Mechanisms of fertility restoration in allotetrapolyploid rape, *Brassica napus* L.

Thanina AZIBI

Funding
Tassili
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2016-2019

UMR IGEPP
Institute for Genetics, Environment and Plant Protection
Inra - Agrocampus ouest -Université de Rennes 1 - Université des Sciences et de la Technologie Houari Boumediene - Alger

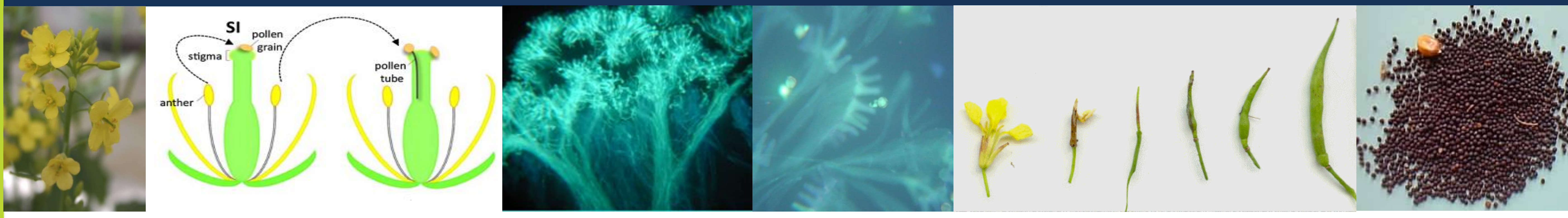
Team names
Biodiversity and Polyploidy (BP)
Laboratoire de Biologie et Physiologie des Organismes (LBPO)

Direction
Chèvre Anne-Marie
Rousseau-Gueutin Mathieu
Hadj-Arab Houria

Partners
Campus FRANCE

Keywords
Self-incompatibility, Synthetics, *Brassica napus*, *S-locus*

Agro ecology
Plant health
Genome and diversity



Social-economic context

Genome duplication (polyploidy) is the main mechanism of plant evolution. Several crops, such as oilseed rape, are still polyploid but the mechanisms involved in their stabilization are poorly understood and especially the regulation of floral biology and of seed development. The understanding of such processes could open new avenue to elucidate evolutionary forces of speciation but also to optimally use the large diversity available in progenitors in breeding programs.

Scientific context

A newly formed polyploid individual, derived from the hybridization and duplication of the genome of related species, must develop strategies to isolate itself from its progenitor species and ensure its evolutionary success. It is clear that its floral biology and seed development will play a key role in this speciation process. We propose to study these phenomenona on the *Brassica* model and more precisely in rapeseed (*Brassica napus*, AACC, 2n = 38), an interspecific hybrid between turnip (*B. rapa*, AA, 2n = 20) and cabbage (*B. oleracea*, CC, 2n = 18). Despite the fact that its two progenitors are self-incompatible, rapeseed is self-compatible. Self-incompatibility is characterized by the rejection of self-pollen. This mechanism is controlled by a multi-allelic *S-locus,* which consists of 3 genes (*SRK*, *SLG* and *SCR*). The rejection reaction takes place when the pollen and pistil have the same *S*-allele. In addition, several genes are involved in the regulation of the self-incompatibility locus. Thus, the self-incompatibility of natural rapeseed could be explained by structural and/or functional modifications for one or several genes involved in this mechanism.

Irrespective of the level of self-incompatibility, a newly formed oilseed rape also shows a very low fertility (~ 1 to 3 seeds / pod) compared to a natural rape (20 to 30 seeds / pod). The origin of this low fertility remains to be elucidated, and maybe either due to the presence of pre- (leading to a lower number of ovules formed) or post-zygotic (leading to the production of a small amount of seeds) barriers.

Objectives

The aim of this study is to determine the mechanisms that allow the restoration of fertility in *B. napus*, which leads us to ask the following questions during my PhD:

I. What is the role of self-incompatibility in the restoration of fertility in the newly formed allopolyploid *B. napus?* Are there structural or functional changes in the *S*-locus or in its regulatory genes, allowing a return to self-fertility?

II. Could the post-zygotic barriers explain the low seed set observed in the first generations? Does the development of the seed after fertilization play a role in the restoration of fertility?

Results

The study of the mechanisms involved are performed by comparison of G2 and G3 synthetics (from two genetic backgrounds) with their diploid parents as well as with natural oilseed rape varieties.

I. Impact of self-compatibility regulation

The diploid used to produce synthetics show different levels of self-incompatibility, with S-alleles belonging to different classes. The diploid parents, the 23 synthetics selected (3 replicates/plant) and two natural *B. napus* will be phenotyped for self incompatibility (nb. of pollen tubes/flower pollinated at the anthesis). In order to detect the impact of structural modifications, we have physically localized the genes of the *S*-locus and its regulatory genes (*Thl-1, Thl-2, Arc1, Mlpk, Exo70a1*) in *Brassica* genomes (Tab 1). We have searched for structural rearrangements in each *B. napus* plant (using a 60k SNP array) in order to determine if copies of these genes may have been lost (e.g. Fig 1 for S2 plants). The functional regulation of S-locus and its regulatory genes will be analyzed from cDNA of the same plants.

