SMART: An open and affordable plant phenotyping system for leaf-specific color analysis of the Arabidopsis Rosette

Computational Plant Science Lab

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Abstract

Challenge :

Most plant imaging systems focus predominantly on monitoring morphological traits. The challenge is to relate color information to measurements of physiological processes.

Question:

Can the color of individual leaves be measured and quantified over time to infer physiological information about the plant?

Solution:

We developed the open source and affordable plant phenotyping software pipeline for *Arabidopsis thaliana*. SMART (Speedy Measurement of Arabidopsis Rosette Traits) that integrates a new color analysis algorithm to measure leaf surface temperature, leaf wilting and zinc toxicity over time.

Data Collection:

We used public datasets to develop the algorithm [1] and validate morphological measurements. We also collected top-view images of the Arabidopsis rosette with the Open-Leaf imaging robot [2] and top-view setups for heat stress to validate physiological measurements.

Figure 1: (a) The OPEN Leaf imaging robot developed at the DMC lab at the University of Missouri [2] using Allied Vision Mako G-503B 1/2.5" Monochrome CMOS Camera, it can capture RGB images automatically over time and upload the data to collaborators using shared online storage Cyverse [3]. (b) The Raspberry Pi imaging system used at Rothamsted Research to capture RGB top view image data; it enables the real time monitoring the growth of rosette with high resolution in growth chamber. (c) The imaging setup used at Hofstra University, developed in the Tara Enders lab using a NIKON D7200 digital cameras controlled by laptops. to A standard color palette was used to setup a reference color when comparing the color change of plant surface.

SMART pipeline overview

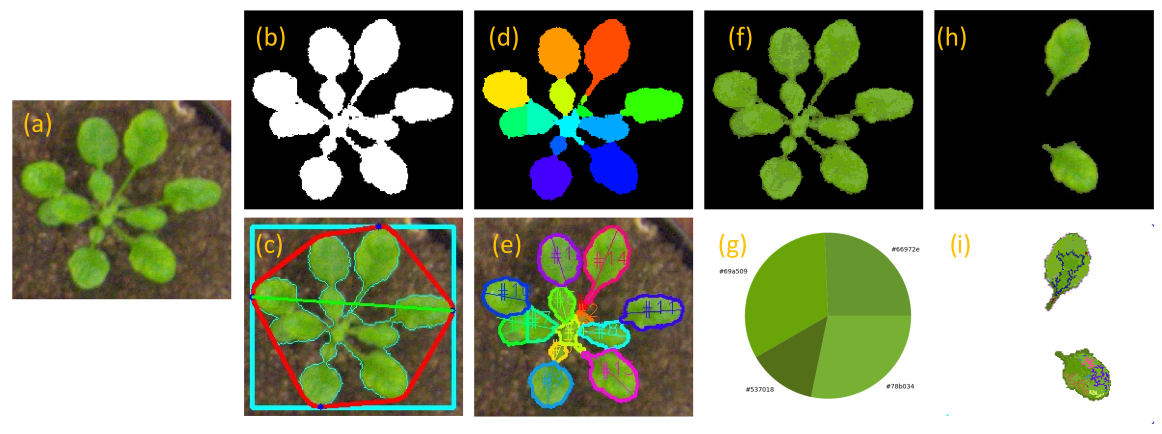

Figure 2: Schematic overview of the SMART pipeline. (a) A top-view plant image taken from [1]. (b) Segmentation of the plant into white foreground and black background (c) Geometric traits describing the whole rosette such as convex hull, diameter and eccentricity. (d) segmentation of the rosette into individual leaves. (e) Measuring individual leaf traits such as area and length. (f) Color distribution of the four dominant greens of the whole plant and (g) the four dominant shades of green represented as a pie chart with its corresponding hex value. (h) The two largest leaves were selected to show the original color distribution of the leaves (i) Visual representation of the color quantization of the two leaves shown in (h) computed with the dominant color analysis method.

