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**Estimate Stomatal Conductance of Corn and Soybean Plants in Greenhouse via Imaging and Pot Weighing**

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**Abstract.**

*Plants control the transpiration and photosynthetic efficiency by regulating the opening and closing of stomata. Optimizing those efficiencies enable growers to conserve water and sustain crop yield under drought conditions. Stomatal conductance ( $g_s$ ) is a crucial parameter that represents the rate of water loss for transpiration and carbon dioxide uptake for photosynthesis. Therefore, understanding the variation of  $g_s$  can help researchers comprehend the plant capabilities of water regulation during periods of water stress. Typically,  $g_s$  is quantified using handheld devices that require users to go to each plant and attach sensors on each leaf to get the reading, which demands a substantial amount of time and effort. To minimize the amount of manual effort and automate the data collection, we have created a pipeline that employs image processing algorithms and a weighting system to estimate the  $g_s$  with high precision. The experiment was conducted at the Greenhouse Innovation Center at the University of Nebraska-Lincoln, with two different genotypes of maize and soybean. A high-throughput phenotyping system was used to precisely apply water to the plants and track their weight. This study included three different water treatments: 75% field capacity (FC), 50% FC, and drought recovery. By utilizing image analysis, the fresh weight and projected area of each single plant were estimated. Those estimations, along with the daily pot weight before and after watering process, can be used to derive  $g_s$  of plants. Throughout various stages of growing season, we gathered the daily  $g_s$  of plants for both species and under different water treatments. After comparing the ground truth  $g_s$  and estimated  $g_s$ , it has a  $R^2$  value of 0.58 for maize and 0.70 for soybean. The result demonstrated that this pipeline effectively estimates plants'  $g_s$  at various stages. It can be used as a suitable alternative approach for monitoring plants'  $g_s$  in a greenhouse setup.*

**Keywords.**

*Drought, Image Analysis, Plant Phenotyping, Stomatal Conductance, Transpiration*

## Introduction

Stomata are pores consisting of guard cells on the leaf surface. They play a crucial role in gas exchange during the processes of transpiration and photosynthesis. The opening and closing of stomata regulate the flux of carbon dioxide uptake and water release, the rate of those gas flux is measured as stomatal conductance ( $g_s$ ). Therefore, monitoring  $g_s$  for plants can help researchers to understand how plants adapt to environmental changes. However, the sensors used for measuring  $g_s$  require individuals to visit each plant and take the measurements, which is time-consuming and labor-intensive. The objective of this study is to develop an automated pipeline to estimated  $g_s$  for large scale phenomics platforms in controlled environments.

## Material and Methods

The experiment was conducted at the Greenhouse Innovation Center of the University of Nebraska-Lincoln using the LemnaTec3D Scanalyzer system (LemnaTec GmbH, Aachen, Germany). The RGB (Red Green Blue) imaging chamber of the system has the capability to capture RGB images from top view and 10 different angles of side view. Each view has a resolution of 6576 x 4384 pixels. This system also incorporated an automated watering and weighting module that could measure the pot weight before and after the watering process.

Two maize genotypes (B73 and OH43) and two soybean genotypes (Thorne and Williams) were used in this study, with each genotype subjected to three water treatments (Well-Watered (WW): soil water content (SWC) = 75% of Field Capacity; Drought (D1): SWC = 50% of Field Capacity; and Drought Recovery (D2) that keep SWC at 25% of Field Capacity for 7 days during growth stage V6). Each group has 8 plants, 96 plants in total. In addition, there are 4 empty pots containing only soil to estimate the evaporation. With in each group, there were three additional plants under same environment and destructively sampling was conducted on these plants at three dates between V4 and VT stages to get plant shoot fresh weight (FW).

The plant RGB images were converted to binary images by separating plant pixels from the background. Fig 1. A and B show the example maize RGB image and the resultant binary image. The pixel counts of plants from multiple views were averaged to derive the plant projected area, and plant FW were modeled by results from destructive sampling to estimate FW for other dates (Ge et al., 2016).

