Floral Developmental Stages and Stress-induced Reversions from Asexual to Sexual Seed Formation in *Boechera* (Brassicaceae)

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Abstract

- In the apomictic process, plants produce a genetic copy of themselves by forming genetically-unreduced eggs (2*n* instead of 1*n*) that produce embryos parthenogenically, i.e., without fusion of egg and sperm nuclei.
- In some plants such as the *Boechera* species studies have shown that stressing the apomictic plants caused a reversion from apomeiosis to meiosis[1].
- The object of my study is to more fully understand the stage of floral development at which an apomictic plant must be exposed to a stress signal in order to revert from apomictic to sexual reproduction or vice versa.
- Apomixis could be used to cheaply produce commercial quantities of crop seed of heterozygous genotypes, such as superior yielding hybrids. Such plants would remain hybrid from one seed generation to the next.

Methods

- *B. retrofracta x stricta* bud clusters were cultured *in vitro* with MS media and increasing concentrations of PEG (polyethylene-glycol) (Figure 1B) to simulate drought stress.
- The concentrations used were 20gL^{-1}, 40 gL^{-1}, 60 gL^{-1}, and a control that lacks PEG.
- The bud clusters were fixed in clearing solution and individual pistils were dissected and analyzed cytologically via differential interference contrast (DIC) microscopy to determine mode of reproduction.
- The results of the analysis were graphed based on percentages of total ovules (Figure 2)

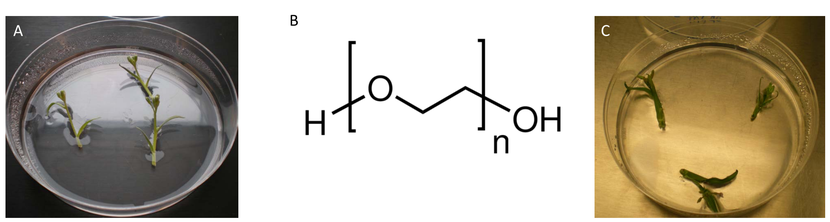

Figure 1. A) Bud clusters in MS-PEG 40 starting treatment. B) Chemical structure of polyethylene-glycol (PEG) C) Post 48 hour exposure (bud clusters from same group treated in PEG 40)

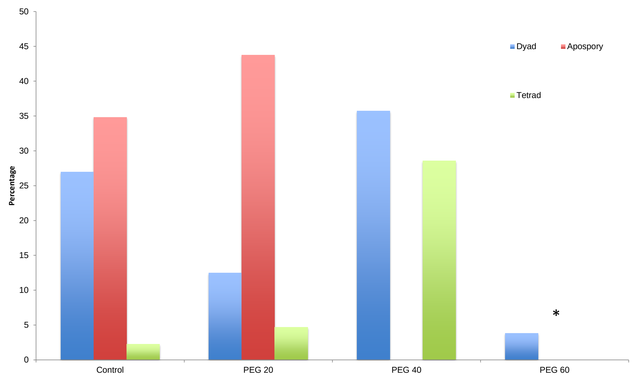

Figure 2. PEG treatment (gL^{-1}) effects on tetrad and dyad formation frequencies in apomictic *B. retrofracta* x *stricta*. Percentages generated represent ovules observed that were in a diagnostic stage. *Note the PEG 60 gL^{-1} clusters were unclear and diagnosis via DIC microscopy was impossible with the exception of 2 malformed dyads.

Results

- In the control group the relative expected values of dyad and aposporous development were observed for an apomictic plant such as *B. retrofracta x stricta.*
- An increase in tetrad development seen in PEG 20gL^{-1} , but more extensively in PEG 40gL^{-1} indicates the level of stress was sufficient to begin to cause a switch from the apomictic reproduction to sexual reproduction.
- PEG 40gL^{-1} showed a significant increase in the number of tetrads but many pistils also showed abnormal cell membranes in the ovules. (Figure 3E) This could be possible dehydration of the cells.
- The pistils cultured at PEG 60gL^{-1} were unclear and impossible to determine mode of reproduction.

Discussion

- The lack of data for PEG 60gL^{-1} necessitates a repeat trial to determine the affect of higher concentrations on pistil development before further conclusions can be drawn.
- For determination of the optimal, intermediate PEG concentrations (i.e. 30gL^{-1} and 50 gL^{-1}) should also be tested.

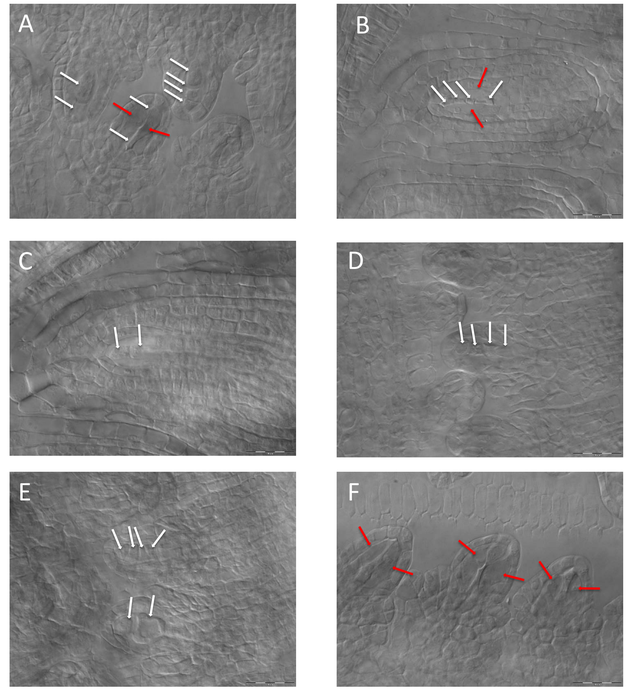

Figure 3. DIC microscopy pictures. Red arrows indicate aposporous cell development. White arrows indicate megaspore cells. A) PEG 20gL^{-1} ; dyad, tetrad and apospory. B) Control; apospory and diplospory. C) Control; dyad. D)PEG 20gL^{-1} ; tetrad. E) PEG 40gL^{-1} ; abnormal development with dyad and tetrad under possible dehydration conditions. F) PEG 20gL^{-1} ; apospory.

Future Directions

- After determining the optimal level of PEG required for a apomictic to sexual switch, I plan to culture pistils between the length of 0.8-1.0 mm. For a control I will allow the pistils to grow for 72 hours in MS media. One group will be exposed to the defined optimal PEG for the first 24hours then placed in MS media to complete the 72 hour growth. Likewise, a second group will be cultured in MS media then placed in the PEG after 24 hours and returned to MS media at 48 hours. The last group will be cultured in MS media and placed in the PEG after 48 hours to complete the term. Following this procedure should allow me to determine the stage of development at which a meiotic switch is occurring.

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