

# A synthetic biology approach to investigating arbuscular mycorrhizal symbiosis in *Marchantia paleacea*

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## The Idea

D14-LIKE (D14L) encodes an alpha/beta hydrolase receptor that has been well characterized for its role in the perception of the smoke constituent karrikin; whilst in recent years it has been heavily studied for functions in development and light responses. Recently however it has also been identified as being vital for the establishment of arbuscular mycorrhizal (AM) symbiosis in rice (*Oryza sativa*). Mutation of this gene results in a complete breakdown in communication between the plant and fungus (Gutjahr et al 2015). The evolutionary origin of the AM symbiosis coincides with the occurrence of the early land plants with affinity to liverworts approximately 450 million years ago. The liverwort lineage includes members of the Marchantiaceae of which some species, such as *Marchantia paleacea*, engage in AM symbioses; whilst others, including *Marchantia polymorpha*, do not. Here, we propose to determine the relevance of the ancient D14L for AM-symbiosis. The approach is two-fold and involves (1) genetic complementation of the rice d14l mutant with synthesized homologs of *M. paleacea* and *M. polymorpha*. (2) the CRISPR-Cas9-based editing of the *M. paleacea* locus to assess the functional requirement of MpD14L for AM symbiosis. The project utilizes gene synthesis, Golden Gate cloning, the CRISPR/Cas9 system and established protocols for liverworts available in the Oldroyd laboratory.

## Who we are

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## Implementation

Open Plant funding will facilitate a set of experiments; first of which would involve the synthesis, cloning and transformation of D14L constructs into the rice d14l mutant background (table 1). Once constructs are successfully expressed, transformants will be screened for complementation of the mycorrhizal phenotype with AM fungus *Rhizophagus irregularis*.

Open Plant funding will facilitate the synthesis and cloning of D14L constructs for genetic complementation of the rice d14l mutant (table 1).

The *Marchantia paleacea* has recently been sequenced by the Oldroyd group. This means D14L sequences for both *M. paleacea* and *M. polymorpha* are now readily available for synthesis. Considering that D14L is over 2kb in length, synthesising provides a more attractive option than PCR amplification to ensure that the gene modules are accurately produced. The Golden Gate assembly method will be used to introduce the synthetic D14L sequences into plasmids for cloning. Andrew Breakspear in the Oldroyd group, an expert of the Golden gate system, will aid in setting up this

process. The implementation of this cloning method will provide an extremely valuable tool, allowing for the simply assemble up to nine sequence fragments at a time in a recipient plasmid; which in this case would include modules for the gene of interest, native promoter and resistance. Importantly, the plasmid modules designed in this process will then be open-sourced for other researchers to utilise. Synthesised constructs will be introduced to the rice d14l mutant background via agrobacterium-mediated transformation, routinely performed at Cambridge. Successfully transformed plants will be screened for mycorrhizal colonisation.

## Table 1. Summary of transformations

The first transformation will act as a control to confirm that expression of rice D14L CDS restores normal mycorrhizal colonisation (table 1). *Arabidopsis thaliana* provides a well-characterised non-host control, whilst an empty vector will be used as a negative control. Transformations 3-8 will then assess the ability for copies of D14L from two closely related liverwort species, *Marchantia paleacea* (AM-host) and *Marchantia polymorpha* (non-host), to complemented the mycorrhizal phenotype in rice. There are several conceivable outcomes from this analysis -

Outcome 1 - Neither *M. paleacea* or *M. polymorpha* successfully complement. This would show that the role of D14L in AM symbiosis is not conserved from rice to basal liverworts.

Outcome 2 – Some copies of D14L from either *M. paleacea* or *M. polymorpha* complement. This would show that the function of D14L in AM symbiosis is evolutionarily ancient.

Outcome 3 – Both *M. paleacea* and *M. polymorpha* D14L complement. This would show that whilst the function of D14L in AM symbiosis is evolutionarily ancient, there are not features of D14L itself that confer mycorrhizal specificity.

The second, reciprocal component of this project examines AM colonisation in *M. paleacea* itself. Utilising the CRISPR/Cas9 system in *M. paleacea*, edited D14L knockouts will be produced. Guru Radhakrishnan, who has established a CRISPR/Cas9 system in liverworts, will provide expertise for this work. Three CRISPR knockouts will be produced for study –

## Table 2. Summary of CRISPR knockouts

Mutant	Gene(s) knocked out
1	D14La
2	D14Lb
3	D14La and D14Lb

The resulting alleles will be molecularly characterised, and where appropriate phenotyped for mycorrhizal colonization. Loss of susceptibility of *M. paleacea* to *Rhizophagus irregularis* would confirm functional conservation of D14L across 400 million years of AM symbiosis. In addition, we would also be able to assess how symbiosis function is split (or not) between the two copies of MpD14L.

## Benefits and outcomes

This project offers multiple scientific and strategic benefits. Scientifically, our results will equally impact evolutionary, developmental and organismic interaction biology. Strategically, the proposed synthetic approaches will generate Golden Gate modules which will not only be useful for our project, but also provide valuable tools that can be shared with a wider community of plant researchers. The production of D14L CRISPR lines in *M. paleacea* will be highly beneficial for further investigations into the broader roles of this gene in symbiosis, development and light signaling.

Additionally, the Oldroyd and the Paszkowski lab have existing collaborations along their shared interest in endosymbioses which will be further fostered via the proposed work.

Transformation	Objective	Acceptor plants	Promoter	Selection marker
1	<i>Oryza sativa</i> D14L cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
2	<i>Arabidopsis thaliana</i> D14L cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
3	<i>Marchantia polymorpha</i> D14La cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
4	<i>Marchantia polymorpha</i> D14Lb cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
5	<i>Marchantia polymorpha</i> D14La and D14Lb cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
6	<i>Marchantia paleacea</i> D14La cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
7	<i>Marchantia paleacea</i> D14Lb cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta
8	<i>Marchantia paleacea</i> D14La and D14Lb cDNA	<i>d14l</i> mutant (Nihonmasari)	Native	Basta

## Budget

Golden gate cloning- £2500

Includes gene synthesis and production of modules for all constructs in this project, followed by transformations

CRISPR/Cas9 knockouts - £1000

Includes synthesis of CRISPR constructs and subsequent production of mutants

Arbuscular mycorrhizal phenotyping - £500

Including consumables, biological material, growth space, phenotyping and molecular confirmation of D14L expression

Total budget- £4000