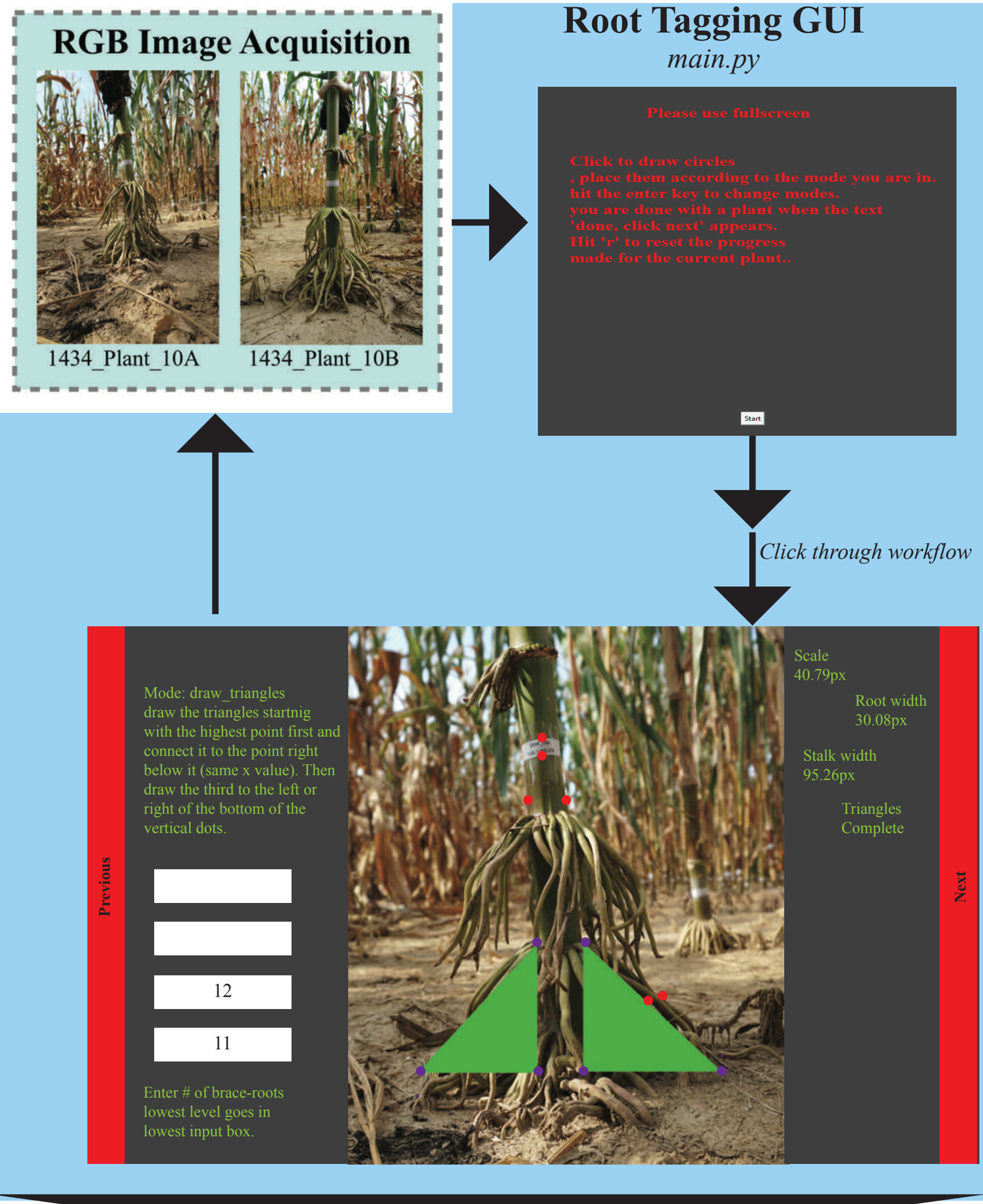
Figure S1. A ground-based brace root phenotyping robot (BRobot) was used for image capture. BRobot is a modified Superdroid LT2 Tracked ATR Robot Platform with a custom controller. The red arrow highlights the side-mounted FLIR 3.2 MP Color Blackfly camera that captures images.



Pixel Data

process_rootpixel_data.py

Scaled Phenotypes

Number of roots/whorl (not shown)
2 = Single root width
3 = Stalk width
4 = Height of whorl (*a*)
5 = Stalk-to-root grounding (*c*)
6 = Root angle (B)
7 = Spread width
8 = Number of whorls in soil

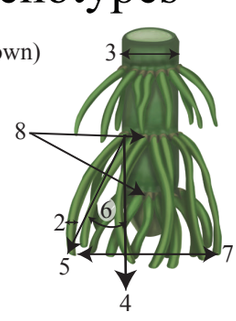


Figure S2. A semi-automated root tagging workflow was developed to optimize image processing. After RGB images are acquired, a python script (*main.py*) launches the root tagging graphical user interface (GUI). A tag of known size (0.5 inches) was placed on the stem prior to image acquisition for scale. First, the tag is selected for a pixel (px) scale, then each additional screen will prompt the user to click and/or record/identify specific regions in the image. The red dots shown on the image highlight the following phenotypes that have been tagged: 1) the number of pixels within a 0.5-inch region, 2) the stalk width, and 3) the single root width. The purple dots shown on the image highlight the right triangle that is used to identify the following phenotypes: 1) the height of the whorl, 2) the stalk-to-root grounding, and 3) the root angle. The number of roots within each whorl was counted and typed into the white boxes (shown on the left). This records the total number of roots per whorl and the number of whorls in the ground. After recording or identifying phenotypes, the user will hit "enter." After all phenotypes have been recorded, the user will hit "Next" (red bar on the right of the screen) to begin the next image. After completing all images, a python script (*process_rootpixel_data.py*) is used to convert pixel data to scaled phenotype data. All data is exported to a .csv file for processing and analysis.

