A Whole Cell Biosensor (*GlnLux*) to Measure Symbiotic Nitrogen Fixation (SNF) in Legumes

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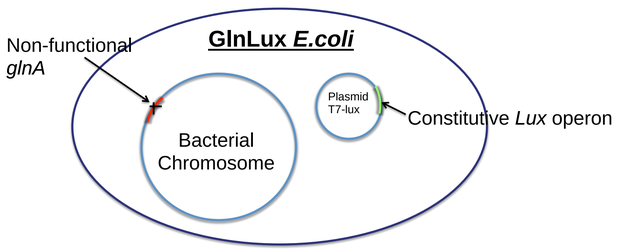
Abstract

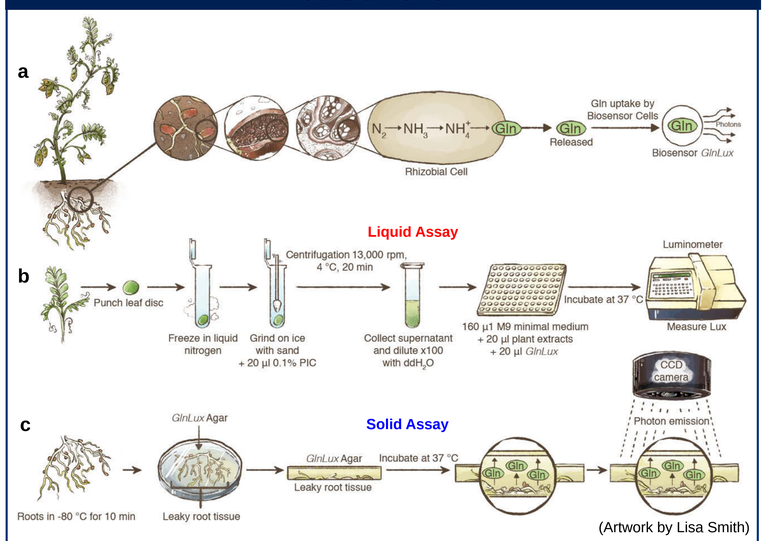
Measuring symbiotic nitrogen fixation (SNF) of large numbers of legume plants is a challenge in terms of time, labor, and cost. Here we present a whole cell biosensor to measure SNF efficiently in legumes. A whole cell biosensor (*GlnLux*) for glutamine (Gln) was constructed by transforming a bacterial Gln auxotroph with a constitutive lux reporter. Extracts from single legume leaf punches were sufficient for measuring SNF by the *GlnLux* method, wherein leaf extracts are incubated with *GlnLux* and luminescence is measured using a luminometer in 96 well plates. This method has potential to measure SNF in amide exporting legumes (lentil) as well as ureide exporting legumes (cowpea). Further the *GlnLux* method can be used to identify the effect of different rhizobia and plant genotypes on SNF. Interestingly the *GlnLux* method permitted localization of active sites of SNF in nodulated legume roots. For imaging of Gln in whole plant organs, tissues were freeze-thawed to cause Gln leakage, placed on agar pre-embedded with *GlnLux* (*GlnLux* agar), and then imaged using a photon capture camera.

Construction of *GlnLux* Whole Cell Biosensor



***GlnLux* cells are auxotrophic for Gln** (Tessaro, Raizada, 2012)

GlnLux Whole Cell Biosensor Assays to Measure SNF



(Artwork by Lisa Smith)

Figure 1 | (a) Overview of concept: Rhizobia inside the nodules (bacteroids) convert atmospheric nitrogen into NH_4^+, which is assimilated into Gln. In *in vitro* bioassays, Gln is taken up by *GlnLux* biosensor cells, allowing them to multiply, replicate the lux plasmid and release photons. (b) 96-well liquid assay: Leaf punches are sampled from the youngest full expanded leaves and immediately frozen in liquid nitrogen. Individual frozen leaf punches are ground using a micropestle with silica sand in a microcentrifuge tube placed on ice with protease inhibitor cocktail (PIC). Plant extracts are centrifuged, and the supernatant is transferred to a microcentrifuge tube on ice. White opaque 96-well reader plates are loaded with M9 minimal medium to which each plant extract is added. Finally, an aliquot of 14 h-Gln-depleted *GlnLux* culture is added to each well. Plates are sealed with sterile film, mixed, and incubated at 37 °C without shaking. For lux quantification, plates are read in a luminometer. (c) Solid assay (tissue imaging): Legume roots are frozen in -80 °C and thawed at room temperature for 1 min. Roots are placed in contact with agar containing *GlnLux* cells (*GlnLux* agar) and tissues are pressed down. Inverted plates are imaged at the zero time point, then incubated at 37 °C with hourly imaging with 300-600 s exposure times using a ChemiProHT luminescence imaging system.

