The use of the barley desynaptic mutants to study recombination in cereals

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Introduction

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 sequence of events also ensures the fundamental process of recombination that underpins much of genetics as well as applied technologies such as plant breeding. 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 these crops.

As part of the EU FP7 project 'MeioSys', information and data derived from the model plant *Arabidopsis thaliana* 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.

The barley collection consists of 14 non-allelic desynaptic (*des)* mutants that have been backcrossed into a common cltv. Bowman background (J.D.Francowiak) using phenotypic selection for the semi-sterility (Fig.1) exhibited compared to wild type.

Figure 1 *des7* and *des10* show various degrees of fertility/sterility. Scale bar = 1cm

These lines, in particular *des7* and *des10* are being analyzed genetically and cytologically (FISH and Immunocytology) to assess the effect of the mutation on chromosome pairing and recombination.

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Homologous Synapsis of *des7* and *des10*

Synapsis is being assessed with the proteins ASY1 and ZYP1 in the mutants *des7* and *des10.* In the wild type, ASY1 loads onto the chromatin at G2/leptotene to fully coat the chromosomes at zygotene. At mid-zygotene, the homologues are «zipping » together from one side of the nucleus via the protein ZYP1 which loads in between the pairs (Fig.2a). At Pachytene, the homologues are fully synapsed (Fig.2d).

ASY1 appears normal in d*es7* and *des 10* (Fig.2b,c) but the chromatin in both mutants look somewhat disorganized compared to the wild type exhibiting regular «round» shaped meiocytes. Despite ZYP1 appearing to load slightly later than in the wild type, *des7* exhibits normal synapsis at later stages with the chromosome threads labelled with ASY1 linked together via ZYP1 (Fig.2e) forming seven bivalents. 3D-SIM microscopy also reveals the presence of interlocks in *des7* (Fig.2e, green arrow). In *des10,* the chromosomes appear to align correctly and ZYP1 starts polymerizing at one side of the nucleus (Fig.2c), however the signal is non-linear which would indicate that some areas of the chromosomes are not properly linked together and therefore the strand invasion for DSBs repair could be compromised. This would explain the unpaired chromosomes observed at Metaphase I.

In most of the desynaptic mutants, such as *des7*, normal pairing between homologous chromosomes occurs at early stages of prophase but the chromosomes fail to remain paired in the later stages. However *des10* clearly shows an imperfect synapsis earlier on suggesting that this mutant is not in fact «desynaptic».

