MEICOM Marie Curie ITN 2018 ESR Progress Summary

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ESR Start Date: 16/04/2018

Workpackage Title: Break-Rec; WP3

Research aims and progress for the period:

i) CRISPR/Cas9 constructs will be designed and built to cleave specific sites in differing chromosomal contexts (hetecro/euchromatin, centromere/arm/telomere…) during meiosis.

In order to try to assess the effect of CRISPR/Cas9-mediated DSBs on homologous recombination frequencies and repair outcomes, gRNAs were designed to cleave different sites between fluorescent pollen markers using bioinformatic tools.
The enzyme chosen for this purpose, among the different variants, is the codon-optimized variant of Cas9 (hCas) developed by George Church lab (Mali et al., 2013). This variant, according to preliminary results (not published) in our lab, is able to cleave and cut in *Arabidopsis thaliana*. Three different promoters were chosen to drive the expression of the hCas9: DMC1 promoter to, according to DMC1 gene expression profile, achieve meiosis specific expression; RAD51 promoter, expressed in all dividing cells, being pollen mother cells among them; and a estradiol inducible promoter, for inducible expression.
To study the effect in homologous recombination, gRNAs were designed inside chromosomal intervals delimitated by insertions of genes encoding for different fluorescent proteins expressed in pollen grains, the so-called fluorescent tagged lines (FTLs; Francis *et al* 2007. *PNAS* 104:3913–3918; Yelina *et al* 2012. *PLoS Genet* 8:e1002844) and kindly provided by G. Copenhaver and I. Henderson. Three different intervals were chosen, located in three different chromosomes and three different subchromosomal domains: I1bc, located in a mid-arm of chromosome 1; I2fg, located in a subtelomeric region of chromosome 2; and CEN3, the covers the centromere and pericentromeric regions of chromosome 3. Two different gRNAs were design inside each interval. Countings of the different combinations of colours of the pollen grains forming tetrads (each tetrad being product of the meiosis of one pollen mother cell) allow the inference of the homologous recombination frequency during meiosis inside the interval, expected to be modified by the presence of cuts by the CRISPR/Cas9 system.

Once the constructs were designed, the next step was the performance of different steps of cloning in bacteria to achieve the final expression vectors that can be transformed into the plant. hCas9 was already in a Gateaway-compatible entry clone and the different promoters in a Gateway-compatible destination vector. hCas9 gene was mobilized through a Gateway LR reaction into the destination vectors, creating 3 different expression clones containing hCas9 under the 3 promoters.

The cassettes encoding the gRNA fragment were ordered to be synthesised, six in total. Through Gateway reactions, they were placed into binary expression vectors under a U6 promoter. All constructions were tested via diagnostic restriction digestions.

Once the cloning was finished, they were transformed into *Agrobacterium tumefaciens* in order to be able to transfer them into *Arabidopsis thaliana*. FTL plants were co-transformed with both the hCas9 and gRNA expression clones in order to obtain all the possible combinations between the constructs with the different promoters and the different gRNA constructs. Seeds have been collected from the transformed plants and are currently being selected to identify the transformants.

Skills Training received:

- **Bioinformatics**: Genome browser management, cloning design, CRISPR/Cas9 design.
- **Molecular biology**: Bacterial culture and transformation (*E.coli* and *A.tumefaciens*), Gateway plasmid cloning in *E.coli*, diagnostic restriction digest, PCR, western blot.
- **Plant biology**: *Arabidopsis thaliana* culture in soil and *in vitro*, plant transformation by *A.tumefaciens,* plant crossing, selection of transformant plants.
- **Imaging**: Preparations of fresh plant anthers for fluorescent imaging, preparation of fixed seedlings for histochemistry (GUS assay), confocal microscopy, fluorescent pollen preparations and counting.
- **French Language:** French language courses provided by the University.

Meetings attended:

- Plant genome stability and change; EMBO workshop. IPK Gatersleben, Germany; June 3-6, 2018.
- MEICOM inaugural meeting & workshop on recombination/plant breeding. Birmingham, UK; July 9-11, 2018.

Outreach activity: