Development and Application of Priority Trait Evaluation Protocols and Terpene Analysis Method

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Abstract

Morphological and secondary metabolic data can determine a plant's overall horticultural and agronomic quality. By exploring these phenotypic protocols, hemp researchers can better assess and identify unique and valuable hemp (*Cannabis sativa* L.) for subsequent breeding efforts and cultivar improvement. Hemp is an emerging crop that is utilized for fiber, grain, and diverse secondary metabolic compounds. Evaluation of phenotypic variation across the hemp genetic resources held within the Plant Genetic Resources Unit (PGRU) provides value to breeders and researchers. This project applies morphologic and secondary metabolic protocols to support this collection, supply diverse datasets, and provide readily identifiable germplasm. In conjunction with molecular markers, these datasets may contribute to mapping studies and identifying genetic controls of priority traits. In this study, diverse hemp genetic resources are assessed for plant architectural traits and secondary metabolites. The USDA Plant Genetics Resources Unit (PGRU) in Geneva, New York is conducting an ongoing replicated phenotypic evaluation of 104 fiber, grain, cannabinoid, and feral accessions. Individual plants (N = 2,412) were evaluated for plant height, stem diameter, flowering date, and sex. Plant height was measured directly and using UAV (drone) phenotyping approaches. In this study, secondary metabolites with a primary focus on terpenes are also assessed. Terpenes are naturally occurring chemical compounds that contribute to aroma in hemp and were evaluated using gas chromatography at Cornell University. These compounds provide unique scent profiles and may have implications for pest resistance. The molecular trial analyzed 20 terpenes commonly found in hemp across 6 cultivars. We used a mini-GC equipped with flame ionization detection to identify each terpene based on retention time and peak height. We also explored the relationship between the boiling point of a terpene and its retention time. Overall, these trials have provided valuable method development and data to support hemp genetic resource conservation, evaluation, and distribution of genetic resources to the international hemp community.

Materials and Methods

Experiment 1 (**Plant Height Analysis):** We transplanted 104 hemp cultivars from the PGRU hemp collection on June 5, 2022, in Geneva, NY. These plants were grown under standard research conditions (Stack, 2021) in a randomized complete block design using three replicates. Plants were measured every seven days for height according to the USDA Hemp Phenotyping Handbook (Stansell, 2021) using an Android phone running FieldBook (Rife and Poland, 2014) and a grade rod. All data were processed in R version 4.2.0 (R Core Team, 2022) and visualized using the ggplot2 functions (Wickham, 2016).

Experiment 2 (Terpene Analysis): We analyzed six hemp cultivars from PGRU using the Lucidity Mini-GC provided by the Hemp Processing Lab. The GC is equipped with flame ionization detection to identify each terpene based on retention time and peak height. 200 mg of terminal inflorescence and leaf tissue from three plants were pulled from each cultivar using a standard protocol (Storm, 2018). These samples were then analyzed using GC and all data was processed in R version 4.2.0 to explore terpene variance between cultivars and tissue type. We also investigated the relationship between boiling point and retention time, allowing us to identify unknown terpene compounds outside of the terpene standard mix prepared.

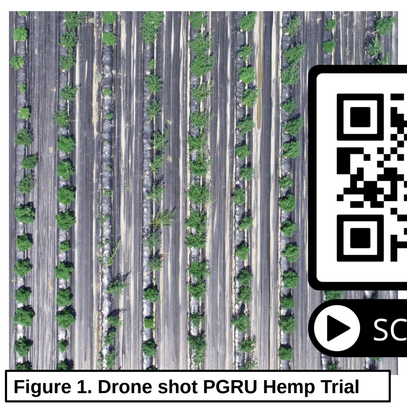

Figure 1. Drone shot PGRU Hemp Trial

Figure 2. USDA-ARS PGRU Hemp Trial

Results

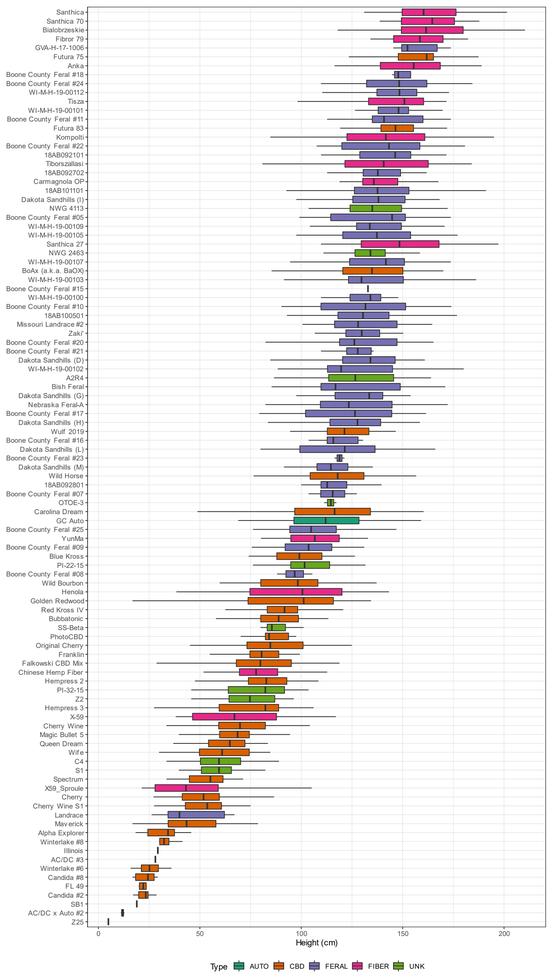

Figure 1. Plant Height Variance by Cultivar at 45 DAP

Experiment 1: We were able to ascertain plant height across four cultivar groups: FIBER, cannabinoid (CBD), FERAL, and previously uncharacterized germplasm (UNK).