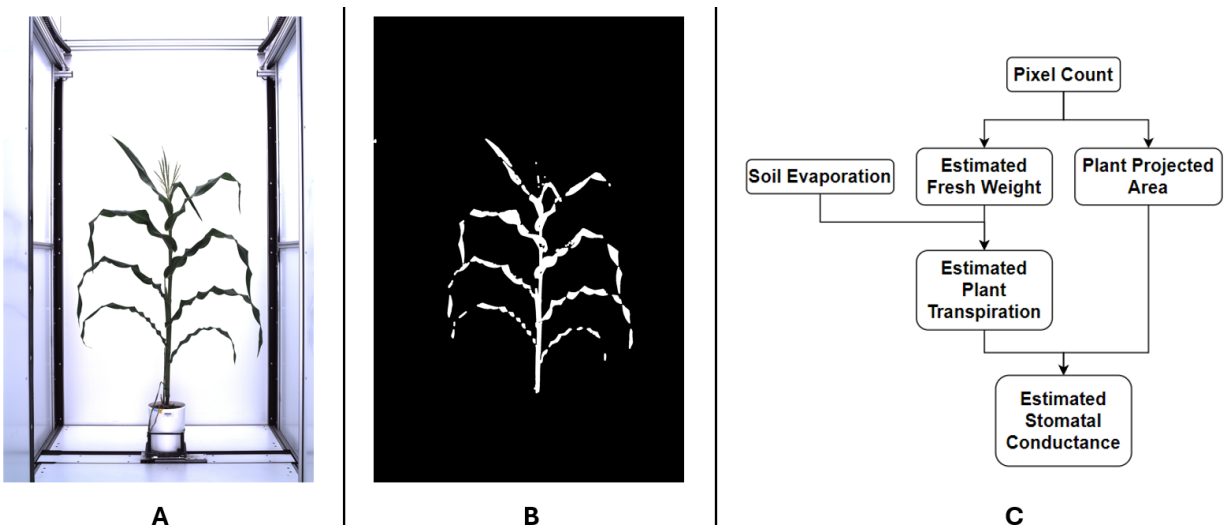


Fig 1. (A) Example RGB image of maize. (B) Example binary image of plant without background. (C) Workflow of

The daily soil evaporation was calculated based on weight difference between empty pots in two consecutive days. This information and estimated FW were then used to calculate the plant daily transpiration.

$$Evaporation = W_{i,After} - W_{i+1,Before} \quad (1)$$

$$\text{Transpiration} = (W_{i,After} - FW_i) - (W_{i+1,Before} - FW_{i+1}) - \text{Evaporation} \quad (2)$$

Where  $W_{i,After}$  is the pot weight after the water application at day  $i$ ;  $W_{i+1,Before}$  is the pot weight before the water application at day  $i+1$ ;  $FW_i$  is the estimated plant fresh weight at day  $i$ ; And  $FW_{i+1}$  is the estimated plant fresh weight at day  $i+1$ .

The plant's  $g_s$  can be estimated based on daily transpiration and its projected area. The overall estimation workflow is depicted in Fig 1. C. The ground truth  $g_s$  was collected using a LI-600 instrument (LI-COR, Lincoln, NE), at 7 days throughout the growing season. The ground truth  $g_s$  was then compared with estimated  $g_s$  to evaluate the accuracy of the pipeline.

## Results and Discussion

Fig 2. A shows the correlation between the number of plant pixel and Fresh weight. There is a significant linear correlation between these two values for both maize and soybean. Maize has a higher FW than soybean for the same number of pixels. This discrepancy may arise due to the contrasting structural characteristics of the two species, as well as the weight disparity between the maize stem and soybean stem.

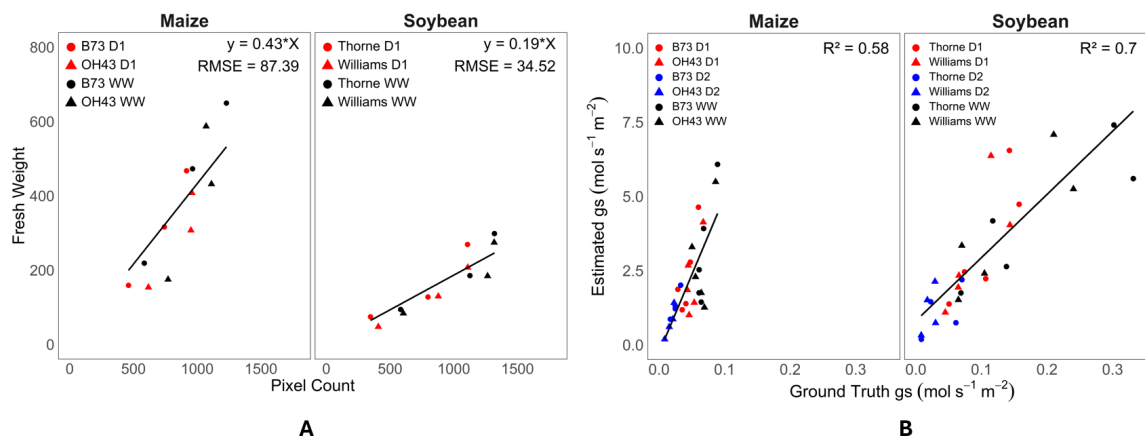


Fig 2. (A) Relationship between the average Pixel Count and fresh weight for both maize and soybean. (B) Daily measured  $g_s$  vs estimated  $g_s$

Fig 2. B shows the relationship between daily measured  $g_s$  and estimated  $g_s$  for both maize and soybean in the group of two genotypes and three water treatments, both plants demonstrate a satisfactory level of estimate accuracy ( $R^2 = 0.58$  for maize and  $R^2 = 0.70$  for soybean). Same as the FW model, the slope for maize is higher than soybean, it might be because the difference in the relationship between real leaf area and projected area.

## Conclusion

The result shows the viability of large-scale automated collection of  $g_s$  with high-throughput phenotyping platform. However, additional investigation is required to explore the relationship between projected area and actual leaf area.

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## References

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