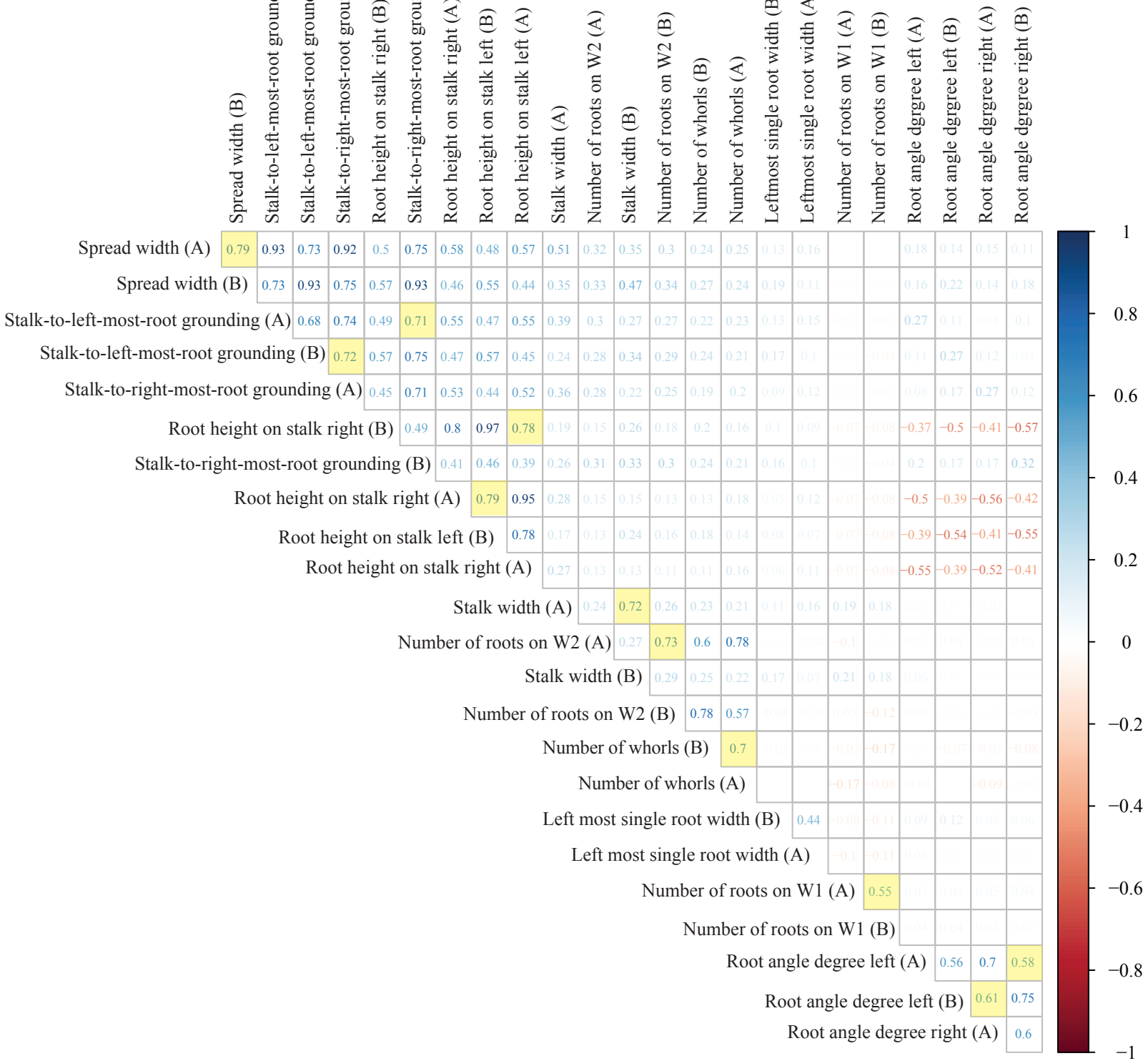

Spread width (B)
Stalk-to-left-most-root grounding (A)
Stalk-to-left-most-root grounding (B)
Stalk-to-right-most-root grounding (A)
Root height on stalk right (B)
Stalk-to-right-most-root grounding (B)
Root height on stalk right (A)
Root height on stalk left (B)
Root height on stalk left (A)
Stalk width (A)
Number of roots on W2 (A)
Stalk width (B)
Number of roots on W2 (B)
Number of whorls (B)
Number of whorls (A)
Leftmost single root width (B)
Leftmost single root width (A)
Number of roots on W1 (A)
Number of roots on W1 (B)
Root angle dgrgree left (A)
Root angle dgrgree left (B)
Root angle dgrgree right (A)
Root angle dgrgree right (B)
Spread width (A)
Spread width (B)
Stalk-to-left-most-root grounding (A)
Stalk-to-left-most-root grounding (B)
Stalk-to-right-most-root grounding (A)
Root height on stalk right (B)
Stalk-to-right-most-root grounding (B)
Root height on stalk right (A)
Root height on stalk left (B)
Root height on stalk right (A)
Stalk width (A)
Number of roots on W2 (A)
Stalk width (B)
Number of roots on W2 (B)
Number of whorls (B)
Number of whorls (A)
Left most single root width (B)
Left most single root width (A)
Number of roots on W1 (A)
Number of roots on W1 (B)
Root angle degree left (A)
Root angle degree left (B)
Root angle degree right (A)
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure S3. Phenotypes extracted from left and right images were highly correlated. Left and right images (labeled A and B) were acquired for each plant and phenotypes were extracted from images with the root tagging GUI. A Pearson correlation analysis was run to determine the precision of our root tagging GUI. Cells highlighted in yellow indicate the same phenotype from both sides of the plant.

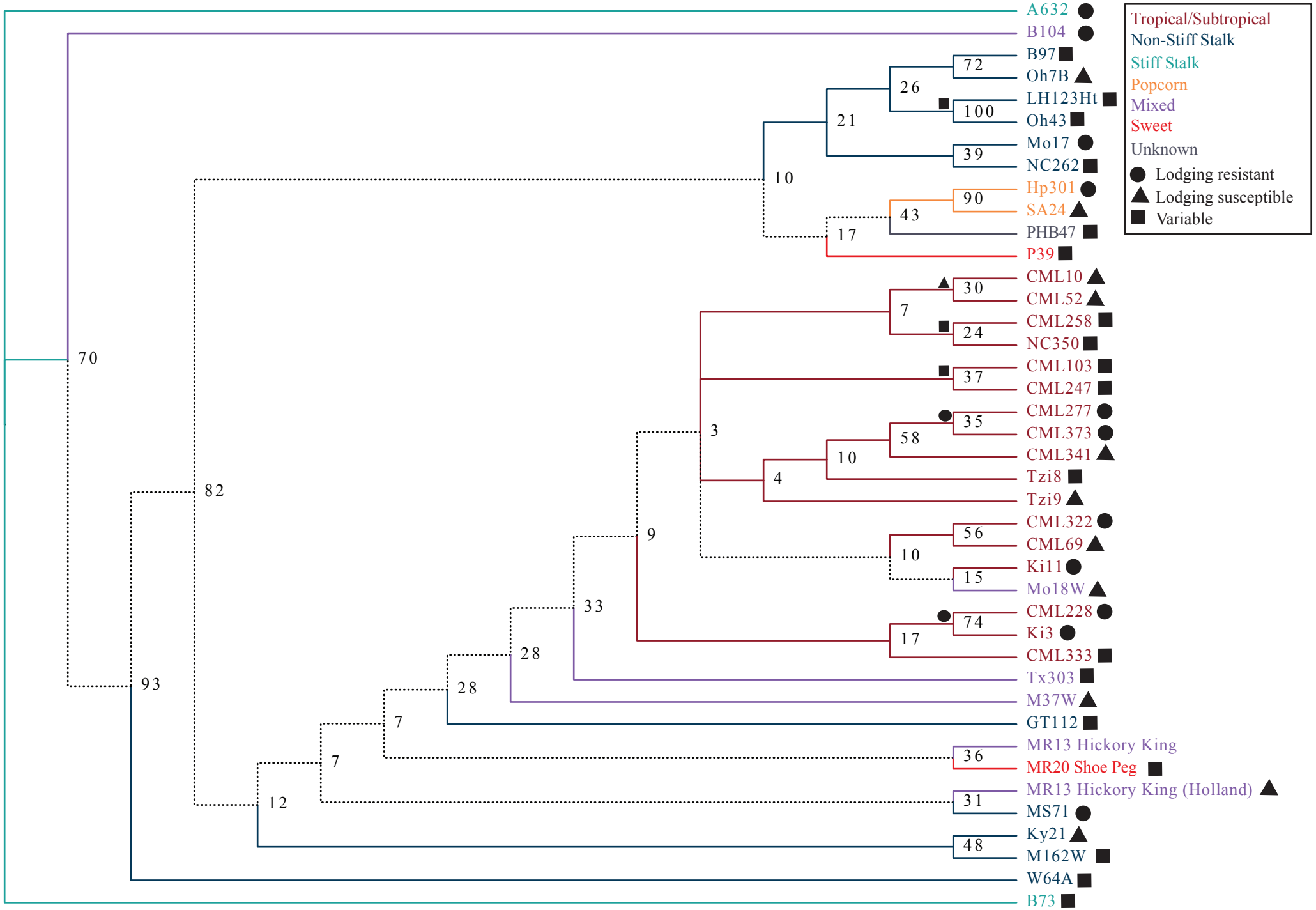


Figure S4. Root lodging is not monophyletic. A species tree was generated for 41 of the 52 maize inbred genotypes included in this study. Genotype names are color coded according to their assigned subpopulation information [Flint-Garcia et al (2005) and Liu et al (2003)]. If all genotypes within a clade are from the same subpopulation, branches are solid and colored with the respective subpopulation. Branches are dotted if the clade includes genotypes from more than one subpopulation. Genotypes were identified as lodging resistant or variable per Figure 2. The assigned lodging classification is illustrated to the right of the genotype with a circle or square. Clades where all genotypes within the clade are a part of a single lodging classification are highlighted with the corresponding shape at the node.

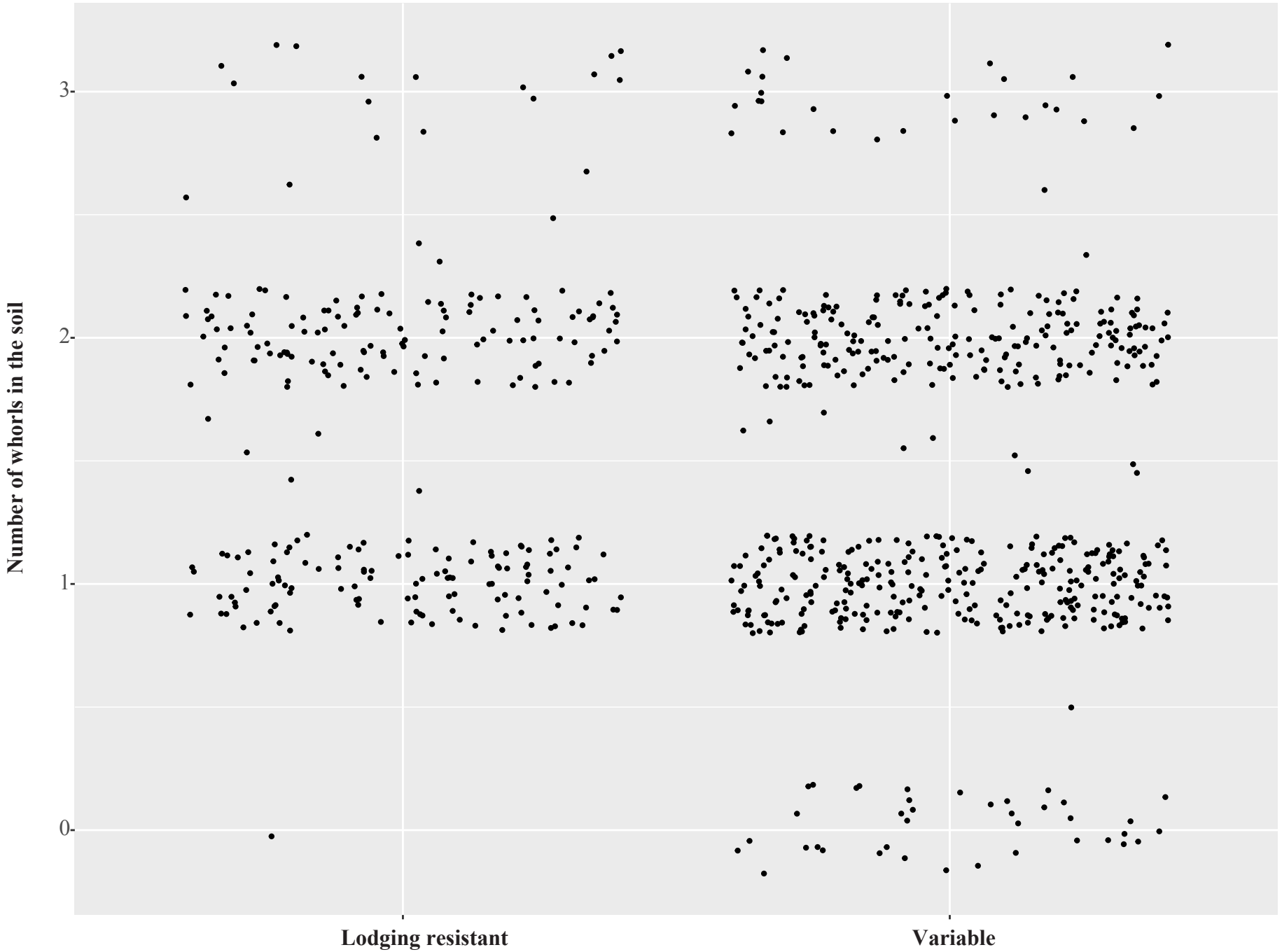


Figure S5. Root lodging resistant genotypes had more brace root whorls in the ground. Images were acquired with BRobot one day before Tropical Storm Isaias. From the RGB images, the number of brace root whorls that entered the ground was extracted. Genotypes were classified as lodging resistant or variable according to Figure 2. Genotypes that were root lodging resistant had more brace root whorls in the ground compared to those that were root lodging susceptible ($p<0.05$).

