SOFTWARE NOTE

EASY LEAF AREA: AUTOMATED DIGITAL IMAGE ANALYSIS FOR RAPID AND ACCURATE MEASUREMENT OF LEAF AREA

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• Premise of the study: Measurement of leaf areas from digital photographs has traditionally required significant user input unless backgrounds are carefully masked. Easy Leaf Area was developed to batch process hundreds of Arabidopsis rosette images in minutes, removing background artifacts and saving results to a spreadsheet-ready CSV file.
• Methods and Results: Easy Leaf Area uses the color ratios of each pixel to distinguish leaves and calibration areas from their background and compares leaf pixel counts to a red calibration area to eliminate the need for camera distance calculations or manual ruler scale measurement that other software methods typically require. Leaf areas estimated by this software from images taken with a camera phone were more accurate than ImageJ estimates from flattened scanner images.
• Conclusions: Easy Leaf Area provides an easy-to-use method for rapid measurement of leaf area and nondestructive estimation of canopy area from digital images.

Key words: Arabidopsis; digital images; leaf area; Python.

Accurate, rapid, and nondestructive leaf area estimates are critical in many plant physiological and ecological experiments. Now-ubiquitous digital scanners and cameras, in conjunction with digital image processing software, have largely replaced older methods using light obstruction to estimate leaf area. ImageJ, the most common software used for leaf area measurement, uses a threshold-based pixel count measurement to calculate leaf area (Orsini et al., 2010; Warman et al., 2011; Juneau and Tarasoff, 2012; Carins Murphy et al., 2012; Schneider et al., 2012; Easlon et al., 2014). ImageJ, however, can require significant user input and often has difficulty in distinguishing leaves from their background using thresholding alone (Davidson, 2011). Physical masking of soil using paper collars before photographing leaves or software removal of background from images (using, e.g., GNU Image Manipulation Program; Kimball et al., 2012) can remove background artifacts from images before ImageJ analysis, but these approaches add considerable processing time to leaf area measurements (Campillo et al., 2008; Warman et al., 2011; Juneau and Tarasoff, 2012).

We developed Easy Leaf Area software to rapidly estimate leaf area from Arabidopsis (DC.) Heynh. images against complex backgrounds with little user input. Easy Leaf Area uses a combination of thresholding, color ratios, and connected component analysis to rapidly measure leaf area in individual images in seconds or batch process hundreds of images in minutes; results are saved to a spreadsheet-ready CSV file. Each analyzed image is also saved in lossless TIFF format to provide a visual record of leaf area measurement and to facilitate additional analyses (Figs. 1C, F; 2C, E). Easy Leaf Area was written in Python (http://www.python.org/), a free and open-source programming language with image processing and mathematical tools, and is easy to modify to suit specific experimental requirements; e.g., a "Crop Cover" version of the program was written to facilitate measurement of projected leaf area and percent crop canopy cover.

METHODS AND RESULTS

Easy Leaf Area uses a red calibration area of known area in each image as a scale to calibrate leaf area estimates regardless of image source, eliminating the need for assessing camera distance and focal length or measuring ruler length manually (Baker et al., 1996). Total counts of green leaf pixels and red calibration pixels are used to estimate leaf area, according to: leaf area = (green pixel count) × (calibration area/red pixel count). When possible, the calibration area should be kept in the same plane as the leaves to avoid perspective distortion. Leaf area and calibration area should also be located in similar regions of the image to minimize errors from lens distortion. Errors due to camera set up and lens distortion can be quantified by analyzing area of squares in photographs of the ‘distortion sheet’ of green squares surrounding a red square of the same area (available for download at https://github.com/haslon/Easy-Leaf-Area/blob/master/DistortionSheet.jpg). A camera phone (iPhone 4, Apple, Cupertino, California, USA) image of the ‘distortion sheet’ taken without a tripod at a camera distance of 20 cm had a mean distortion of 0.17% (standard error [SE] ± 0.006). A digital single-lens reflex (DSLR) camera (18–55-mm lens, 25-mm focal length = f/4; EOS Rebel T2i, Canon, Melville, New York, USA) image of the ‘distortion sheet’ at a camera distance of 30 cm had a mean distortion of −2.94% (SE ± 0.008) due to significant barrel distortion. Alternatively, destructively harvested leaves can be scanned on a flattened scanner to eliminate leaf overlap and minimize perspective and lens distortions. Scanner images (MFC-J425w, Brother International, Bridgewater, New Jersey, USA) had a mean distortion of 0.02% (SE ± 0.003). Leaf area analyses typically rely on thresholding of either grayscale images or the blue channel of RGB (red, green, and blue) images to distinguish leaf and calibration areas from their background (O’Neal et al., 2002; Bylesjo et al., 2008; Davidson, 2011). Easy Leaf Area uses...