Developing novel selection markers for plant transformation to advance live-imaging techniques

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The Idea

We aim to develop a novel visual selection marker for plant transformation that 1) is not based on resistance to antibiotics and herbicides, 2) allows for fast screening of transformed plant tissue, and 3) does not interfere with live cell fluorescence microscopy.

In the Oldroyd lab we study root endosymbioses using the model plant Medicago truncatula. Medicago and other plant species can be transformed transiently by hairy root transformation, a fast transformation technique that can be used to introduce and test a large number of constructs within a few weeks. Because these hairy roots are usually used to assess symbiotic interactions with bacteria and mycorrhiza, the selection markers should not interfere with root development or symbiotic interactions, ruling out markers based on resistance to antibiotics and herbicides. Currently, a dsRed marker under the 35S promoter is used as a visual selection marker, but because it is strongly expressed in all tissues, confocal live imaging of fluorescent markers under endogenous promoters is not feasible.

To solve this problem, we aim to develop tissue-specific visual markers that do not interfere with the region of our interest in the root (the differentiation zone and the proximal elongation zone). In addition, we would like to develop organelle-specific markers that are only present in a very restricted subcellular region of the cells, such as the nuclear envelope. We will test this approach using fluorescent proteins (dsRed and 3XVENUS) as well as chromo proteins, which are visible to the naked eye and therefore allow for a faster screening of transformed tissue without the need of a fluorescence microscope.

The fast hairy root transformation system in combination with the modular Golden Gate cloning approach will allow us to test and compare a large number of different visual markers in a short amount of time. In order to assess whether fluorescent and chromo protein markers can also be useful for other plant transformation systems, we will test the constructs in Marchantia polymorpha under appropriate promoters.

Who We Are

Fernan Federici, is from Argentina, currently an assistant professor at Department of Molecular Genetics and Microbiology, director of the Synthetic Biology Centre at PUC University-Chile and research associate at Cambridge Univ. Trained as a biologist and working on SynBio for the last 6 years (on characterization of components, engineering form in bacterial colonies, CRISPR, iGEM, teaching resources). Experience in microscopy, DNA assembly, morphogenesis, plant development and bacterial engineering.

Katharina Schiessl is a Post Doc working on nodule organogenesis in the Oldroyd Group at the John Innes Centre in Norwich; interested in post-embryonic organogenesis in plants; experience in microscopy, hairy root transformation, nodulation assays.

Leonie Luginbuehl is a PhD student in the Oldroyd group at the John Innes Centre; studying the transcriptional regulation during symbiosis signalling in Medicago; experience in Golden Gate cloning, hairy root transformation, nodulation and mycorrhization assays.

Guru Rhadakrishnan is a PhD student working with Giles Oldroyd at the John Innes Centre on the evolution of signalling events during plant-microbe interactions; expertise in Marchantia transformation.

Implementation

In order to develop tissue-specific visual selection markers, we will express the fluorescent proteins dsRed, mKate2, TdTomato, and mNeonGreen and the chromo proteins cjBlue, AmilCP, and aeBlue under the promoter of the BEARSKIN gene that has been shown to be specifically expressed in the Arabidopsis lateral root cap (Bennett et al., 2010; for example pMt/AtBEARSKIN:dsRed and pMt/AtBEARSKIN:mNeonGreen). In addition to testing the tissue-specific expression of chromo proteins, we will also assess the use of constitutively expressed chromo proteins as selection markers.

For an organelle-specific visual marker with very confined expression patterns we will test fluorescent proteins fused to the NUP85 protein that has been shown to be exclusively expressed in the nuclear envelope (Groth et al., 2010; for example p35S:MtNUP85-mCherry and p35S:MtNUP85-dsRed).

To achieve this we will design and synthesise the DNA sequences as level 0 GOLDENGATE components (promoters, terminators, and coding sequences of fluorophores and chromo proteins). We will assemble these components using GOLDENGATE cloning and we will test the constructs using hairy root transformation in *Medicago truncatula*. In order to test whether the generated visual selection markers interfere with endogenous reporter genes we will test the root specific marker *SHORTROOT* (pSHR:SHR-GFP) in combination with the visual transformation markers. In addition, we will test whether these visual selection markers have any negative effects on root development or on the capability to enter symbioses with rhizobia and mycorrhizal fungi. The visual selection markers will also be tested in *Marchantia polymorpha*.
The synthesised DNA parts will be made publically available as GOLDENGATE Level 0, 1 and 2 components.

Design and synthesis of constructs: Leonie, Katharina, Fernan (3 months)
GOLDENGATE cloning: Leonie, Katharina (2 weeks)
Hairy root transformation of Medicago: Leonie, Katharina (1 month)
Transformation of Marchantia polymorpha: Guru (2 months in parallel to hairy root transformation)
Symbiosis assays: Leonie, Katharina (1 month)
Microscopy and imaging: Fernan, Katharina (2 weeks)

Benefits and outcomes

Novel visual tissue- and organelle-specific selection markers will benefit the research community working on legume root symbiosis including model plants like Medicago and

Lotus but will also benefit engineering efforts in important crop species like soybean, beans and peas. Furthermore, these selection markers will benefit a wider research community using hairy root transformation systems in a variety of dicotyledonous plant species for example in tomato, poppy and different medicinal plants. Moreover, novel visual selection markers will be an alternative to selection markers that are based on resistance to antibiotics and herbicides or can be used for double selection. Hence, our novel visual selection markers will be useful components in plant engineering. Particularly for crop species, providing a transformation marker system that does not require the use of antibiotics and herbicides will also be saver for the environment.

The marker constructs will be available as Level 0, Level 1, and Level 2 GOLDENGATE components and can therefore easily be adapted into different cloning and transformation protocols of various plant species.

Budget

DNA synthesis of level 0 components for GOLDENGATE cloning
(promoters, terminators and coding sequences): £2400
Golden Gate cloning and sequencing: £200
Transformation and bioassays (nodulation and mycorrhization): £200
Microscopy and imaging: £1200