Principle of dominant color clustering

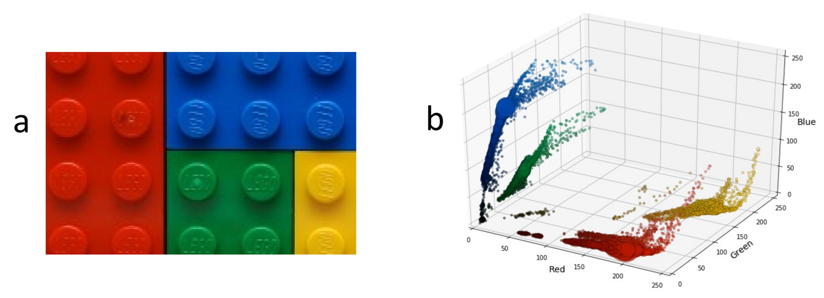

Figure 3: Dominant color clustering was to partition data points into different clusters C_k. For example, (a) is an RGB image, and (b) shows all colors of (a) in RGB space. Every point in (b) corresponds to a pixel and its color in (a). The size of each point is proportional to the number of pixels of that color. In dominant color clustering process, each of the RGB color points will be assigned to a cluster with the spatially closest mean in a Cartesian space equipped with a Euclidean metric. The mean of each cluster is defined as its centroid. Color data points inside a cluster C_k are assigned to the same cluster centroid. This method uses an iterative refinement technique to their corresponding cluster centroid by minimizing the least-squares of distances between points. The optimal number of clusters is estimated by the Elbow method [4]. The result of the dominant cluster method applied to (a) would result in four clusters denoting an average red, green, blue and yellow.

Color transformation to compute dominant shades of green

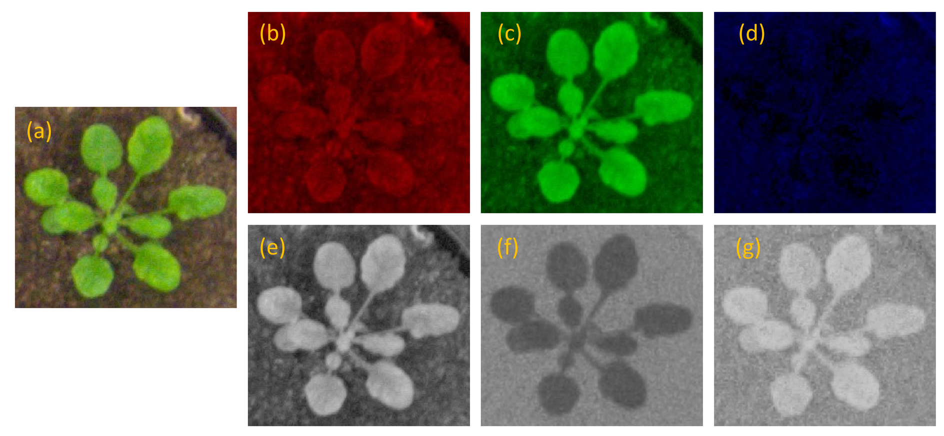

Figure 4: Color transformation from RGB space to LAB space. (a) A top-view image of Arabidopsis thaliana in RGB color space. We extracted the R, G, and B channels and display them as individual images in (b),(c), and (d). We convert the image in (a) from RGB color space to CIE L*a*b* color space. L*, a*, and b* channels are extracted and displayed in (e), (f), and (g) respectively.