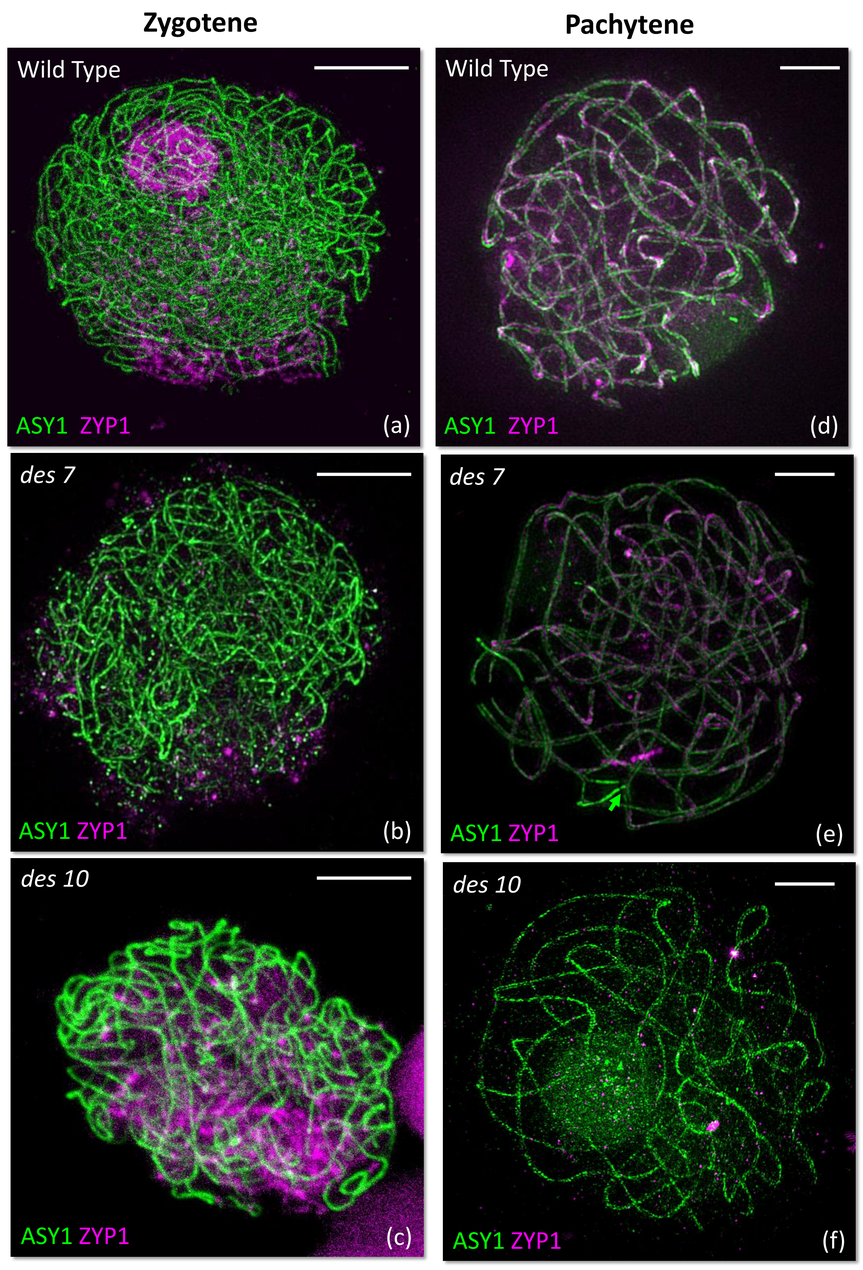

Figure 2 3D-SIM and confocal images of meiocytes at prophase labeled with the synapsis proteins ASY1 and ZYP1 Scale bars = 5µm

Metaphase I and Chromosome segregation of *Des7* and *Des10*

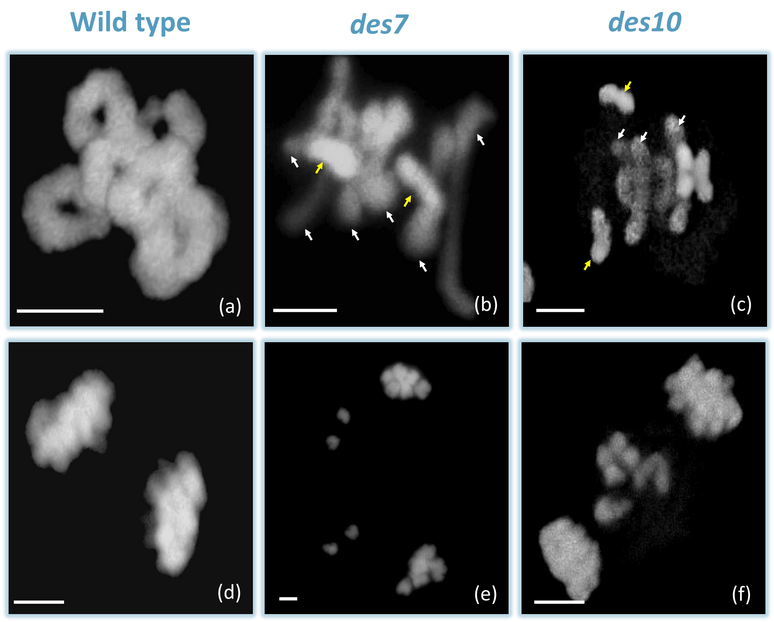

Figure 3 Metaphase I and chromosome segregation comparison of des7 and des10 using DAPI staining. Scale bars = 10 µm

Barley generally exhibits seven ring bivalents at metaphase I (Fig.3a), resulting in equal reduction of the homologues at Anaphase I (Fig3b). However, both mutants show abnormal metaphases (Fig.3c,e,g,i), exhibiting varying number of rod-bivalents (white arrows) and univalents (yellow arrows) resulting in aberrant segregation and chromosome loss (Fig.3d,f,h,j).

Despite a complete synapsis (Fig.2e), *des7* has an average of 9 chiasma per cell (63%) instead of 14 suggesting that *des7* is likely defective for recombination protein rather than synaptonemal complex formation. *Des10* has an average of 11 chiasma per metaphase (76%), which seems to be a direct consequence of the abnormal synapsis (Fig.2f). The 2 mutants differ in the number of univalent with 34% of cells having 2 univalents in *des7* but only 11% of the cells for *des10*.

Conclusions

In this study, cytological tools from Arabidopsis are used to assess synapsis and recombination in various barley desynaptic mutants, in particular *des7* and *des10*. Their difference in synapsis subsequently resulted in differences in chromosome segregation and degree of fertility suggesting two different mechanisms. Mapping data reinforced this hypothesis with the mapping of *des7* on 3HL towards the centromere while *des10* lies on 5HL. A potential candidate gene for *des10* is currently being investigated for mutations.

The use of the desynaptic mutants in barley and the transfer of knowledge from Arabidopsis to the crop raises new opportunities to develop novel resources with altered meiotic phenotypes in barley. These will provide the means of improving our understanding of chromosome pairing, synapsis and recombination in cereals and allow manipulation of the frequency and distribution of recombination to enable breeders to access variation in the low-recombinogenic regions of the genome.