Control of Meiotic Recombination in Barley

Isabelle Colas[1], Luke Ramsay[1], Robbie Waugh[1] and the MeioSys Consortium[2].

1- Scottish Crop Research Institute, Errol Road, Invergowrie, Dundee, DD2 5DA, UK.
2- www.meiosys.org: University of Birmingham, University of Vienna, Research Institute of Molecular Pathology (IMP), Institut National de la Recherche Agronomique, Université Paris XI, Universidad Complutense de Madrid, CNR-IGV, Radboud Universiteit Nijmegen , Scottish Crop Research Institute.

During meiosis, homologous chromosomes recognize each other, align and pair via chiasmata, ensuring correct segregation at metaphase and thus avoiding aberrant chromosome numbers within gametes. This process also ensures the fundamental process of recombination between parental alleles within progeny that underpins much of genetics and breeding programmes. In crops such as wheat or barley, the distribution of chiasmata is markedly skewed towards the telomere, meaning that a considerable proportion of the genome rarely recombines. An ability to modify the pattern of recombination in these species could therefore have profound impact on the breeding of the crops.

As part of the EU FP7 project 'MeioSys' information and data derived from the model Arabidopsis is providing a basis for the development of reverse genetics strategies to understand and thus modify recombination in barley. In addition, a collection of barley desynaptic mutants provides a complementary forward genetics strategy for understanding the control of meiosis in cereals. These lines are being assessed cytologically and also form the basis of segregating populations that will allow the assessment of the mutations on recombination.

Identification of meiotic genes using barley mutants

The barley desynaptic mutants were originally identified as showing an abnormal metaphase, exhibiting varying numbers of univalents and rod bivalents instead of the seven ring bivalents typically shown by the wild type, with chiasmata in both chromosome arms. This abnormal metaphase phenotype results in aberrant chromosome segregation which leads in turn to varying degrees of semi-sterility.

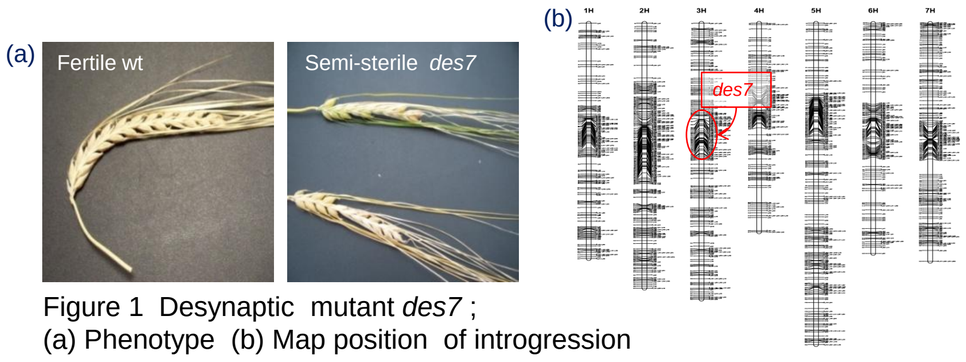

Figure 1 Desynaptic mutant *des7* ;
(a) Phenotype (b) Map position of introgression

14 non-allelic *des* mutants have been backcrossed into a common Bowman background (J.D. Francowiak) and the position of the introgressions and thus the putative map position of the mutations delineated through SNP genotyping (A. Druka). These backcross lines have been used to construct further segregating populations that are being used for fine mapping.

Cytological assessment of meiotic behaviour

In interphase, telomeres and centromeres are in the Rabl configuration (Fig2a) but at the onset of meiosis the centromeres pair in 7 sites (Fig2b) and prior to synapsis, telomeres cluster at one end of the nucleus to form the telomere bouquet (Fig2c). Cryosection of barley anthers (Fig3a) is being used to differentiate tapetal cells and meiocytes (Fig3b) for specific labelling of novel meiotic proteins such as ASY1 (Fig3b,c).

