GrasVIQ: An Image Analysis Framework for Automatic Quantification of Veins in Grass Leaves

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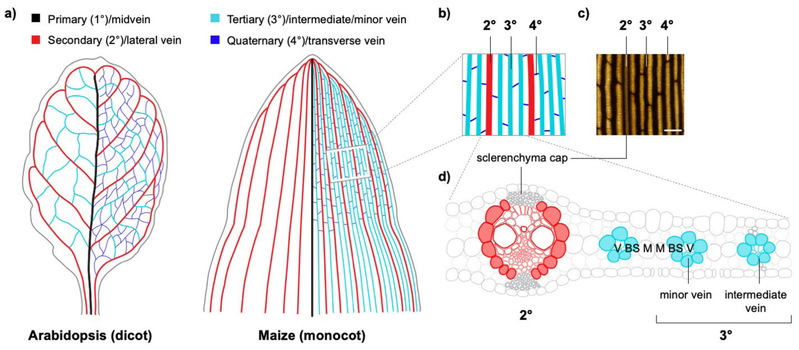
Abstract

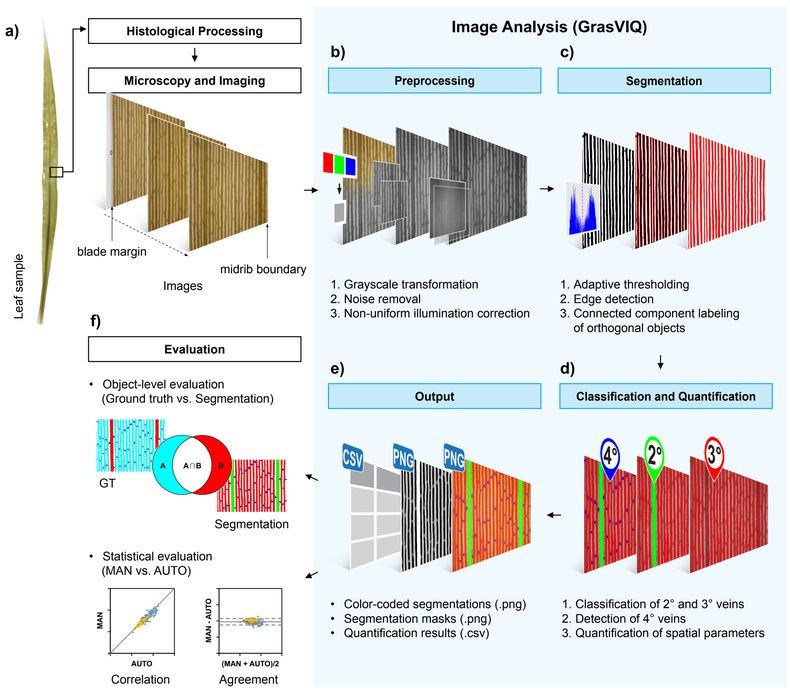
Leaf veins facilitate transport and provide mechanical support to the leaf and have critical implications for the performance and productivity of the plant and the ecosystem. Computational image analysis programs have been developed to extract and quantify vein traits from the reticulate venation of dicots, but a dedicated program for the parallel venation of monocots, particularly grasses, has yet to be developed. To address the need for high throughput vein phenotyping in grass species, like *Oryza sativa* (rice) and *Zea mays* (maize), we developed the Grass Vein Image Quantification (GrasVIQ) framework which automatically segments and quantifies vein patterns from images of cleared leaf pieces using classical computer vision techniques. Using image datasets from inbred lines and auxin mutants in maize, we demonstrate that GrasVIQ can perform high throughput quantification of vein traits, including vein density, vein width, and interveinal distance, with a precision on par with manual quantification. Further, we show that the framework can be used to identify previously undetected phenotypes, and measure vein patterning defects, which is advantageous for both basic and translational research. We envision GrasVIQ to be adapted for vein phenomics in maize and other grass species.

