MEICOM Marie Curie ITN 2018 ESR Progress Summary

ESR Name: Francesco Blasio	Supervisor: Mónica Pradillo Orellana

Workpackage title: Analysis and modulation of double-strand breaks (DSBs) in meiosis of bread wheat (*Triticum aestivum*).

Research aims and progress for the period:

The main focus of the project is to analyse the homologous recombination process on hexaploid wheat. This species has slow growth (only one generation per year) and last October I have sowed the plants to conduct the first analyses next spring (see below). In addition, during these months I am also participating in other lines of research that were being carried out in the lab and that have points in common with the aim of my thesis: study of chromosome behaviour in different mutants of Arabidopsis in which the chromosome number has been doubled. Arabidopsis grows fast, in a month and a half a generation can be obtained. This situation offers me the advantage of being able to expand my knowledge in plant genetics, in two very different species. The following is a brief summary of my progress in the different lines of research in which I have participated:

A. Analysis of meiotic chromosome behaviour after a colchicine treatment in mutants affected in chromosome dynamics (*Arabidopsis thaliana*)

Previous results obtained in the laboratory demonstrated that the inner nuclear envelope proteins AtSUN1 and AtSUN2 are essential for normal meiosis, since their absence produces a delay in meiotic progression and defects in synapsis and recombination (Varas *et al*., 2015). The double mutant *Atsun1-1 Atsun2-2* presents unresolved interlock-like structures, defects in telomere dynamics and a significant reduction in the mean cell chiasma frequency. It is possible that the reduction in chiasma frequency presented by *Atsun1-1 Atsun2-2* is a consequence of failures in chromosome movement (due to problems in the anchoring of the telomeres to the nuclear envelope). For this reason, it could be interesting to determine what would be the chiasma frequency when there are four homologous chromosomes instead of two in this background. To achieve this objective I have applied colchicine to *Atsun1-1 Atsun2-2* plants.

To conduct this analysis I have become familiar with the spreading technique to get chromosome preparations. I have also learned to identify the different meiotic stages under the fluorescence microscope, and to carry out fluorescence in situ hybridisations (FISH) with specific probes that allow to identify the different Arabidopsis chromosomes.

B. Meiotic characterisation of several natural and synthetic polyploid accessions (*Arabidopsis thaliana*).

Chiasma frequency varies among different accessions, although crossovers are usually located distally (López *et al*., 2012; Sanchez-Moran *et al*., 2002). Recently, in the laboratory, meiosis has been characterised in a European accession. This ecotype presents bivalent morphologies in which crossovers seem to be more interstitial than in the ecotypes most commonly used in

laboratories (e.g. Columbia). Interestingly, there is also a natural tetraploid accession from a close location where the diploid accession grows. We plan to analyse chiasma frequency and distribution in both, the diploid and the tetraploid accession, and also in synthetic autotetraploids obtained by means of a colchicine treatment applied to the diploid accession. Since there is a tendency for modulation of chiasma position during autotetraploid evolution (terminal localization of chiasmata might facilitate regular quadrivalent segregation) (Bomblies *et al*., 2016), it should be interesting to compare multivalent configurations at metaphase I between the natural and the synthetic autopolyploids from this accession.

Currently, the plants have been treated with colchicine. During the next month, I will collect the buds for a further cytological analysis.

C. Consequences of artificial DSB production by cisplatin on wheat meiosis (*Triticum aestivum*)

Cisplatin is known to create DNA inter-strand and intra-strand cross-links. Removal of ICLs results in DNA DSBs, which can be repaired by the non-homologous end joining or by homologous recombination, the latter being favoured in the germline. To asses an effect of exogenous DSBs we have treated wheat spikes with cisplatin following a similar protocol to that described by Corredor and Naranjo (2007) for the use of colchicine. Until now we have checked the effect of two cisplatin doses (7,5 and 15 μM) on chamber-grown plants, which have a high percentage of aborted spikes. We have applied these doses in plants of the Chinese Spring landrace and also in mutant plants for the *Ph1* gene, which is essential to avoid the pairing between homeologous chromosomes in wheat (Sears et. al. 1977). I have obtained chromosome preparations of anthers from these plants and I am currently analysing the results obtained.

On the other hand, due to the problems in fertility observed in the plants grown in the chamber I have also sowed these plants in the greenhouse. During these months we will have information on the most appropriate cisplatin dose and we will improve the protocol of application of the drug so that it is ready when spring arrives and the plants in the greenhouse are ready for the corresponding analysis. In addition to the Chinese Spring and *ph1* plants, in the greenhouse I have also sowed *ph2* mutant plants and rye plants to complete the study.

I will check the meiotic stage in the spikes and collect the anthers when the plants grown in the greenhouse glean. We also plan to analyse the dynamics of proteins involved in homologous recombination by immunolocalization to detect the recombinase RAD51 and the crossover marker MLH1.

Bibliography

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Skills Training received:

a. Cytological analysis (chromosome spreads) in Arabidopsis and wheat
b. Fluorescence in situ hybridisation (FISH) in Arabidopsis and wheat
c. DNA extraction, genotyping by PCR, electrophoresis
d. Polyploidisation of Arabidopsis using colchicine
e. Fluorescence microscopy
f. Identification of meiotic stages in Arabidopsis and wheat
g. Set up the protocol to apply cisplatin to the wheat spikes

Meetings attended:

- Kick-off MEICOM meeting in Birmingham (July, 2018)
- Next month I will attend a meeting (related to the COST action INDEPTH in which my supervisor is involved) in Prague where I will present a poster.

Outreach activity:

- Participation in “Semana de la ciencia” (science week) at the Universidad Complutense. Preparation of a poster related to the research I carry out in the laboratory.

During the next month I will participate in:

- Bioinformatics course at the University of Wageningen (Netherlands).
- Think tank activity in Birmingham.