To N or not to N: Changes in Chemistry Allocation with Limiting Conditions in *Heterotheca subaxillaris*

Alicia Marmolejo[1], and Sara Fuentes-Soriano[2]

[1]Department of Plant and Environmental Sciences; [2]NMSU herbarium and Department of Animal and Range Sciences,
New Mexico State University[1] e-mail alice12@nmsu.edu

Introduction

Heterotheca subaxillaris (camphorweed), a member of the Asteraceae family, is an annual, biennial, or perennial herb and a widely distributed native of North American[1] since the 1950s and 1970s, respectively, has become a critical aggressive invasive species in Argentina and Israel. In the Holy land, the species was introduced intentionally as a dune stabilizer[2] and, in the last 30 years, has remarkable adaptive reproductive strategies reflected in numerous vegetative and reproductive morphological features[2]. The overall goal of this project is to tackle three main questions 1) what the identity of the founding population in Israel is, 2) what the extent of the genetic diversity in native & invasive distributions is, and 3) what the genetic backgrounds are supporting the plasticity & divergence of phenotypic variation in native and non-native populations.

Main Objectives

- To identify the parental origin of the population that was brought from North America to Israel
- To compare and understand how different the genetic diversity of the Israel and North American population.

Specific Objectives

- Create DNA extraction Protocol
- Use protocol to perform DNA extractions
- Analyze DNA

Methods

- Field work Fall 2021 (Fig 6.)
  - *North America* Southwest and Gulf of Mexico in (7 populations total)
  - *Israel* collections along a costal latitudinal gradient (5 populations total)
- Population genomics (Fig. 1)
  - DNA extractions
  - ddRAD library preparation
- Preliminary explorations of the geographic variation of leaves secondary metabolite composition

DNA Extraction Method

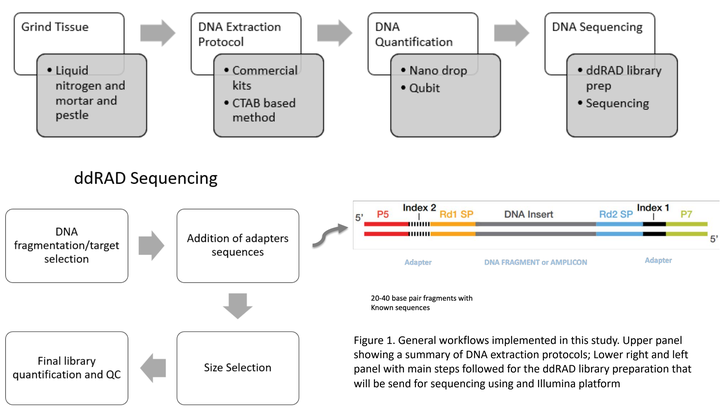

Figure 1. General workflows implemented in this study. Upper panel showing a summary of DNA extraction protocols; Lower right and left panel with main steps followed for the ddRAD library preparation that will be send for sequencing using and Illumina platform

Results

Protocols assayed (Table 1 & Fig. 3)

- Commercial kits
  - Qiagen kit
  - Aqua-genomics
  - Zymo kit
- CTAB based protocols
  - The CTAB modified 2x Chloroform protocol showed the best results throughout all populations
  - During extraction we observed variations in color, smells, and leaf size (Fig. 4).

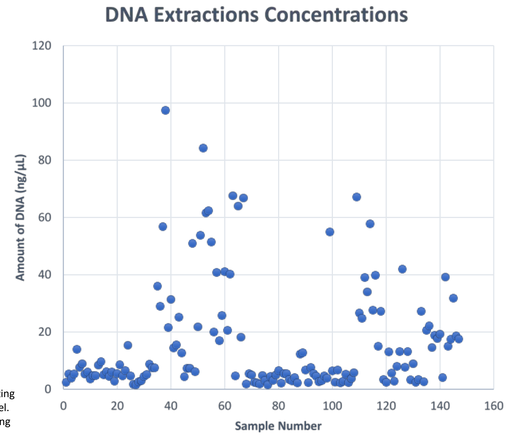

Figure 2. DNA concentrations per Qubit (dsDNA High Sensitivity) across 144 individuals representing eleven populations from North America and Israel. Higher concentrations of DNA were obtained using a modified CTAB protocol (x2 chloroform: isopropanol extractions, 5% PVPP, and high salt concentrations).

DNA Extraction Methods			
Protocols/ Kits	Lysis	Preciptiation	Purification
Aqua-Genomics	60 min at 60° C	100% isopropanol (2-propanol)	70% ethanol
Qiagen Dneasy Plant mini kit	Guanidine	95% ethanol + silica filters	Filters
Zymo Quick- DNA plant mini kit	2 Step Lysis Approach (buffers + silica filter)	2 washes (alchol based) + silica filters	Filters
CTAB Standard	Standard Buffer	100% isopropanol	70 % ethanol + H2O
CTAB + Phenol	Standard Buffer + Phenol	95% ethanol	70 % ethanol + H2O
CTAB + 4x Chloroform	Standard Buffer	95% ethanol	70 % ethanol + H2O
CTAB + 2x Chloroform	Highly Standard Buffer + PVPP (antioxidant)	95% ethanol + 1/2 volume NaCl	Clear Pellet: 70% ethanol+ 1/10 volume NaCl Visibly Colored Pellet: 95% ethanol + overnight Precipitation then 70 % ethanol + H2O

Table 1. Comparative summary of seven DNA extraction protocols tested during the study