Venation patterns and vein order nomenclature

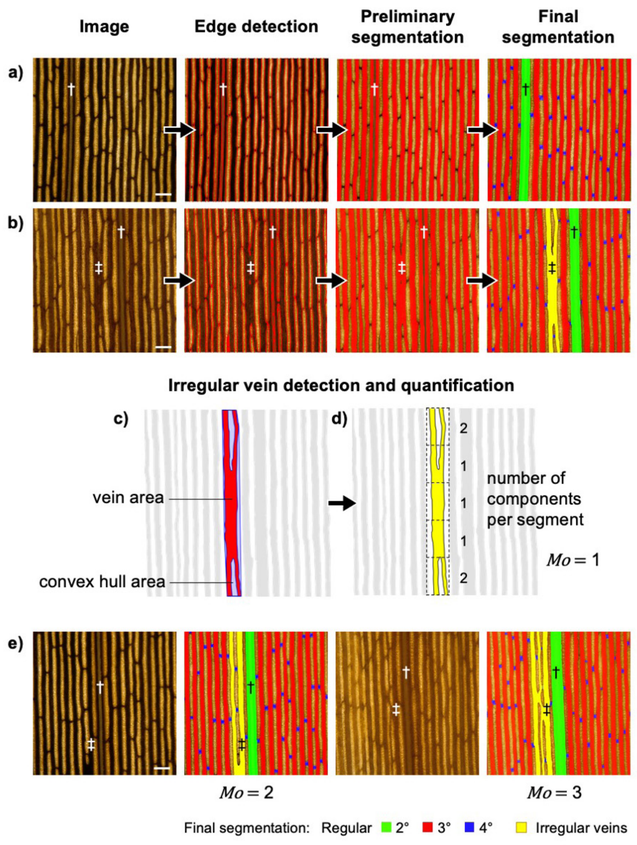


Redrawn from Nelson and Dengler (1997)

Experiment workflow and image analysis framework



Classification of vein order and detection of irregular veins



Object-level evaluation of vein detection and classification

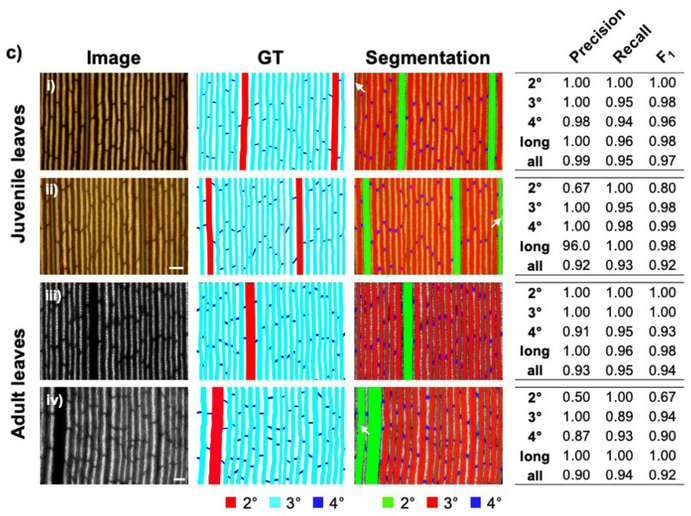
a)

Predicted (GrasVIQ) \ Actual (GT)	Positive	Negative
Positive	True Positive (TP)	False Positive (FP)
Negative	False Negative (FN)	True Negative (TN)

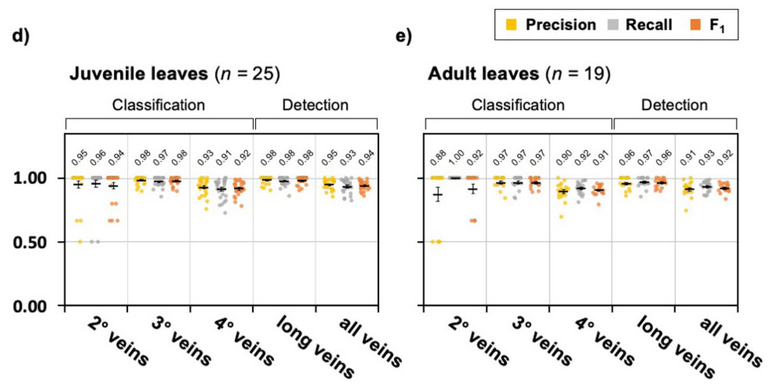
b)

