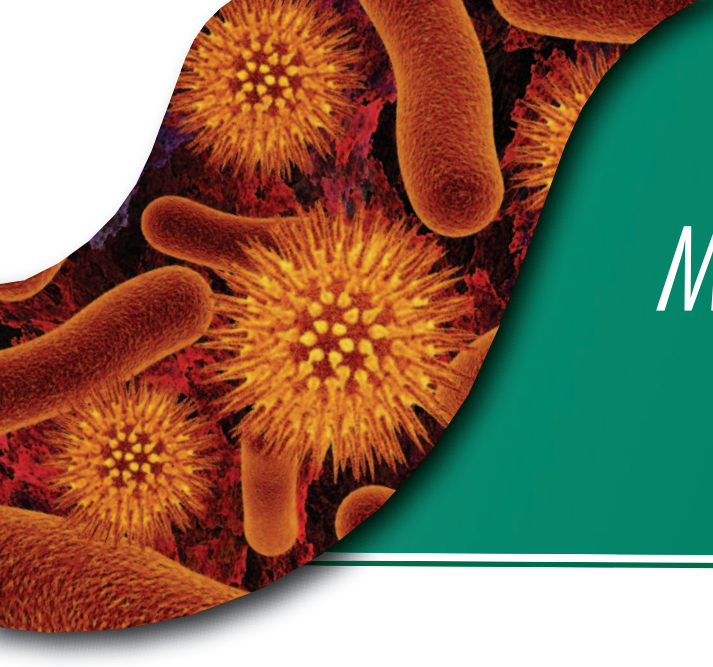

Investigating Phosphate Starvation in Plants Using Bioluminescence

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A reliable food source is essential for life. To meet the requirements of the world's growing population, food production will likely need to double by 2050 [1]. Overcoming this challenge will require the optimisation of crop growth, for example by improving efficiency and driving down costs.

This can be achieved in several ways. Firstly, many crops are affected by pests and disease, reducing the usable fraction of crop yield. Secondly, plants need a plentiful supply of various elements to grow, including sunlight, water and organic and inorganic molecules containing elements such as nitrogen, phosphorous (organically utilised as phosphate) or potassium. They also require space, management and maintenance to grow quickly and efficiently. Hence, strategies to mitigate any harmful or inhibitory effects caused by these factors would increase efficiency and yield.

To aid with this process, researchers in our group and others around the world are investigating how plants respond to drought, nutrient starvation and stress. In this way, we hope to understand how to encourage crops to grow better, even under challenging environmental conditions.

Phosphate and plant growth

Phosphate starvation has a significant impact on plant growth, and is the second most frequently limiting nutrient after nitrogen [2]. This is largely due to the fact that phosphate is a major component of many important biological molecules including DNA, the hereditary material, and adenosine tri-phosphate (ATP), the energy currency of the cell.

Although phosphate is often present in relatively large amounts in the soil, it is often trapped in forms that plants cannot easily utilise. Indeed, most unfertilised soils do not release phosphate sufficiently quickly for optimised crop growth. This creates a problem, as fertilisers rely on the addition of inorganic phosphate, a non-renewable resource harvested from phosphate rock, which takes millions of years to form. Eventually, the use of additional phosphate to drive plant growth will become impossible due to exhausted resources or economically unfeasible as diminishing availability drives up prices.

In an effort to circumvent the limiting effects of phosphate availability on crop growth, our team at the Laboratory of Developmental Plant Biology (funded by the French Alternative Energies and Atomic Energy Commission) is investigating the biological pathways and mechanisms involved in regulating phosphate uptake, utilisation and starvation.

Tracking phosphate pathways

Although our main interest is in investigating the response of plants to phosphate starvation, at the current time we still know relatively little about the genetic mechanisms that control phosphate utilisation during normal plant growth. Much of the work done to understand plant growth, development and responses to environmental stimuli is performed using the model system *Arabidopsis thaliana*. This weed is one of the best studied plants in the world, and was the first to have its genome sequenced in 2000 [3]. Therefore, we also use this system to study phosphate starvation.

