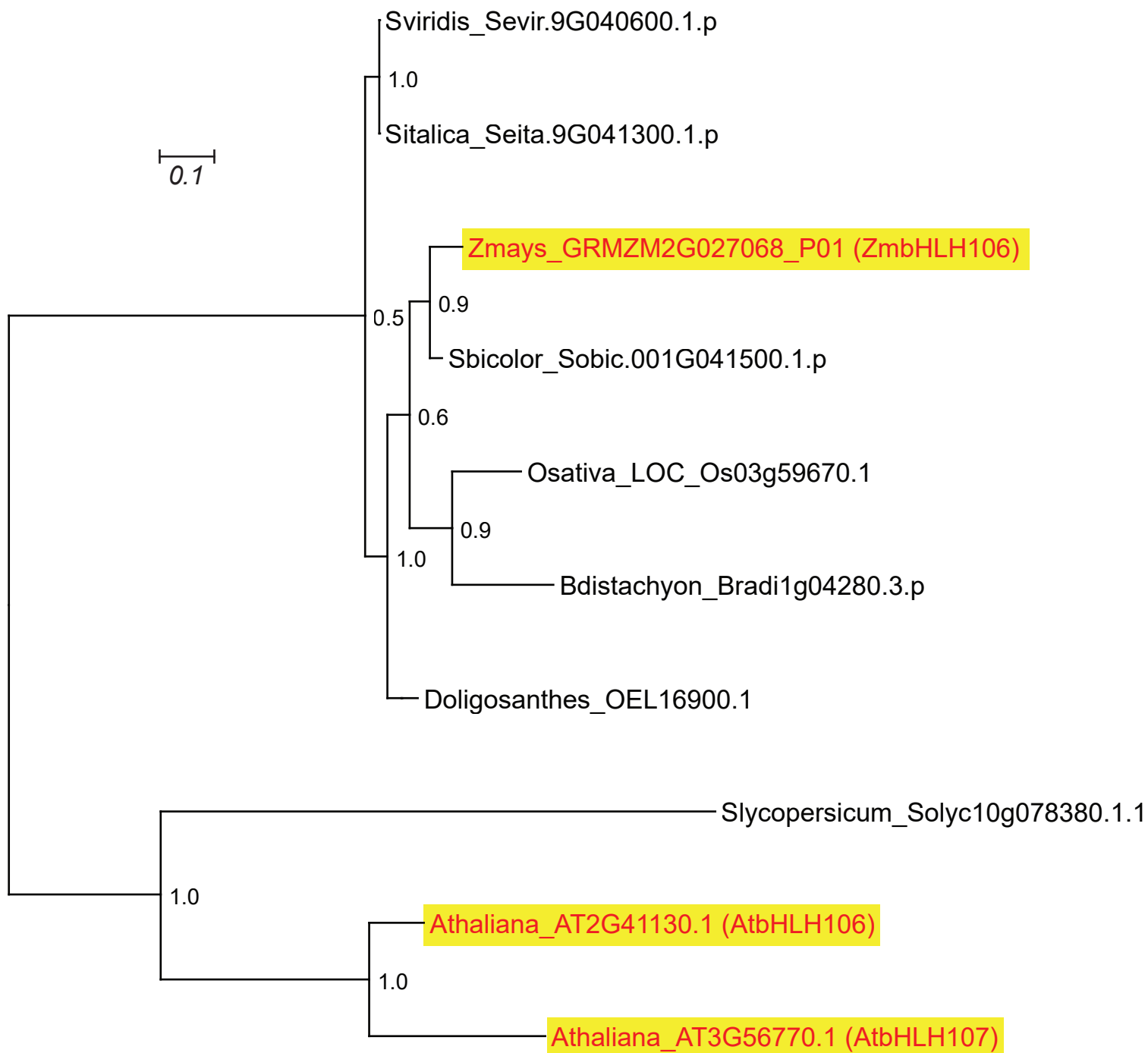
Figure S10. Phylogenetic tree of GRMZM2G377217 (*ZmWRKY12*). Maximum-likelihood tree of one of the sub-groups of the GRMZM2G377217 orthogroup (as defined by Orthofinder²). Numbers at each node are support values based on the consensus tree of 10 bootstrap replicates. Arabidopsis proteins plus the maize protein encoded by the gene analysed in this study are highlighted in yellow. Branch lengths reflect the number of amino acid substitutions per site.

