DNA Methylation and Stress-induced Reversions from Asexual to Sexual Seed Formation in *Boechera* (Brassicaceae)

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Abstract

Apomixis is a form of asexual reproduction that occurs in many plants and animals. Eggs of apomictic plants and animals form by apomeiosis, which means they do not become genetically reduced. Furthermore, these eggs develop into embryos without fertilization. Apomictic plants and animals generally reproduce quickly by making genetic copies of themselves, a trait that would be economically beneficial for perpetuating hybrid vigor if introduced into major seed crops. Metabolic stress induces a reversion from apomixis to sex in many apomicts, and this was recently shown to occur in species of the plant genus *Boechera*.[1] Transcriptome studies strongly suggest that this switch involves epigenetic reprogramming of the genome. I have drought-stressed cultured buds of apomictic *Boechera* in vitro to varying levels to determine an optimum treatment for inducing the switch from apomeiosis to meiosis. I have also exposed clusters of very immature floral buds to a DNA analogue that prevents DNA methylation (Fig. 1). This research is designed to allow me to determine the extent to which genome reprogramming, which involves DNA methylation, is involved in the switch from apomeiosis to meiosis.

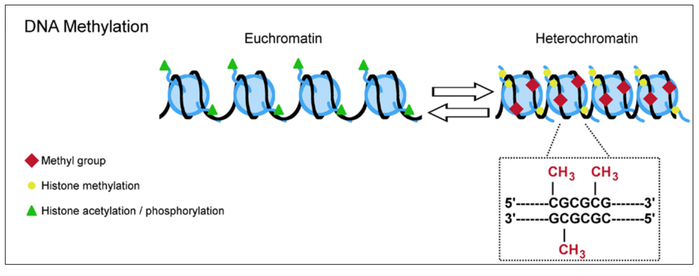

Figure 1: Diagram showing the reversion of euchromatin to heterochromatin caused by methylation of cytosine base pairs in the DNA. Image from Frontiers in Psychiatry.

Introduction

- The reversion from apomeiosis to meiosis have been previously been shown to be caused by both drought and heat stress.[1]
- My research analyzes the effects of plant uptake of the DNA analogue 5-Azacytidine (analogous to Cytosine) on reproduction (Fig. 2).
- It is supposed that preventing DNA methylation of the genome will also prevent a reversion from apomeiosis to meiosis in *Boechera*.

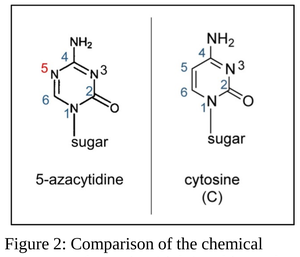

Figure 2: Comparison of the chemical structures of cytosine (right) and its analogue, 5-azacytidine (left)

Methods

- Bud clusters from three different plants of the *B. retrofracta x stricta* species (an apomictic hybrid cross) were picked and grown for 72 hours in MS medium and varying concentrations of 5-azacytidine.
  - Two control petri dishes with three clusters each (0 µM 5-azaC)
  - One petri dish at 50µM 5-azaC with three clusters each
  - One petri dish at 100µM 5-azaC with three clusters each
  - One petri dish at 200µM 5-azaC with three clusters each
- Bud clusters were assigned randomly to each of the three petri dishes that contained the previously mentioned concentrations of 5-azacytidine (Fig. 3).
- After growth for 72 hours *in vitro*, the bud clusters were then fixed, cleared dissected, and mounted for embryological analysis (Fig. 3).
- Ovules from the pistils were analyzed using differential interference contrast (DIC) microscopy in order to determine the ploidy of each ovule.

