

How do plants reduce leaf growth at low humidity

How does plant water loss affect plant growth at high and low h

Student : Flora Manu-Fofie

Supervisor : Jim Rowe

Project location: University of Sheffield

Absciscic acid is a plant hormone that is involved in the regulating water loss from the plant. ABA is especially important during periods of stress and stimulates the closure of the stomata preserving water for the plant. Understanding how ABA functions in plant tis essential to engineer plants for the rapid changes in our climate. This study looks at how absciscic acid affects plant growth at different humidities. We grew *Arabidopsis thaliana* plants that were ABA deficient, ABA over accumulators, ABA signalling mutants or lines engineered to be ABA insensitive in different leaf tissues. We measured how much these different lines grew at different humidities and their leaf temperatures. Leaf temperature is a proxy for water loss as cooler plants are losing more water. We discovered that there was a link between the average temperature of the plants and the growth rate. The cooler the plants the lower the growth rate. These findings could help with increasing food security as climate change intensifies to introduce crops that can withstand stress of a harsher climate.

- Photograph (of you in the lab, or similar)



- What was the aim of your project?

The project aimed to investigate the effect of ABA(abscisic acid) on plant development in both low relative humidity and high relative humidity.

- How did you address the aim?

- In order to address the aim we grew 8 different mutants over a period of 5 weeks from seed. The mutants included tissue specific ABA deficient lines, ABA signalling suppressant lines and double mutants for aba catabolism. Half the samples were grown at 50% relative humidity, and the other half was grown at 70% relative humidity.
- A Raspberry pi camera was utilised to capture an image of the plants every day. By doing so we were able to analyse the morphological differences between the plants over time and calculate the growth rate.
- Widefield and confocal microscopy was used in conjunction with the ABACUS biosensor to visualise the ABA accumulation in the ABACUS leaves on a cellular level. To complement this, we used image analysis software to visualise the location and quantity of ABA in the samples.
- We used a thermal camera to capture thermal images to measure the temperature differences between the samples. This measure was to determine the water loss from each plant.(Figure 1)