Figure S11. Details of activation tagged line M0094935. **a)** Schematic of rice chromosome 3 showing insertion site of T-DNA (red arrow) upstream of Os03g50920 in line M0094935. **b)** Gel images of RT-PCR reactions showing transcript levels of 03g50920 relative to actin in 21 day old seedling shoots of wild-type (WT) and the insertion line (M0094935).

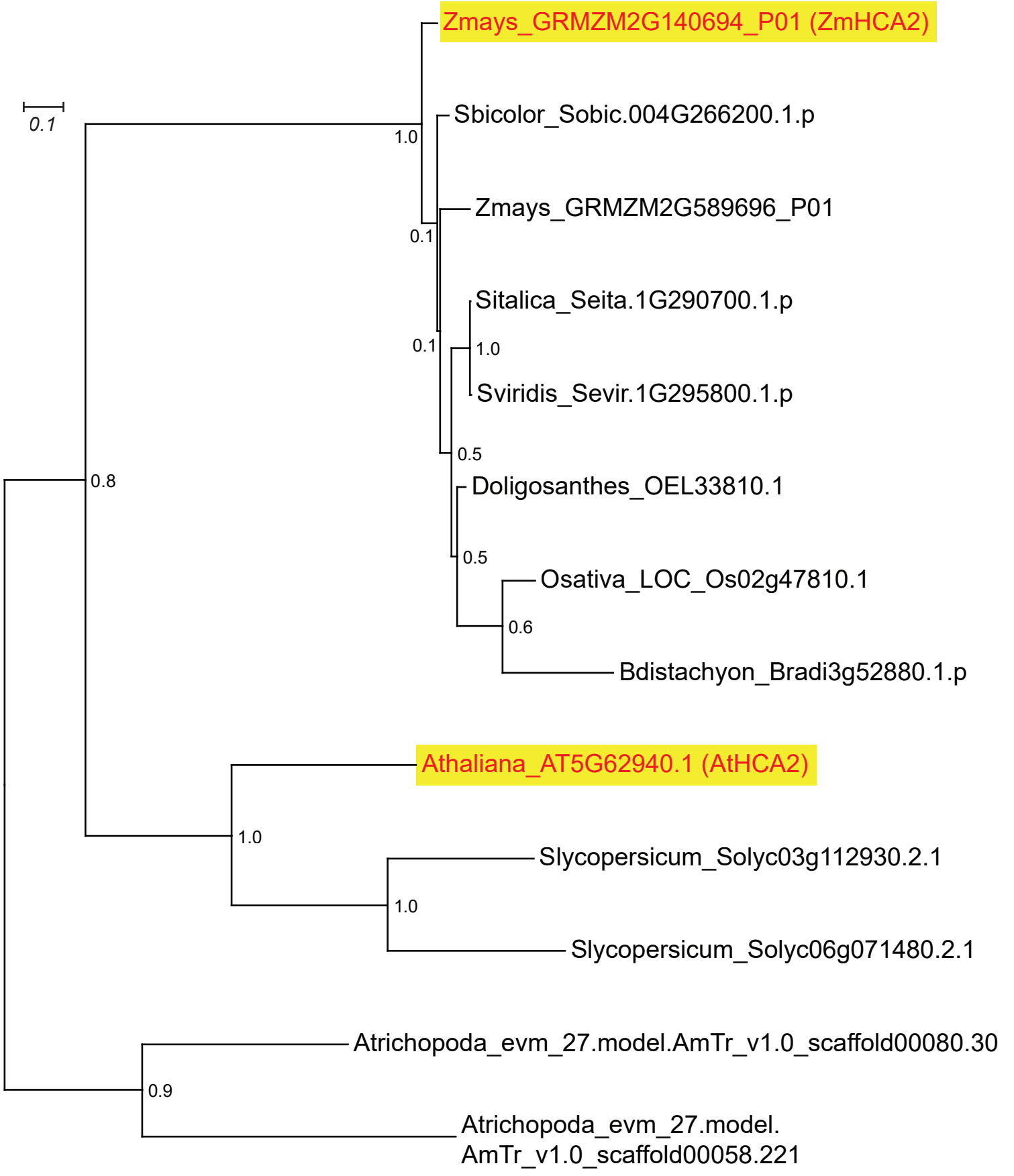
SUPPLEMENTARY FIGURE S1



