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In Brief

# Bypassing reproductive barriers by chemical epimutagenesis

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Plant domestication and modern breeding of elite species have taken advantage of the fact that hybrid offspring arising from two different parents can have superior performance. This phenomenon, known as heterosis or hybrid vigor, is tightly linked to the nature and dosage of the parental chromosomes in the next generation (Hochholdinger and Baldauf, 2018). Similarly, polyploidization or hybridization between parents of different ploidy levels can lead to descendants with enhanced desired crop traits. However, the use of neo-polyploids, interspecies, and interploidy crosses in plant breeding is hindered by strong reproductive barriers and low fertility. Indeed, the latter often results in a "triploid block" characterized by abnormal endosperm development and ultimately seed collapse (Köhler et al., 2021). In this issue of *The Plant Cell*, **Jonathan Huc and colleagues (Huc et al., 2022)** succeeded in bypassing the triploid block in *Arabidopsis* and the interspecies hybridization barrier in *Capsella* by the chemical inhibition of DNA methylation.

The development of the endosperm, an angiosperm-specific embryo-nourishing triploid tissue, relies on the proper balance between maternally and paternally inherited genomes and their pre-established epigenetic features (Dresselhaus et al., 2016). The endosperm is formed by the fertilization of a diploid central cell by a haploid sperm cell leading to an uneven 2:1 maternal:paternal genomic ratio. The expression of maternally and paternally expressed genes (MEGs and PEGs), also referred to as genomic imprinting, plays a key role in balancing transcription in the endosperm. Interestingly, loss-of-function mutations in PEGs and changes in the pollen epigenome were found to suppress the triploid block, suggesting that the paternal epigenome is involved in genomic imprinting (Calarco et al., 2012; Wolff et al., 2015).

To study the contribution of the paternal epigenome in the triploid block in *Arabidopsis*, Huc et al. (2022) made use of the mutant *jason* (Erilova et al., 2009). Self-fertilized homozygous *jason* plants produce about 35% diploid pollen while the female gametophyte remains haploid, which is reflected by a similar percentage of triploid seed abortion. Surprisingly, treatment with 5-azacytidine to chemically inhibit DNA methylation led to a significant decrease of the triploid block (see Figure), confirming that the integrity of the paternal epigenome is important for this process. Furthermore, this effect was inherited in the following plant generation in a paternally dependent manner.

Whole-genome DNA methylation profiling revealed a large number of hypomethylated CG (but not CHG or CHH) regions in protein coding genes as well as in transposable elements (see Figure) in the progeny of 5-azacytidine-treated plants. About 10% of the regions with decreased CG methylation overlapped in different independent lines, suggesting that these genomic paternally inherited locations are important for endosperm development. Indeed, transcriptome analyzes showed that 42 differentially expressed genes overlapped with the identified differentially methylated regions and thus constitute possible candidates to explain the suppression of the triploid block.

Following these exciting results, the authors assessed if chemically induced hypomethylation facilitates bypassing interploidy and interspecific hybridization barriers. They found that inhibiting DNA methylation in tetraploid *Arabidopsis* plants significantly decreases the triploid block. Furthermore, cross-pollination experiments between two different *Capsella* species treated with 5-azacytidine resulted in 30% of hybrid seeds that appeared normal in contrast to control crosses in which all seeds

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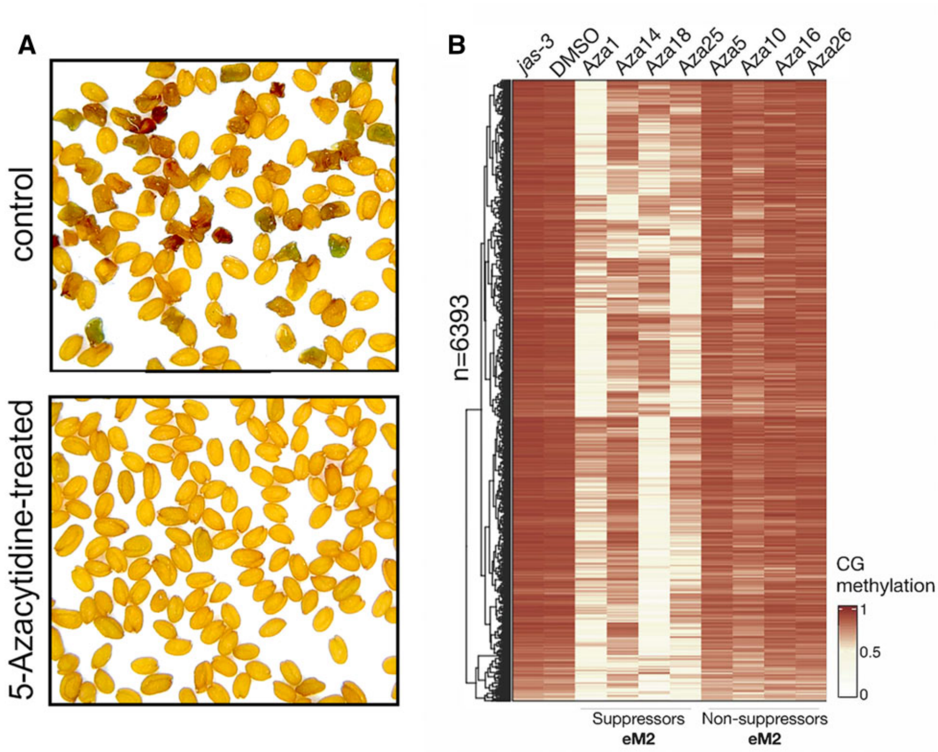


**Figure** Chemical inhibition of DNA methylation decreases the triploid block response in *Arabidopsis*. A, Treatment with 5-azacytidine to inhibit DNA methylation in the *jason jas-3* mutant (upper picture) leads to the suppression of collapsed seeds (lower picture). B, Whole-genome DNA methylation profiling revealed differentially methylated regions in the progeny (eM2) of 5-azacytidine-treated *jas-3* plants. Suppressor lines of the triploid block display a stronger reduction of DNA methylation compared with non-suppressor lines. Adapted from Huc et al. (2022), Figures 1, 2.

collapse. In summary, this new study reported that hybridization barriers can be bypassed by chemically induced mutagenesis of the paternal epigenome. This approach could potentially emerge as a novel breeding strategy for plants with high agronomical interest.

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