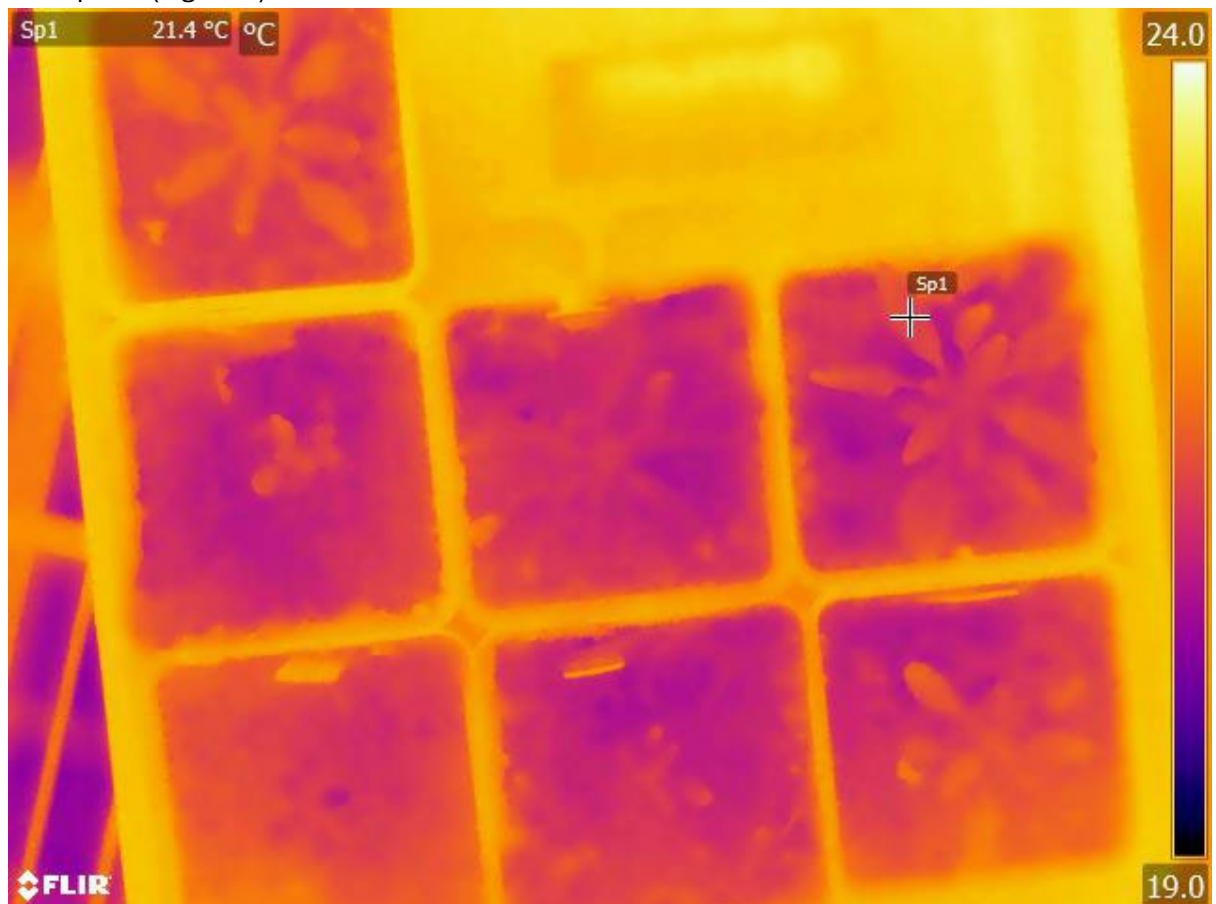


Figure 1. Thermal image of *Arabidopsis thaliana* plants

We discovered that there was a correlation between leaf temperature and plant growth. Specifically, that in the ABA signalling suppression lines (CYP86Apro::abi1-1), there was less transpiration, leading to higher leaf temperatures and preservation of water in the leaves. Additionally, our results reveal that ABA doesn't only affect the stomata opening and closing but

has an impact on the bundle sheaf and mesophyll cells. It is evident that the CAB3pro::abi1-1 (mesophyll) line and MYBpro::abi1-1 (bundle sheaf) line had a notably reduced growth rate compared to the ABA over-accumulators such as cyp707a1cyp707a3 biosynthesis mutant (graph 2). Furthermore, confirming the proposition that ABA impacts the plants morphological development. These patterns held steady whether the plants were grown in high or low humidity

By using multiphoton microscopy, we were able to image the ABACUS biosensor plant at high resolution. In low humidity there was more ABA accumulated at high concentrations. The images reveal that ABA accumulates in *Arabidopsis thaliana* cells at low humidity. Figure 2 portrays this effect. This signal was higher in guard cell nuclei, showing that ABA builds up there under dry conditions to help close the stomata and limit water loss. Nuclei with higher emission ratios are represented as yellow-white spots supporting that ABA accumulates in guard cells at low humidity to facilitate stomatal closure.(Figure 3)