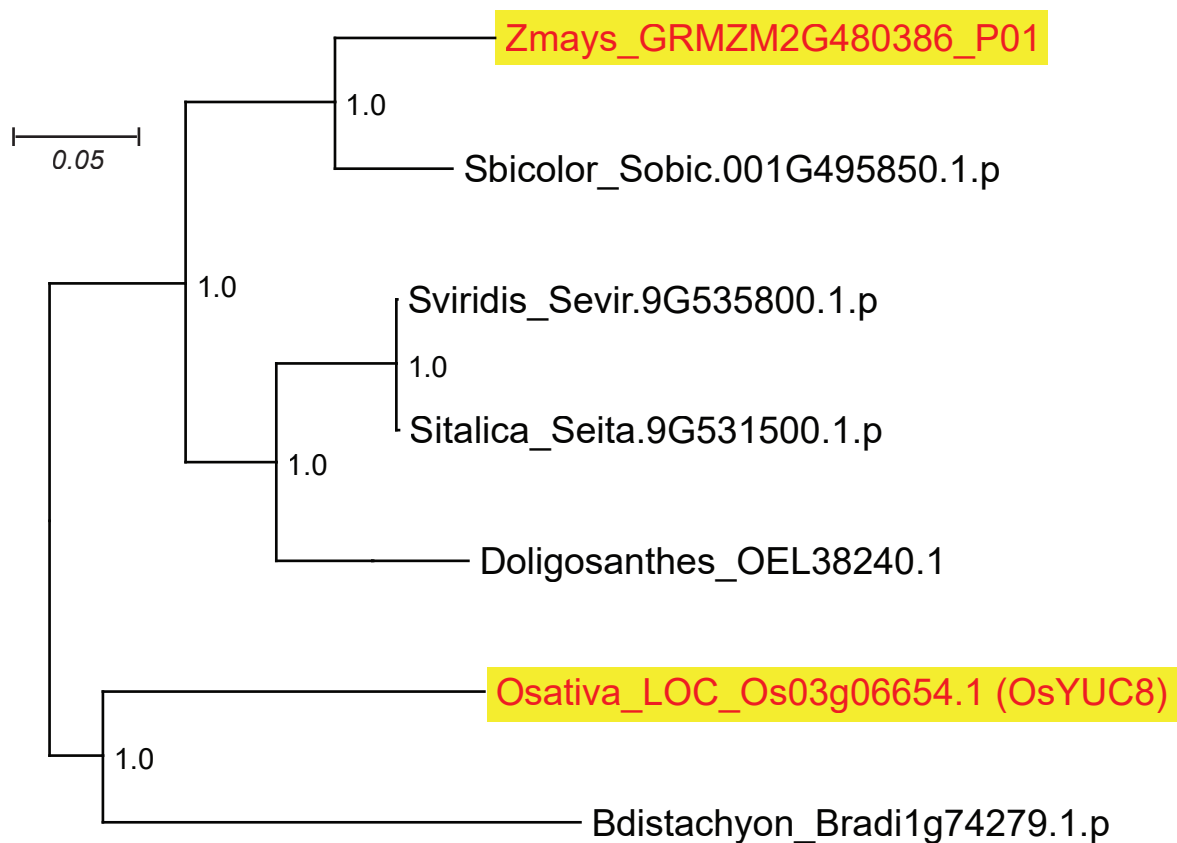
SUPPLEMENTARY FIGURE S2



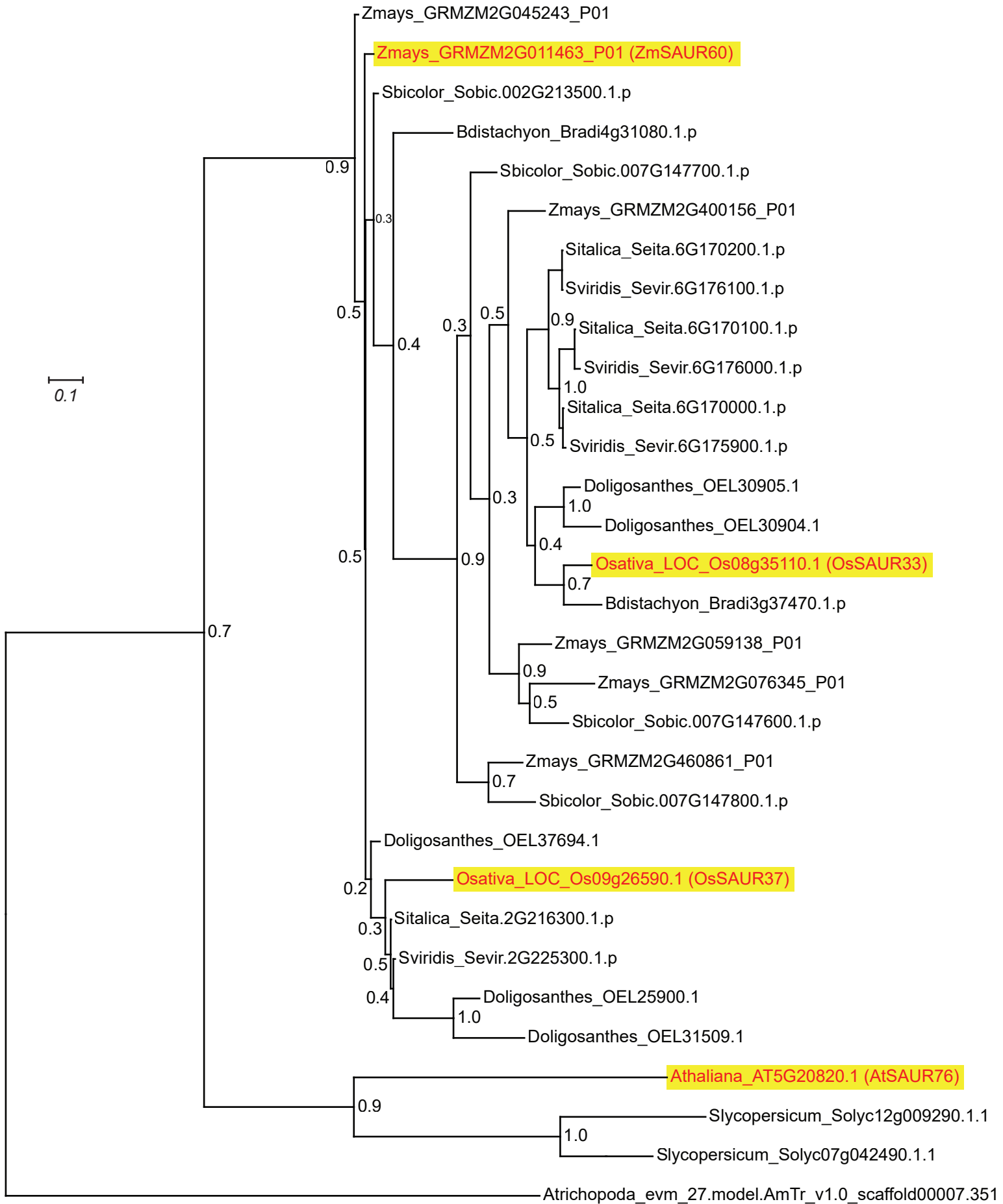
SUPPLEMENTARY FIGURE S3



