Analyzing HEI10 Dosage Effect on CO formation in an allopolyploid species: Camelina sativa

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Analyzing HEI10 Dosage Effect on CO formation in an *allo*polyploid species: *Camelina sativa*

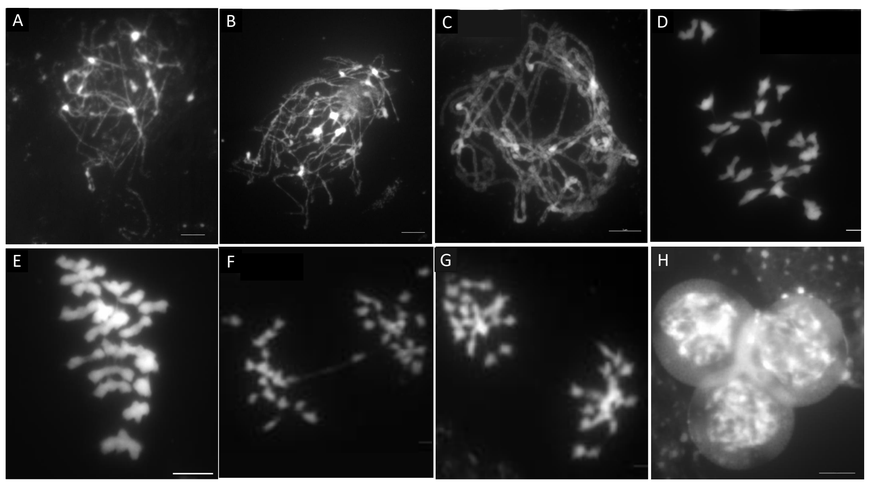
Marie Casado[1], Greta Sandmann[1], Julie Guerin[1], Aurélie Chambon[1], Patrick Grillot[1] and Eric Jenczewski[1]

Abstract

Meiotic recombination is a fundamental process for all sexual eukaryotes; it is also crucial for **plant breeding** as it enables **genetic material to be reshuffled** between individuals (and species) *via* the formation of **crossovers** (COs). To date, most progress in deciphering the mechanisms of CO formation has been achieved through the analysis of *knockout* (KO) mutants in diploid plants such as *Arabidopsis thaliana*. Our working hypothesis is that the study of meiosis in allopolyploid species -which generally contain each gene in multiple copies- could provide additional information, in particular by making it possible to study the effect of **gene dosage**. In this work, we have evaluated the extent to which manipulating the number of functional copies encoding **HEI10** (Enhancer of Cell Invasion n°10), a protein involved in the main COs pathway[1], could modify COs number in *Camelina sativa*, a hexaploid (2n=40) oleaginous species carrying 3 *CsaHEI10* genes. HEI10 is an excellent candidate for this study, as it has been shown that the addition of extra copies of. HEI10 in *A.thaliana* leads to a significant increase in COs number, suggesting a dosage effect[2,3].

As meiosis has never been described in *C.sativa*, we first characterized several key hallmarks of male meiosis in this species using (immuno-)cytology approaches. Then, we modulated the number of functional HEI10 copies downwards by generating (from 0 to 6) CRISPR-Cas9 KO alleles, and upwards by adding extra HEI10 copies from *C.sativa* and *A.thaliana*. Analyzing the meiotic behavior in these different genetic backgrounds - which show varying numbers of functional HEI10 copies - provides further insight into the level of HEI10 required to maintain obligatory CO, and the possibility of compensation between copies.

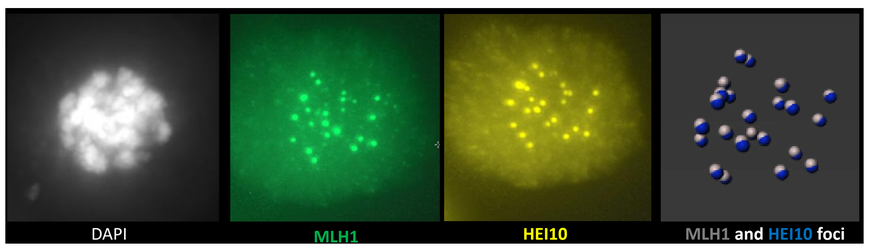
C.sativa shows a diploid-like meiotic behavior with about 1 class I CO per bivalent