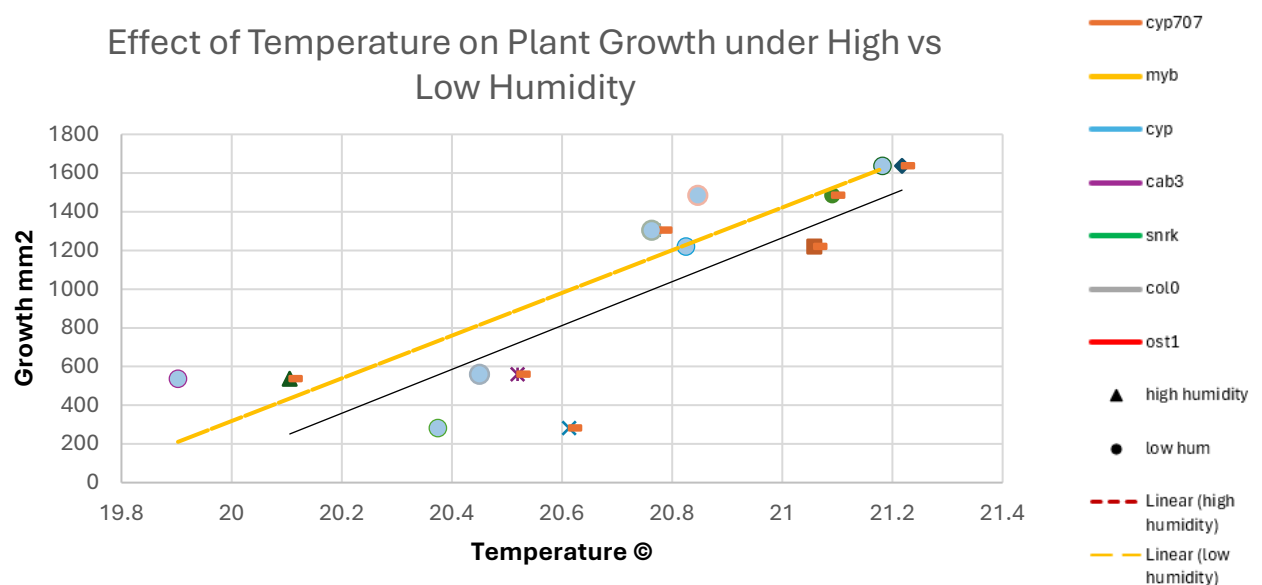


Figure 2. Effect of temperature on plant growth under high and low humidity. Growth area (mm²) of different genotypes was plotted against temperature (°C). Data points are colored by genotype: cyp707 (orange), myb (yellow), cyp (blue), cab3 (purple), snr