Color analysis of individual leaves

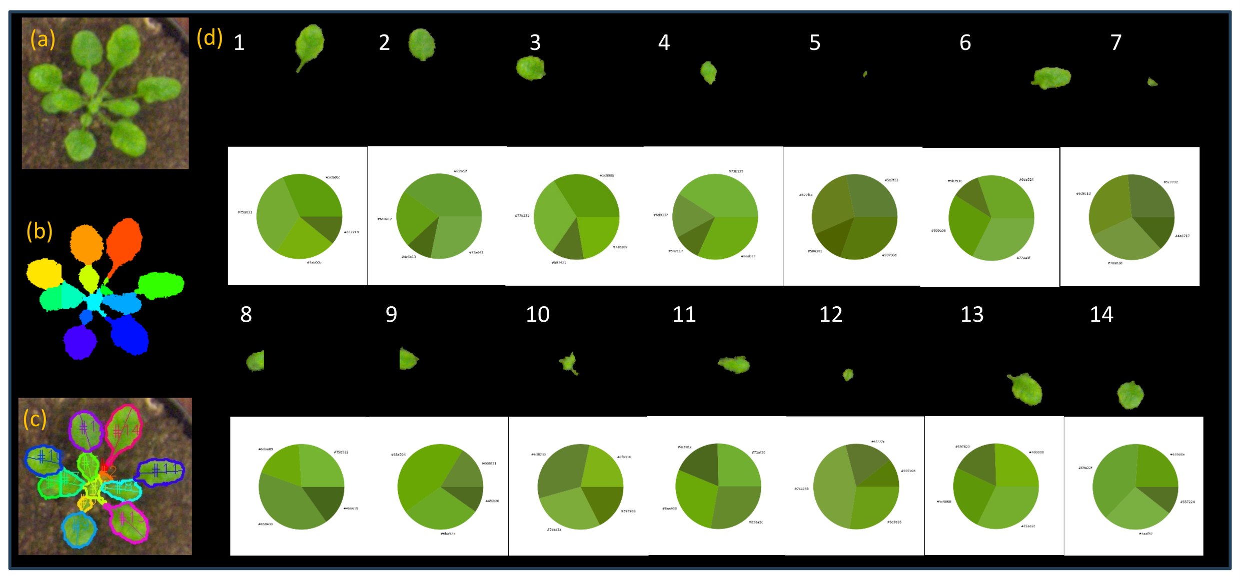

Figure 5: **Individual leaf color distributions**. (a) A top-view image of Arabidopsis thaliana. (b) The segmented individual leaves, each leaf is labelled with a unique color. (c) For each leaf the size is computed. (d) All the leaves are sorted by size and a color distribution pie chart is computed for each leaf.