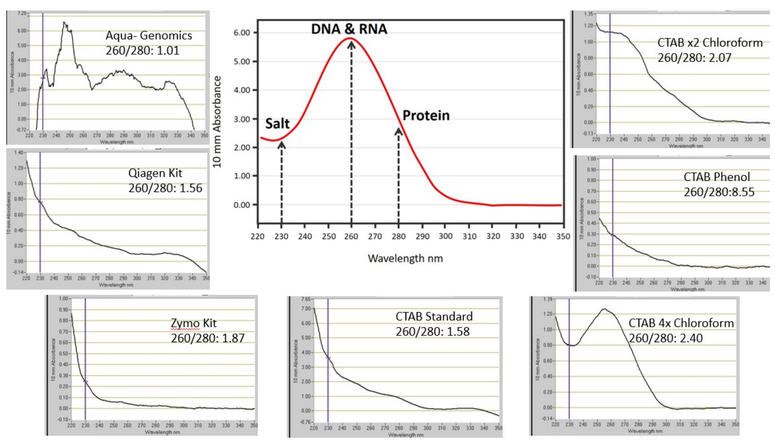

Figure 3. Protocol performance based on measurement of DNA concentrations per Nanodrop readings. All extractions used 0.3 g of silica dried leaves

References

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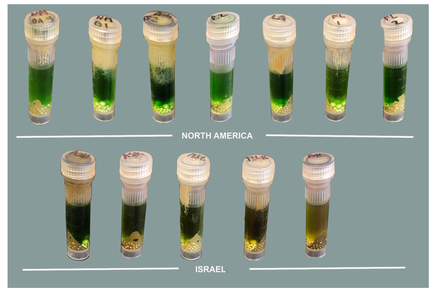

Figure 4. Methanol extracts from dried leaves of *Hetherotheca subaxillaris* populations from North America and Israel.

During DNA extractions, Israel's populations varied remarkably in 1) camphor fragrance and 2) color of aqueous supernatants separated during DNA extractions specially when compared with those from North America (Fig. 4).

We measured color changes, likely related to secondary metabolites composition, from secondary metabolites methanol extracts using 0.3 gr of dried leaves in a spectrophotometer (Spectra Max M_2). Changes in light absorbance reflected variation in chemical secondary metabolite composition across populations (Figs. 4 & 5)

Discussion

- A CTAB modified protocol with 2x chloroform, 5% PVPP and high salt buffer concentrations was the most effective in removing phenolics from DNA extracts of Camphorweed . Although this protocol yield the highest DNA concentrations (Fig. 2) as in all tested protocols yielded suboptimal DNA purity values per $A_{260}/_{230}$ absorbance ratios between 0.6 - 1.7 (2.0) from Nanodrop.
- During DNA extractions while grinding leaves strong camphor (e.g., terpenoid) scents could be smelled especially from the Israel population.
- According to Mihaliak and Lincoln (1985) plants that have little nitrate availability have produced higher amounts of terpenes.[3] We hypothesize that the plants from Israel are experiencing low nitrate availability which can explain the increase of terpenes and possibly other factors that have made it able to become invasive.
- We also believe that *Heterotheca subaxillaris* was able to become an invasive species in Israel based on the Fluctuating resource availability theory of invasibility.[4]

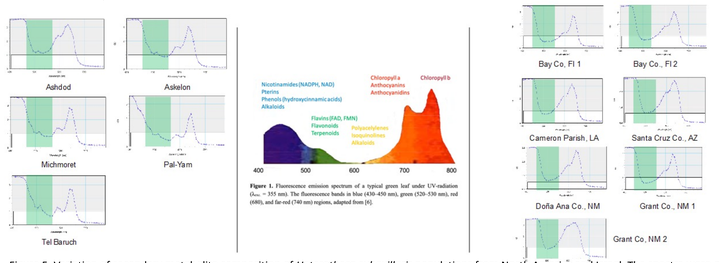

Figure 5. Variation of secondary metabolite composition of *Heterotheca subaxillaris* populations from North America and Israel. The spectra were obtained from methanol leave extracts of silica dried leaves.

Future Directions

- ddRAD sequencing and Population genomic data analyses
- Analysis of genomic and phenotypic variation (reproductive morphology and ecology, shifts in phenology)
- Analysis of significant genomic and metabolomic markers, including secondary metabolites response to the environment and its potential adaptive roles.
- Soil nitrogen effect on amount of terpenoids produced.
- Chemical properties to further help management practices.
- Different types of soil affect the chemistry and composition of camphorweed.

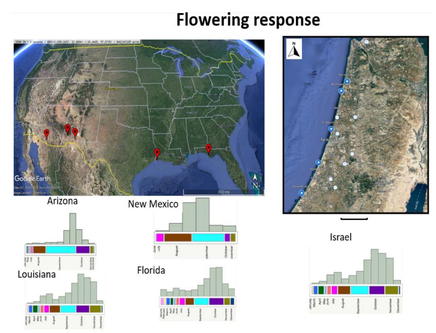

Figure 6. Comparison of flowering responses of populations of camphorweed in North America and Israel. Upper left panel with map of distribution of North American population included in the study; Upper right panel with map of distribution of populations collected in Israel; Lower bottom panel with five charts showing flowering patterns of *Heterotheca subaxillaris* in native and non-native distributions. Phenology information was inferred for North America based on more than 3,000 herbarium records and for Israel based on field observations (Sternberg *comp. pers*).

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