Several Arabidopsis genes can be used as markers of phosphate-related activity. These include genes that were identified as being up-regulated or down-regulated in different parts of the plant in response to phosphate starvation [4]. For instance, the induction of phosphate transporters is a very reliable marker of a plant's response to phosphate depletion. Another example is the induction of several lipid metabolism enzymes that help the plant recycle the phosphate contained in phospholipids. Many genes that we are currently studying are relatively new to this line of research, and as such, their role during phosphate starvation may be of special interest.

Something that is already clear from previous work by our group [5] is that factors controlling phosphate metabolism can have distinct functions apart from this process, as well as short and long distance effects that can be difficult to tease apart [4]. For example, the expression of FRY1 (a bifunctional phosphatase enzyme) in the shoots of Arabidopsis modulates the development of roots by long range signalling, whereas local expression in the root appears to affect the expression of a number of phosphate starvation marker genes. This suggests that FRY1 might regulate the phosphate starvation response via a mechanism distinct from its role in root growth and development.

To understand how complex metabolic mechanisms regulate local phosphate starvation, it is helpful to create a temporal and positional atlas describing the exact expression patterns of important pathway genes. Initially, this involves documenting the regulation of gene expression during normal environmental conditions, before exploring how these patterns change upon the onset of phosphate starvation. In this way, we can identify those genes and pathways that play a vital role specifically during the starvation response.

Visualising gene expression in organs, tissues and cells

In our lab we are using the Olympus LV200 Luminoview microscope system to track gene expression using a bioluminescent reporter system employing the firefly luciferase enzyme. As the name would suggest, this enzyme was isolated from fireflies and is responsible for catalysing the reaction that produces the luminescent glow for which the flies are well known.

Using genetic engineering, it is possible to create artificial gene constructs combining a gene of interest with the firefly luciferase gene. In the presence of a luciferin substrate, the activity and location of gene expression can then be tracked by following photon emission. Alternatively, the control regions for a given gene can be engineered to drive expression of luciferase instead of the endogenous target gene, providing an accurate readout of gene expression without the need to create potentially toxic or biologically atypical chimerical proteins.

Using our reporter and imaging system we have been able to observe gene expression across the whole plant, from organs and general areas right down to the level of individual cells and tissues (*Figure 1*). This is extremely powerful, as it allows us to fine map very precisely when and where individual genes are being expressed. After normal expression has been assessed, we can then reinvestigate the same genes during phosphate starvation, simultaneously identifying those whose expression might be modulated to boost plant tolerance to low phosphate supplies.

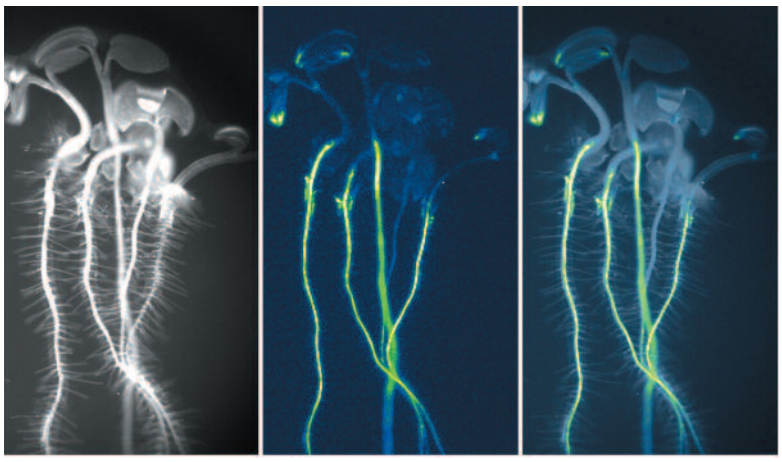

Figure 1. Transmitted light image (A), bioluminescence signal (B) and overlay of the two images (C) showing Arabidopsis plantlets grown in a phosphate-deprived medium. This plant line expresses a luciferase reporter gene driven by the promoter of a phosphatase (5).