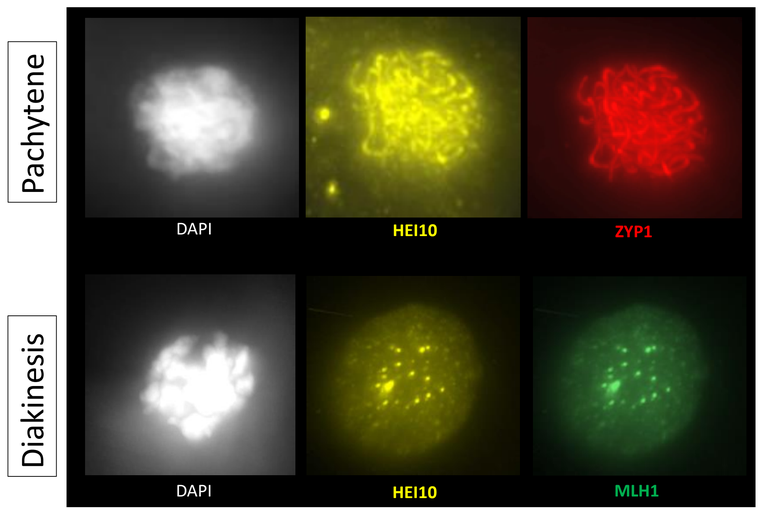
A: Leptotene, B: Zygotene, C : Pachytene, D: Diakinesis, E: Metaphase I, F: Anaphase I, G: Telophase, H : microspore tetrades, scale = 5 µm

Male meiosis in *C.sativa* follows the expected sequence of events[4] : chromosome axes are formed (A,B) and joined by the synaptonemal complex (C) ; 20 bivalents are observed at metaphase I (E). Meiosis II ends up with a tetrad of microspores (H).

3D immunocytological analyses of MLH1 and HEI10 shows that *C.sativa var* Celine only produces **20,8+/-2,2 class I CO/cell.**



In *C.sativa*, HEI10 shows a specific dynamics modelled as **coarsening**



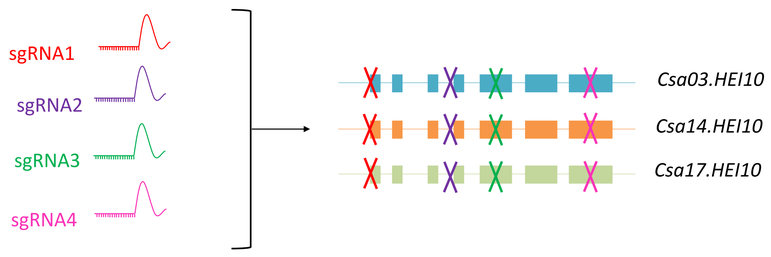
In the early stages, HEI10 shows a multitude of small foci, some being brighter than others. At pachytene, all foci colocalize with the synaptonemal complex (ZYP1).

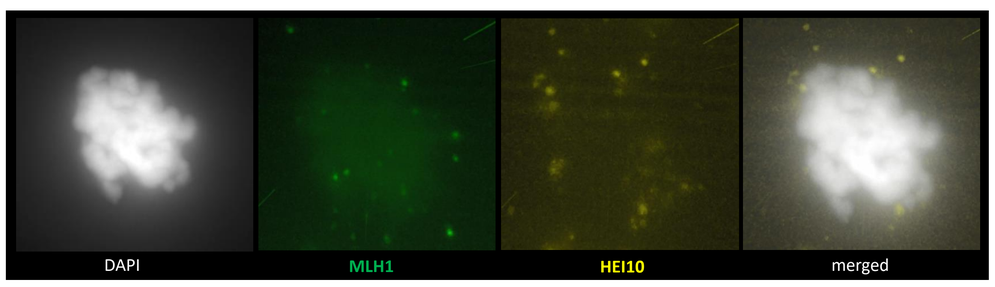
From early to late pachytene, the number of HEI10 foci decreases drastically, to form a small number of large foci. This dynamic could be modeled by a **diffusion-mediated coarsening model**[3,5] in which larger HEI10 foci grow at the expense of smaller ones.

At diakinesis, HEI10 foci **perfectly colocalized with MLH1 foci**, spotting where class I COs are formed.

Generating a series of *hei10 null* mutant alleles in *C.sativa*

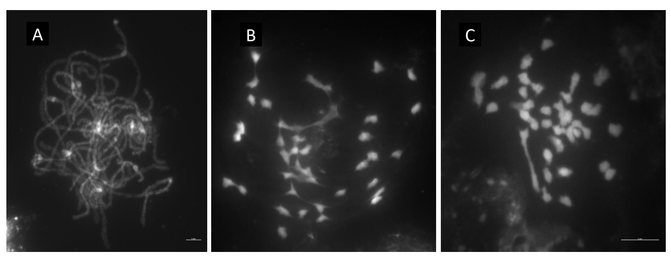
The three genes encoding HEI10 in *C.sativa* var Celine, were succesfully knocked-out using the CRISPR/Cas9 strategy.





