Characterization of the dynamics of expression of the *AGO1* gene and miR168 during the formation of *Brassica* neo-allotetraploids

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Introduction

The interspecific hybridization has played a major role in the evolution of species, particularly in plants, where its success has been mainly linked to polyploidy. Here we aim at identifying epigenetic regulatory mechanisms involved in the formation of a new allopolyploid species.

Our plant model is oilseed rape (*Brassica napus*, AACC), an allotetraploid species originating from interspecific hybridization between *B. rapa* (AA) and *B. oleracea* (CC). Recently, we have demonstrated an immediate response of small non coding RNAs to the allopolyploidy genomic shock, by analyzing newly synthesized *B. napus* allotetraploids in comparison with the diploid progenitors [1]. Our results indicated a PTGS-to-TGS shift involving specific populations of 21-nt and 24-nt, respectively, to control transcriptionally reactivated transposable elements and other non-coding elements (Fig. 1). We thus **undertook the analysis of the expression dynamics of *AGO1* and miR168** as central players of the PTGS pathway [2], along the formation of the *Brassica* neo-allotetraploids.

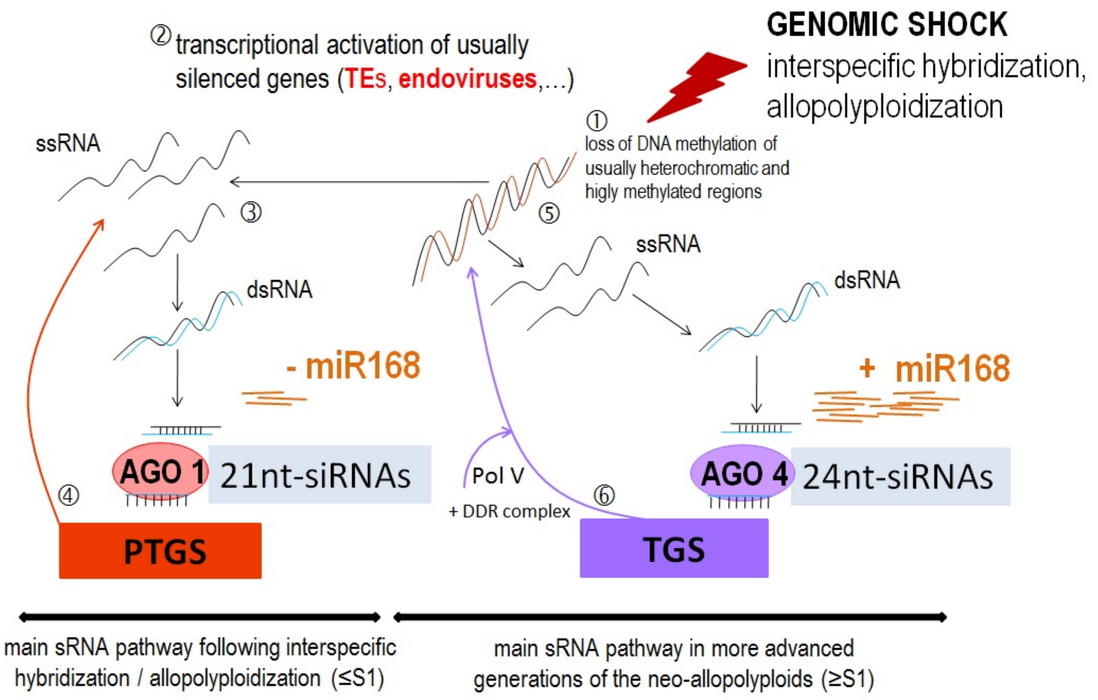

Figure 1. Hypothesized model supporting the PTGS-to-TGS shift in response to allopolyploidization, according to analysis of sRNA library sequencing during the formation of *Brassica* neo-allotetraploids [1]

