MEICOM Marie Curie ITN 2018 ESR Progress Summary

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Workpackage Title: Interplay between the genomic environment and CO formation

Research aims and progress for the period:

1. **Transforming tomato to overexpress HEI10 and increase crossovers.**

HEI10 belongs to a conserved family of meiotic E3 ligases that function within the ZMM pathway to promote crossovers. HEI10 is also highly dosage sensitive and is a limiting factor for Class I crossover formation in Arabidopsis. For example, overexpression of HEI10 leads to increased crossover rate in the genome of Arabidopsis by 2.7 fold compared to wild type. To investigate effect of *HEI10* overexpression in crops, an overexpression vector for tomato SlHEI10 will be created and transformed into the Micro-Tom cultivar.

So far, genomic *HEI10* sequence including 2 kb upstream and downstream has been cloned into the pFGC-RCS binary vector. Tomato transformation has been performed using explants from 1 week old seedlings with AgI1 *Agrobacterium tumefaciens* strain and is being in vitro regenerated to produce transgenic plants.

T_0 *HEI10* transgenics will be screened for copy number using qPCR and/or Southern blot analysis and expression level will be measured using qRT-PCR. In order to visualise crossover modulation either localisation and/or number in *HEI10* overexpression lines, cytological immunostaining with MLH1 will be performed. Later, in order to obtain genome-wide maps of crossover events, these plants will be crossed with a mapping strain (M82). DNA from F2 plants will be extracted and low-coverage sequencing will be performed to identify crossovers.

2. **Varying HEI10 expression levels and timing in *Arabidopsis thaliana*.**

The aim of this objective is to test the impact of HEI10 expression and timing on the formation of crossovers. HEI10 expression dynamically varies from early to late prophase I of meiosis. Transformation of additional HEI10 copies under control of the endogenous promoter into Arabidopsis thaliana elevates crossover levels. This increase correlates with transcript levels, which indicates that HEI10 is a dosage sensitive. Therefore, it is interesting to study the impact on crossovers by altering the expression and timing of HEI10. It will be achieved by overexpressing HEI10 genomic sequence under different meiotic promoters to induce more effective promotion of crossovers.

So far, HEI10 genomic sequence starting from the gene transcriptional start site (TSS) and including the terminator sequence has been cloned into the pFGC-RCS binary vector using Gibson assembly. New promoter sequences have been subcloned into this vector, which are typically around 2 kb in length. Gene annotation and transcript information were used to evaluate promoter regions. Promoters of SPO11-1, MTOPVIB, DMC1, MSH5, REC8, UBI10 and HVA22P were cloned. Plants carrying FTL (Fluorescent Tagged Lines) system that allows to measure recombination rates in T_1 have been sown and will be soon transformed using AgI1 *Agrobacterium tumefaciens* strain using floral dip method.

FTL crossover measurements will be correlated with transcription levels of HEI10 gene in these lines, quantified by qRT-PCR on RNA extracted from ~40 mg of immature flower buds (up to stage 12 of flower development, which will contain all meiotic stages). Quantification of MLH1 foci (a Class I crossover marker) on pachytene stage meiotic chromosomes from at least 25 individuals per construct will allow to compare total number of interfering crossovers between lines. For constructs that positively modulate recombination levels, further mapping of crossovers may be performed by genotyping-by-sequencing of F2 populations.

3. **Analysis of centromere satellite sequences and induction of DSBs in these regions.**

Centromeric and pericentromeric localisation of some genes mostly exclude them from meiotic recombination, which is suppressed in these regions. Even in lines with highly elevated crossovers, like HEI10 overexpressors, these increase do not release recombination in the centromeres. Despite these regions showing suppressed crossover frequency, sequence analysis reveals abundant signatures of tandem and inverted duplications involving satellite sequences. To investigate DSB repair within the centromeres I plan to develop bioinformatics tools to analyse satellite repeats and induce DSBs in these sequences during mitosis and meiosis using CRISPR/Cas9.

So far I have analysed the repeats of *Arabidopsis thaliana* by extracting their positions using Galaxy and Genious software and characterised them by their size, repeats number, pairwise identity and similarity to other repeats. I have started cloning the binary vector for Cas9 overexpression, with gRNAs targeting previously analysed repeats.

Cas9 will be initially expressed under a constitutive promoter, which later can be switched to the promoter of SPO11-1 that should ensure expression when meiotic DSBs occur naturally. Cytology will be performed on somatic tissue as well as young flower buds. Expected results include chromosomal rearrangements or creation of neocentromeres or even neochromosomes. To visualise this FISH probes against various repeats will be used to stain and analyse these rearrangements and counterstained with DAPI. To monitor axial element polymerization during meiosis, ASY1 immunolocalisation will be used.

Skills Training received:

- 10 Oct 2018: Uoc Occupational Health and Safety Service training course: Chemical Safety
- 17 Oct 2018: UoC Bioinformatics training course: Introduction to Unix shell
- 14 Dec 2018: UoC Bioinformatics training course: Analysis of DNA Methylation using Sequencing

Meetings attended:

- 9-11 Jul 2018 Birmingham, United Kingdom: MEICOM Kick Off Meeting

Outreach activity:

- 24-25 Nov 2018 Poznan, Poland: Speech during a biotechnology conference „Biotechnologia Niejedno ma Imie" about meiotic recombination, MEICOM project and Marie-Curie Actions