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Title: "Transformation of rice calli to generate novel resistant rice plants"

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Applicant: Maik Reschke

Host: Emmanuel Guiderdoni, CIRAD Montpellier

Background

In some growing areas of rice, farmers struggle with high harvest losses due to the bacterial leaf blight and bacterial leaf streak diseases, which are caused by the plant pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), respectively (White & Yang, 2009). During an infection of these bacteria they build up a type-III-secretion system and deliver a cocktail of up to 30 different effector proteins into the cytoplasm of the plant cell (White *et al.,* 2009). Among these effectors are so-called transcription activator-like effectors (TALE), which function as specific transcriptional activators in the plant cell nucleus (Boch & Bonas, 2010). These TALEs are highly important virulence factors for the *Xoo* and *Xoc* bacteria. The proteins can bind to a specific DNA sequence via a programmable DNA binding domain consisting of nearly identical amino acid repeats arranged in tandem. Each repeat coordinates the binding of one base pair (bp) in which two adjacent amino acids, called repeat variable diresidue (RVD) determine the base-specificity (Boch *et al.*, 2009; Moscou & Bogdanove, 2009). Furthermore TALEs have eukaryotic motifs like nuclear localization signals and an acidic activation domain in the C-terminal part, as well as a type-III-secretion and translocation signal in the N-terminal part.

Two proteins of this family, the TALEs AvrXa7 and TalC are key virulence factors for the *Xoo* bacteria during infection of rice plants, since strains lacking these genes are no longer able to cause disease (Antony *et al*., 2010; Yu *et al.*, 2011). AvrXa7 and TalC promote the transcriptional activation of the rice *SWEET* gene *OsSWEET14*, which is regarded as a major susceptibility target (Antony *et al*., 2010; Yu *et al.*, 2011). Members of the *SWEET* gene family are sugar transporters and export glucose or sucrose from

the cytoplasm to the apoplastic space where it can be assimilated by the bacteria (Chen *et al*., 2010; Chen *et al*., 2012; Braun, 2012).

In the last few years two novel biotechnological techniques have been developed to edit the genome of higher eukaryotes, including plants. One tool is the TALE-based nuclease (TALEN) system, where the catalytic domain of the *Fok*I endonuclease is fused to a TALE (Miller *et al.*, 2011). The other system is the RNA-guided CRISPR/Cas9 system, where a short guide RNA directs the nuclease protein Cas9 to the DNA (Cong *et al.*, 2012; Jinek *et al*., 2012). Such tools can widely be used to target any specific site in a genome to introduce a DNA double strand break (DSB). The DNA repair machinery corrects those DSBs, by either non-homologous end joining (NHEJ) or homologous directed repair (HDR), which leads to small deletions and/or insertions. This repair mechanism allows the use of TALENs and CRISPR/Cas9 to insert mutations by cutting the genomic DNA without destroying the genome integrity.

Objectives/ Purpose of the visit

One of the possible strategies generating resistant rice plants against infections of *Xoo* and *Xoc* is to mutate the TALE binding sequence, of natural TALEs like AvrXa7 and TalC, in the promoter of major susceptibility genes. Small deletions and/or insertions at those target sequences will prevent the TALE to bind to the promoter and initiate the transcription of the gene. Accordingly, they cannot activate the expression of certain genes that would favor the disease progression. As a consequence, the bacteria would no longer be able to colonize these rice plants (Li *et al.,* 2012).

One goal of my PhD thesis is to generate such resistant rice plants using the TALEN and/or CRISPR/Cas9 system. I intend to mutate known and novel TALE boxes in the promoters of major susceptibility genes.

I already constructed TALENs to mutate the binding sequence of AvrXa7 and TalC. Our collaborators at the CIRAD and IRD in Montpellier (France) currently perform the generation of transgenic rice plants using these TALENs. During the STSM at the CIRAD in Montpellier (France) I wanted to learn the techniques of *in vitro* rice cultivation and callus transformation, that I can establish these methods in our lab in Halle/Saale (Germany) and use it to accomplish the objectives of my PhD thesis.

Description of the work

During my STSM different transformation experiments were ongoing at the CIRAD, so that I could directly attend most of the procedures in my visit. I learned the necessary techniques for *in vitro* rice cultivation and callus transformation mediated by *Agrobacterium*. All necessary solutions and media for rice callus cultivation were prepared and the processes of sterilizing rice seeds, induction of primary calli and multiplication of derived secondary calli were carried out. I was taught how to choose healthy and reactive rice calli and transform those with *Agrobacterium* carrying T-DNAs of TALEN constructs. Critical steps were the selection of non-contaminated rice calli, selection methods for antibiotic resistant cell lines till maturation and regeneration of calli into plants. Finally, I learned the basics of growing rice in the greenhouse, considering parameters like temperature, humidity, light type and intensity, day length, harvesting and seed storage.

Main results

During my STSM I learned all necessary methods to cultivate rice *in vitro* and transform rice calli via *Agrobacterium* mediated T-DNA transfer. Together with our collaborators I transformed different rice cultivars (Kitaake, Nipponbare) with several *Agrobacterium* strains containing TALEN constructs I cloned before. New transformation strategies, like a mix of four *Agrobacterium* strains and transformations for proof of principle reporter assays were done. A new reporter construct was transformed to generate a rice reporter line for analysis of TALEN or CRISPR/Cas9 activity *in vivo*. This reporter contains a non-functional GFFP gene containing an internal duplication. The reading frame is reconstituted after introducing a DSB with TALENs or CRISPR/Cas9 and subsequent intramolecular recombination. With the help of this reporter line we will be able to test different site-specific nucleases and various delivery strategies. The whole procedure from callus co-culture to transfer of young regenerated plants to the greenhouse takes about 3 months, therefore, our collaborators at the CIRAD and IRD in Montpellier (France) will finish the selection and the regeneration process of the transformed rice calli as well as the analysis of the resulting new transgenic rice plants.

The STSM gave me the possibility to learn techniques of rice callus cultivation and transformation and I had important hands on time of the critical steps of this process. The next step will be the establishment of *in vitro* rice cultivation and transformation, from callus induction to selection and regeneration of new rice cell lines in our lab in Halle (Germany).

Future collaboration with the host institution (if applicable)

We collaborate further with the IRD and CIRAD for the development and application of TALEN and CRISPR/Cas9 constructs to mutagenize rice plants and generate pathogen-resistant lines.

Projected publications/articles related to or resulting from the STSM

Publications resulting from the collaborative work between Halle, IRD and CIRAD are being prepared and experiments carried in the frame of the training are part of it.

Confirmation by the host institution of the successful execution of the STSM (this part should be written by the host institution)

The hosting and training of Maik Reschke was clearly a success. Tissue culture is not that easy because beyond the knowledge and protocols, the “feeling” on how to handle the tissues and how to recognize tissues amenable to regeneration / transformation and exhibiting resistance to antibiotics is not share by all individuals. I had reports from technical staff that Maik had from the start that feeling that can make him an autonomous tissue culturist. Maik had the chance to see all the aspects of tissue culture and transformation and also rice growth under greenhouse conditions. Therefore he has the background to determine what is needed in Halle to set up in the lab, culture rooms and greenhouse conditions for a rice transformation pipeline. His integration to the team was also excellent. He gave an impressive presentation in the unit meeting and I got outstanding feed back from the audience. We also took the opportunity of Maik presence to discuss further new experiments. The support of STSM was therefore timely and useful and led to successful training.

E. Guiderdoni

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