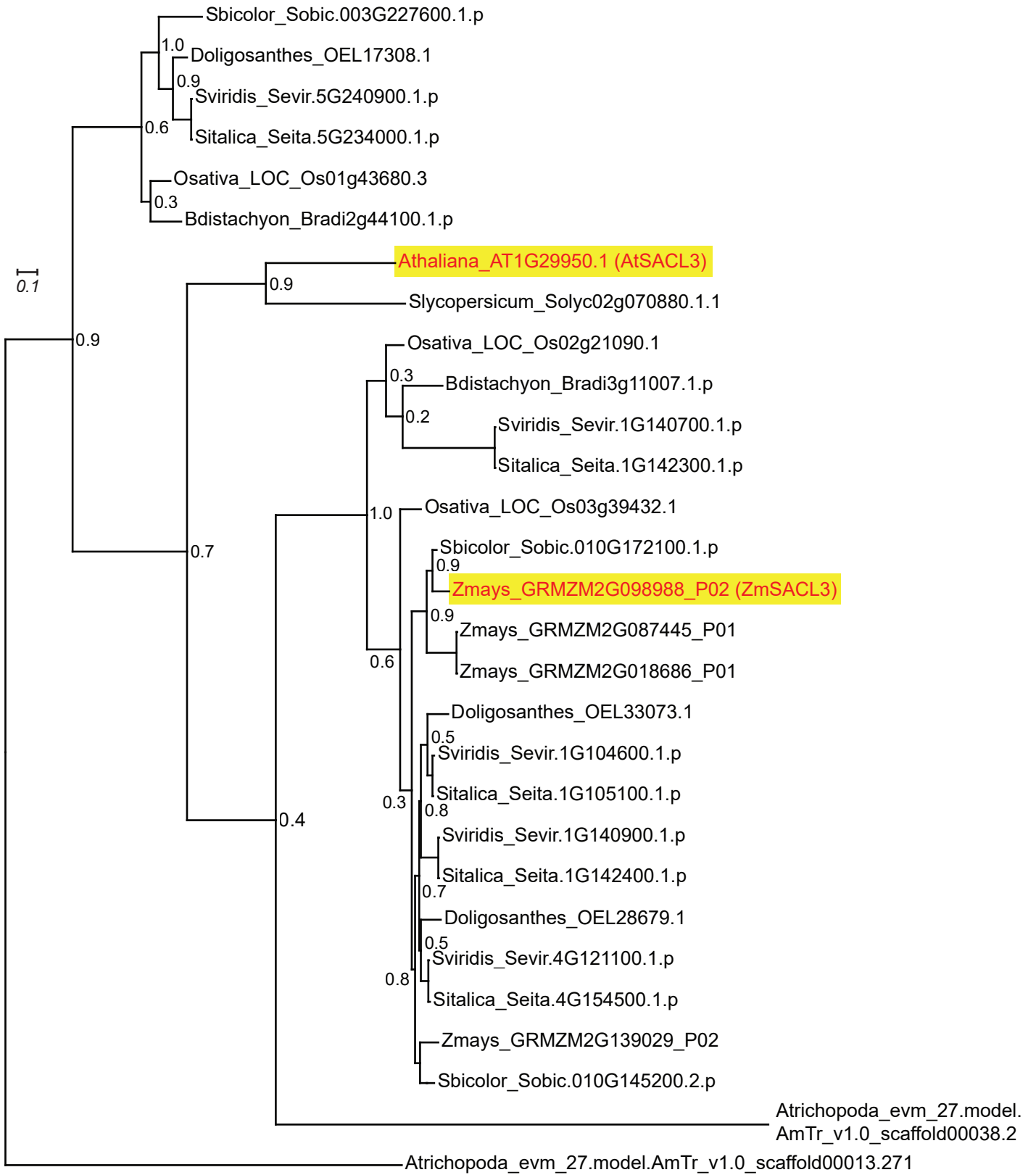
SUPPLEMENTARY FIGURE S4



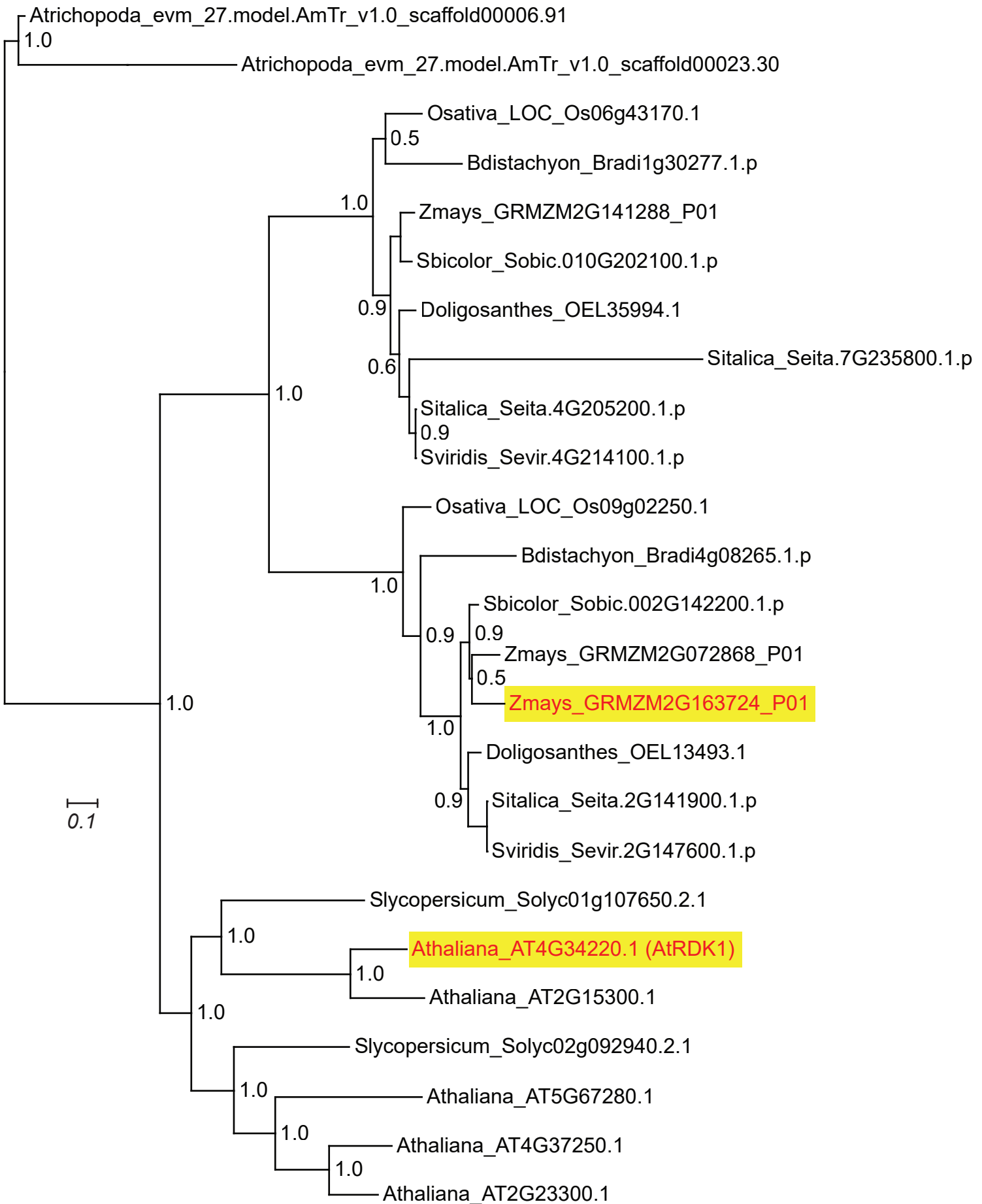
SUPPLEMENTARY FIGURE S5