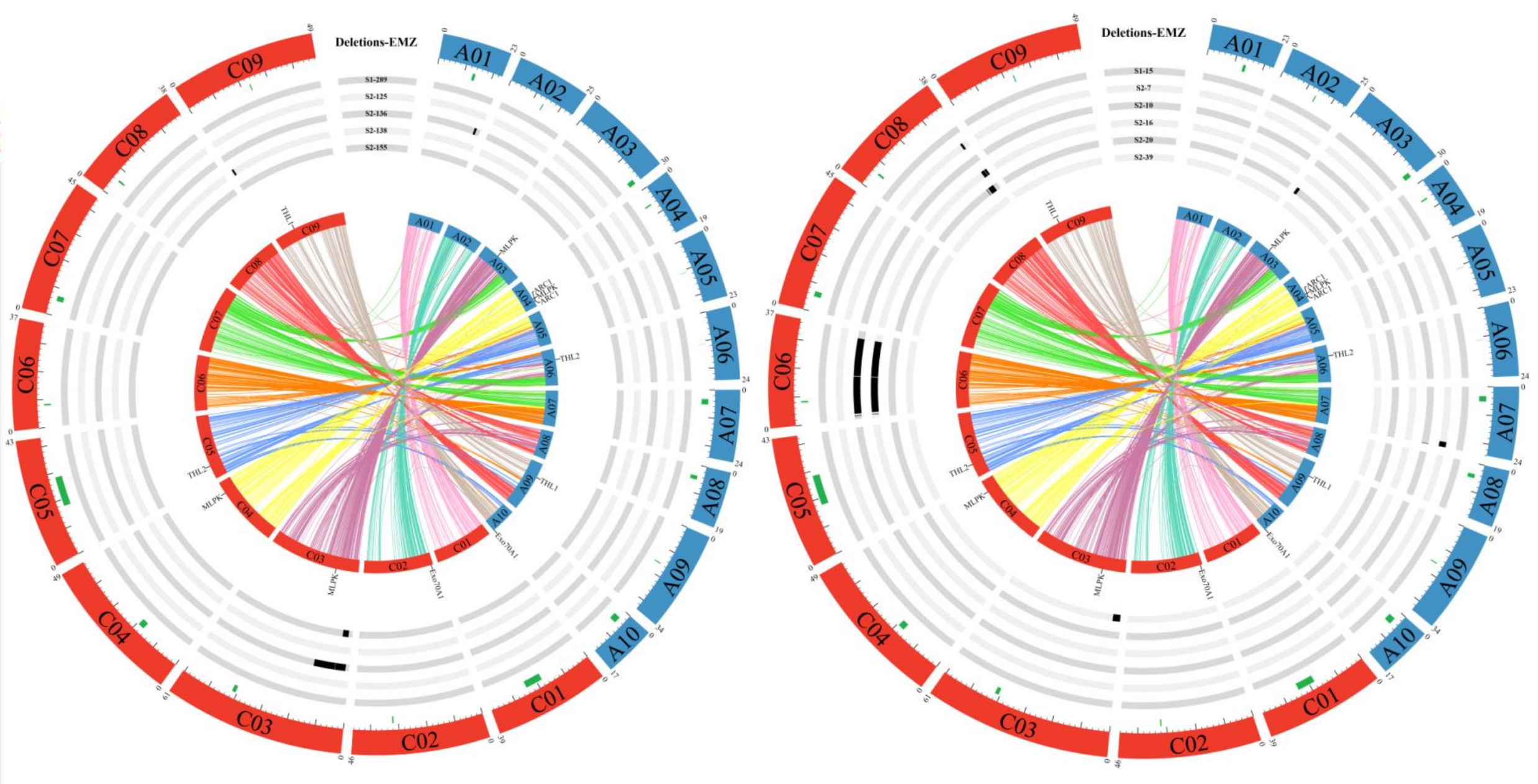


Figure 1: Graphical representation of the physical position of polymorphic SNPs (deletions in black) and of regulatory genes in EMZ-G2 on the rapeseed genome (Chalhoub *et al.*, 2014).

Table 1: Position of the regulatory genes on the two subgenome A and C of *B.napus*

Genes	Positions on the subgenome A	Positions on the subgenome C
THL1	A09	C09
THL2	A06	C05
ARC1	A04	
	A04	
MLPK	A03	C03
	A04	C04
Ex070A1	A10	C02

II. Impact of pre or post-zygotic barriers

On the same plants, the number of ovules and the number of seeds per flower after selfing will be assessed in order to identify the best steps of development to analyze.

Perspectives

Combining structural and functional analyses, we will try to identify which mechanism(s) is involved in the restoration of self-compatibility and fertility in natural *B. napus*.

References

Chalhoub B, Denoeud F, Liu S, Parkin IAP, Tang H, Wang X, Chiquet J, Belcram H, Tong C,Samans B, et al. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345(6199): 950-953