GlnLux Distinguishes Amide-Exporting Plants Inoculated with Diverse Rhizobia

GlnLux Liquid Assay with leaf punches

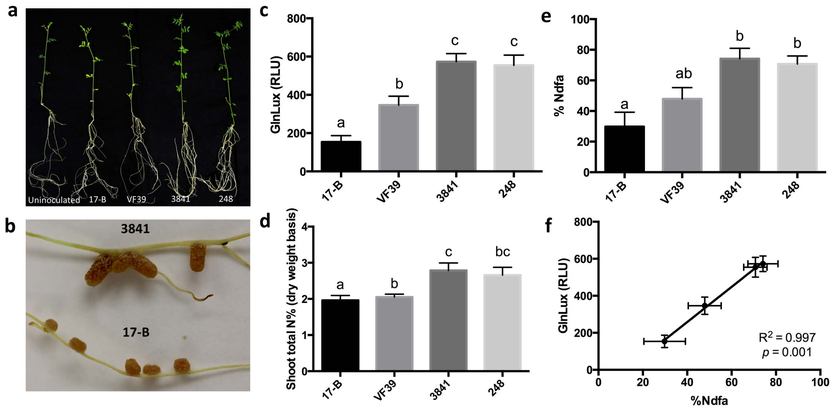


Figure 2 | (a) Lentil plants inoculated with four different strains of *R. leguminosarum* bv. *viciae* (wild type: 3841, VF39, 248; mutant 17-B) and uninoculated control. (b) Mutant strain (17-B) produced smaller nodules compared to the plants inoculated with wild type strains. (c) Shoot Gln content of lentil plants tested using the *GlnLux* assay (RLU, relative light unit). (d) Shoot total N% of lentil plants. (e) The % of nitrogen derived from atmosphere (%Ndfa) of lentil plants calculated using ^{15}N isotope dilution technique. (f) The correlation between *GlnLux* and %Ndfa.

GlnLux Solid Assay with Roots

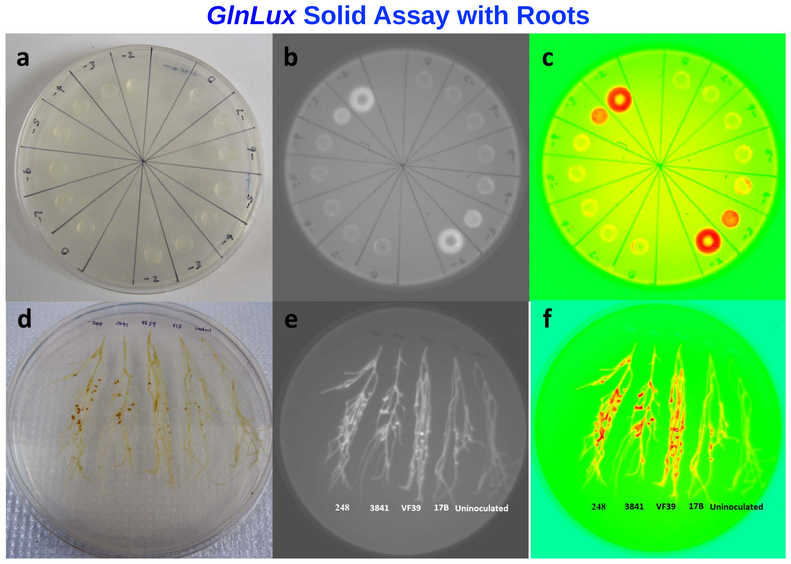


Figure 3 | Luminescence imaging of pure Gln liquid standards and lentil root Gln using *GlnLux* agar. (a-c) Agar discs were prepared with different concentrations of Gln (0, 10^{-7} – 10^{-2} M) and placed on *GlnLux* agar. The opposite agar surface was then imaged using a photon capture CCD camera. (d-f) Four-week-old lentil roots inoculated with four different strains of *R. leguminosarum* bv. *viciae* (VF39, 248, 3841, 17-B) or control (un-inoculated) were freeze-thawed to cause Gln leakage, and then placed on *GlnLux* agar. The opposite agar surface was then imaged using a photon capture CCD camera. (a,d) The light image, (b,e) white lux image, and (c,f) false-colored lux images after 3 hours of incubation and 300 s exposure.