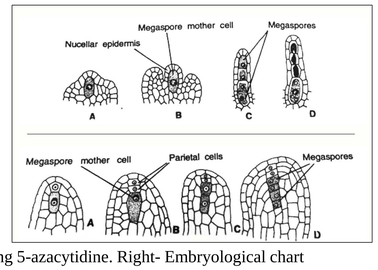

Figure 3: Left- Image of bud clusters placed in petri dishes containing 5-azacytidine. Right- Embryological chart showing tetrad formation (upper diagram) and dyad formation (lower diagram)

Results

- Bud clusters exposed to 5-azacytidine at all three tested concentrations were able to grow *in vitro* with similar results (Table 1).
- Embryological analysis indicated that none of the ovules showed signs of an apomeiotic to sexual reversion, which would be expected if under stress.
- Many ovules were found to be aposporous, which may or may not have a correlation to the treatment (Fig. 4).
- It was noted that the stems of the bud clusters bent out of the medium before 72 had elapsed, thus the growth of the pistils may have been slowed as a result. Replication of the experiment is necessary.

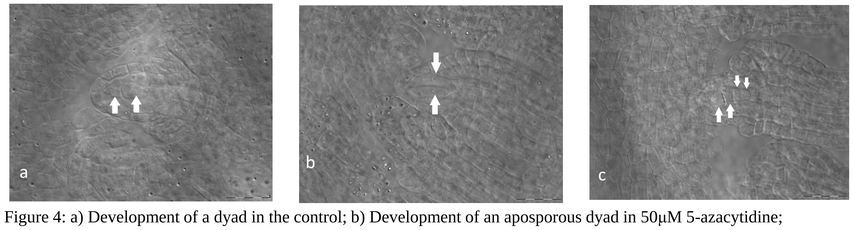

Figure 4: a) Development of a dyad in the control; b) Development of an aposporous dyad in 50µM 5-azacytidine; c) Development of a tetrad in 200µM 5-azacytidine

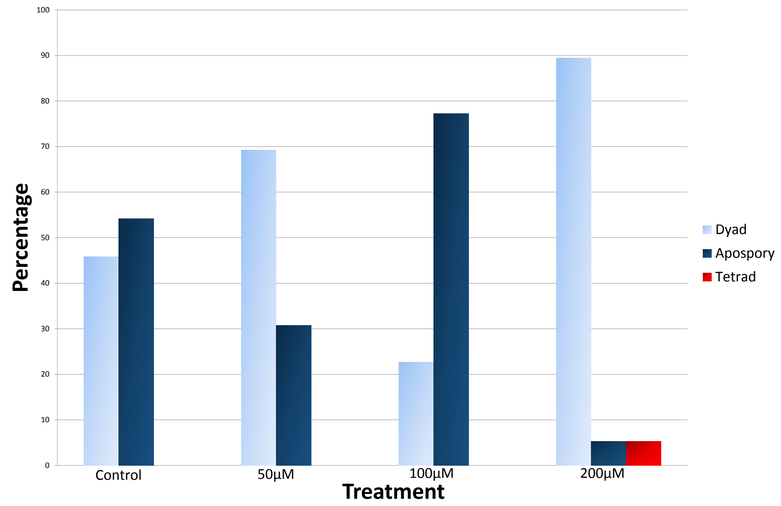

Table 1: Percentages of Dyads, apospory, and tetrads found in ovules of *B. retrofracta x stricta* in varied concentrations of 5-azacytidine. The control contained 0µM of 5-azacytidine. Both light and dark blue are indicative of apomictic plants, while red is indicative of a sexual mode of reproduction.

Future Research

- Since none of the plants showed a shift from apomeiosis to meiosis when exposed to 5-azacytidine, it would be beneficial to observe if the shift occurs when plants are exposed to this chemical in addition to a drought stressing agent, such as polyethylene-glycol (PEG).
- It is hypothesized that 5-azacytidine will prevent the plants from using epigenetic regulation in order to shift, even under drought-stressed conditions.
- It may be advantageous to test stronger concentrations of 5-azacytidine in order to further de-methylate the DNA.
- My lab partners and I have begun to grow several hundred plants from three different species (*B. lignifera*, *B. gunisoniana*, and *B. stricta*) in order to test the previously mentioned hypothesis over the summer.

Acknowledgements

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References

1. Mateo de Arias, M. 2015. Effects of plant stress on facultative apomixis in Boechera (Brassicaceae). PhD Dissertation, Utah State University, Logan, UT, USA