Isoëtes butleri: Assessing factors influencing population dynamics in a rare plant

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Introduction

Butler's quillwort (*Isoëtes butleri*) species are mainly found in Alabama, Arkansas, Georgia, Illinois and Kentucky. It is an endangered species in IL and it could be destroyed by drought or by invasive non-native species. Within IL it grows in dolomite prairies in four locations: Midewin, Lockport, Blodgett Road, and Waterfall Glen. *Isoëtes butleri* is a seasonal species that grows only from May to June. This species is a tufted fern ally, grass-like in growth form, and perennial. It leaves are 6-20 cm long, erect, twisted, and gray green and pale reddish near the base (IL Natural Heritage Database1999). Currently, very little is known about the species, and no DNA extractions or analysis have been performed on this species.

Research Project

Biodiversity is decreasing in rare and endangered species are susceptible due to anthropogenic disturbance, genetic drift, and environmental catastrophes (Shaffer 1981). *Isoëtes butleri* is an endangered species that is understudied, and little known about its demographics, ecology and possible contributions to ecosystem function (Zedler and Kercher 2005). From this overall study, we will determine if populations of *Isoëtes butleri* show inbreeding and if most genetic variation will occur among populations or within populations. It will also compare soil properties by soil analysis as well as plant diversity and composition differences among different *Isoëtes butleri* populations. At the end of 10 weeks research experience, I can compare the difference between CTAB and Qiagen DNA extraction methods and gene expression ratios from samples that we collected from different prairies through DNA analysis.

Methods

Primary methods include CTAB DNA extraction, Thermo Nanodrop for DNA concentration, PCR, soil analysis and vegetative surveys.

1. CTAB DNA extraction- Adapted from Doyle & Doyle (1)
2. Qiagen- Adapted from Qiagen Kit.
3. Thermo Nanodrop- Thermo Nanodrop 2000 machine reports the concentration of the DNA
4. PCR & Gel electrophoresis- Polymerase Chain Reaction was used to amplify the gene based on the protocol adapted from Yuan-yuan, C., et.al. 2012 (2). Primers IH1-IH9 works best with a touchdown program using 61-46 ^{o}c for 31 cycles.

