

Quantification and Measurement of Veins in Maize Leaves through Image Analysis

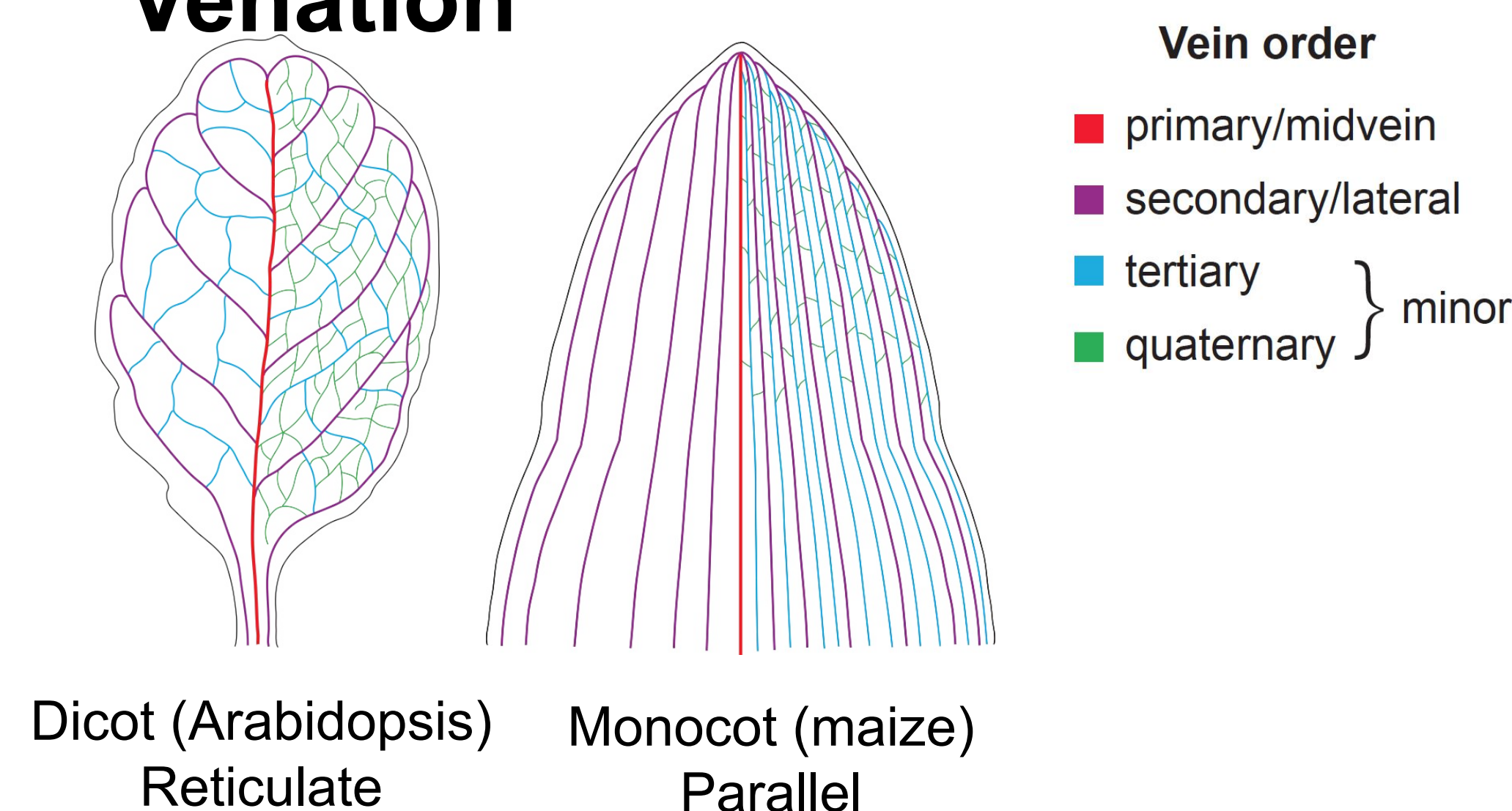
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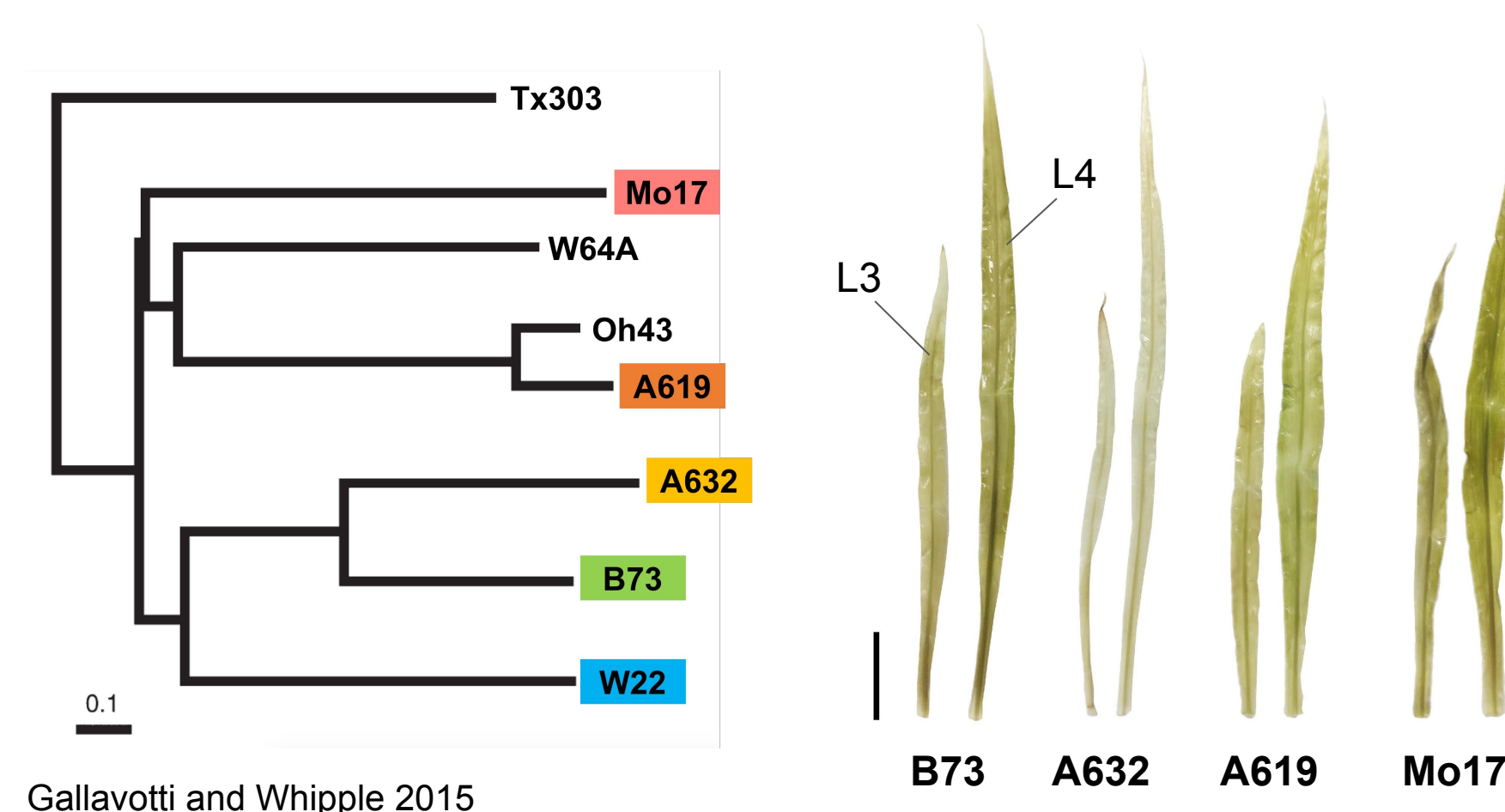
Abstract