The strengths of bioluminescence

When wishing to visualise gene expression or promoter activity *in vivo*, researchers have a number of options to choose from including the β-galactosidase system, green/red fluorescent proteins (GFP/RFP) and their derivatives, as well as light emitting systems such as firefly luciferase. Each approach has its own strengths and weaknesses influencing its suitability for use in each assay. For our research we opted to use a bioluminescence reporter, as it has several advantages over similar fluorescent techniques.

Fluorescent assays are plagued by background auto-fluorescence, increasing noise and reducing the sensitivity of GFP/RFP reporter systems. In contrast, bioluminescent reporter assays require no excitation with fluorescence illumination, instead producing photons directly as the result of a chemical reaction. This eliminates background auto-fluorescence and maximises signal to noise ratio [6]. The approach is also up 40 times more sensitive than fluorescent microscopy, as the physical components of a bioluminescent microscope system can capture a higher proportion of the photons emitted by the sample. For the user, this means that labelled features which might be difficult to differentiate from background using fluorescent approaches can now be clearly distinguished and analysed. Therefore, the technique is ideal for measuring genes/proteins expressed at very low levels and is capable of detecting a handful of molecules per cell, while remaining flexible enough to detect and quantify gene expression levels across several orders of magnitude.

Luciferase-based methods are also better reporters of response dynamics than most fluorescent proteins, as they can provide detectable but transient readouts. Modern synthetic luciferases have been engineered to have very short half lives, such that the production of photons ceases soon after the reporter gene becomes inactive [7]. The low background offered by bioluminescence approaches also means that they can provide usable data even when expressed at low levels. Therefore, even if the expression time window is short, a signal will still be detected. In contrast, fluorescent proteins take time to mature after the onset of expression and tend to be relatively long lived, continuing to fluoresce for several hours after their initial expression. They are subsequently limited in terms of the temporal resolution they can provide. This factor is particularly important when investigating living cells, where gene expression patterns can change very quickly. Using the Olympus LV200 Luminoview microscope system we have been able to reliably detect transient gene expression using luciferase reporters, even at very low levels.

Conclusions

In the future, we aim to further develop our bioluminescence assay for detecting dynamic gene expression in developing plants, using it to create an atlas of expression patterns for genes important in the phosphate starvation response. Once we have accurately analysed these patterns in plants grown under optimal conditions, we can go on to investigate how these change in low phosphate environments.

Along the way, we hope to identify those genes that play a key role in the starvation response, as it may be possible to manipulate their expression or activity to boost plant tolerance to low phosphate supply. It is hoped that such genetic approaches might help optimise the growth of important crop species, by minimising the need for external phosphate harvested from non-renewable sources.

References

1. Parry M.A.J. and Hawkesford M.J. (2010). Food security: increasing yield and improving resource use efficiency. Proceedings of the Nutrition Society, 69: 592-600.

2. Schachtman D.P. et al. (1998). Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiology, 116: 447-453.

3. Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature, 408(6814):796-815.

4. Thibaud M.C. et al. (2010). Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. The Plant Journal, 64:775-789

5. Hirsch J. et al. (2011). A novel fry1 allele reveals the existence of a mutant phenotype unrelated to 5'->3' exoribonuclease (XRN) activities in Arabidopsis thaliana roots. PLoS One, 6(2):e16724.

6. Lewis J.C. and Daunert S. (2000). Photoproteins as luminescent labels in binding assays. Fresenius Journal of Analytical Chemistry 366: 760–768.

7. Fan F. and Wood K.V. (2007). Bioluminescent assays for high-throughput screening. Assay and Drug Development Technologies, 5(1):127-36.

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