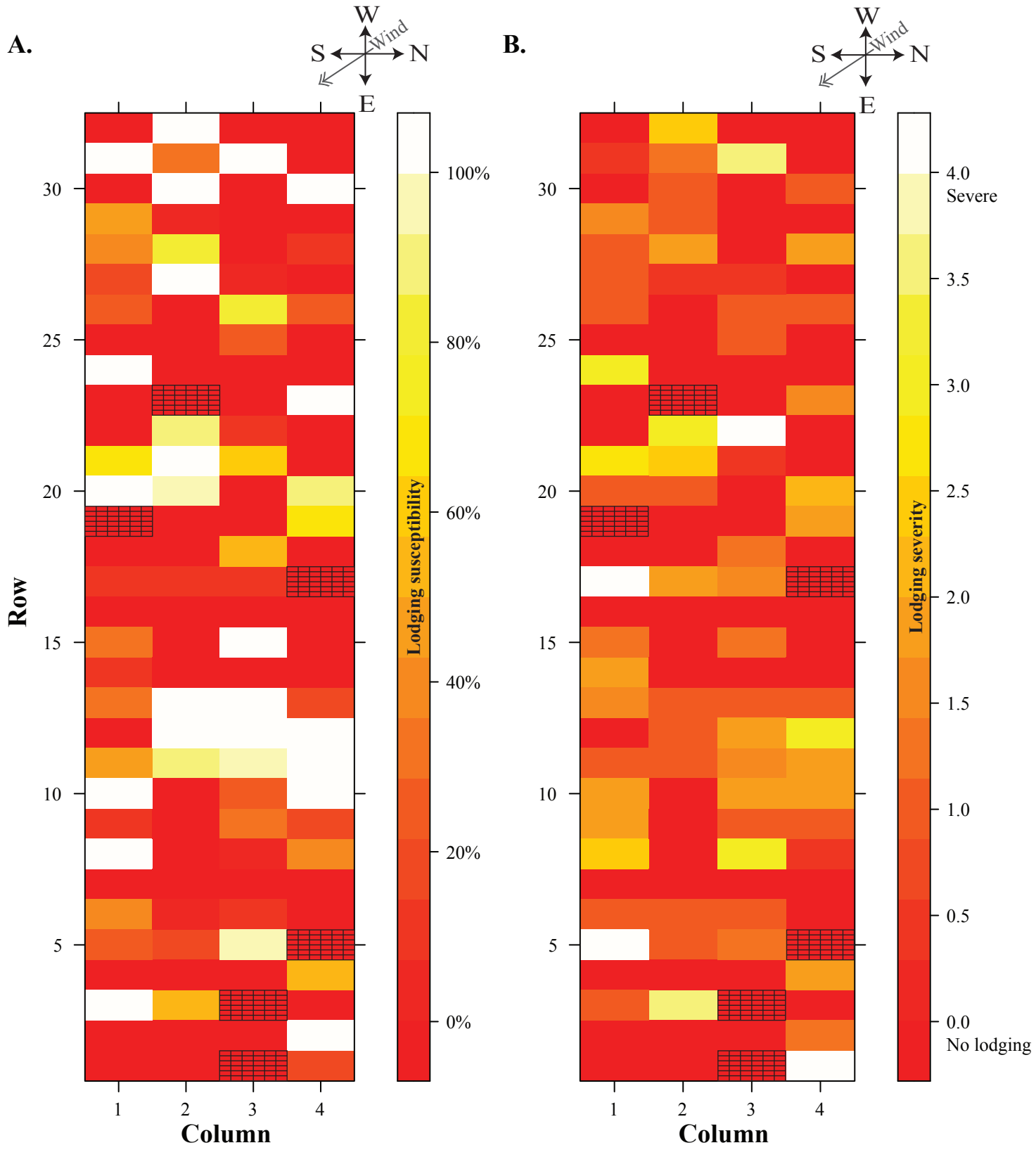
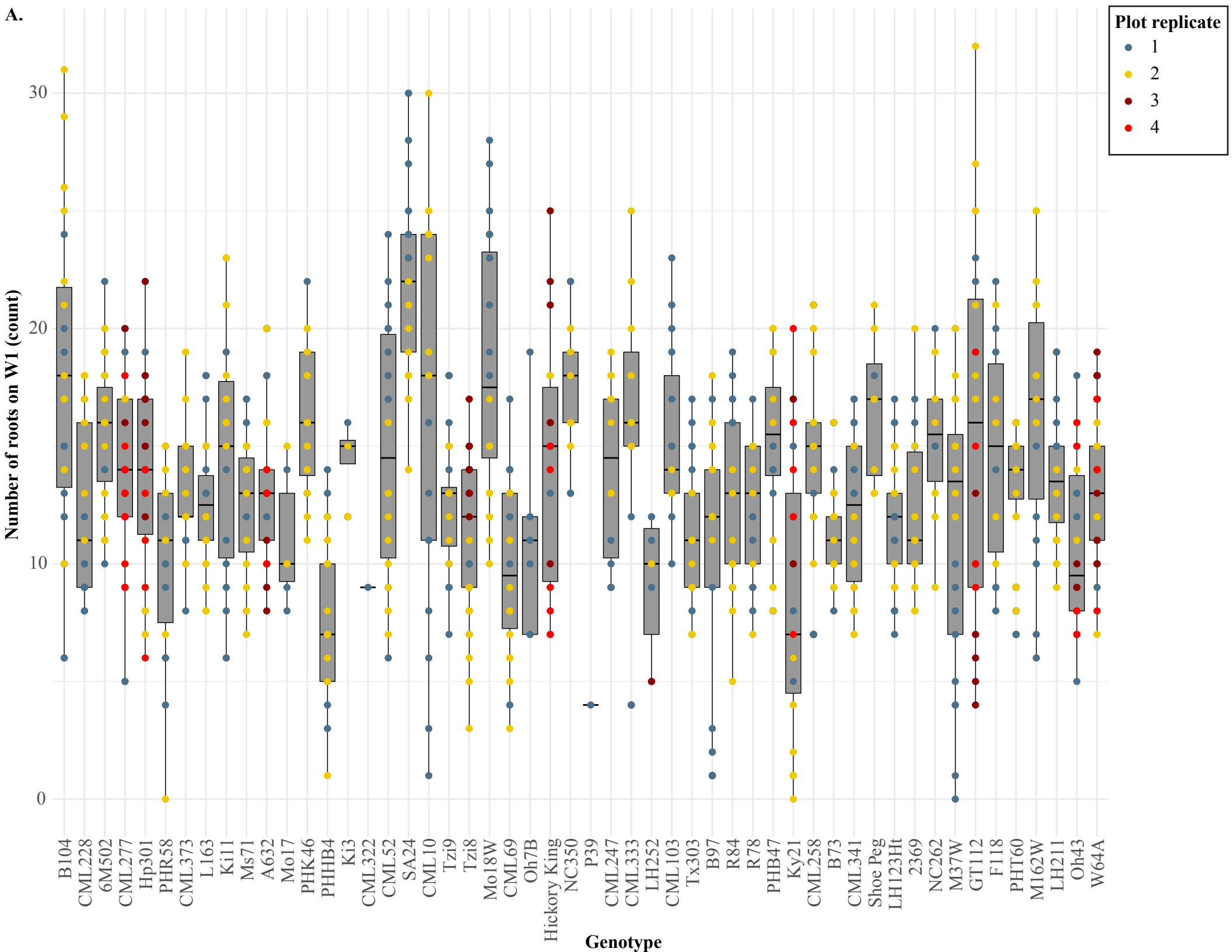
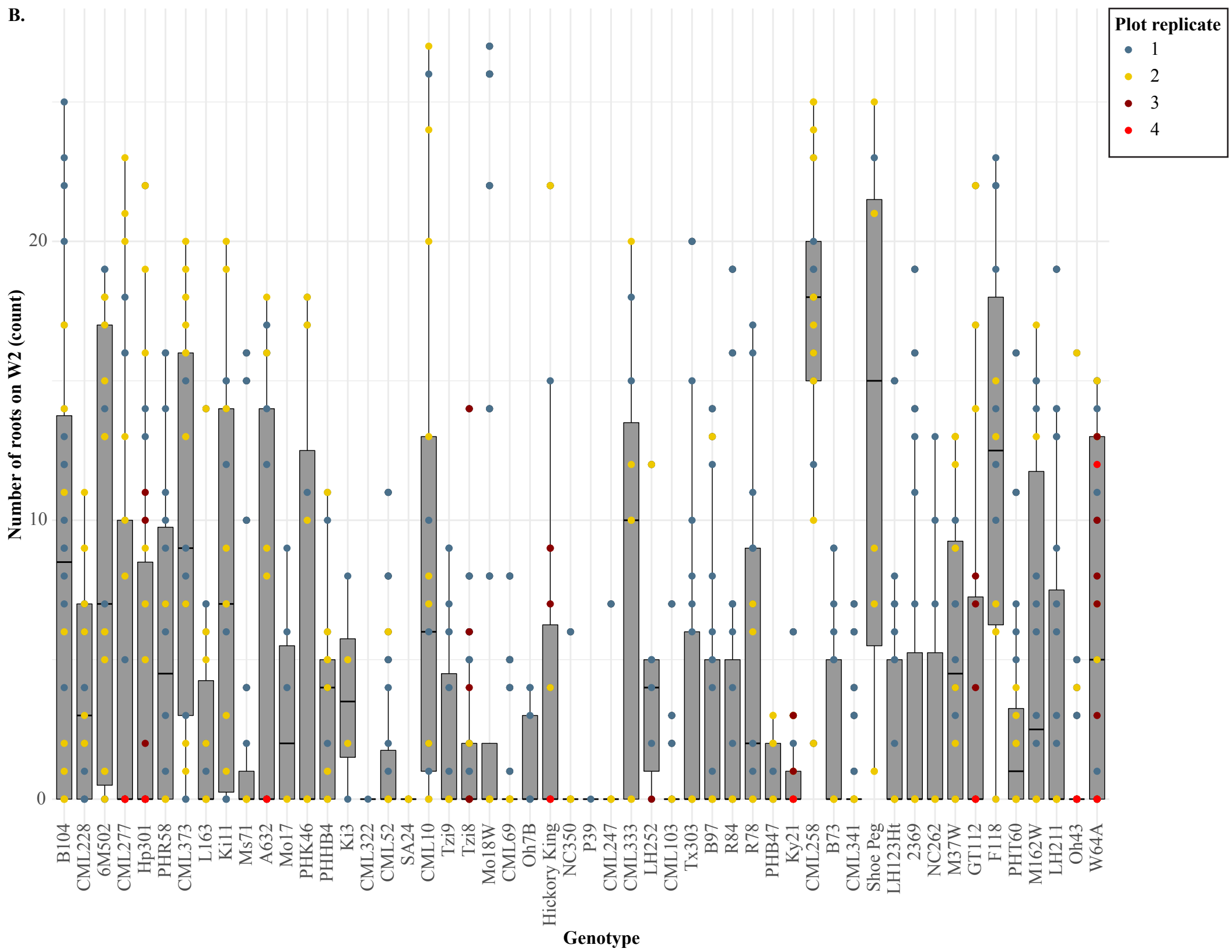
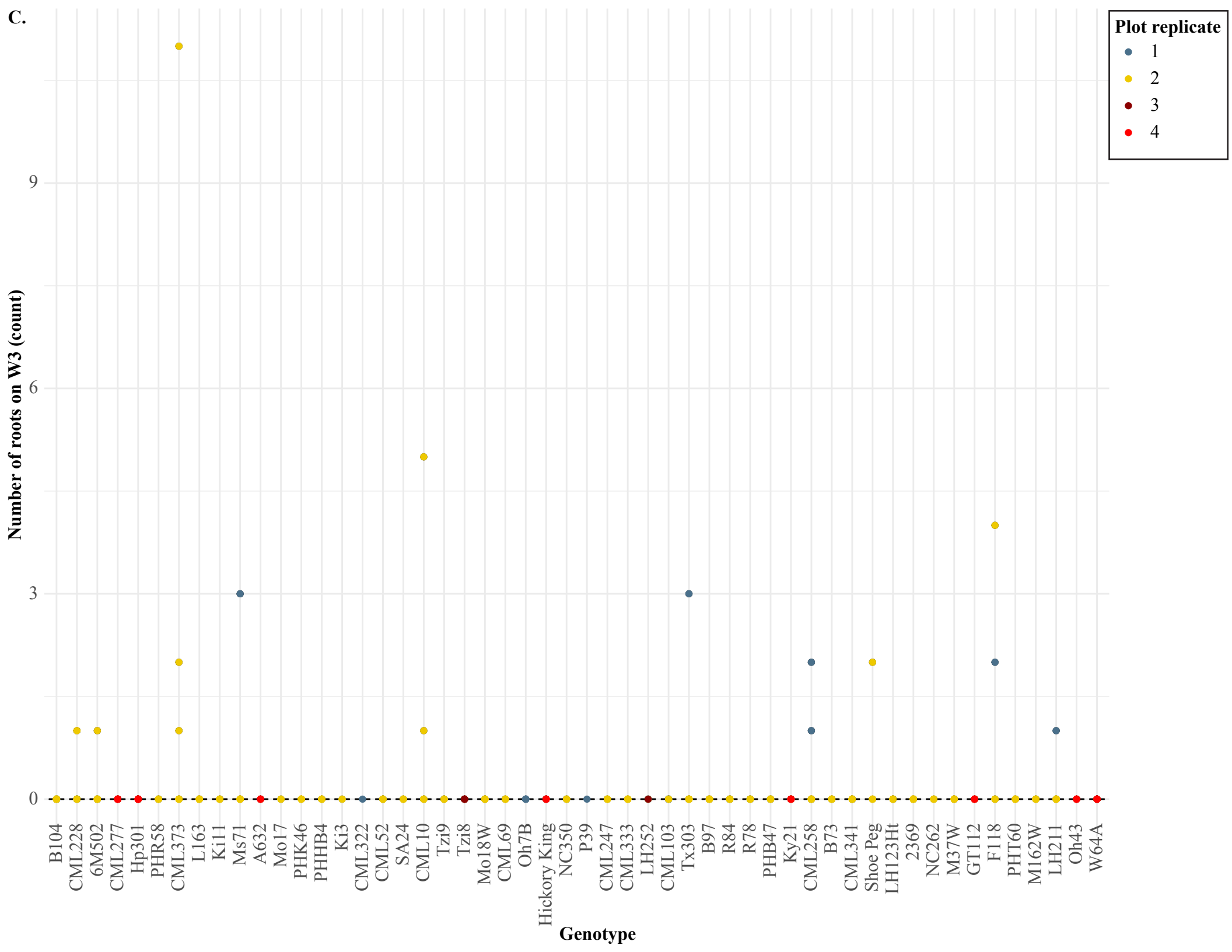
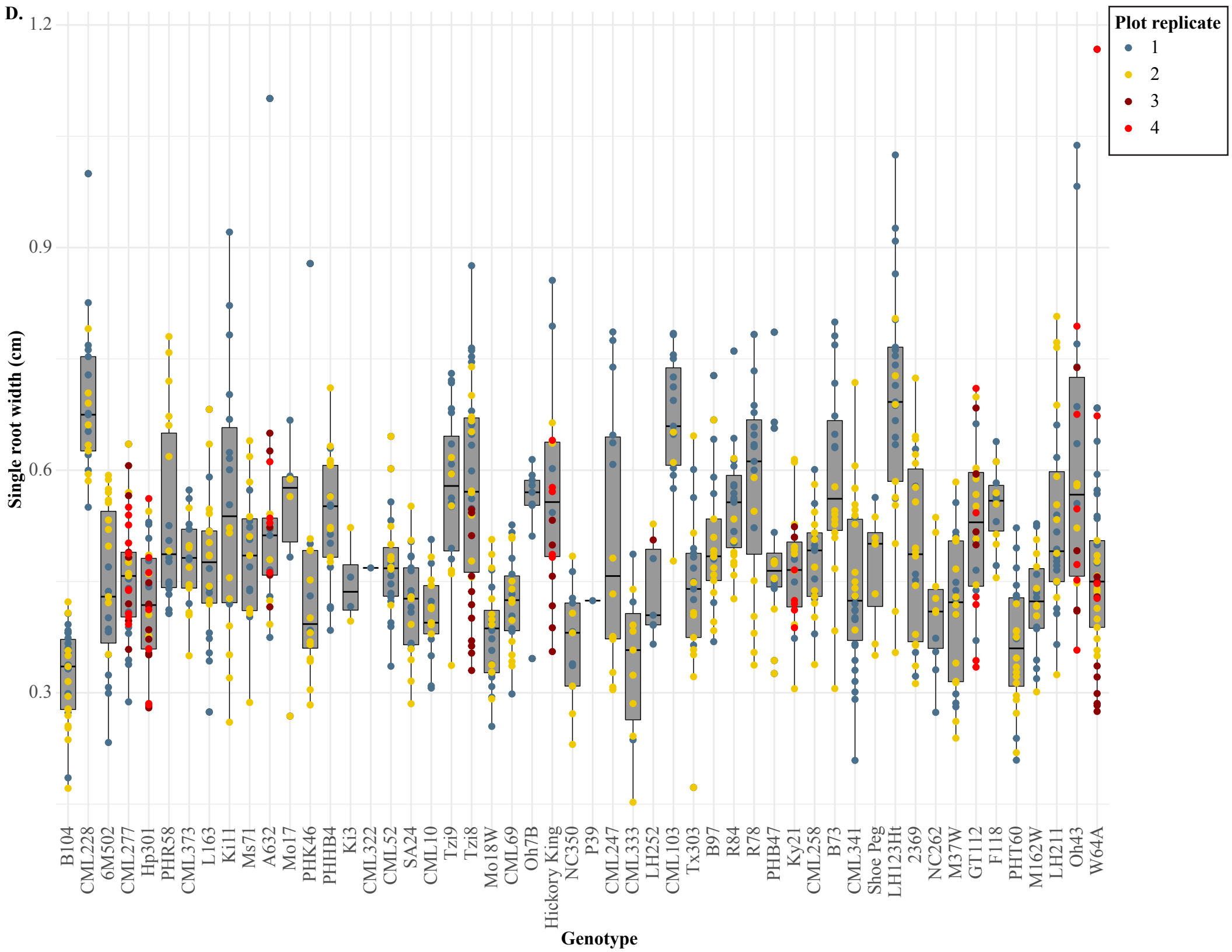


