OpenPlant Fund Application Form

Application Deadline: Midnight on 24 November 2017

Please submit an application containing the following sections by email to colette.matthewman@jic.ac.uk in an editable format such as .odt or .docx (not PDF) and attach any images separately to the email.

Title of Project: Towards an efficient transformation system for legumes.

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Team

1. Dr Abhimanyu Sarkar (abhimanyu.sarkar@jic.ac.uk) will clone the somatic embryogenesis genes, make constructs, help with the grasspea transformation experiments, analyse the data and write the manuscript.
2. Dr Julia Russell (julia.russell@jic.ac.uk) will perform grasspea transformation, maintain and analyse the grasspea transformants and write the manuscript.
3. Dr Susana Sauret-Gueto (ss2359@cam.ac.uk) has supplied the information regarding the Loop Assembly cloning system.

Preface

Grasspea (*Lathyrus sativus*) is a hardy legume that originated in the Balkans. It produces seeds that are high in protein (25- 30 % by weight). In spite of it containing a neurotoxin (b-ODAP) that can cause paralysis of the lower limbs (lathyrism) when consumed as a staple food over an extended period of time, it is grown by farmers in the Indian subcontinent and Africa as an insurance against crop failure, for use as a food of last resort. Grasspea has the potential to be developed as an important source of protein to fulfil the needs of the growing population in Africa and Asia in a sustainable manner in the face of climate change. Efforts to produce a grasspea plant that lacks the neurotoxin have been handicapped by the lack of resources and tools to transform the plant.

Summary

Legumes (family Fabaceae) are a family of plants that are of tremendous importance to humans due to their role in fixing atmospheric nitrogen (in symbiosis with nitrogen fixing bacteria) and in agriculture due to the high protein content of their seeds. However, plant improvement in legumes is handicapped by the difficulties in achieving routine transformation; this makes the use of genetic modification and genome editing for improving legumes very difficult to achieve. We will develop an

efficient transformation platform for a legume, grasspea (*Lathyrus sativus*), by co-expressing genes that positively regulate somatic embryogenesis with our transformation/gene editing constructs. This work can be subsequently extended to other legumes of economic and academic importance, as well as other groups of plants.

Proposal

Legumes in general and grasspea in particular is difficult to transform routinely/at high efficiencies. This is the chief reason holding back the application of the advances in transgenic and genomic editing technologies in legumes. One of the major bottlenecks in the transformation process is the lack of somatic embryogenesis from legume explants in tissue culture. Studies in *Arabidopsis*, a model plant, has identified a number of genes that positively regulate somatic embryogenesis (reviewed by Elhiti *et al*., 2013). *LEAFY COTYLEDON* (*LEC1, LEC2*) genes were shown to be required for somatic embryogenesis (Harada, 2001; Karami, *et al*., 2009). Overexpression of a transcription factor gene *WUSCHEL* (*WUS*) has been shown to enhance somatic embryogenesis in *Arabidopsis* (Zuo *et al*., 2002). We will clone the homologues of these genes from grasspea using standard molecular biology techniques and plant expression/transformation constructs will be assembled using the Loop Assembly system developed in Dr Jim Haselhoff's lab. Constructs will be verified by sequencing and *Nicotiana tabacum* transformation before using them to transform grasspea in tissue culture (by *Agrobacterium tumefaciens* mediated transformation). Somatic embryogenesis will be observed by light microscopy and stable transformants selected using BASTA and confirmed by inverse PCR and the presence of the scorable marker GFP.

Intellectual Property position and Freedom to Operate

While there exist patents on the genes that we propose to use for enhancing somatic embryogenesis in grasspea, we are not restricted from using them for research purposes **i.e. we have freedom to operate for research purposes**. Thus, the tools developed by us should be shareable within the legume research community. Also, the "*Leafy cotyledon1* genes and their uses" patent (WO 1999067405 A2/ US 6320102 B1) is set to expire on 24 June 2018. The "*Leafy cotyledon 2* genes and their uses" (WO 2001070777 A3) will expire on 16 March 2020 while the *WUSCHEL* patent "Promotion of somatic embryogenesis in plants by wuschel gene expression" (US 20030082813 A1/ WO 2003037072 A3) will expire on 29 Oct 2021.

Aims

1. Clone grasspea genes that positively regulate/enhance somatic embryogenesis from explant tissue. Three grasspea genes, *LEAFY COTYLEDON* (*LEC1, LEC2*) and the transcription factor gene *WUSCHEL* (*WUS*) will be cloned.