SMART Application 1

Quantifying toxicity levels of zinc accumulation in individual leaves over time

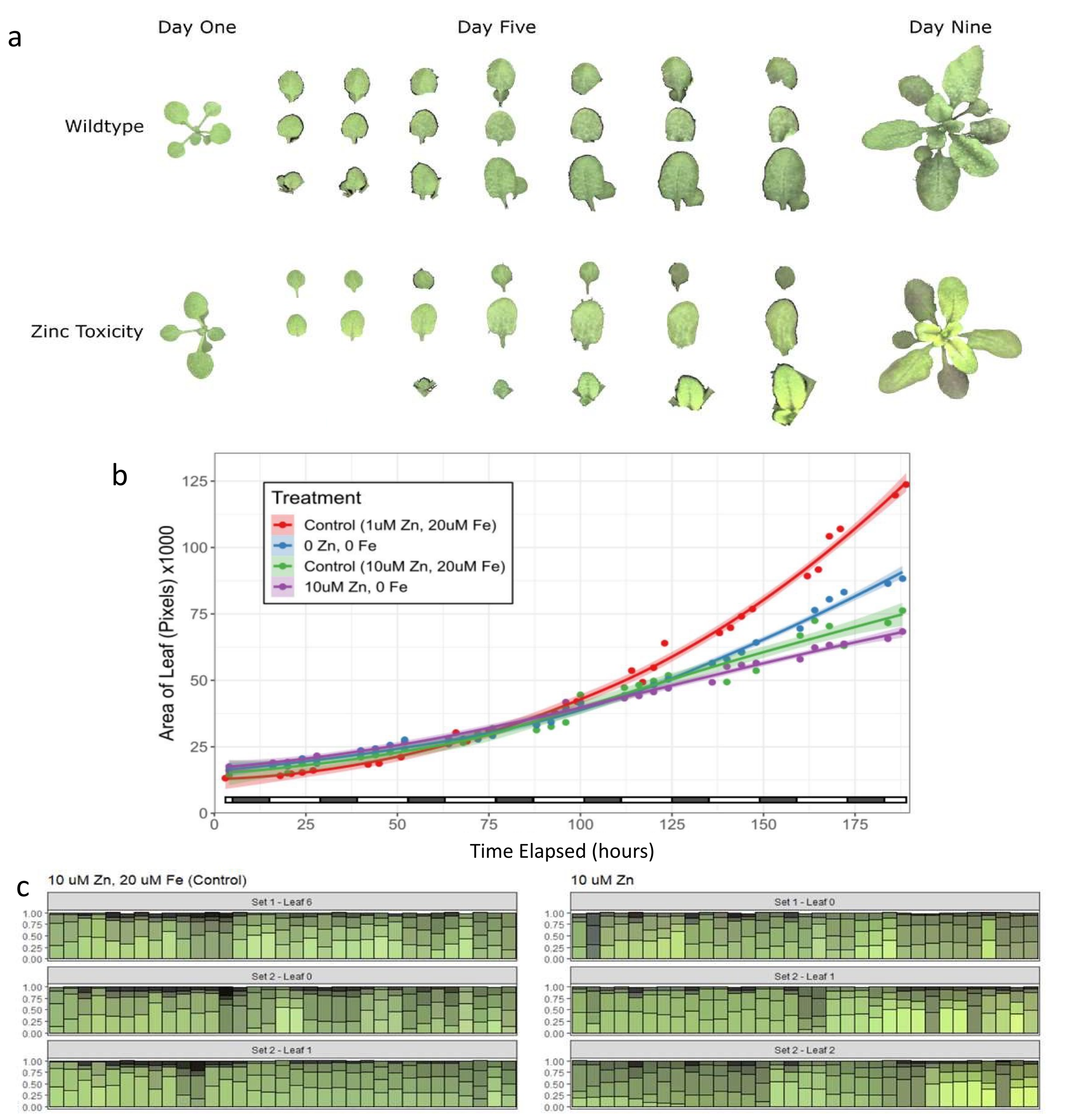

Figure 6: Availability of nutrients dictates the fate of individual leaves within the Arabidopsis rosette. (a) SMART pipeline can track individual leaves over plant development. Changes in the Fe/Zn ratio have a severe impact on (b) individual leaf size and (c) color. The leaf color clustering results generated by SMART were consistent with the leaf developmental stage [2]. Examples of individual leaf color clustering into four dominant colors shown in (c) allowed for dynamic tracking of color transitioning over time. Each bar plot represents the proportional distribution of each dominant color in individual leaves. The plots are representative of one experiment and similar results were obtained in three independent experiments.

SMART Application 2

Estimating the average leaf surface temperature of the rosette

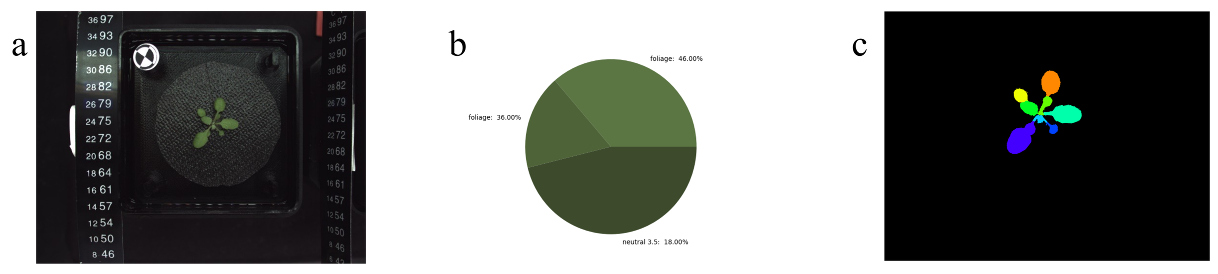

Figure 7: SMART can estimate the temperature of Arabidopsis over time using the color difference between temperature sticker and the plant surface color. (a) One sample top view RGB image of an Arabidopsis with a temperature sticker. (b) Color distribution of the whole plant in a pie chart, with the percentage of each color and its color name. (c) Individual leaf detection result.