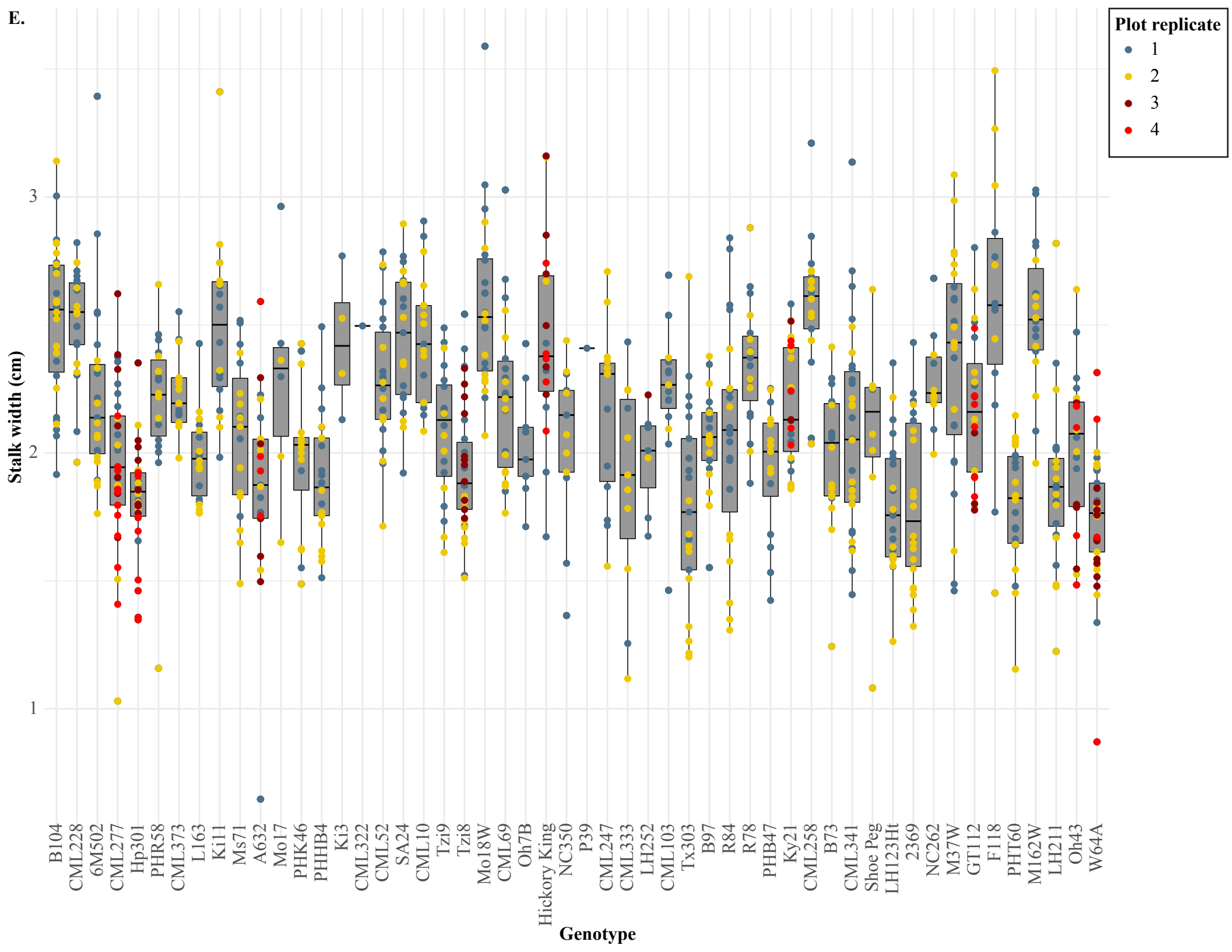
Figure S6. Root lodging was not related to field position. Winds from the West and North-West induced root lodging. (A) Plots were colored according to the susceptibility of root lodging within each plot. Plots with 100% root lodging were highlighted with white, whereas plots that had 0% root lodging were highlighted with dark red. (B) Plots were colored according to the average severity score within the plot. Plots that are highlighted in white are plots where plants were on the ground (90-degree displacement), whereas plots that are highlighted in dark red are plots where plants remained vertical (lack of root lodging). (A-B) Plots with a grid overlaid indicate plots that did not germinate.