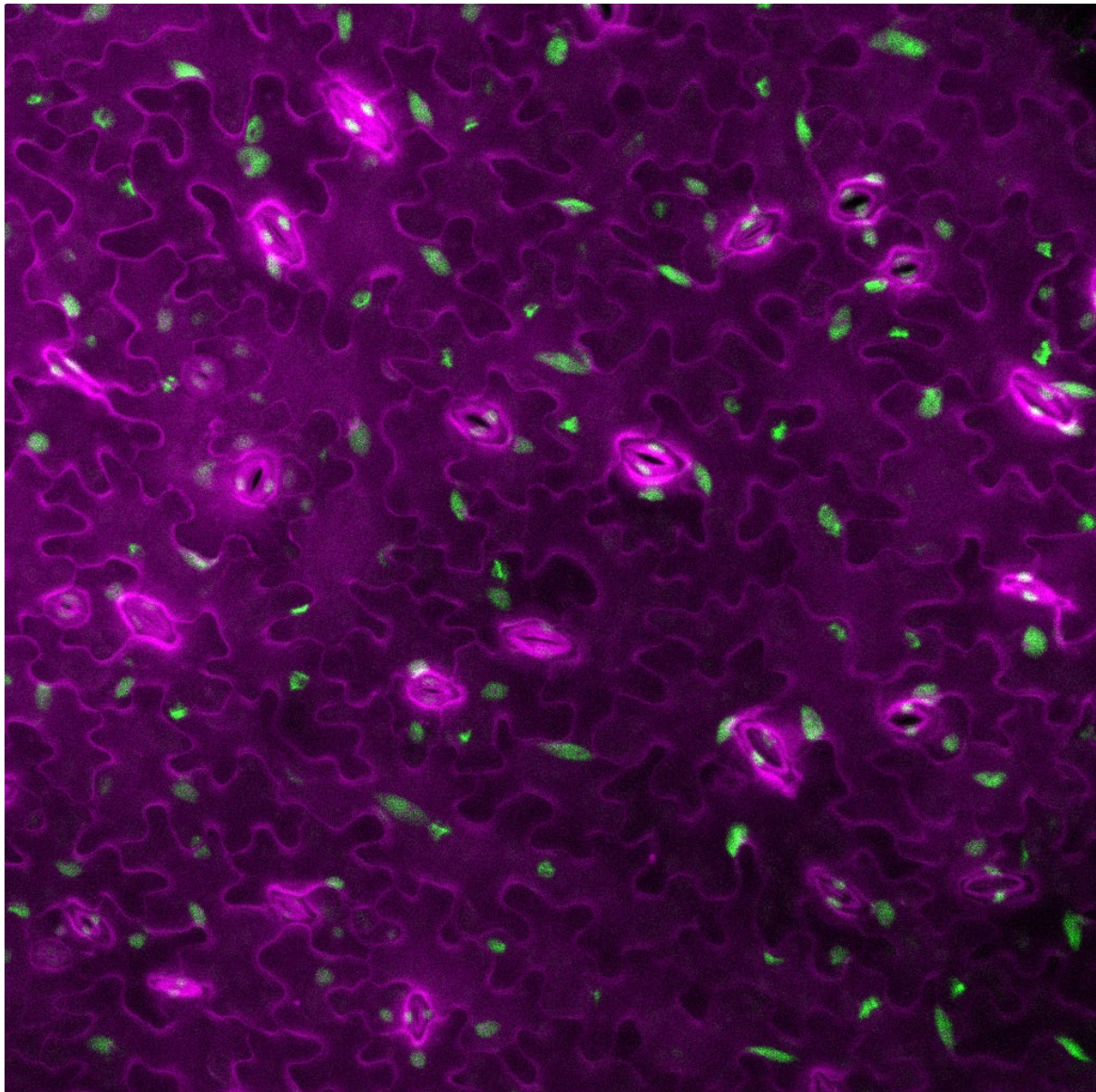


Figure 4. Multiphoton microscopy of Arabidopsis epidermal cells showing stomata, pavement cells, and nuclei. Abscisic acid biosensor (ABACUS) fluorescence is shown in green, highlighting stomata, pavement cells, and nuclear regions. Cell walls and nuclei were counterstained with propidium iodide (red), providing structural contrast. Images were acquired using multiphoton excitation and edited in image analysis software to represent biosensor and stain channels.

I learnt a range of new techniques from participating in this project which will greatly aid me in academia and my future endeavours in science. This included learning how to effectively mount samples onto microscopes as well as performing special techniques on the microscope such as Z-stacks, and multichannel acquisition. Learning how to use and operate new technology such as the confocal microscope, the thermal cameras and 3D printer was highly insightful. The most rewarding thing I learnt from this project was how to analyse my images from the microscope using the image J software, My confidence using the initially intimidating programme increased greatly which I am grateful for. This allowed me to use the images from the biosensor to identify the effect ABA was having on the guard cells and stomata opening. This taught me the importance of automated pipelines and how efficient they make research. I learnt

how to make tables in Image J which quickly gave me the multiple rows of information on the samples I needed in a short amount of time. I especially enjoyed learning more plant science during the project including how to grow and propagate *Arabidopsis thaliana*. I also learnt how to use a CAD software and how to design basic 3D models to 3D print to facilitate and enhance the experiment.