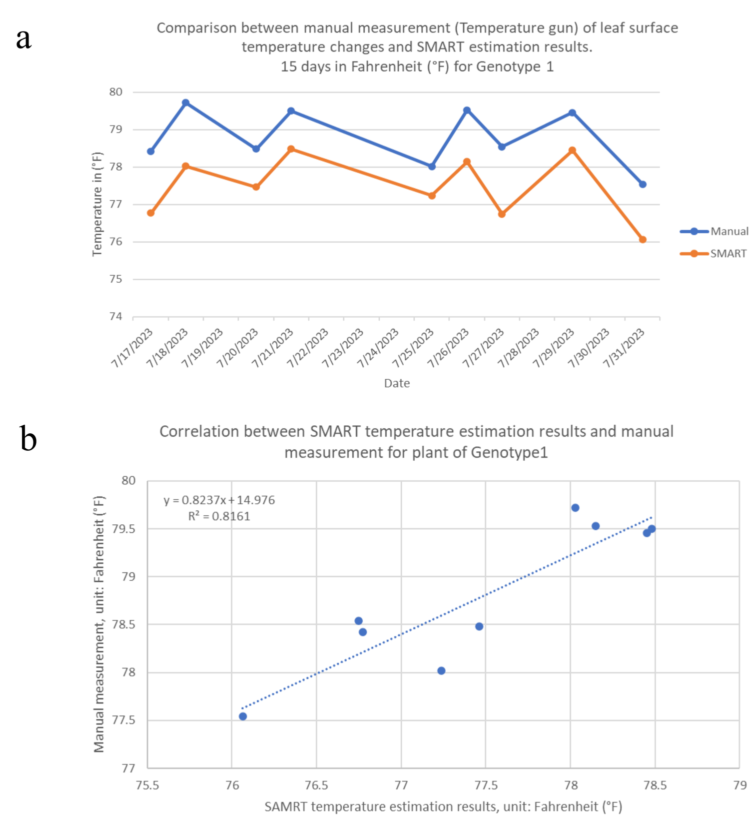

Figure 8: Temperature validation results between manual measurements and SMART computation results. The image data was collected using OPEN Leaf imaging robot in Figure 1 over 15 consecutive days (a) Comparison of manual temperature measurement with a temperature gun (blue line) and the SMART temperature estimation (orange line) for genotype 1. Each manual measurement point is the average of five temperature measurements at different locations per plant over four replicates. (b) correlation analysis between manual measurement versus SMART computation results revealed R^2> 0.81 for this genotype.

