Non-invasive, real-time mapping of metabolite secretion from roots of *Vicia hirsuta* during nodule formation using Lux biosensors

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Background

Metabolite secretion by plant roots is a relatively unexplored area. Being able to image plant roots and examine the presence of metabolites during growth and development is an exciting prospect.

We have recently developed maps of bacterial transcription in *Rhizobium leguminosarum* 3841 which reveal the presence of specific plant metabolites released by roots (1). To capitalize on this information we have developed bacterial *lux* fusions to the promoters of many of these genes.

A suite of 15 *lux*-expressing bioreporters were developed to detect

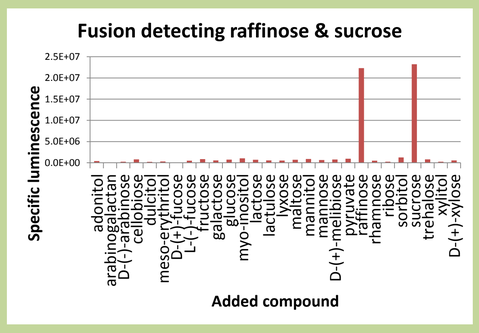
- specific sugars (mono- and di-saccharides),
- polyols,
- organic acids (mono- and di-carboxylic acids),
- amino acids
- flavonoids.

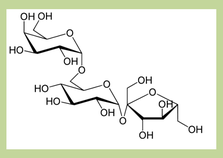
Detection of Lux with a specialised charge-coupled device (CCD) camera (NightOWL) is non-invasive and this system provides spatial and temporal information on the presence of metabolites on roots of growing plants.

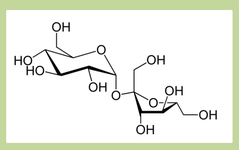
R. leguminosarum 3841 forms nitrogen-fixing nodules on the roots of the legume *Vicia hirsuta* (vetch). The time course of this developmental process was followed with fusions to detect different metabolites, including flavonoids.

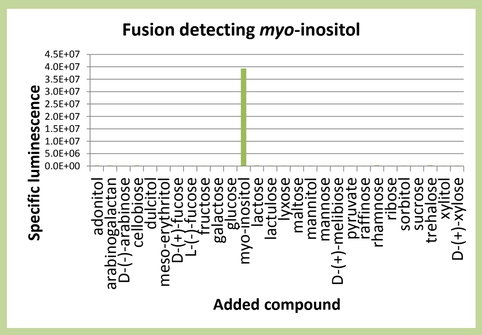
Determining induction profile

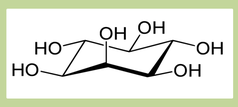
Promoter-containing fragments were amplified by PCR and cloned into a *luxCDABE* reporter vector (2). Following conjugation into *R.leguminosarum* 3841, the induction profile of each fusion was determined following growth in the presence of different metabolites (10mM).

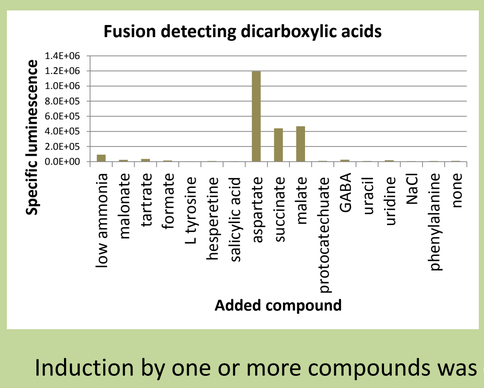


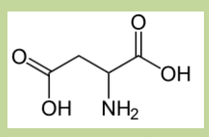


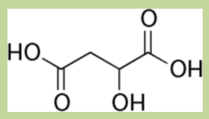








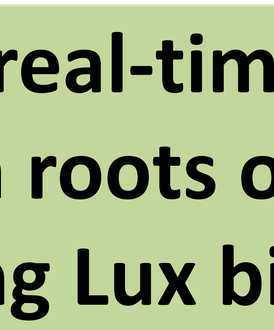




Induction by one or more compounds was dependent on chemical structure, with closely related compounds often causing induction of the same fusion. Level of expression was dependent on the strength of each individual promoter.

Results on plates and peas

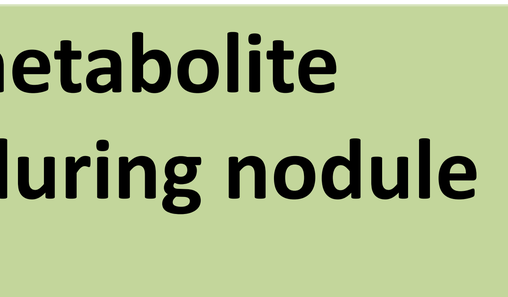
Bacterial growth of a biosensor on an agar plate containing its inducer resulted in *lux* expression.
An example is a fusion to detect phenylalanine. The same fusion is shown investigating the rhizosphere of a pea seedling, 1dpi.



