Quantification and measurement of veins in maize leaf through image analysis

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. 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.