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## Have a break: how to decipher Spo11.2 role in *Triticum aestivum*?

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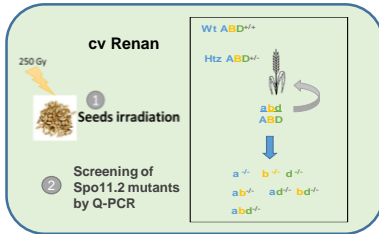
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## Introduction

Spo11.2 protein is part of the Spo11 complex that initiates meiotic recombination in plant (1). Spo11.2 role is well-documented in diploid species but remains unknown in polyploid species (3). The objectives of this study were to decipher the Spo11.2 protein's role in the hexaploid bread wheat (*Triticum aestivum* L., AABBDD; 2n = 6X=42) using simple, double and triple mutants. We compared them between each other at different meiotic scales: phenotype, pollen integrity and meiosis progression (Part 1); homologous recombination (Part 2); genomic and transcriptomic sequences (Part 3).

## Materials and Methods / Results



### Part 1

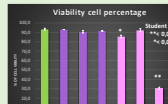
#### Are mutants fertiles?

##### 1.A. Ears phenotype



Mutant Spo11.2 *abd<sup>-/-</sup>* is sterile.

##### 1.B. Viability of pollen (%)

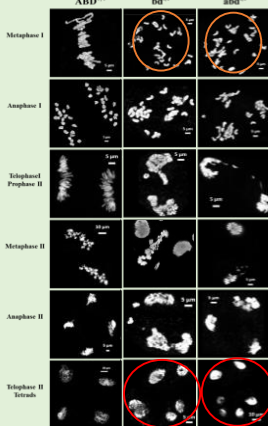


The percentage of pollen viability is very significantly reduced in Spo11.2 *bd<sup>-/-</sup>* (70%) and *abd<sup>-/-</sup>* mutants (88%).

#### How takes place meiosis?

##### 1.C. Comparison of meiosis atlas

ABD<sup>+/+</sup> vs mutants Spo11.2 *bd<sup>-/-</sup>* and *abd<sup>-/-</sup>*



Mutants *bd<sup>-/-</sup>* and *abd<sup>-/-</sup>* show anomalies of meiosis with appariement defaults in metaphase I (presence of singletons) and polyades formation.

Spo11.2 in hexaploid *Triticum aestivum* is essential to successfully meiotic recombination as in diploids species.

#### References

- (1) Hartung, F., et al 2007, The Plant Cell 19, 3090-3099.
- (2) Wang, Y., et al 2018, Annual Review of Plant Biology 69, 577-609.
- (3) Lambing, C., et al 2018, Frontiers of plant Science 9, 829.
- (4) Lloyd A.H., et al 2014, Molecular Biology Evolution 31, 1724-1727.

### Part 2

#### How is meiotic behavior of Spo11.2 mutants?

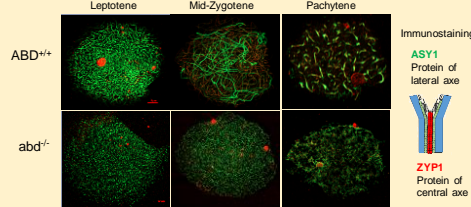
##### 2.A Number of chiasmata / cell (metaphase I stage)



The number of chiasmata is strongly reduced (X8) for *bd<sup>-/-</sup>* mutants. The copy A conserved a slight activity while *abd* mutants cannot pair.

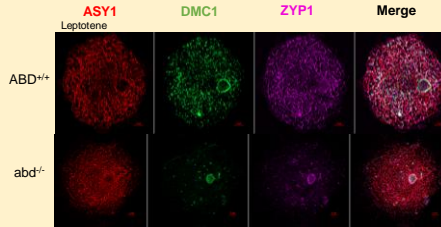
#### Can homologous chromosomes recognize each other?

##### 2.B Establishment of synaptonemal complex (SC)

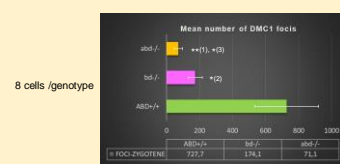


ABD<sup>+/+</sup>: normal synapse formation until complete co-localisation of ASY1/ZYP1 at pachytene stage. *abd<sup>-/-</sup>* mutants: alteration of the SC with ZYP1 protein aggregates leading to synapse failure. Spo11.2 is essential to formation of SC in the hexaploid wheat.

#### How recombination occurs?



##### 2.C Mean number DMC1 Focis at mid-zygotene



ABD<sup>+/+</sup>: 727 foci were counted on average (more or less 193) *bd<sup>-/-</sup>* and *abd<sup>-/-</sup>* mutants: the number of DMC1 foci is strongly reduced by a factor of 4 and 10 respectively.

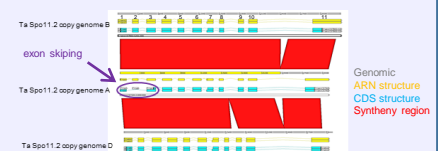
Ta Spo11.2-A copy does not compensate for the absence of B and D copies.

### Part 3

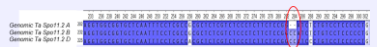
#### Why genome A copy of Spo11.2 remains active?

Comparison of the 3 Spo11.2 copies (A, B and D) with annotation / prediction software Ab-Initio (Triannot, Science 2017)

##### 3.A Visualization of datas with ACT (Artemis Comparison Tools)



##### 3.B Exon 2 comparison for the 3 copies Ta Spo11.2



Transcript: Result of translation. Deletion of 2 nucleotides leads to a frameshift, resulting in a premature codon stop at the beginning of exon 3.

##### 3.C Protein fate hypothesis

3 hypothesis: an exon skipping (3.A), a deletion (3.B) or a splicing site (3.C)?



Previous work of RNA seq Spo11.2 datas (L. Pingault 2011) supports the alternative splice site hypothesis.

The deletion of 2 nucleotides and the presence of splicing site in Ta Spo11.2-A copy lead to an impaired protein.

## Conclusion

*Triticum aestivum* Spo11.2 has the same role in recombination as in diploid species. The *abd<sup>-/-</sup>* mutants were sterile, present meiosis anomalies leading to synapse failure while sterile *bd<sup>-/-</sup>* mutants conserved again the capacity to form some chiasmata. However the loss of Ta Spo11.2-A function could constitute the first step to the wheat genome fractionation (4).

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