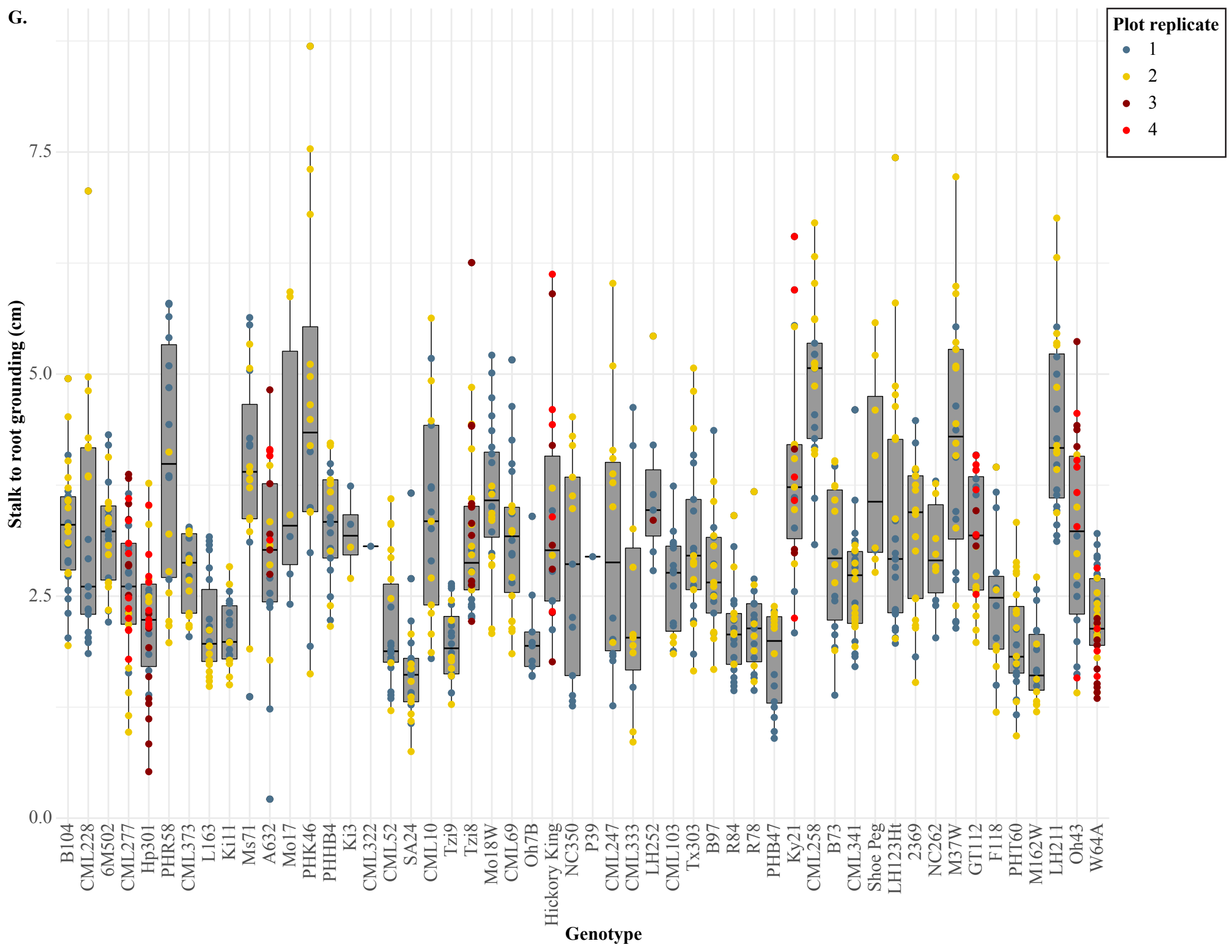

A.
Plot replicate
1
2
3
4
Number of roots on W1 (count)
0
10
20
30
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype