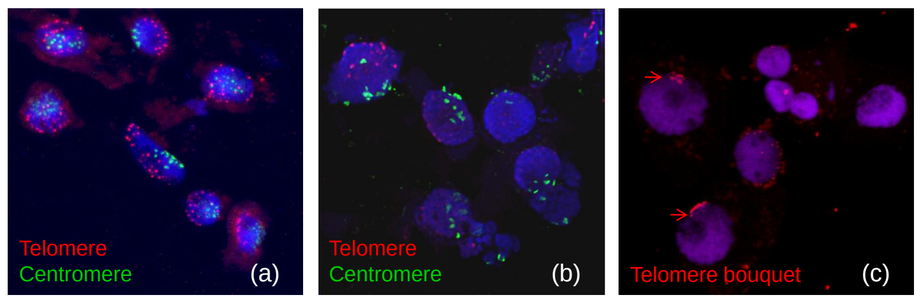

Figure 2 - Barley FISH

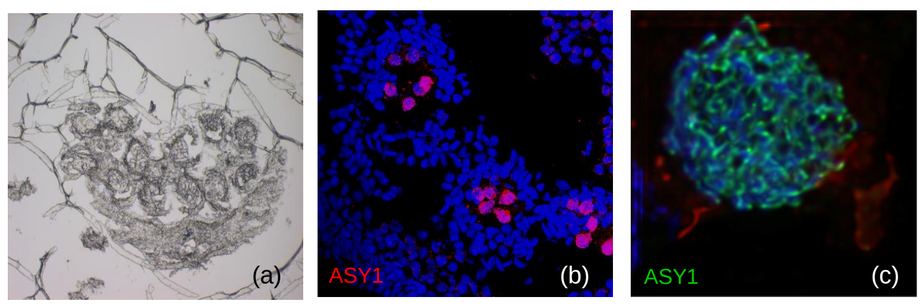

Figure 3 - ASY1 labelling on barley flower Cryosection

Initial investigation of the meiotic behaviour in barley mutant *des7*

It has been shown that the protein ASY1 starts loading on the chromosome at G2/leptotene to fully coat the chromatin at zygotene (Fig4a). At this stage the protein Zyp1 loads on the chromosomes to bring the homologues together. At pachytene, the homologues are fully synapsed (Fig4b) and chromosomes condensed for metaphase. In barley, the homologues are seen as 7 ring bivalents on the metaphase plate (Fig4c).

The mutant *des7* initiates synapsis with ASY1 loading on the chromosomes threads (Fig4d,e,f,g,h,j). However at pachytene, the threads look much thinner (Fig4j) than in the wild type (Fig4b), suggesting the chromosomes are not fully paired. At the diplotene-diakinesis transition, the chromatin looks disorganized (Fig4k). As a result, metaphases generally contain rod bivalents (Fig4l) instead of the ring bivalents of the normal cell (Fig4c). These bivalents appear to be fragile as incorrect chromosome segregation is observed subsequently (Fig4m,n,o) leading to abnormal pollen and semi-sterility.

Further immunolocalization with meiotic proteins such as Zyp1 as well as studies of the chromosome organization using FISH and BrdU labelling will help to further delineate the effect of the *des7* mutant on prophase length and synapsis as well as the progression of meiosis.

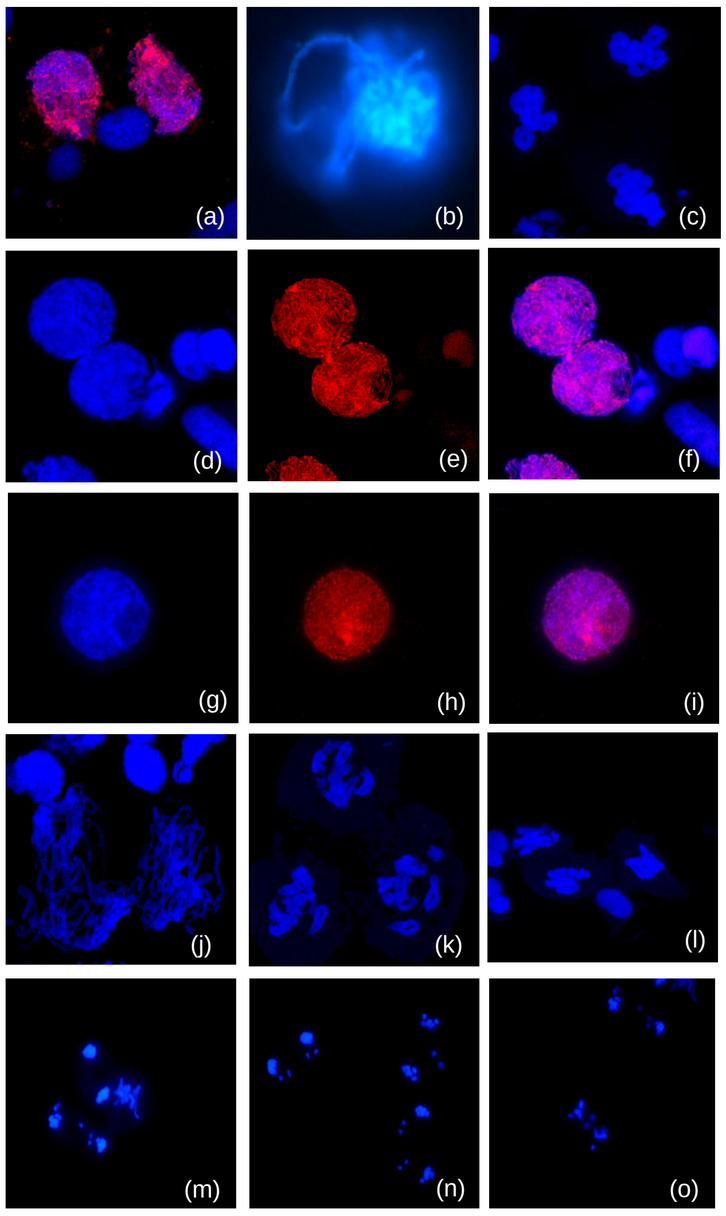

Figure 4 - barley *des7* cytology

In parallel to the cytological investigation the des7 mutant is being fine mapped using a segregating F_2 population derived from a Bowman backcross line (BW246) x Morex cross using in-house SNP genotyping facilities.

MeioSys Project

The EU FP7 project MeioSys, brings together nine participants and combines approaches in genomics and systems biology with the aim of obtaining a detailed understanding of the factors that control recombination.

An important strand of the project is the utilization of knowledge derived from the model Arabidopsis to provide a basis for the development of strategies to modify recombination in barley.

The transfer of knowledge to the crop will involve the development of novel resources with altered meiotic phenotypes. These will provide the means of improving the understanding of chromosome pairing, synapsis and recombination in cereals and allow the manipulation of the frequency and distribution of recombination to enable breeders to access variation in these low-recombinogenic regions of the genome.

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