2nd Call for Funding: Dr Matt Jones – Institute of Molecular, Cell, and Systems Biology University of Glasgow

October 8, 2020 (updated October 8, 2020)

Project Title: *Measuring the consequences of abiotic stress in vivo using fluorescent and bioluminescent probes*

Total Fund Requested – £23 687

Project Summary If we are to understand how plants respond to drought and increased temperatures we need to measure both the immediate changes in metabolism inflicted by stress and the consequences of these changes. Improved instrumentation is needed to reveal how the underlying dynamics of redox homeostasis that govern plants' responses to stress contribute to gene expression. This will enable the signalling pathways initiated by stress to be observed.

We need to measure changes in redox homeostasis and gene expression over time so that we can understand how plants' perceive and adapt in response to drought and high temperature. Our combination of fluorescence and bioluminescent imaging alongside lighting for plant growth will enable a step-change in our ability to observe the relationship between the immediate changes in metabolism inflicted by stress and the consequences of these changes by monitoring gene expression. As part of a new collaboration with the Imaging Concepts Group (led by Prof. Harvey, School of Physics and Astronomy, University of Glasgow) we will design and build a prototype imaging system to simultaneously measure both redox homeostasis (using ratiometric fluorescent probes) and gene expression (using luciferase reporter fusions) over several days. These in vivo measurements will allow us to understand the underlying dynamics of redox homeostasis that govern plants' initial responses to stress.

Background If we are to understand how plants respond to drought and high temperature stress we need to measure both the immediate changes in metabolism inflicted by stress and the consequences of these changes. Immediate consequences of metabolic stress include production of Reactive Oxygen Species (ROS), which are generated as a consequence of perturbed photosynthetic electron transport (Mullineaux et al., 2018). ROS have additionally been co-opted as signaling molecules, thereby allowing the integration of metabolic signals into the regulation of nuclear gene expression (Mullineaux et al., 2018). We already know that the sensory and metabolic pathways initiated by stress are integrated into the circadian system, a pervasive biological oscillator that synchronises plant behaviour with daily rhythms (Millar, 2016; Lai et al., 2012). We now want to understand how ROS signaling is integrated by the circadian system to govern plants' responses to stress.

Improved instrumentation is needed to reveal how underlying dynamics of redox homeostasis govern plants' responses to stress. This project will enable a new collaboration between Plant Science (PI Jones) and the Imaging Concepts Group (PI Harvey) at the University of

Glasgow. We will demonstrate the technical feasibility of our new imager and perform experiments to measure the consequences of abiotic stress over different spatial and temporal scales in planta.

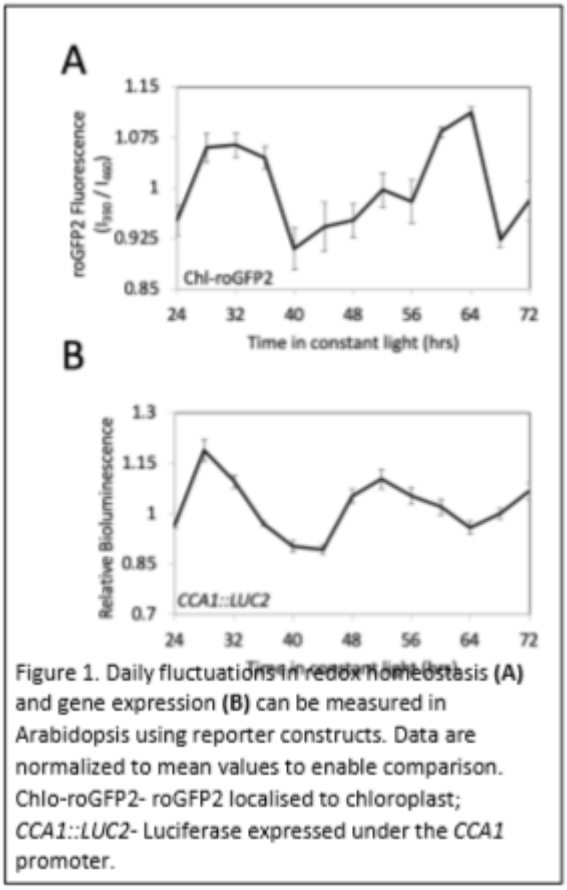

Figure 1. Daily fluctuations in redox homeostasis **(A)** and gene expression **(B)** can be measured in Arabidopsis using reporter constructs. Data are normalized to mean values to enable comparison. Chlo-roGFP2- roGFP2 localised to chloroplast; *CCA1::LUC2*- Luciferase expressed under the *CCA1* promoter.

We need to measure changes in redox homeostasis and gene expression over extended timeframes so that we can understand how underlying dynamics of redox homeostasis contribute to plants' tolerance of stress. Changes to redox homeostasis can be measured in vivo using redox-sensitive versions of GFP [roGFP2; (Nietzel et al., 2019)] whereas changes in gene expression are routinely measured

Figure 1. Daily fluctuations in redox homeostasis (A) and gene expression (B) can be measured in Arabidopsis using reporter constructs. Data are normalized to mean values to enable comparison. Chlo-roGFP2- roGFP2 localised to chloroplast; CCA1::LUC2- Luciferase expressed under the CCA1 promoter.

using promoter fusions expressing firefly luciferase (Battle and Jones, 2020, Jones et al., 2019, Litthauer et al., 2018). We are able to measure changes in both roGFP2 and luciferase activity over several days in vivo (Figure 1)- however measurements of roGFP2 are currently completed using a 96well plate reader that is not amenable to the automation required to measure over several days- nor can we resolve variation between different tissues (e.g. leaves vs. roots). We also observe endogenous fluorescence from wild type controls that limit the accuracy of our measurements.

We will use hyperspectral imaging deconvolution to improve our measurement of roGFP2 fluorescence emission in whole plants, alongside bioluminescent imaging of luciferase activity. This project will deliver a prototype to enable the automated measurement of cellular redox stasis and gene expression in vivo over extended timescales.

Objectives Our combination of fluorescence and bioluminescent imaging alongside lighting for plant growth will enable a step-change in our ability to observe the relationship between the immediate changes in metabolism inflicted by stress and the consequences of these changes by monitoring gene expression.

We will design and build a prototype imaging system to simultaneously measure both redox homeostasis (using ratiometric fluorescent probes) and gene expression (using luciferase reporter fusions) over daily timescales in vivo to understand the underlying dynamics of redox homeostasis that govern plants' responses to stress contribute to gene expression. Our initial efforts of imaging roGFP2 fluorescence have met with limited success due to endogenous plant fluorescence [presumably from kaempferol and other endogenous carotenoids (Lagorio et al., 2015)]. We have designed a combination of narrowband LEDs (400/480/667nm) and optical bandpass filters (532/595/630nm) that we will use in combination with a CCD camera to specifically measure roGFP2 fluorescence. The system will also be capable of imaging luciferase bioluminescence and will be integrated with an incubator adapted with a separate array of LEDs to provide growth illumination. Synchronisation of lighting and fluorescence/bioluminescence image capture will be completed using MicroManager (Edelstein et al., 2014), with image processing being completed using ENVI (L3Harris Ltd). This novel combination of imaging and growth lights will be used to characterise Arabidopsis expressing both roGFP2 and

luciferase reporter constructs (in-hand). Initially we will assess changes in redox homeostasis and gene expression in response to osmotic stress (in support of BBSRC BB/S005404/1). However, the tools developed as part of this project will enable new strands of research within the Jones and Harvey labs as we seek to characterise how plants respond to environmental stress.