+Phe

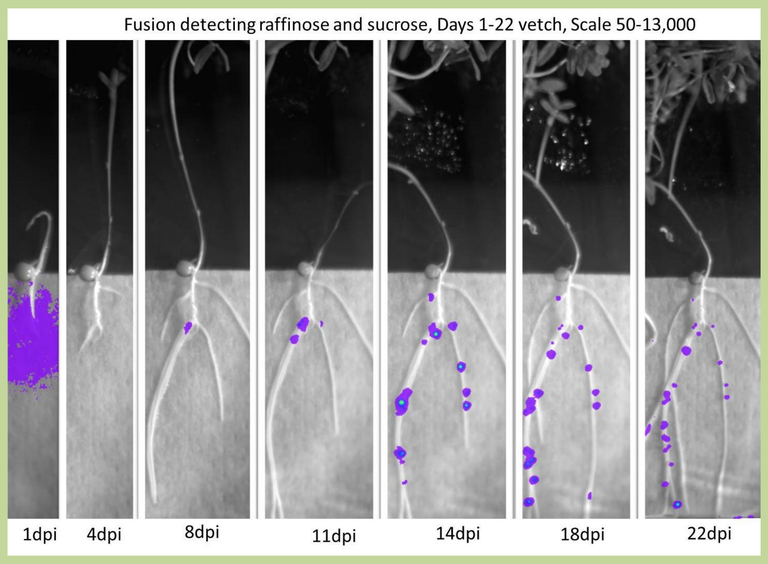
-Phe

Phenylalanine is released from root hairs and from the pea itself.



*RL1860

Detection in the rhizosphere and on roots of vetch

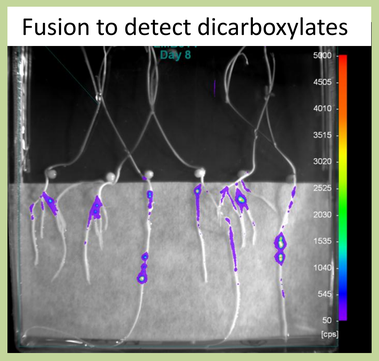


Sucrose/raffinose is present in the rhizosphere of vetch (1dpi).

Nodules on vetch roots are visible by eye at 8dpi.

It is not until 11-14dpi that sucrose/raffinose is detected in vetch nodules.

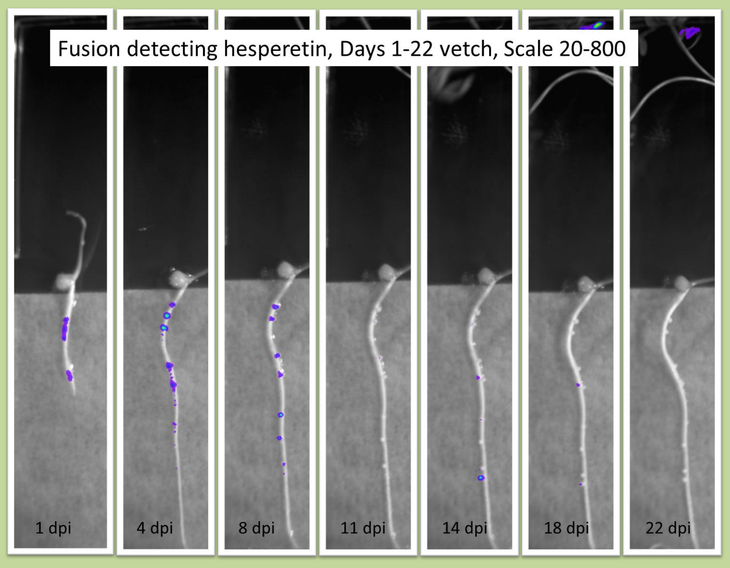
Fusion to detect dicarboxylates



At 8dpi the presence of dicarboxylates is clearly visible in the young developing nodules, as well as on regions of the root.

Fusion to detect inositol (4dpi), Scale 50-10,000 cps

Inositol is present on vetch roots at 4dpi.



Using a fusion to detect the flavonoid hesperetin, the position at which the nodules will later develop is visible at 1dpi.

At 4 and 8 dpi the nodules are detected using the fusion, before the are visible by eye.

As new nodules emerge they are briefly lit up as the presence of flavonoid is detected.

Summary

This suite of fusions has been used to investigate the rhizosphere in a non-invasive manner, providing spatial and temporal information on the metabolites present on plant roots.

References

(1) Ramachandran, V., East, A.K., Karunakaran, R., Downie, J.A. & Poole, P.S. (2011) Adaptation of *Rhizobium leguminosarum* to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. Genome Biology 12:R106.

(2) Frederix, M., Edwards, A., Swiderska, A., Stanger, A., Karunakaran, R., Williams, A., Abbruscato, P., Sanchez-Contreras, M., Poole, P.S. & Downie, J.A. (2014) Mutation of *praR* in *Rhizobium leguminosarum* enhances root biofilms, improving nodulation competitiveness by increased expression of attachment proteins. Mol Microbiol DOI 10.1111/mmi.12670.