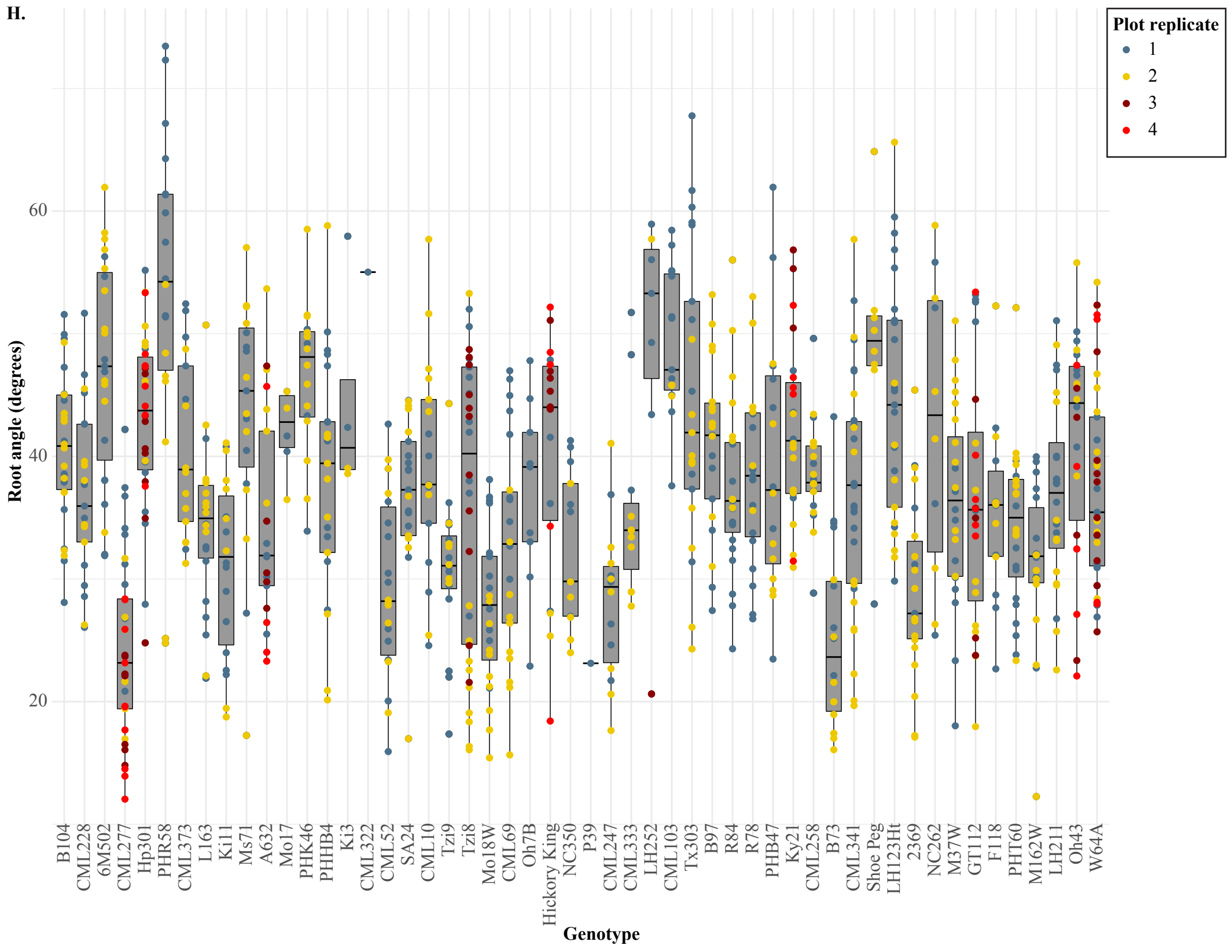

B.
Plot replicate
1
2
3
4
Number of roots on W2 (count)
0
10
20
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype

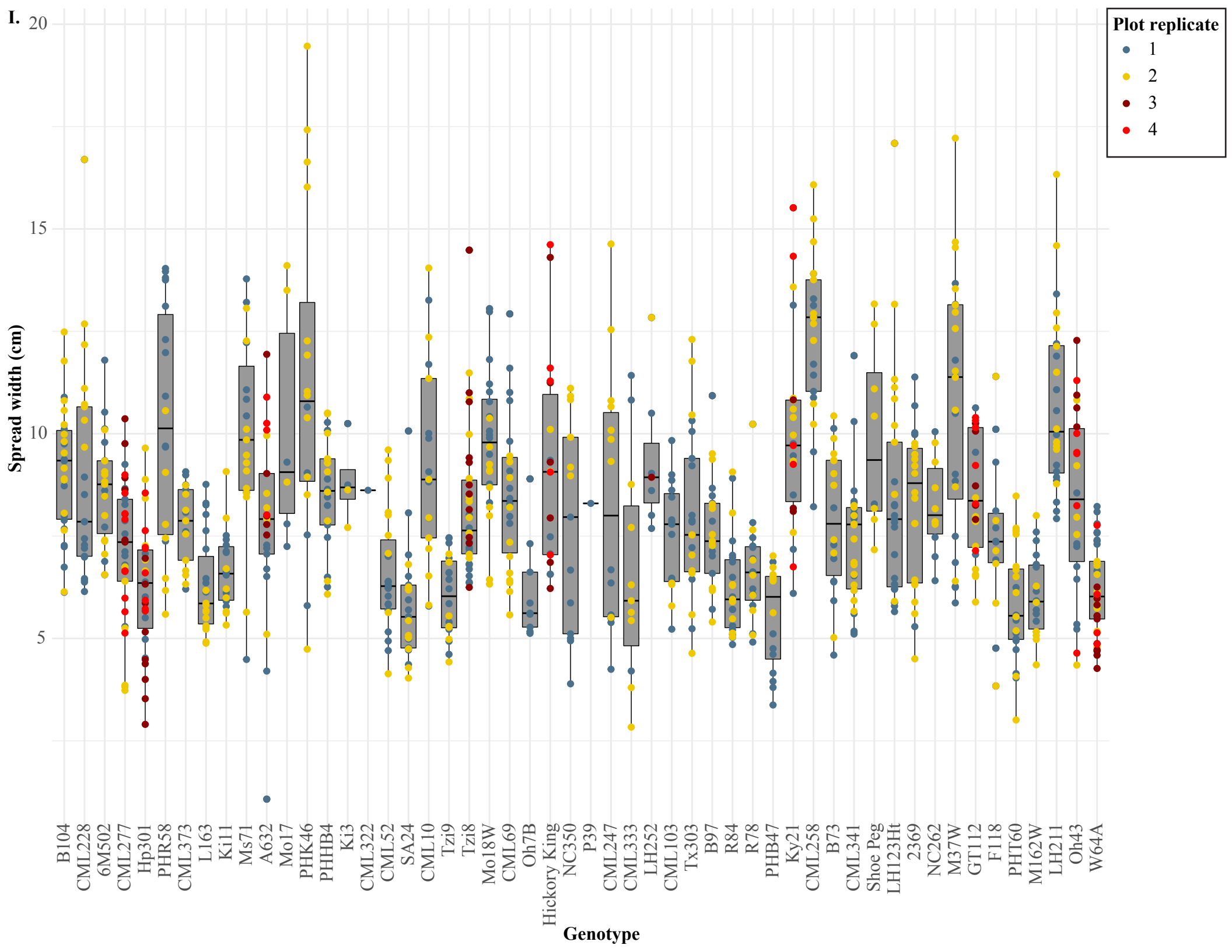

C.
Plot replicate
1
2
3
4
Number of roots on W3 (count)
0
3
6
9
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype

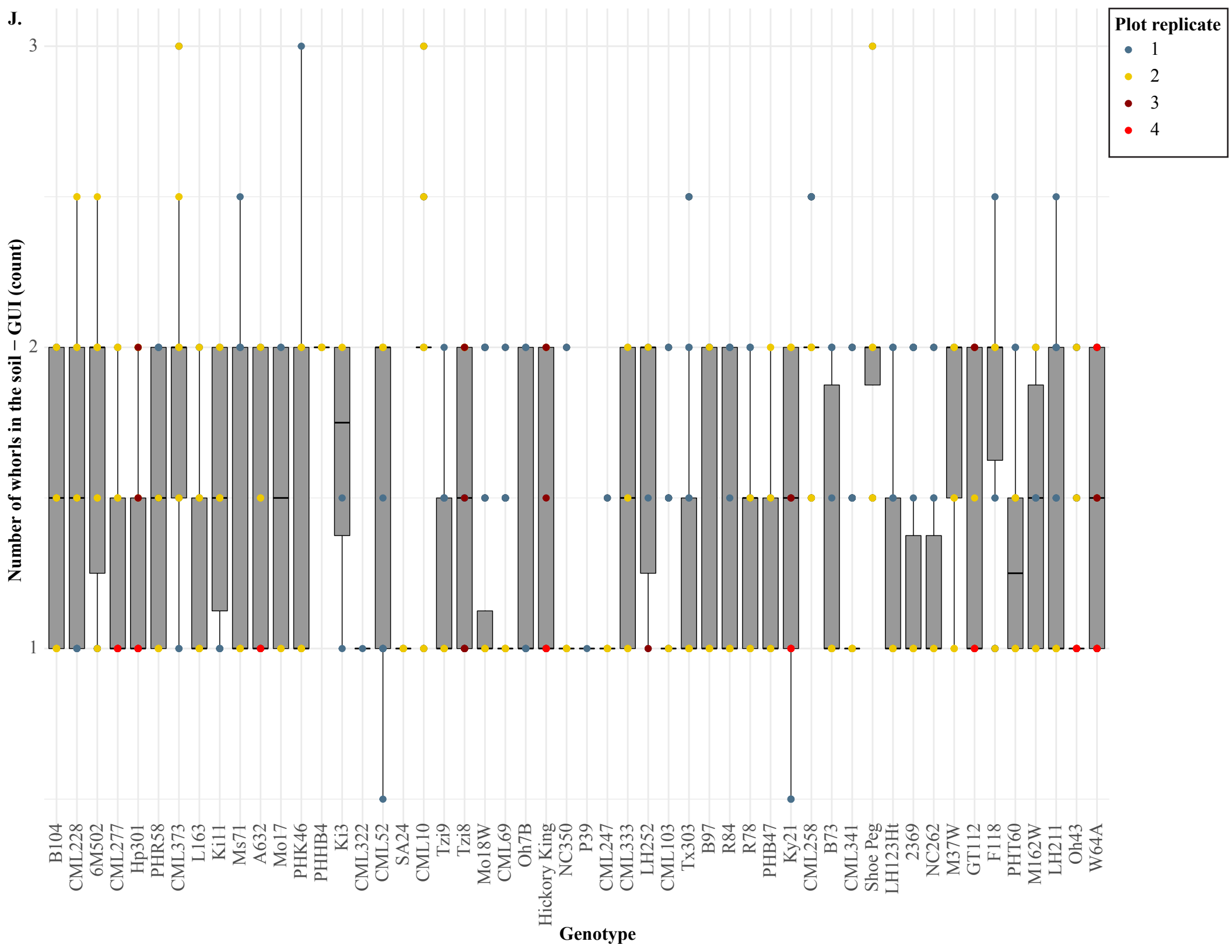

D.
Plot replicate
1
2
3
4
Single root width (cm)
1.2
0.9
0.6
0.3
Genotype
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A

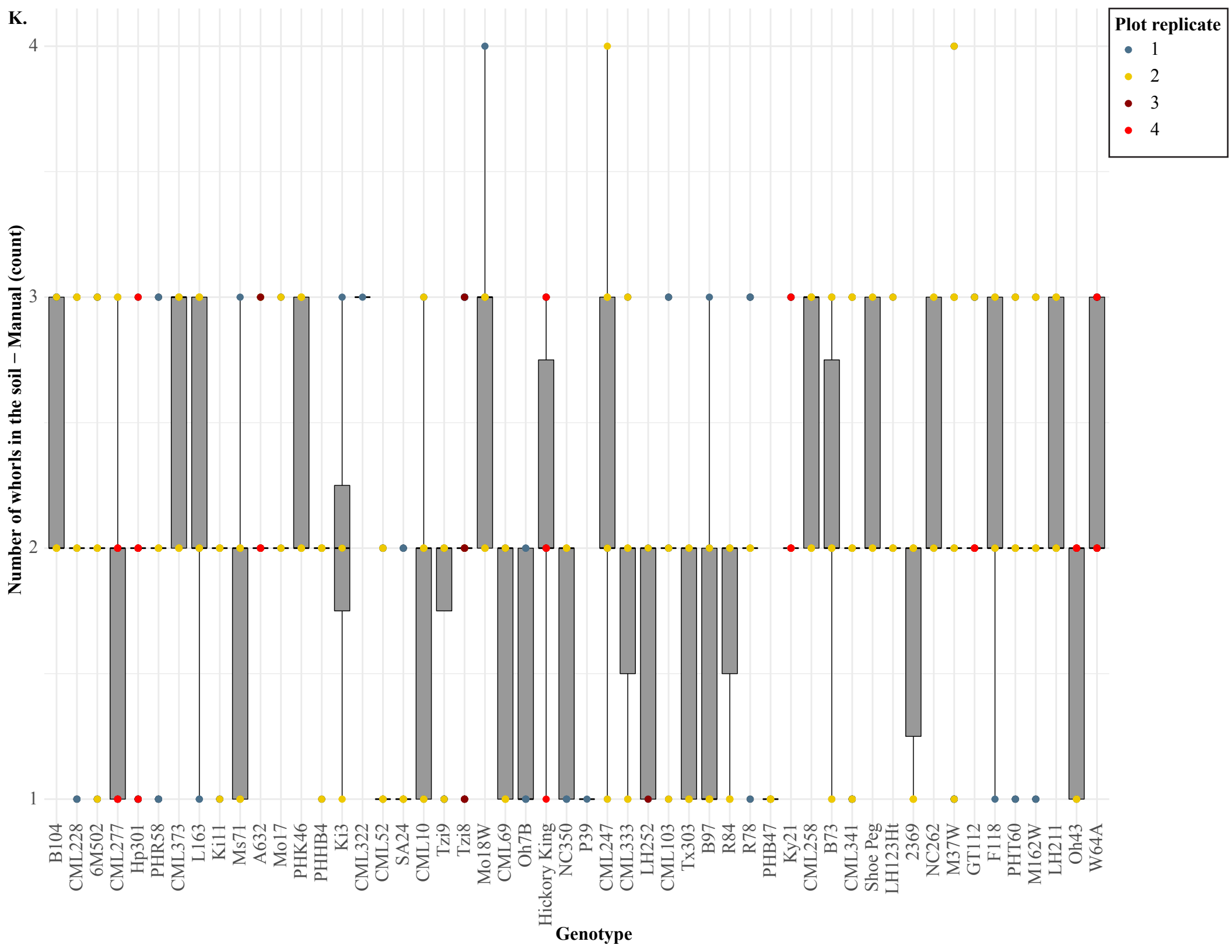

E.
Plot replicate
1
2
3
4
Stalk width (cm)
3
2
1
Genotype
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A


G.
Plot replicate
1
2
3
4
Stalk to root grounding (cm)
0.0
2.5
5.0
7.5
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype


H.
Plot replicate
1
2
3
4
Root angle (degrees)
60
40
20
Genotype