CTAB DNA extraction vs. Qiagen DNA extraction

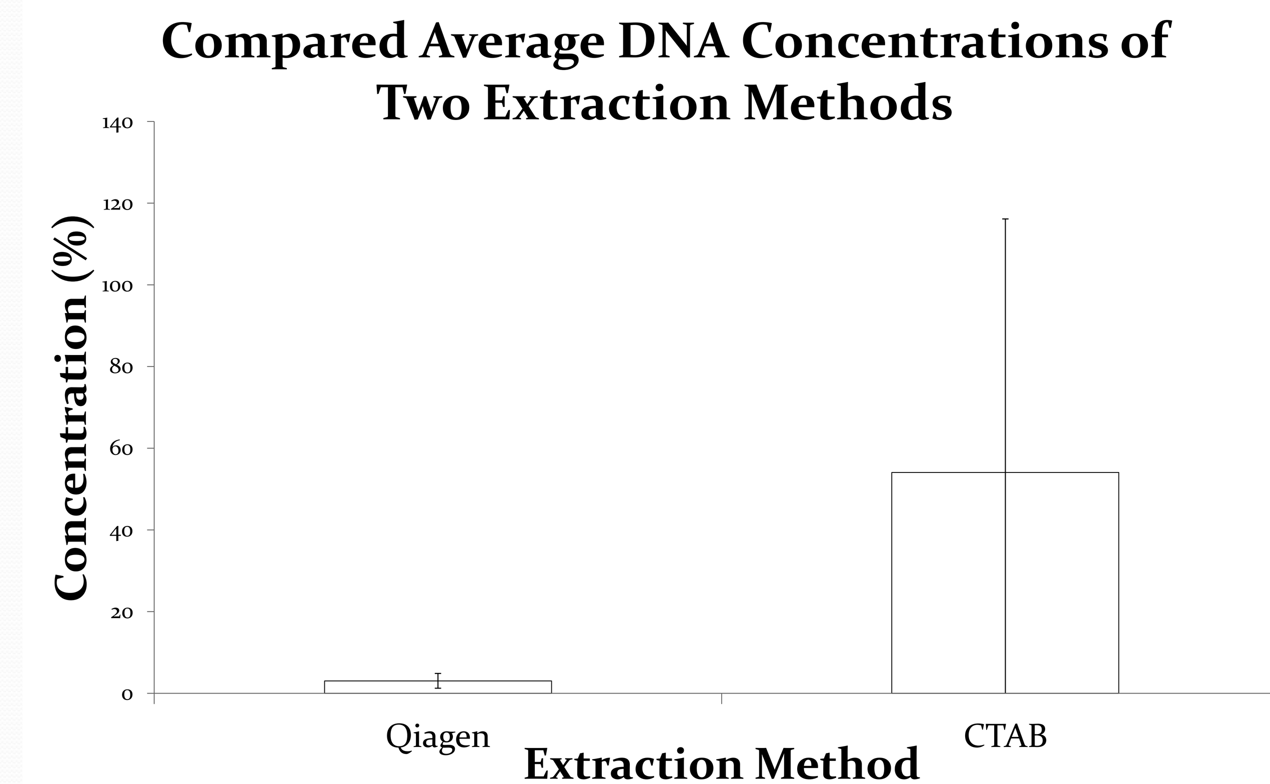


Fig 1. Comparison of Average DNA concentrations of CTAB and Qiagen DNA extractions. From our experiments, we found out that CTAB DNA extraction methods provides higher DNA concentration than Qiagen extraction method.