C4 plants, like maize, are more efficient in photosynthesis primarily due to higher leaf vein density. Since high density of veins is key to efficient photosynthesis, it is imperative to develop efficient methods to visualize and analyze vein traits in this plant group. Here, an analysis of vein density and other vein traits in five maize inbred lines (B73, Mo17, A619, A632 and W22) was performed using a starch staining procedure. Leaves three (L3) and four (L4) of juvenile plants were cleared and stained with iodine potassium iodide (IKI) to visualize and quantify the venation pattern. There is an indication that variation exists within the five inbred lines. Since the plant hormone auxin is known to control vein development, vein densities of L3 and L4 of an auxin-deficient mutant, *vanishing tassel2* (*vt2*), were compared to those of normals. Surprisingly, *vt2* possesses a higher vein number and density than normal. This indicates an auxin dependent regulation of vein development in maize and warrants further investigation into the role of the hormone in the process. Together with the variation seen in the inbred lines, this could give insight into the complex systems and pathways underlying vein development in C4 plants. An image analysis tool was developed to quantify veins semi-automatically through the use of multiple image analysis techniques which include edge detection, convolution smoothing, connected component labeling, and signal processing. For future investigations, the image analysis tool will be used to annotate images that can be used to train a deep learning neural network for high-throughput quantification of veins that will allow for a greater number of inbred lines and maize mutants to be analyzed.