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A


I.
Spread width (cm)
20
15
10
5
Plot replicate
1
2
3
4
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype


J.
Plot replicate
1
2
3
4
Number of whorls in the soil – GUI (count)
3
2
1
Genotype
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A


K.
Plot replicate
1
2
3
4
Number of whorls in the soil – Manual (count)
4
3
2
1
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype

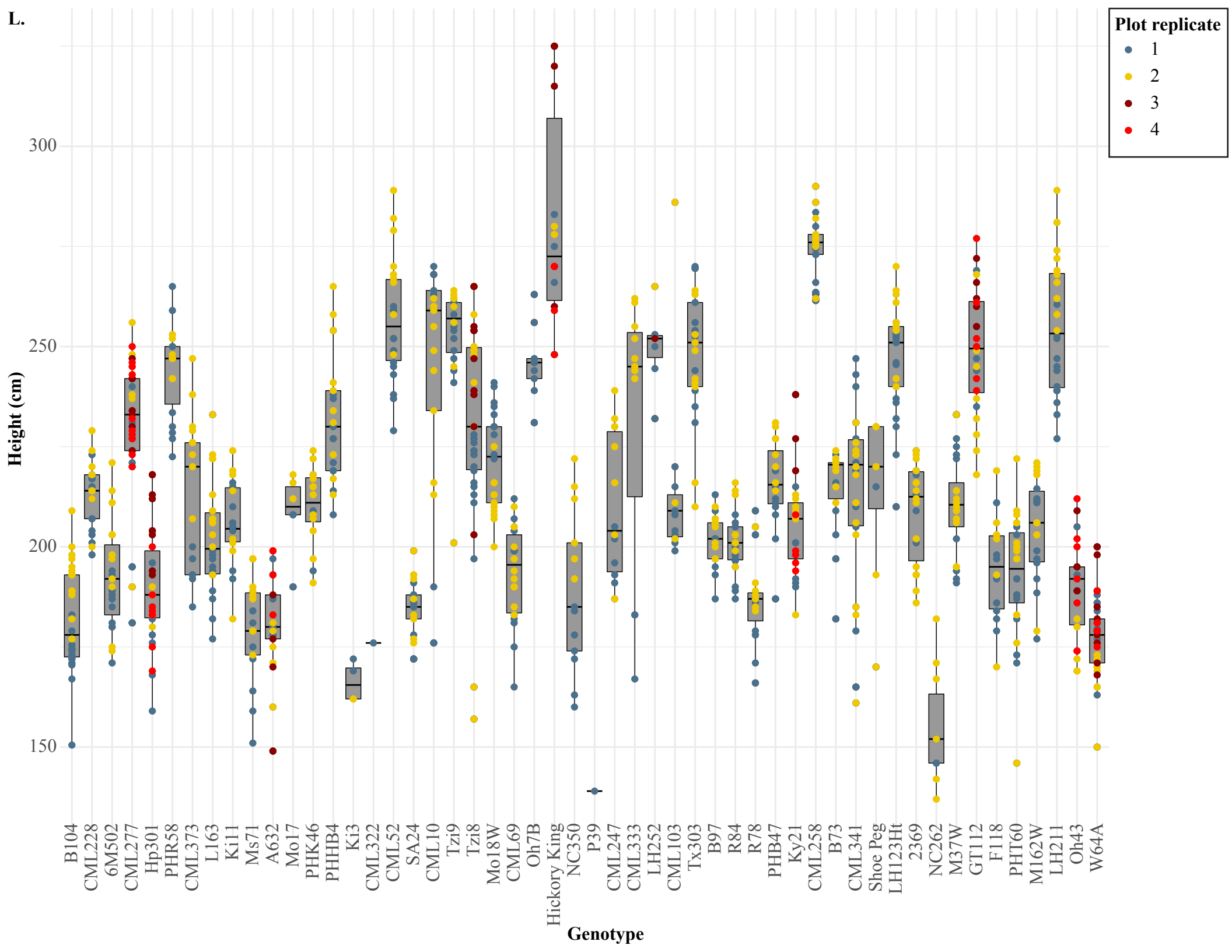

L.
Plot replicate
1
2
3
4
Height (cm)
300
250
200
150
B104
CML228
6M502
CML277
Hp301
PHR58
CML373
L163
Ki11
Ms71
A632
Mo17
PHK46
PHHB4
Ki3
CML322
CML52
SA24
CML10
Tzi9
Tzi8
Mo18W
CML69
Oh7B
Hickory King
NC350
P39
CML247
CML333
LH252
CML103
Tx303
B97
R84
R78
PHB47
Ky21
CML258
B73
CML341
Shoe Peg
LH123Ht
2369
NC262
M37W
GT112
F118
PHT60
M162W
LH211
Oh43
W64A
Genotype