1. Bioanalysis of the *Brassica AGO1* genes

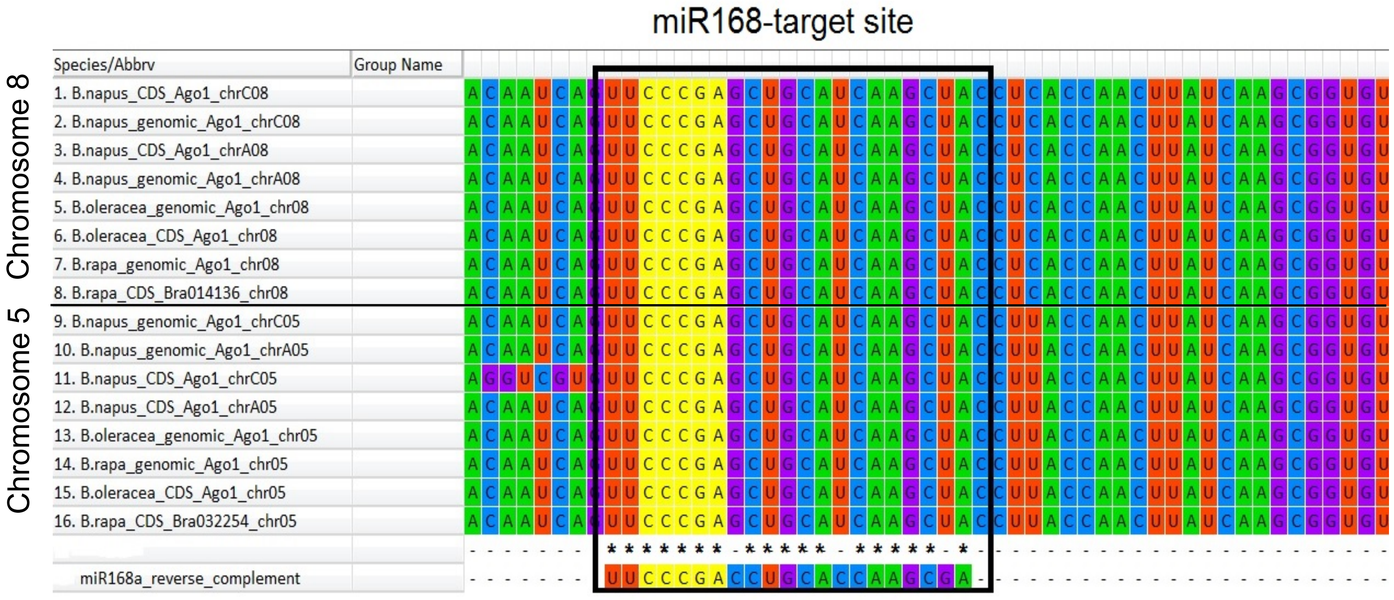

Figure 2. Multiple sequence alignment of all the *AGO1* gene sequences and CDS retrieved from the *Brassica* database (http://brassicadb.org/brad/) and identification of the miR168 target site [3]

✓ We retrieved two copies of *AGO1* gene in each of the diploid genomes that are located on chromosome 5 and chromosome 8; the four copies are maintained in *B. napus*.

✓ **All the *AGO1* copies are highly conserved within the *Brassica* genus**; in particular, the predicted miR168-target sites are perfectly identical between all the annotated *AGO1* sequences of *B. rapa, B. oleracea* and *B. napus* (Fig. 2).

We aimed at **evaluating the expression levels for all the *AGO1* copies** during the neo-allopolyploid formation, by designing primers in the highly conserved regions, not common to the other *AGO* genes (data not shown) to be used in qRT-PCR experiments.