$$Precision = \frac{TP}{TP + FP} \qquad Recall = \frac{TP}{TP + FN}$$

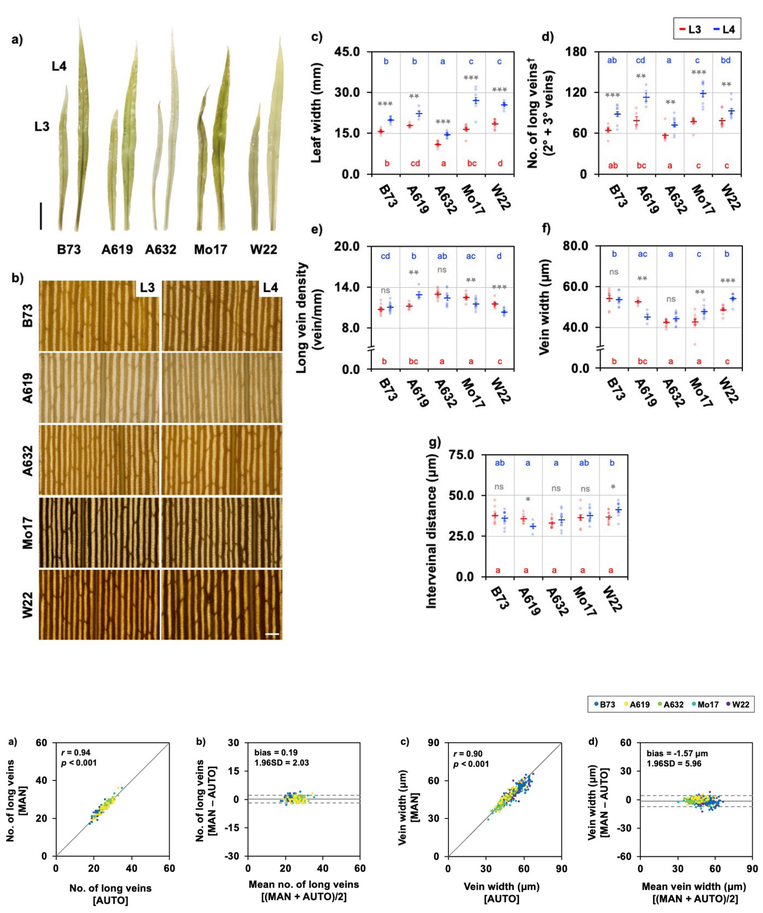
$$F_1 = 2 \cdot \frac{Precision \cdot Recall}{Precision + Recall} = \frac{TP}{TP + \frac{1}{2}(FP + FN)}$$