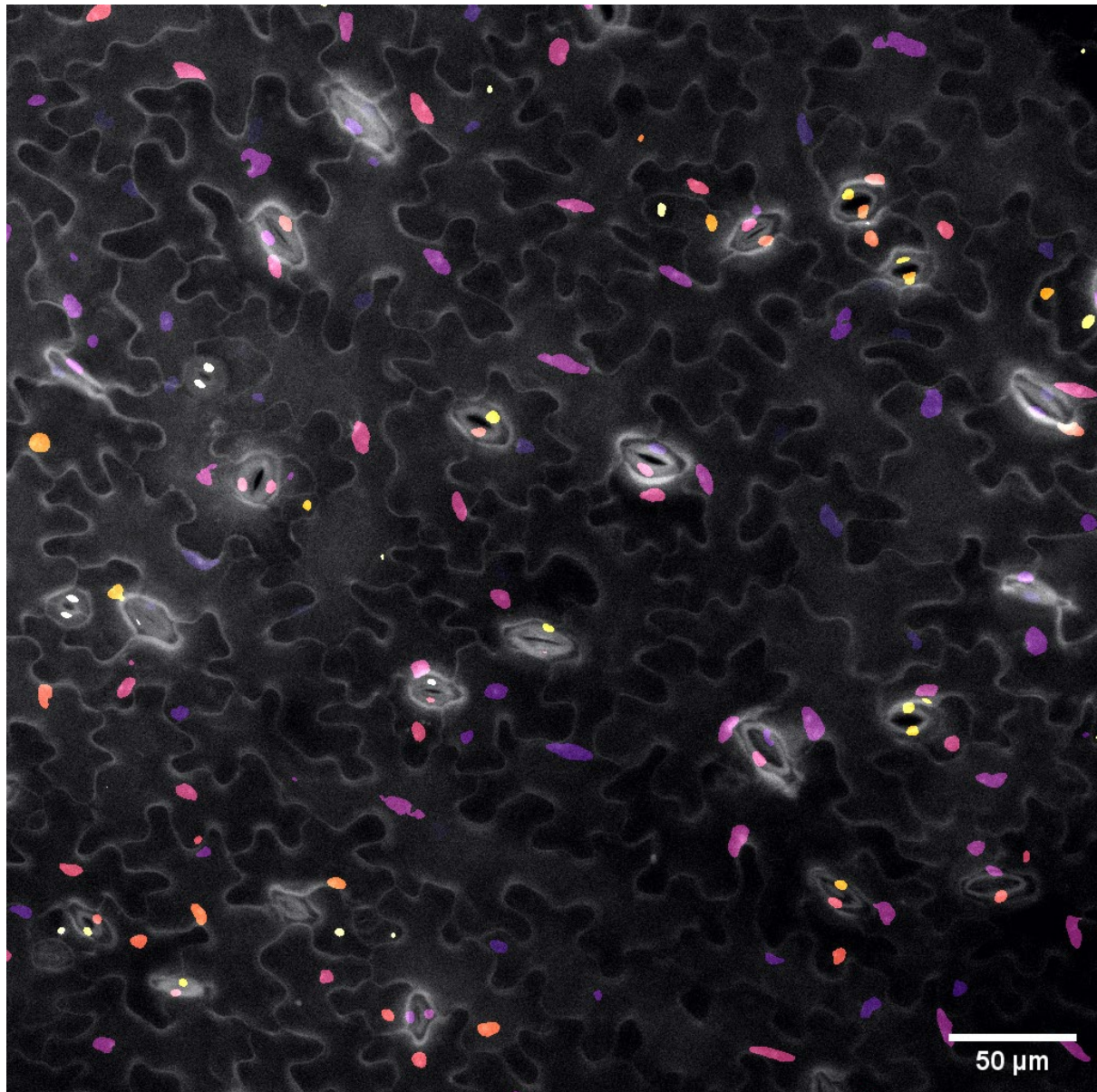


Figure 3-Image of stomata ,pavement cells and guard cells with emission ratios of the nuclei edited as a representation of the ABA levels in each cell.

This project has made me more interested in microscopy. I aim to use the imaging techniques I have learnt to enhance my research during my final year research project. Looking forward I'm excited to use microscopy in areas such as biotechnology and drug discovery.