2. Primers designed to characterize *AGO1* expression

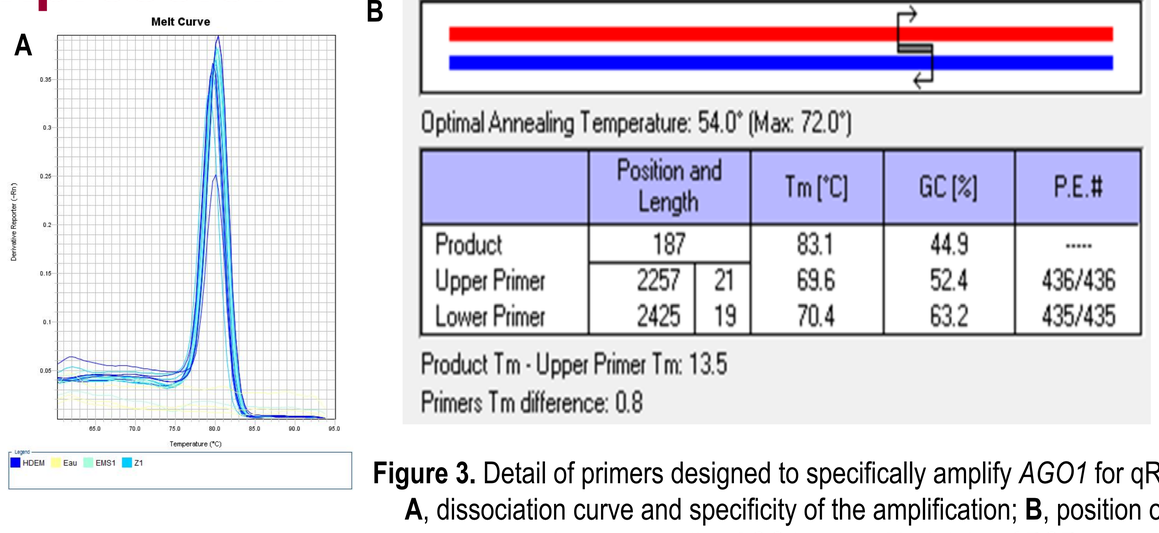

Figure 3. Detail of primers designed to specifically amplify *AGO1* for qRT-PCR: **A**, dissociation curve and specificity of the amplification; **B**, position of the primers along the *AGO1* gene with details of PCR.

✓ *AGO1* specific primers were designed based on the CDS of *B. rapa* (Bra032254) to amplify 187bp-long conserved sequences from all the annotated *AGO1* genes (Fig. 3). The primers were tested on *Brassica* DNA samples and then validated by sequencing.