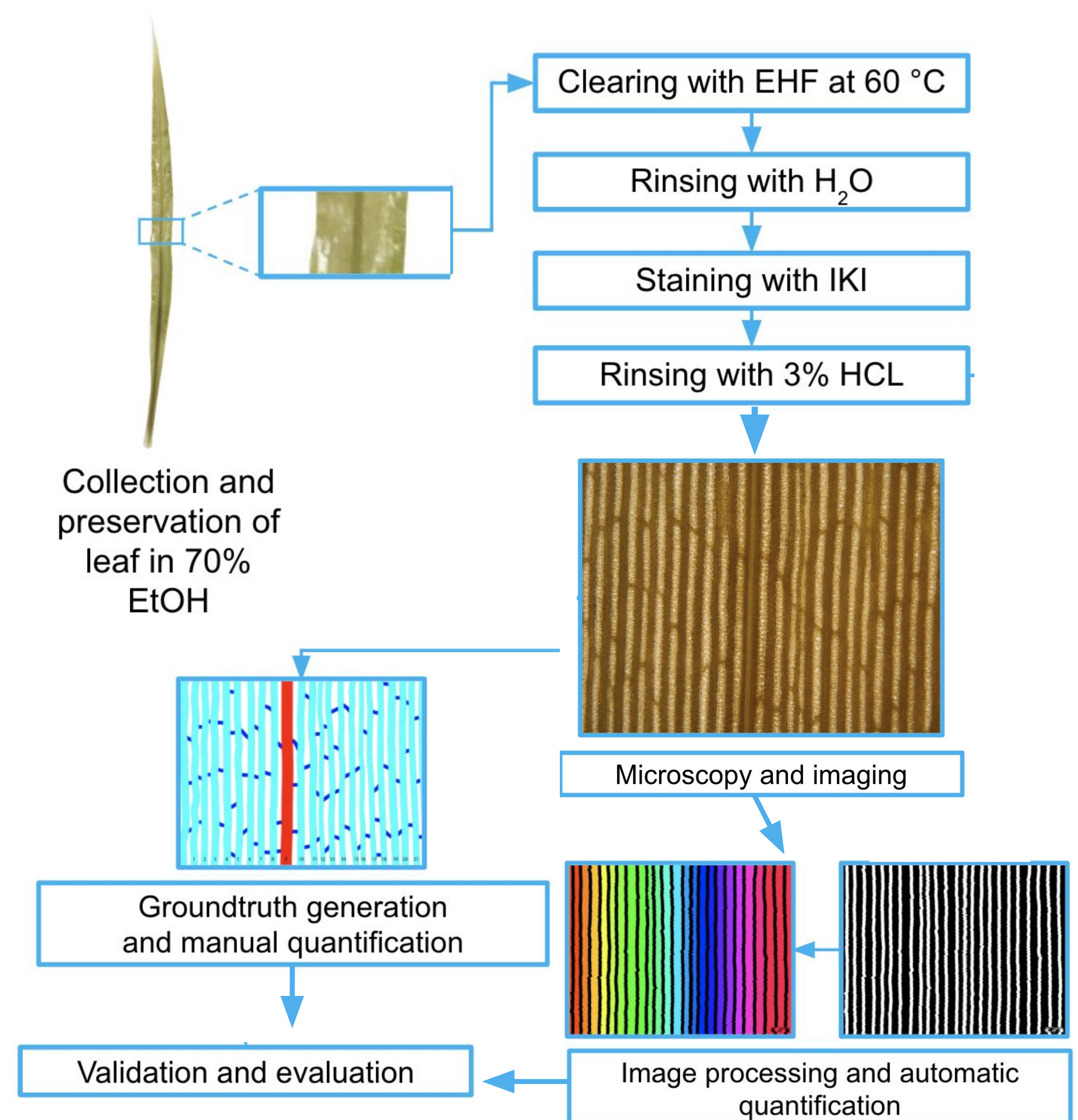
Monocot and Dicot Venation



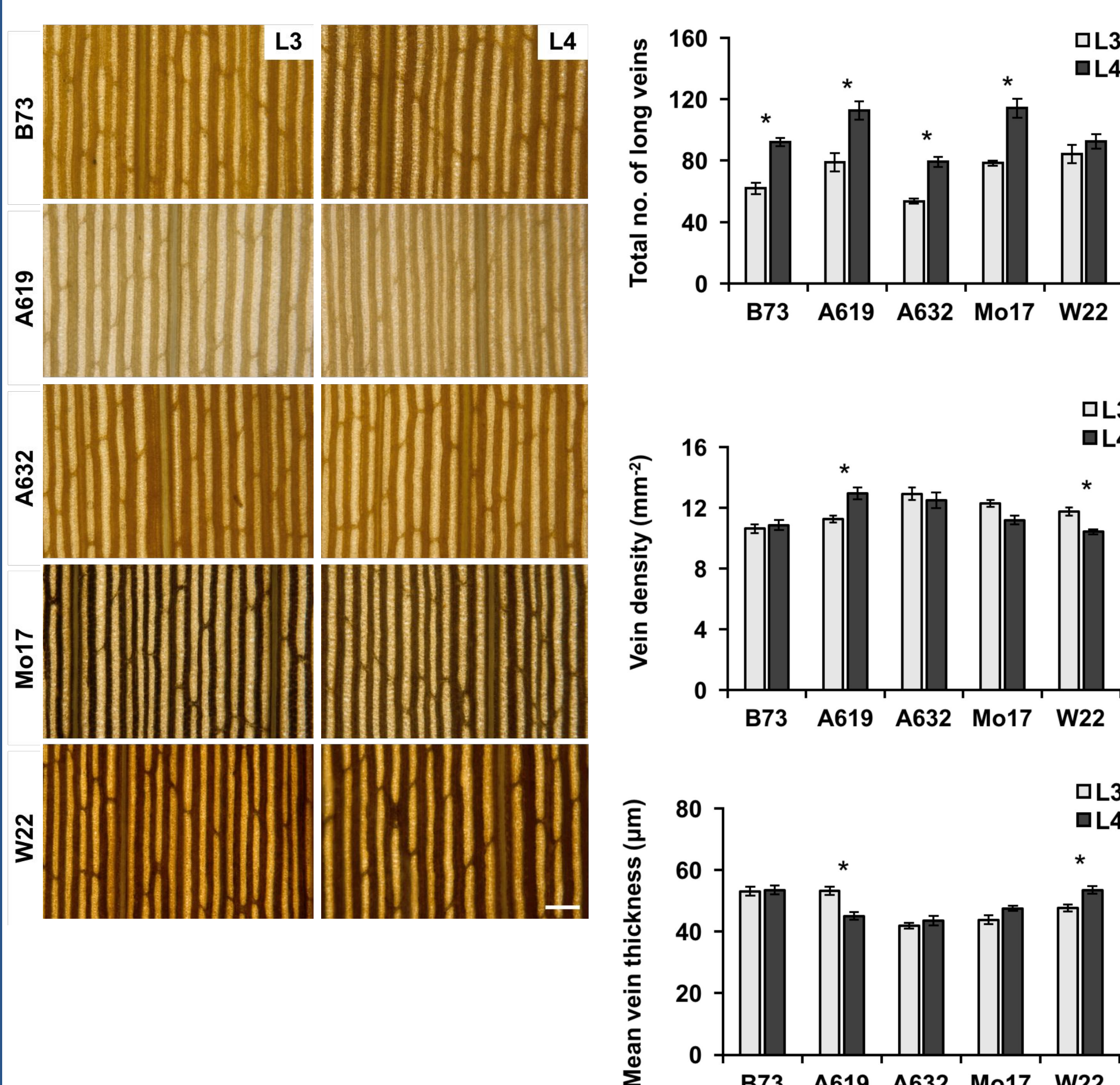
Common Maize Inbred Lines



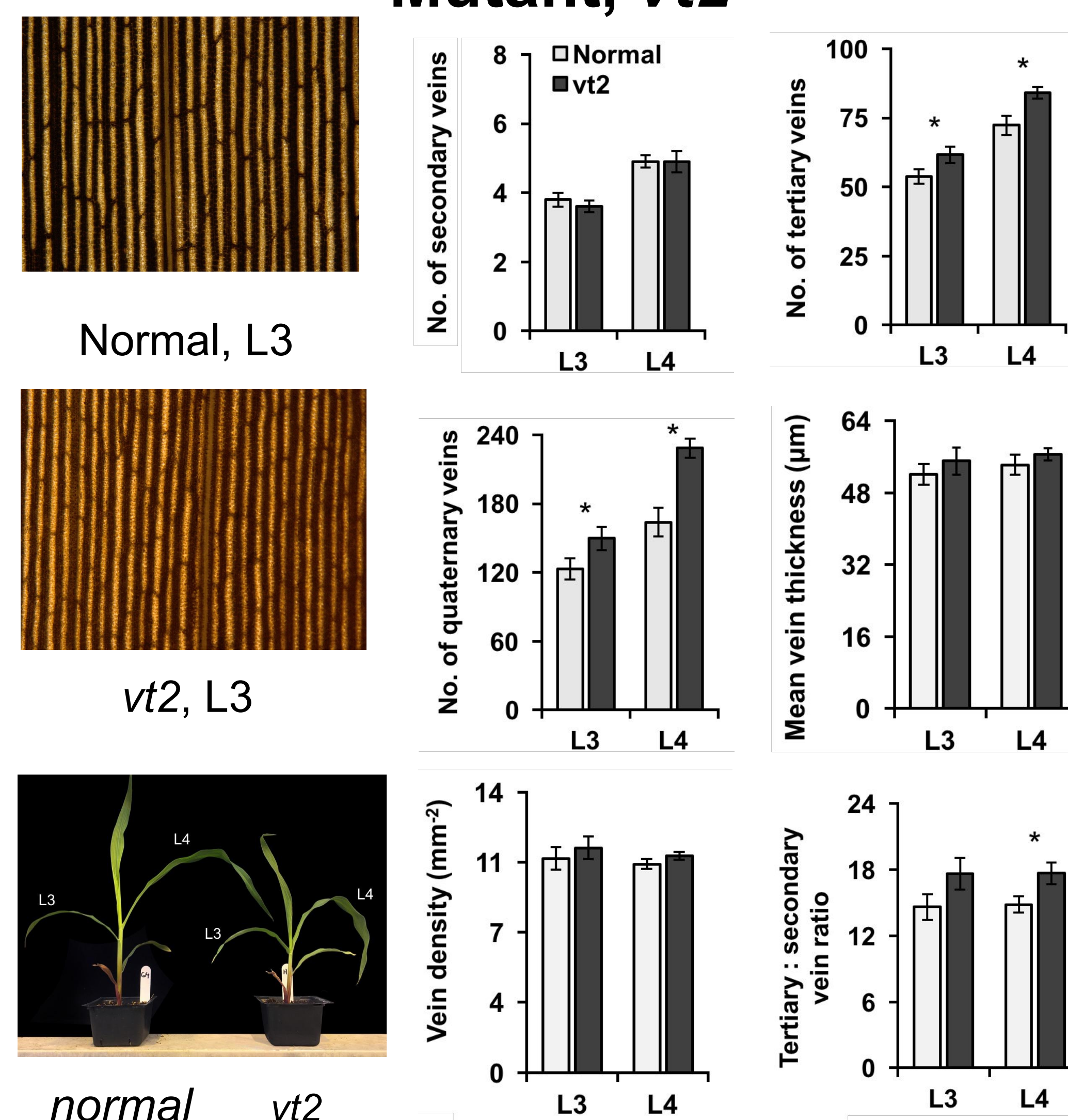
Leaf Clearing and Imaging Process



Vein Analysis of Common Maize Inbred Lines



Vein Analysis of Auxin-deficient Mutant, *vt2*



Conclusions and Future Directions

Maize Inbred Lines

- Constant vein thickness and density in B73
- Inverse correlation between vein thickness and density in A619 and W22

Auxin Mutant

- Greater number of higher order veins in *vt2* than normal
- High throughput technology for phenotyping
- Image analysis software for monocot species

References

Gallavotti, A., & Whipple, C. J. (2015). Positional cloning in maize (*Zea mays* subsp. *mays*, Poaceae). *Applications in plant sciences*, 3(1), 1400092.

Gallavotti, A., Barazesh, S., Malcomber, S., Hall, D., Jackson, D., Schmidt, R. J., & McSteen, P. (2008). *sparse inflorescence1* encodes a monocot-specific YUCCA-like gene required for vegetative and reproductive development in maize. *Proceedings of the National Academy of Sciences*, 105(39), 15196-15201.

Phillips, K. A., Skirpan, A. L., Liu, X., Christensen, A., Slewinski, T. L., Hudson, C., ... & McSteen, P. (2011). *vanishing tassel2* encodes a grass-specific tryptophan aminotransferase required for vegetative and reproductive development in maize. *The Plant Cell*, 23(2), 550-566.

Acknowledgments