Figure S7. Plant phenotypes vary among genotypes. The following phenotypes vary among genotypes: (A) The number of roots on whorl 1 (the whorl closest to the ground, bottom whorl), (B) the number of roots on whorl 2 (middle whorl), (C) the number of roots on whorl 3 (top whorl), (D) the single root width, (E) the stalk width, (F) the root height on stalk, (G) the stalk-to-root grounding, (H) the root angle, (I) the spread width, (J) the number of whorls in the ground, quantified from the GUI, (K) the number of whorls in the ground, quantified manually, and (L) plant height. (A-L) Genotypes are ordered by root lodging classification as in Figure 2. The color of each dot illustrates the replicate plot where the phenotype data is from. Outliers are outlined in black.

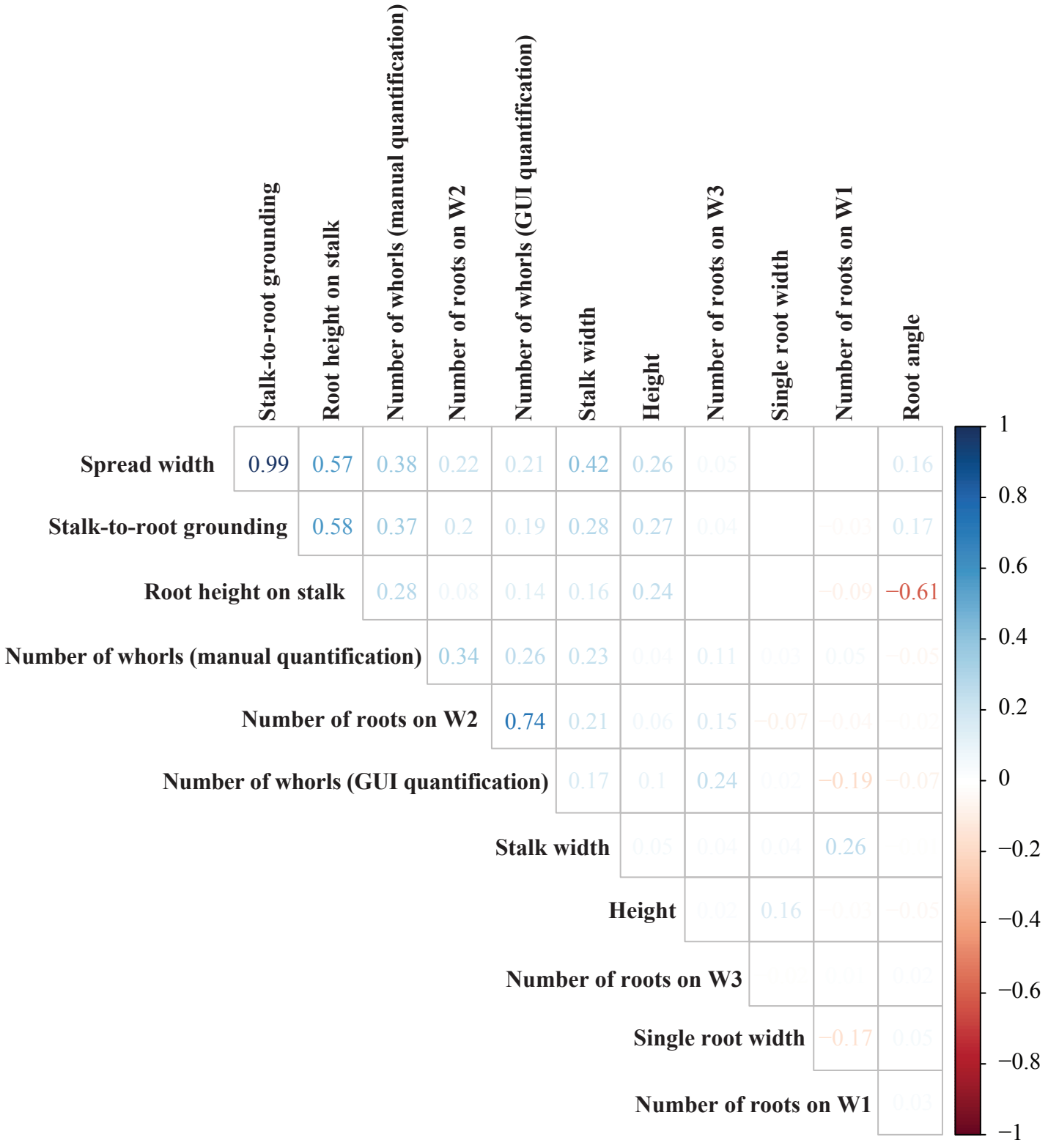


Figure S8. Brace root phenotypes are correlated with each other. A Pearson correlation analysis shows which phenotypes are highly correlated.

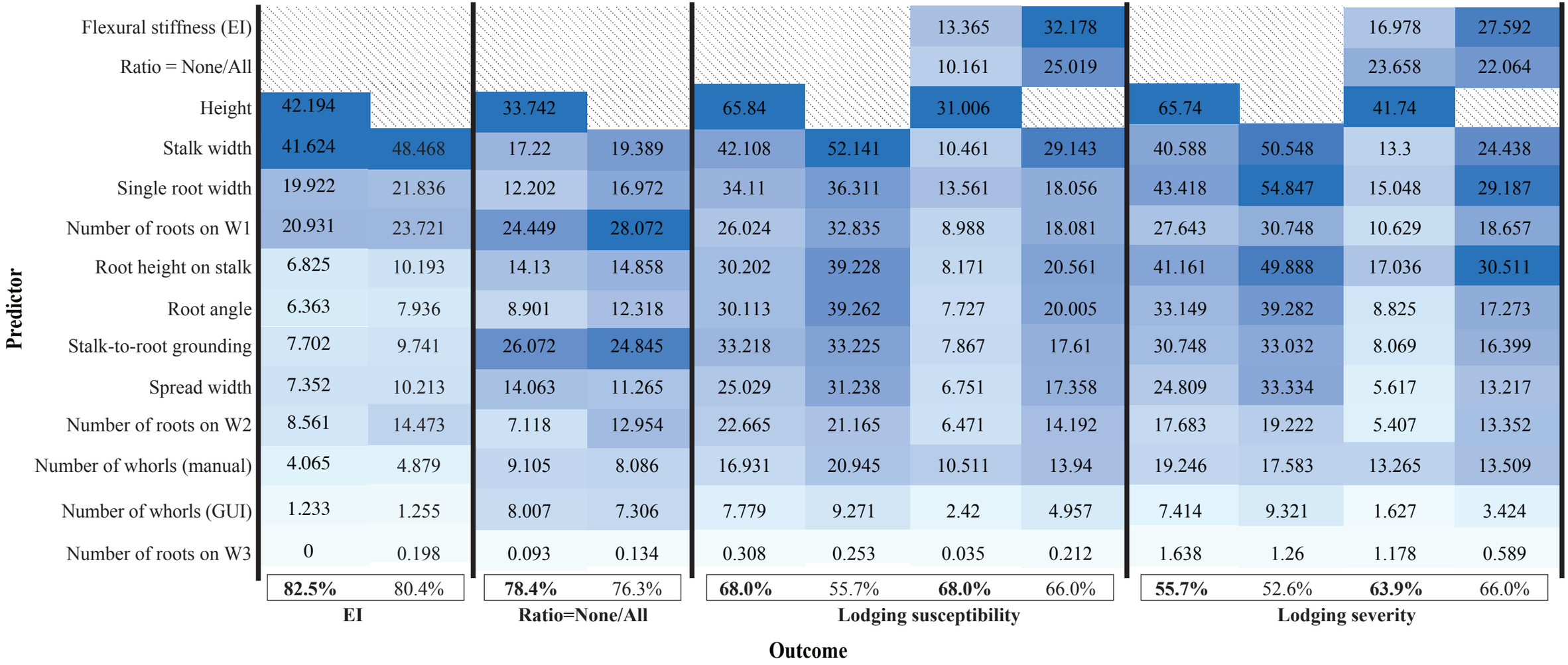


Figure S9. The inclusion of plant height does not increase model prediction accuracy. A random forest model was generated to predict stalk flexural stiffness, the None/All ratio, root lodging susceptibility, and root lodging severity. For each of the models generated, predictors with a higher mean decrease in Gini value were more important compared to those with a lower mean decrease in Gini value. A darker shade of blue indicated predictors that were more important, whereas a lighter shade of blue/white indicated predictors that were less important in successfully predicting the outcome. Although there was not an increase in model accuracy, plant height became the most important predictor.