3. Depicting the expression levels of *AGO1* & miR168 by quantitative RT-PCR

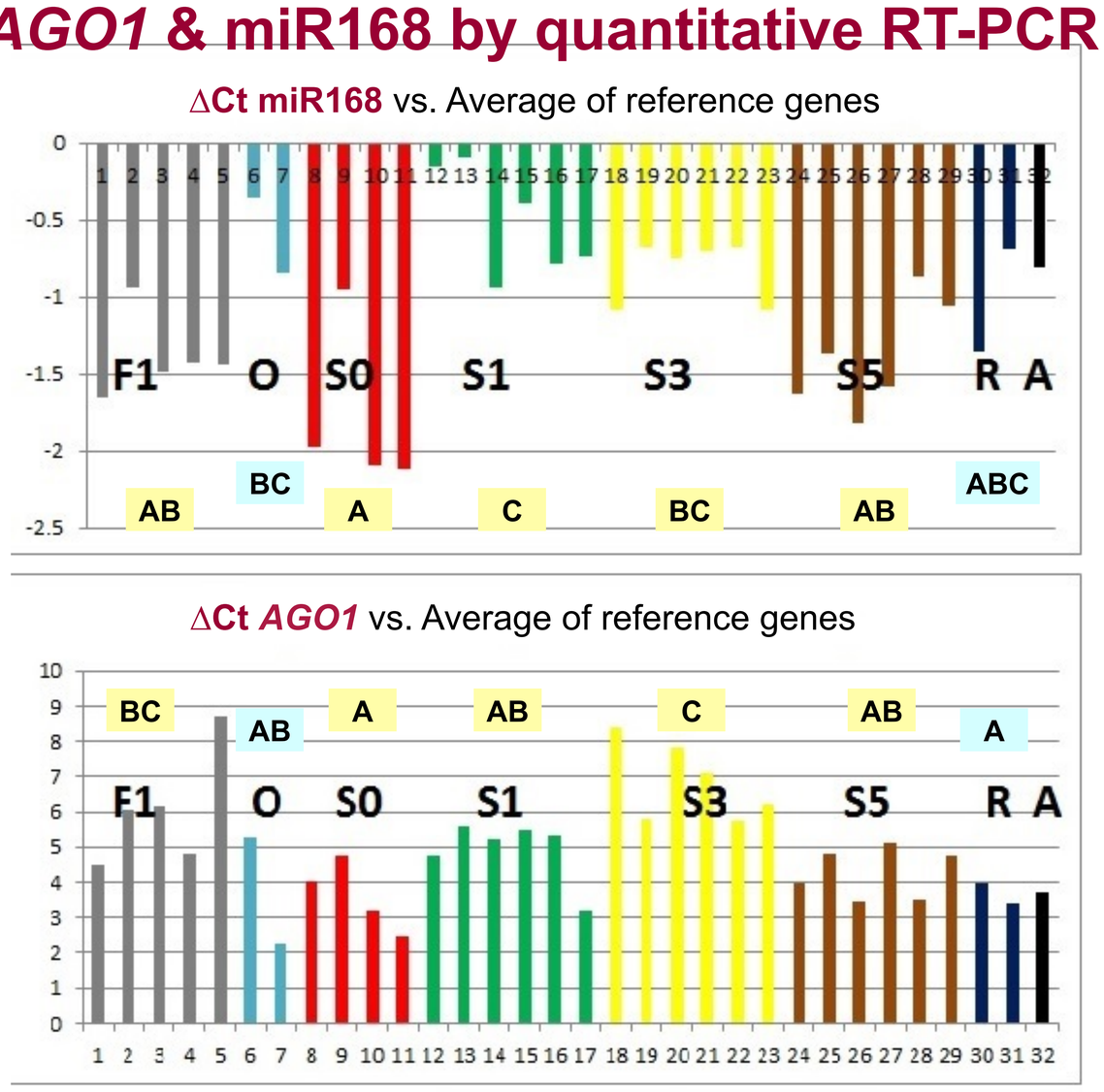

Figure 4. The different expression levels of miR168 and *AGO1* during the formation of the *Brassica* neo-allotetraploids (including the F1 hybrids) in comparison to the diploid parents (O- *B. oleracea*, R- *B. rapa*), estimated by qRT-PCR. ANOVA was used to test for significant expression differences by Holm's grouping (A, B, C letters) across the generations. A: highly expressed, C: less expressed.

✓ **miR168 and *AGO1* show both different expression levels along the formation of the *Brassica* neo-allotetraploids** (Fig. 4). In particular, miR168 dynamics is in accordance with the model of a PTGS-to-TGS shift, with very low levels of expression in the first generations of the neo-allotetraploids to allow AGO1-mediated PTGS pathway against reactivated elements (like TEs, EVEs).

Conclusion & Perspectives

Our preliminary data are in accordance with **a PTGS-to-TGS shift in response to the genomic shock triggered by allopolyploidisation**, as supported by the miR168 dynamics we depicted. Further analysis of the AGO1 protein accumulation but also of the expression dynamics of *AGO4* (responsible for the 24-nt based TGS pathway) during the neo-allotetraploid formation has to be performed to support such a hypothesis.

Our previous work [1] and ongoing study indicate that **the immediate mobilisation of small non coding RNAs** to control TEs and other usually silenced sequences that were observed as being reactivated during allopolyploidisation **is necessary for ensuring the stability of the neo-allopolyploid genome** and thus the success of the allopolyploid event.

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References

1. Martinez Palacios 2014. GQE-Le Moulon, PhD thesis.
2. Mallory and Vaucheret, 2009. EMBO Reports 10: 521-526.
3. Gursinsky et al. 2015. Plant Physiology 168: 938-952.