No HEI10 nor MLH1 foci are observed in the triple *hei10* mutant
➔ **No more class I COs**

hei10 triple -/-



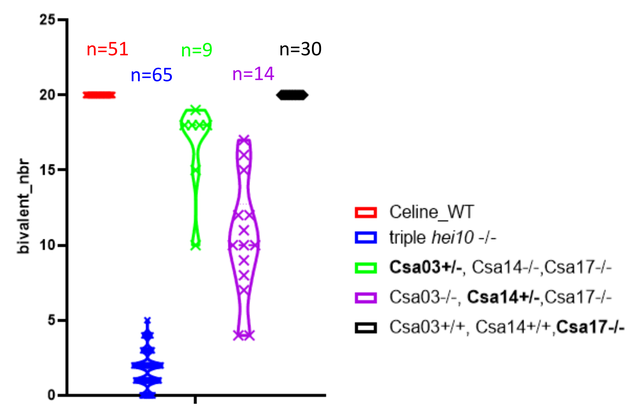
A: Pachytene, B: Diakinesis, C: Metaphase I

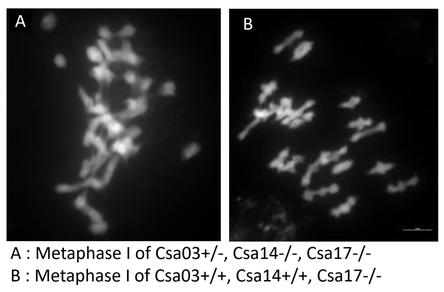
The triple *hei10* mutant shows a **huge reduction in bivalent number**, down to 2 bivalents per cell on average

HEI10 shows a dosage effect on the number of COs in *C.sativa*

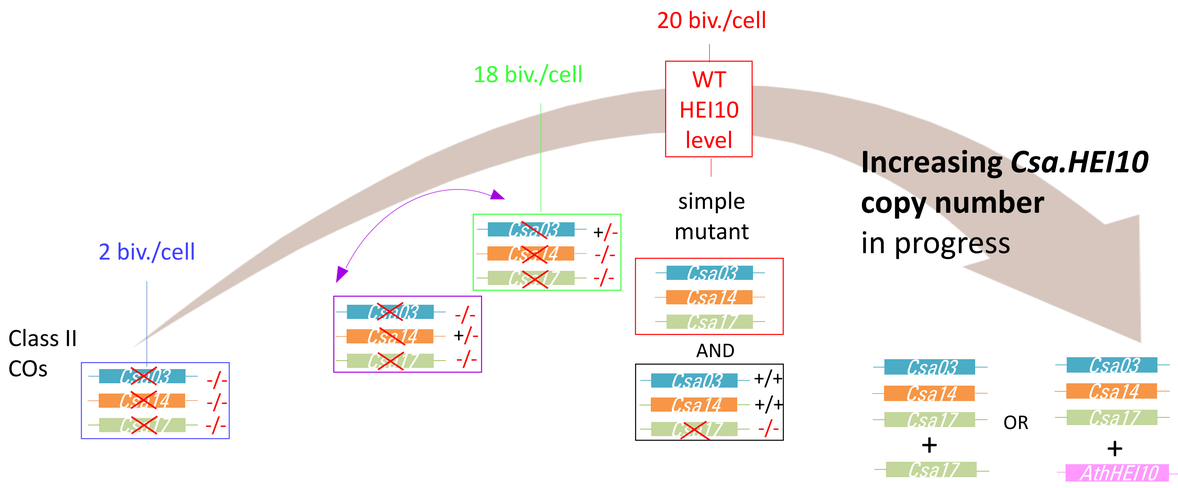
Decreasing *CsaHEI10* copy number

Using different combinations of WT and KO mutant alleles, we show that a single functionnal allele of HEI10 is not sufficient to safeguard the obligatory CO.





A : Metaphase I of Csa03+/-, Csa14-/-, Csa17-/-
B : Metaphase I of Csa03+/+, Csa14+/+, Csa17-/-



TAKE HOME MESSAGES

- ❑ WT *C.sativa* shows about 1 class I CO per bivalent
- ❑ As in other species, HEI10 is required for the Class I CO pathway
- ❑ In contrast to *A. thaliana*, one single functional allele of HEI10 is not sufficient to safeguard the obligatory CO
- ❑ One missing copy (out of 3) does not affect the number of bivalents

❑Hypothesis to test next :

- ○ How many functional HEI10 copies are required to restore the mandatory CO ?
- ○ Does the addition of extra HEI10 copies enhance COs numbers ?
- ○ Is this enhancement proportional to the number of HEI10 copies added or will it be very small or null compared to WT ?
- ○ Do the 3 copies contribute differently to COs formation ?

Bibliography :

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