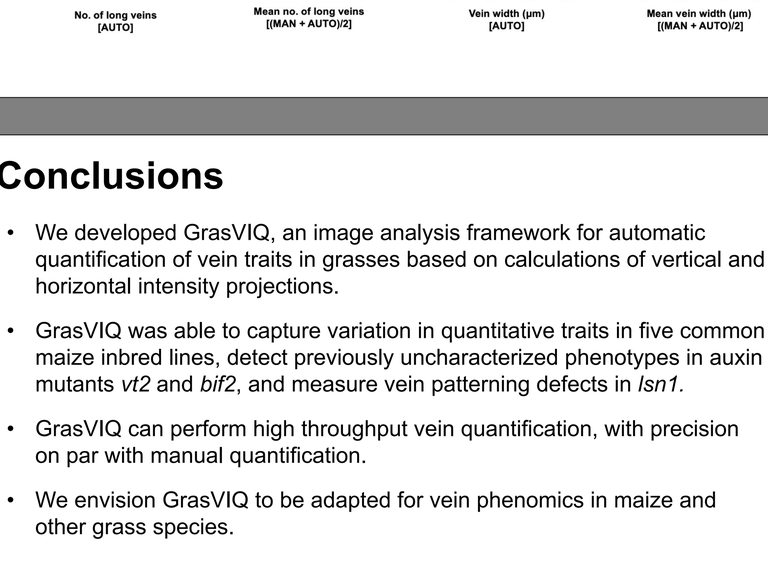


	Precision	Recall	F_1
2°	1.00	1.00	1.00
3°	1.00	0.95	0.98
4°	0.98	0.94	0.96
long	1.00	0.96	0.98
all	0.99	0.95	0.97
2°	0.67	1.00	0.80
3°	1.00	0.95	0.98
4°	1.00	0.98	0.99
long	96.0	1.00	0.98
all	0.92	0.93	0.92
2°	1.00	1.00	1.00
3°	1.00	1.00	1.00
4°	0.91	0.95	0.93
long	1.00	0.96	0.98
all	0.93	0.95	0.94
2°	0.50	1.00	0.67
3°	1.00	0.89	0.94
4°	0.87	0.93	0.90
long	1.00	1.00	1.00
all	0.90	0.94	0.92

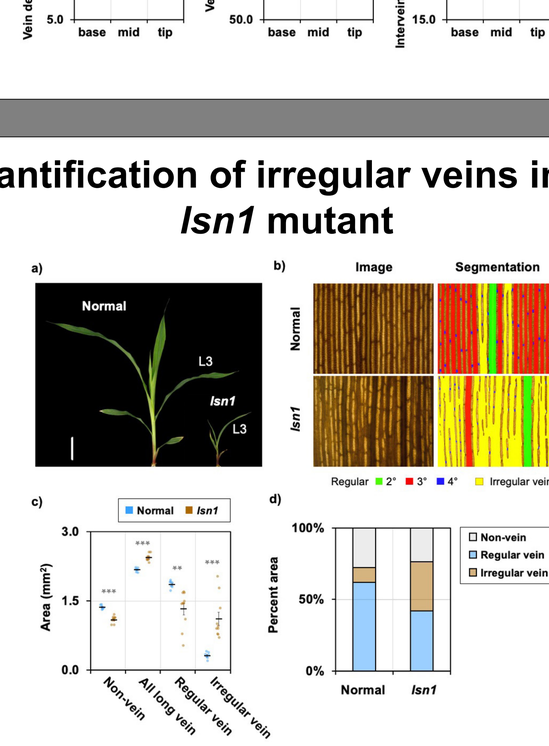


Analysis of quantitative vein traits in common maize inbred lines

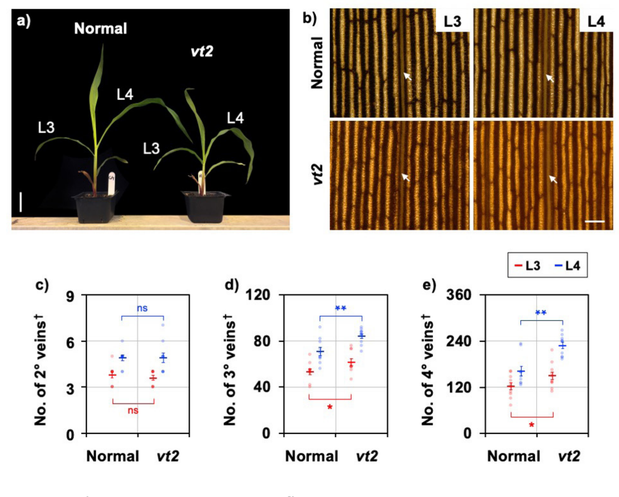




Analysis of quantitative vein traits in different regions of adult leaves in *bif2*



Analysis of quantitative vein traits in the auxin-deficient mutant, *vt2*



Quantification of irregular veins in the *lsn1* mutant

Conclusions

- We developed GrasVIQ, an image analysis framework for automatic quantification of vein traits in grasses based on calculations of vertical and horizontal intensity projections.
- GrasVIQ was able to capture variation in quantitative traits in five common maize inbred lines, detect previously uncharacterized phenotypes in auxin mutants *vt2* and *bif2*, and measure vein patterning defects in *lsn1*.
- GrasVIQ can perform high throughput vein quantification, with precision on par with manual quantification.
- We envision GrasVIQ to be adapted for vein phenomics in maize and other grass species.

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