SUPPLEMENTARY FIGURE S6

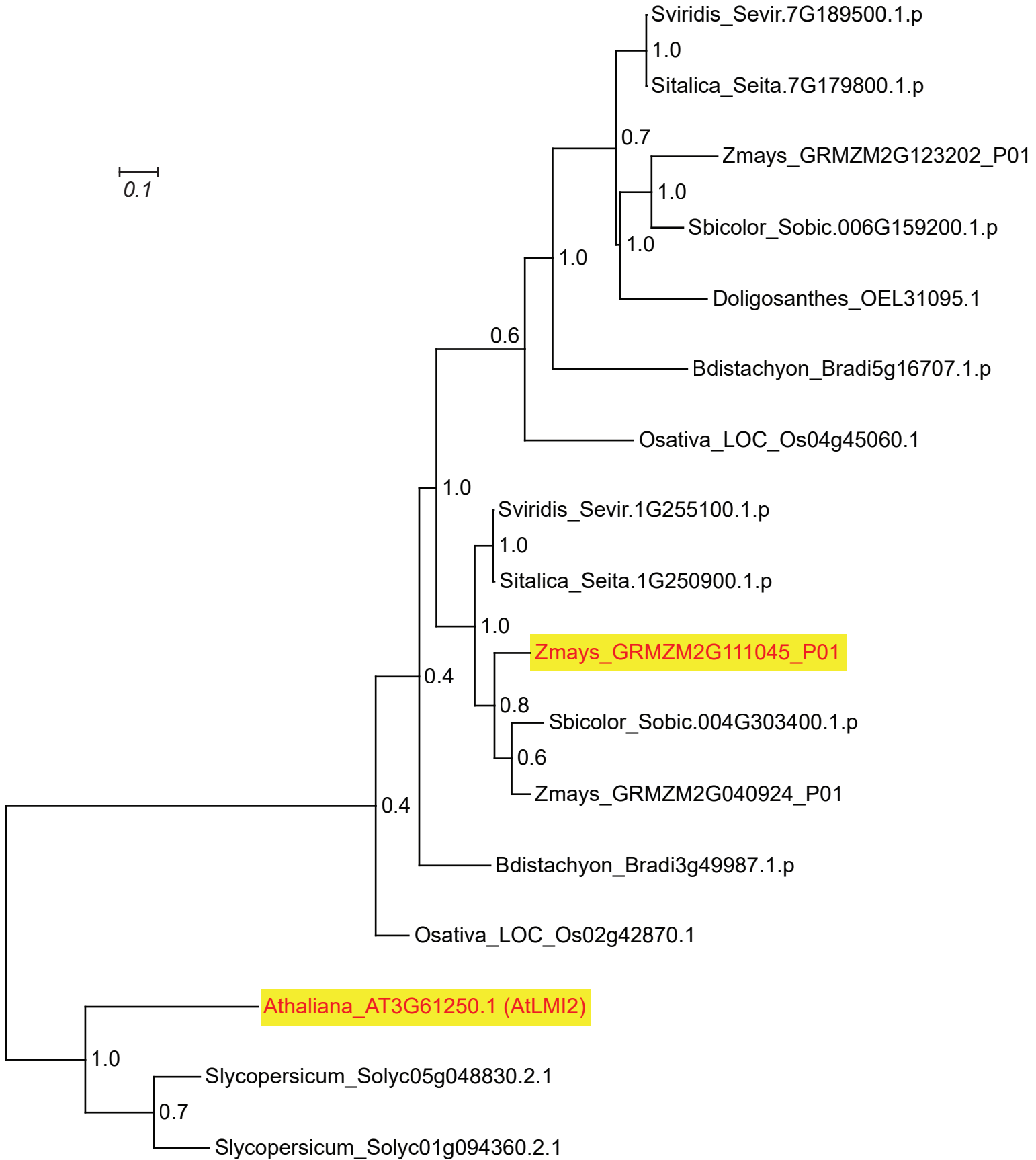


SUPPLEMENTARY FIGURE S7

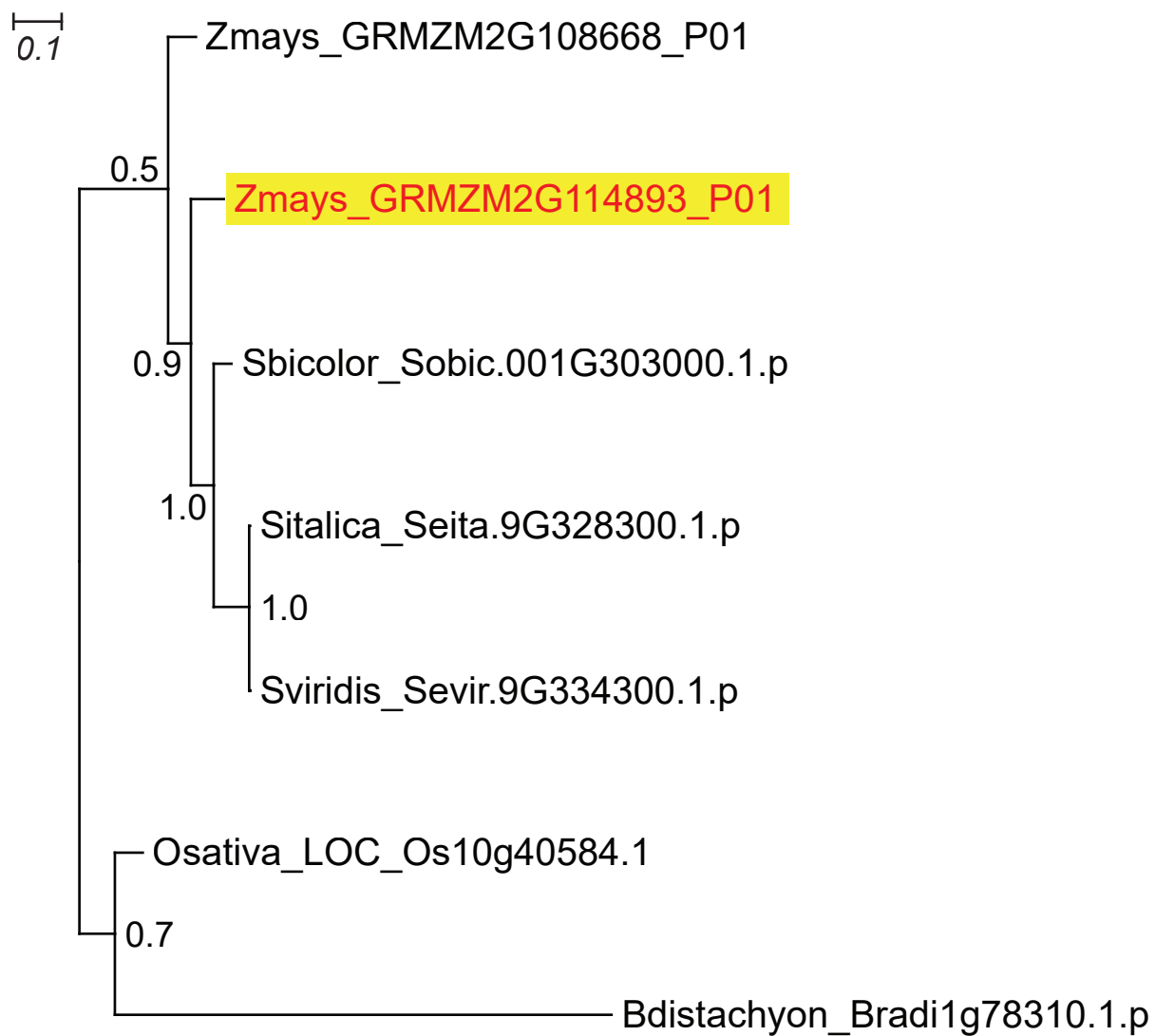


SUPPLEMENTARY FIGURE S8

