

A Breakthrough in Rapid Stomatal Phenotyping

Stomata are small pore-like structures found on the epidermal layer of aerial plant organs. They are key determinants of photosynthetic capacity as they regulate gaseous exchange between the leaf interior and surrounding atmosphere. Therefore, stomatal behavior plays a major role in balancing CO₂ uptake to maintain photosynthesis whilst minimizing water loss to avoid dehydration. Better understanding how stomata develop and respond to the environment will help identify photosynthetic and drought tolerance traits that can be utilized in breeding wheat that is better adapted to a warmer climate. A project, “**Stomata Signaling Pathways for Increasing Yield Potential in Wheat**”, led by Nora Foroud at Agriculture and Agri-Food Canada (AAFC), with other colleagues in Canada, the United Kingdom and Australia is targeting genes involved in pathways that control stomatal development, density and function, in order to determine their impact on grain yield, biomass and water-use efficiency (WUE). This project is employing functional genomic and crop physiology techniques in both lab and greenhouse/field settings and brings together complementary expertise in wheat stomata development/activity, photosynthesis, functional genomics, biotechnology, breeding and agronomy. The current knowledge on stomatal variation is limited because phenotyping these structures is labor-intensive, time-consuming, and costly. Therefore, a rapid non-destructive method for stomata phenotyping is needed for providing information on stomatal numbers and behavior to optimize associated traits, including photosynthesis.

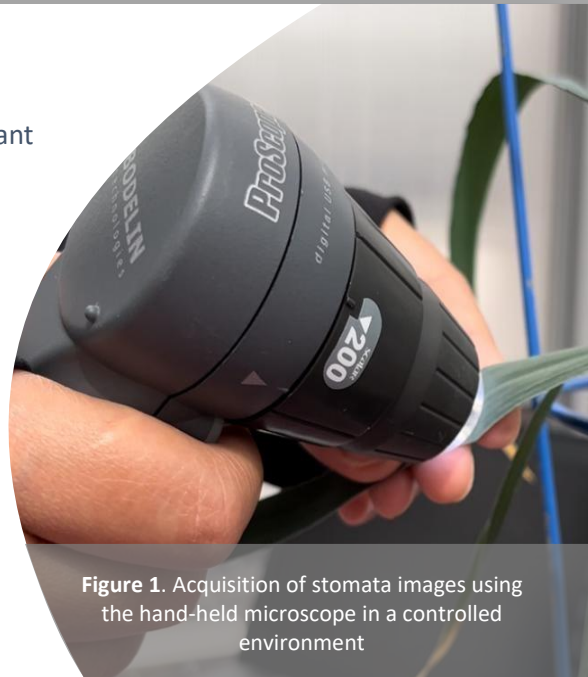


Figure 1. Acquisition of stomata images using the hand-held microscope in a controlled environment



Figure 2. Acquisition of stomata images using the hand-held microscope in the field

What Solutions have been Identified?

- The development of a new method to accelerate stomatal phenotyping using a handheld microscope in combination with machine learning technology¹.
- Non-destructive imaging of stomatal features can now be obtained within seconds, in both the greenhouse and field (**Figures 1 & 2**) requires no tissue fixation or expensive microscopes.
- A machine learning model has been created for detecting, counting and measuring stomatal size in wheat that is highly correlated with manual measurements for stomatal number ($R^2 = 0.99$), stomatal size ($R^2 = 0.87$) and stomatal aperture ($R^2 = 0.94$) (**Figure 3**).

Anticipated Impact of this Research

- Wheat (and other plant species) can now be phenotyped for stomatal traits in < 1min, thus opening the option for large scale screening of stomatal traits in diverse germplasm sets, GWAS panels, bi-parental mapping populations and transgenic lines as components of yield.
- Stomatal phenotyping complemented with molecular analyses will identify alleles involved in regulating stomatal patterning and activity, an important step in marker and germplasm development to improve yields by optimizing respiration, photosynthesis and water loss.

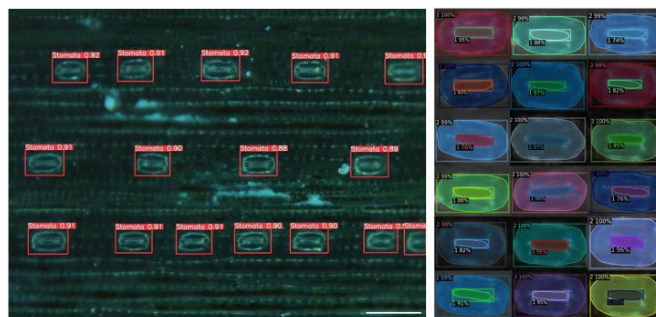


Figure 3. Automated detection and measurement of stomata in wheat.
¹Pathomthong *et al.* 2022 Rapid non-destructive method to phenotype stomatal traits, BioRxiv doi.org/10.1101/2022.06.28.497692