There was substantial variance of height among cultivars (Figure 1). Cultivar group explained much of the variance between cultivars. Within population type, replicate explains additional variation in height. There was a significant replication effect ($p < 0.001$; Figure 2).

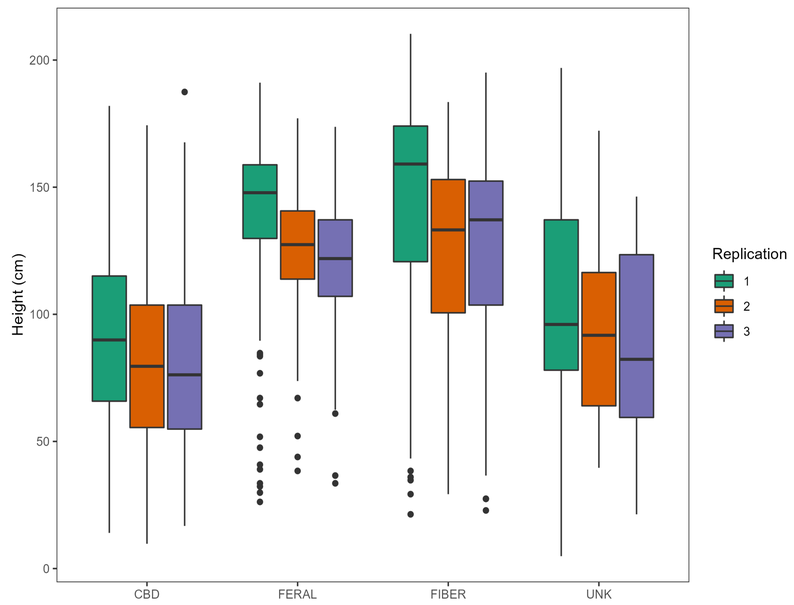

Figure 2. Plant Height Variance by cultivar group and rep at 45 DAP

Experiment 2: Identification of prevalent terpenes in six hemp cultivars (Alpha Explorer, Anka, Hempress, Henola, Maverick, X59-Sproule). Beta-caryophyllene and beta-myrcene were abundant across all cultivars. Cultivars had distinct compounds in both the inflorescence flower and leaf tissue. Some terpenes showed correlation between leaf and flower (e.g., carene and unknown 11.23) but only in certain cultivars not others.

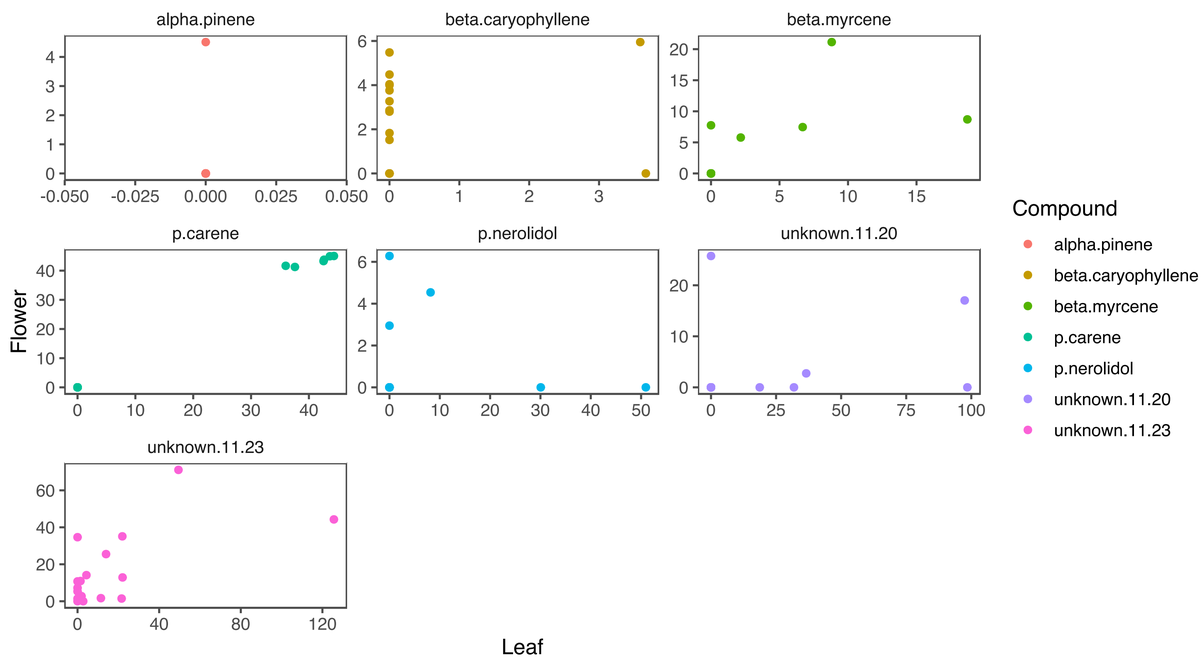

Figure 3. Terpene profiles compared between floral tissue (y axis) and leaf tissue (x axis) in standard peak area

Conclusions

- Preliminary analysis shows plant height varies by cultivar, establishing how diverse the USDA-ARS PGRU Hemp collection is. Fiber and feral cultivars are not distinguishable by height, and likely share a genetic background.
- Terpene levels and profile vary by cultivar and tissue type. Terpenes vary by cultivar and, when prevalent, may have a direct relation between leaf and flower tissue.

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