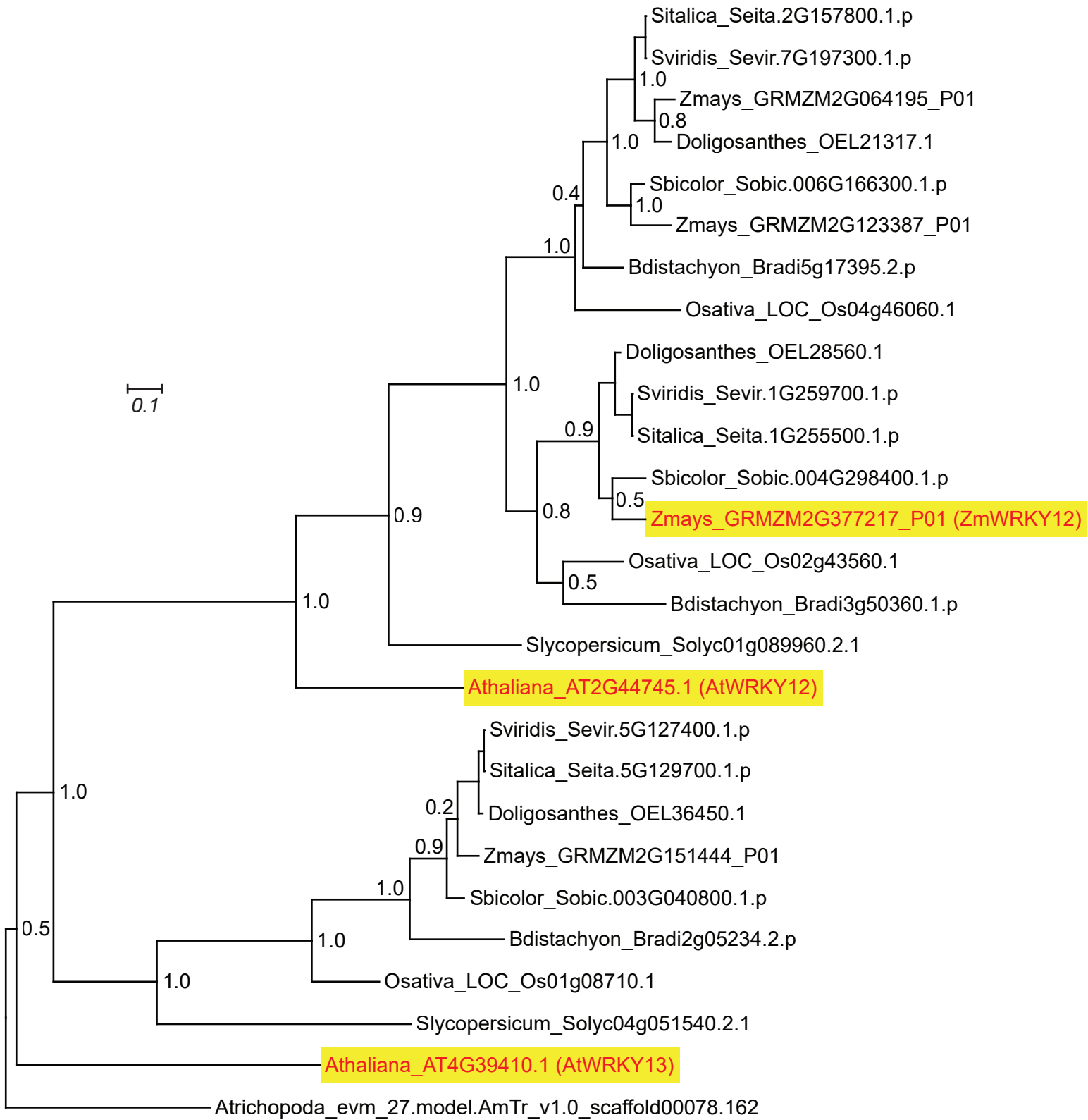
0.1



SUPPLEMENTARY FIGURE S9



SUPPLEMENTARY FIGURE S10



SUPPLEMENTARY FIGURE S11