Images from gel electrophoresis with IH1-IH9 primers

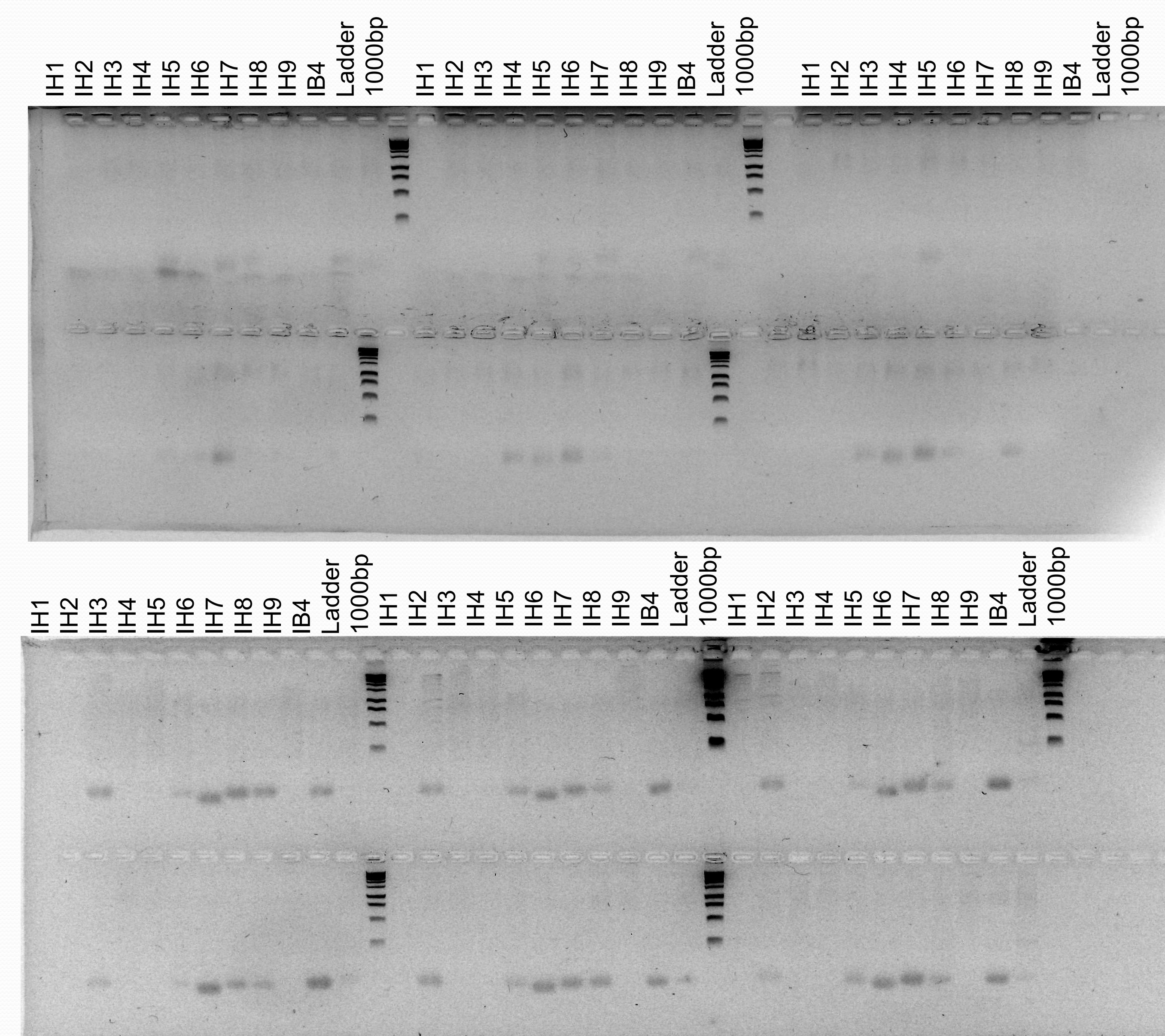


Fig 2. Images from PCR gel electrophoresis with primers IH1- IH9 & IB4. PCR was done on 6 different DNA samples and 10 different primers. New primers include IH1-IH9. IB4 was a positive control primer. DNA ladder of 1000 base pairs were placed in between the DNA samples.

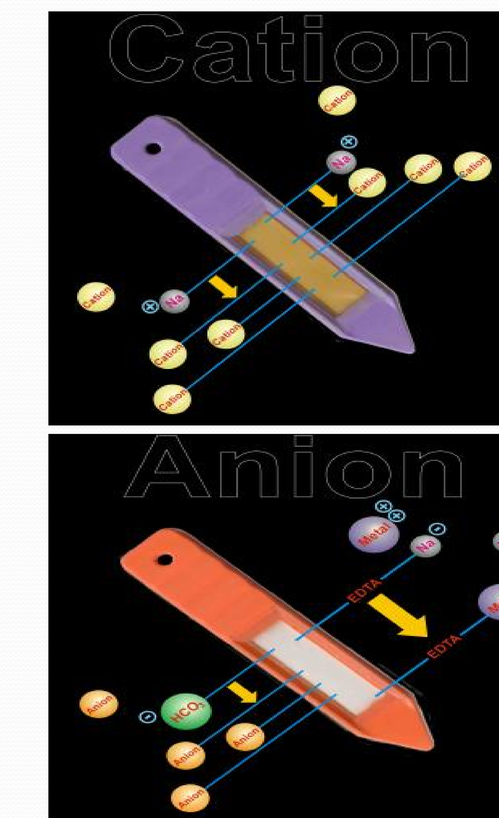
Results

My results indicate that CTAB DNA extraction produces higher concentrations of DNA than Qiagen extractions. The graphs above show higher concentration of DNA are obtained from the CTAB extractions. The CTAB itself is time consuming; however, the resulting higher concentrations of DNA are important because more DNA can be analyzed using PCR with limited amount of tissue. The PCR products were visualized by agarose gel electrophoresis. All nine of the primers we tested works with *Isoëtes butleri*. The ability of the primers to generate reliable PCR products was tested with the genomic DNA of 12 individuals in 4 geographically distinct populations. In vegetative surveys, we found out that many of the species the seven populations within the dolomite prairies were similar. Common plants in our survey were *Allium cernuum*, *Apocynum sibiricum*, *Solanum carolinense*, *Asclepias verticillata*, *Daucus carota*, *Satureja arkansana*, *Melilotus alba*, *Schizachyrium scoparium*, *Monarda fistulosa* and *Rhamnus cathartica*. Soil analysis will be completed later this August by Western Ag.

Discussions

Further study:

1. Analyzing DNA fragments on Beckman Coulter 8000 and comparing genetic diversity and structure using GenAlEx in Excel. Also need to use the data from multiple years and different subpopulations to find their genetic diversity.
2. In addition, more vegetative surveys have to be done in various times of year to find out the changes in the vegetation. Also, a comparison needs to be made using Shannon-Weiner diversity index and community composition among sites.
3. Plant root simulator soil probes will be used to compare soil chemistry inside and outside population boundaries. Gravimetric soil moisture analysis, temperature analysis, and also depth measurement will be completed.



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