2. Achieve increased somatic embryogenesis in grasspea by co-expressing (individually) the cloned genes with transformation constructs using *Agrobacterium tumefaciens* mediated transformation.
3. Achieve a stably transformed grasspea.

Methods

1. Three grasspea genes, *LEAFY COTYLEDON* (*LEC1, LEC2*) and the transcription factor gene *WUSCHEL* (*WUS*) will be cloned by PCR amplification from the genomic DNA of grasspea and standard molecular biology techniques. Plant expression (under a constitutive promoter) and transformation constructs (containing a selectable marker, *BAR*, conferring resistance to the herbicide BASTA, and a visually scorable marker, GFP) will be made using the Loop Assembly system developed in Dr Jim Haselhoff's lab. (***A. Sarkar and S. Sauret-Gueto***).
2. Standard *Agrobacterium tumefaciens* mediated transformation techniques will be used to transform *Nicotiana* and grasspea with transformation constructs overexpressing the somatic embryogenesis genes (individually). Tissue culture techniques developed for grasspea regeneration by our (JIC) lab will be used to grow grasspea transformants (***J. Russell and A. Sarkar***).
3. Stable transformation of grasspea will be confirmed by the presence of marker genes (BASTA resistance and GFP expression) and inverse PCR to confirm genomic integration of the transgenesis construct in the genome of the grasspea. (***A. Sarkar and J. Russell***).

Outcomes

The relative efficiencies of the cloned genes *LEAFY COTYLEDON* (*LEC1, LEC2*) and *WUSCHEL* (*WUS*) in promoting somatic embryogenesis in grasspea will be assessed as a result of these experiments. The **major outcome of the proposed project** will be the development of a method to efficiently transform grasspea in particular and legumes in general. This will be extremely useful in helping set up a genome editing platform in legumes. **Other outcomes** of the project will be to verify that the homologues of the *Arabidopsis* genes involved in somatic embryogenesis are also involved in regulating somatic embryogenesis in grasspea. The results and the method developed for achieving enhanced somatic embryogenesis in tissue culture may be extended to other plant systems as well.

References

Elhiti M, Stasolla C, and Wang A (2013) In Vitro Cell.Dev.Biol.—Plant 49:631–642. *doi 10.1007/s11627-013-9547-3*.

Harada JJ (2001) Role of *Arabidopsis LEAFY COTYLEDON* genes in seed development. J Plant Physiol 158:405–409.

Karami O, Aghavaisi B, Pour AM (2009) Molecular aspects of somatic to-embryogenic transition in plants. J Chem Biol 2:177–190.

Zuo J, Niu QW, Frugis G, Chua NH (2002) The *WUSCHEL* gene promotes vegetative-to-embryonic transition in Arabidopsis. Plant J 30:349–359.

Benefits and outcomes

- The proposed project will utilise an **open technology** (Loop Assembly for plasmid construction) developed in Dr Jim Haselhoff's lab (**Cambridge**) by Bernardo Pollak to develop an efficient transformation system in grasspea at the John Innes Centre (**Norwich**).
- **This interdisciplinary knowledge exchange** (before the publication of the technology) between a synthetic biology (**Cambridge**) and a plant biology laboratory lab (**Norwich**) is critical in allowing the execution of the project in the proposed timeframe.
- Achieving improved transformation in grasspea, a legume, will provide a **valuable contribution to the field of plant biology** by establishing a method that will allow efficient transformation in legumes, an economically and academically important family of plants. The *concept can be extended* to other groups of plants.
- The methodology developed under this project will be **published in an open access journal**, and the **resources developed shared freely**, enabling efficient transformation and genome editing in legumes and other plants by other groups.
- The proof of concept of increased somatic embryogenesis and transformation in grasspea is **realistic and achievable** in the six month time frame with the requested resources.

Sponsor for the research and cost centre

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I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

We (***John Innes Centre, Norwich***) request **£4000** to cover the costs of laboratory reagents, molecular biology kits, PCR, sequencing of constructs, tissue culture plastic ware and media. *No monies for travel or salary costs are requested*. The salary of Dr Abhimanyu Sarkar (JIC) is paid from the Detox Grasspea grant, an Indo-UK (BBSRC, UK and DBT, India) funded grant to improve grasspea.