SMART Application 3

Quantifying leaf wilting in response to heat stress

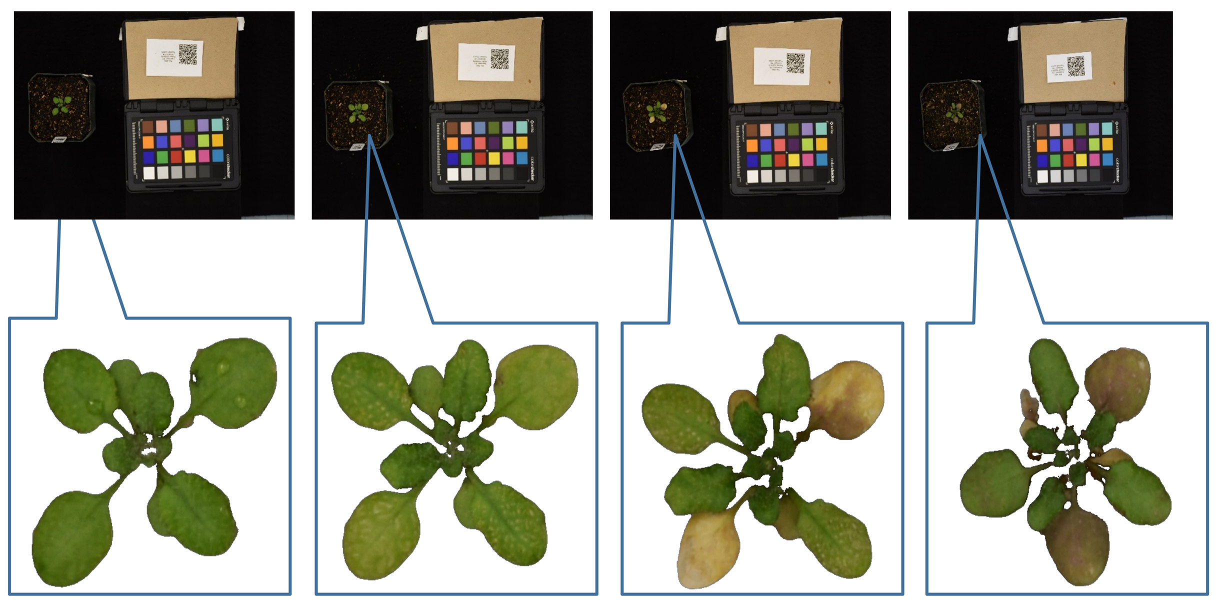

Figure 9: SMART can capture leaf color changes over time to quantify wilting by matching individual leaf colors to a standard color palette.

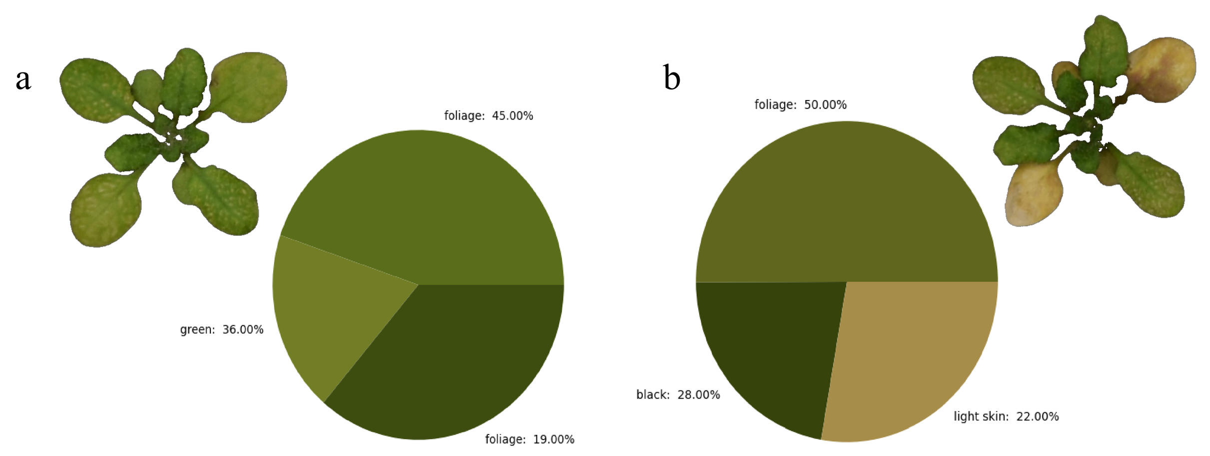

Figure 10: SMART can detect unusual colors. (a) SMART can detected different shades of green on the leaf surfaces of the healthy plant. (b) SMART detected a brown color, which helped to quantify the heat stress exposure of the plant.

Conclusion

1. **The SMART software demonstrated that physiological information can be inferred with detailed color analysis**. Here we showed that zinc toxicity levels, leaf surface temperature and the degree of leaf wilting under heat stress as applications.
2. We observed a likely predictable and correctable bias in the leaf surface temperature measurements.

Software Download

Funding

NSF CAREER AWARDS

NSF CAREER Award No. 1845760

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