Testing the AFLP protocol to determine how genetic diversity may influence the climate change response of Pale Poppy (*Papaver alboroseum*) at Portage Glacier, Alaska

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INTRODUCTION

Pale poppy (*Papaver alboroseum*)

- White/pale pink flower
- Self-fertilization
- Native to Kamchatka, Alaska, the Yukon Territory of Canada, and British Columbia, Canada (See Figure 1)
- Found in areas affected by volcanic eruption and de-glaciation
- 8 samples of pale poppy used for this study
- Specific area of study was in Site B of the Portage Glacier Valley in Alaska (Collet, 2005)

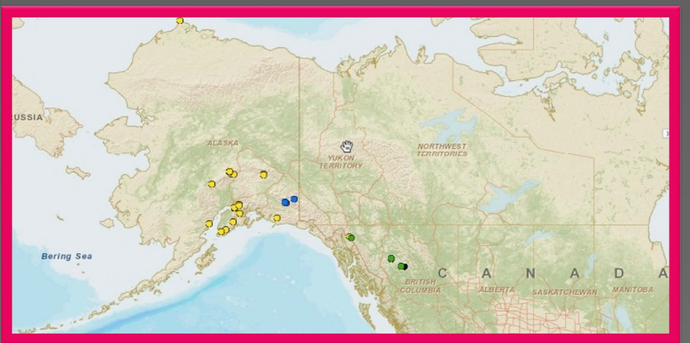

Figure 1: Distribution of *Papaver alboroseum*. Map. *E-Flora BC: Electronic Atlas of the Plants of British Columbia*. 2012.

METHODS

In this project, the Amplified Fragment Length Polymorphism (AFLP) method was used (Vos *et al*, 1995). Here are the steps:

1. Restriction Enzyme Digest
2. Adaptor preparation & ligation
3. PCR 1- Pre-amplification
4. PCR 2 - Selective amplification
5. Formamide and denature sequencing plate
6. Data analysis

RESULTS

Size Standard	LIZ500		
Concentration	6.25%	12.5%	25%
Primer Set Colors (success rate): **Blue** ECOR1-1 x MSE1-5	1:8	7:8	6:8
Green ECOR1-2 x MSE1-2	1:8	5:8	6:8
Yellow ECOR1-3 x MSE1-4	0:8	0:8	1:8

Figure 2: Three different concentrations were used to sequence the restriction enzyme fragments from the eight pale poppy samples using the LIZ500 size concentration.

Size Standard	LIZ600		
Concentration	12.5%	25%	50%
Primer Set Colors (success rate): **Blue** ECOR1-1 x MSE1-5	7:8	7:8	6:8
Green ECOR1-2 x MSE1-2	7:8	7:8	6:8
Yellow ECOR1-3 x MSE1-4	7:8	3:8	1:8

Figure 3: Three different concentrations were used to sequence the restriction enzyme fragments from the eight pale poppy samples using the LIZ600 size concentration.

Size Standard	LIZ500	LIZ500	LIZ500
Concentration	6.25%	12.5%	25%
Ratio (success rate)	2:24	12:24	13:24
Size Standard	**LIZ600**	**LIZ600**	**LIZ600**
Concentration	12.5%	25%	50%
Ratio (success rate)	21:24	17:24	13:24

Figure 4: The ratios of successful primer pairs were analyzed after the AFLP protocol.

RESULTS (cont.)

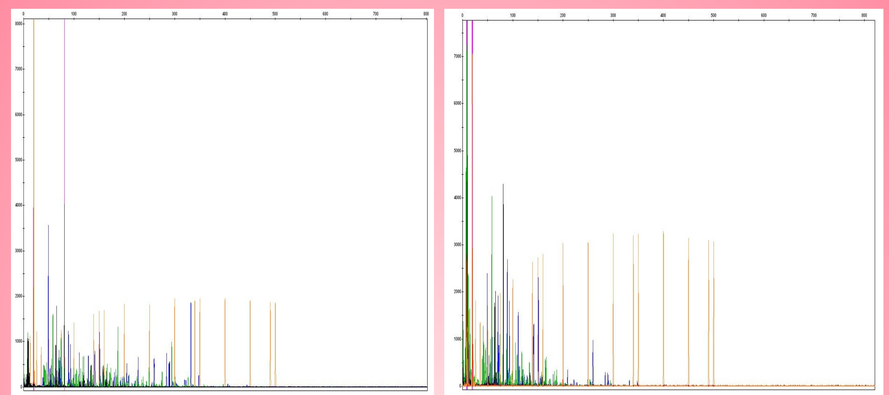

Figure 5: *Left:* Graph shows a successful range of restriction enzyme fragments for sample 25 at 25% DNA concentration using the LIZ500 size standard *Right:* Graph shows a wide range of overshot to flat line levels of DNA at the LIZ500 size standard for sample 35 at 25% DNA concentration.

DISCUSSION

After testing the AFLP method with two size standards (LIZ500 and LIZ600) and four different DNA concentrations (6.25%, 12.5%, 25%, and 50%), the DNA dilution at 25% in the LIZ500 (primer pairs ECOR1-1 x MSE1-5 and ECOR1-2 x MSE1-2) and at 12.5% in the LIZ600 (all 3 primer pairs) were the most successful.

In the LIZ500 size standard, the yellow primer pair (ECOR1-3 x MSE1-4) lacked any success and was declared as a failed primer.

CITATIONS

Collet, Dominique M. "A Survey of Two Sensitive Plant Taxa *Papaver alboroseum* and *Arinca lessingii* ssp. *Norbergii*, in Portage and Bear Valleys, Chugach National Forest, Alaska." *USDA Forest Service*. (2005): 3-18.

Vos, Pieter, Rene Hogers, Marjo Bleeker, Martin Reijans, Leo van de Lee, Miranda Hornes, Adrie Frijters, Jerina Pot, Johan Peleman, Martin Kuiper and Marc Zabeau. "AFLP: a new technique for DNA fingerprinting." *Oxford University Press*. **23**.21 (1995): 4407-13.

ACKNOWLEDGEMENTS

I would like to thank BioPrep, INBRE, the University of Alaska Fairbanks, the Rural Alaska Honors Institute, Dr. Jonathan Rupp, and Jenna Schmidt. This publication was funded by the National Center for Research Resources and the Division of Program Coordination, Planning, and Strategic Initiatives of the National Institutes of Health through grant number 8R25D011159-5.