GlnLux Distinguishes Ureide-Exporting Plants Inoculated with Diverse Rhizobia

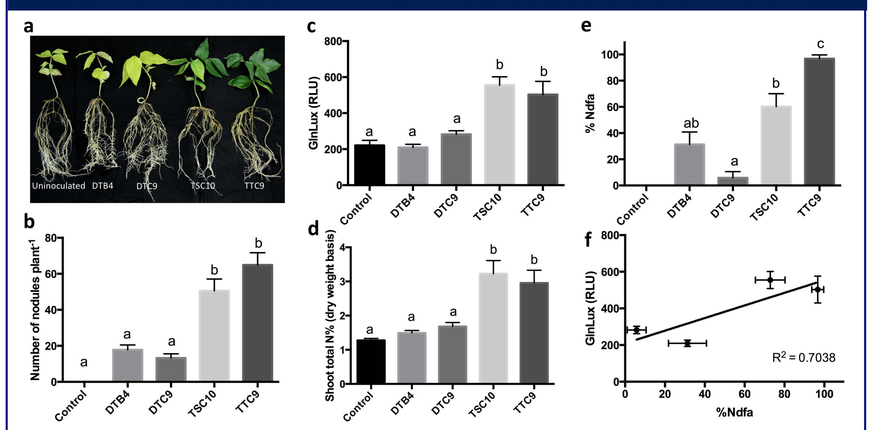


Figure 4 | (a) Cowpea plants inoculated with *Bradyrhizobium yuanmingense* (TTC9, TSC10), *B. japonicum* (DTB4), *B. elkanii* (DTC9), or an un-inoculated control. (b) Number of nodules per plant. (c) Shoot Gln content of lentil plants tested using *GlnLux* leaf punch assay (RLU, relative light unit). (d) Shoot total N% of lentil plants. (e) The %N derived from the atmosphere (%Ndfa) in lentil plants calculated using ^{15}N isotope dilution. (f) The correlation between *GlnLux* and %Ndfa.

Active Sites of Nitrogen Fixation can be Visualized Using *GlnLux*

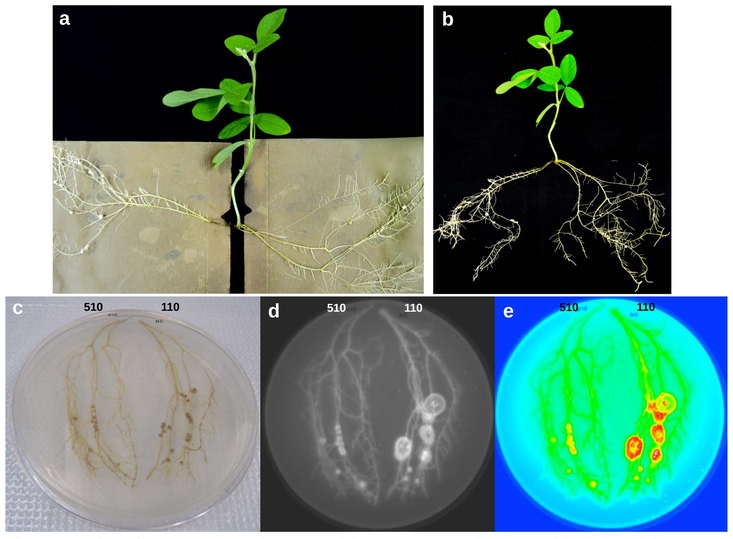


Figure 5 | Split root experiment with soybean plant. (a-b) One side was inoculated with *B. japonicum* 110 (WT), while the other side was inoculated with 510 (MT). (c-e) Luminescence *in vivo* imaging of Gln in soybean roots using *GlnLux* agar. Freeze-thawed roots were placed on *GlnLux* agar. The opposite agar surface was then imaged using a photon capture CCD camera. (c) The light image, (d) white lux image, and (e) false-colored lux images after 2 hours of incubation and 600 s exposure.

Conclusions

- *GlnLux* biosensor is a new method to measure SNF in both ureide- and amide-exporting legumes.
- *GlnLux* 96-well liquid assay uses single leaf punches to measure relative SNF in plants growing without exogenous N, making it a rapid, low-cost, high throughput screening method.
- *GlnLux* solid assay permits visualization of active sites of nitrogen fixation.
- *GlnLux* method can be used to screen plants inoculated with different rhizobia strains to optimize SNF.
- *GlnLux* method is being tested for its ability to distinguish plant genotypes that vary in SNF.

References

Tessaro, M.J., Soliman, S.S.M. and Raizada, M.N. 2012. Bacterial Whole-Cell Biosensor for Glutamine with Applications for Quantifying and Visualizing Glutamine in Plants. *Appl. Environ. Microbiol.* **78,** 604–606.

Thilakarathna, M.S. and Raizada, M.N. 2015. Bacterial Whole-cell biosensor (*GlnLux*) to Measure Symbiotic Nitrogen Fixation in Legumes. (in preparation)

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