Analysis of Chemotypes in Retail *Cannabis* Strains

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ABSTRACT

Although research on the chemical constituents of *Cannabis*, including cannabinoids and terpenes, is abundant, relatively few genetic studies have been conducted, and the origins and genetic identities of most *Cannabis* strains are largely unknown. There are even fewer strain specific *Cannabis* studies assessing genetic and chemical aspects together. The recent legalization of medical *Cannabis* and decriminalization in many states, has led to a surge of new strains that vary widely in appearance, taste, smell, psychotropic effects, and cannabinoid levels. As more people look to *Cannabis* for medical and recreational purposes, it is important to further explore the inconsistencies seen in the genotypes within *Cannabis* strains to determine if there are similar inconsistencies observed in the chemotype within *Cannabis* strains. This study will use previously gathered genetic data in concert with GC and HPLC on samples from 28 popular *Cannabis* strains acquired from different sources to determine if: (1) genotype and chemotype display parallel Principle Component Analysis clustering patterns, (2) strains acquired from different sources have similar chemical profiles, (3) it is possible to determine *Cannabis* strains using a chemical profile, (4) chemical profiles match publicly available online descriptions and (5) the major cannabinoid (THCA, THC, CBDA and CBD) reported on retail *Cannabis* are accurate.

INTRODUCTION

The legalization of medical *Cannabis* and decriminalization has led to a surge of new strains. Growers are hybridizing and selecting plants that produce flowers with varying chemical profiles. Consumers are confronted with an overwhelming number of *Cannabis* strains that vary widely in appearance, taste, smell, psychotropic effects, and cannabinoid levels.

The Leafly database has grown to more than 2200 strains in March 2017 (Leafly, 2016). However, *Cannabis* cultivation has been underground for so long, and in the absence of regulation, many interested parties question whether the integrity of named strains has been maintained over time (Green 2014, Stockton 2015).

Cannabis is not afforded this protection as the USDA considers it an ineligible commodity (United States Department of Agriculture,2016). Without regulation there is the potential for erroneous misidentification and mislabeling of plants, renaming plants of unknown origin, as well as more deceitful actions such as renaming/relabeling products with a more catchy or popular name to increase sales. Consumers have a right to be informed as protection from misleading claims and mislabeling practices.

Morphology alone is not enough to accurately identify or verify the thousands of strains of *Cannabis* available. Registration and verification of common cultivars is often conducted with molecular genetic tools, such as species-specific microsatellite markers (Rongwen et al. 1995). The chemical profile (chemotype) of *Cannabis* is determined by a combination of both genotype and environmental factors. While the chemical profile is inherited from the parents, the expression levels of the various chemicals within each plant can vary under different growing conditions.

To date, only 21 samples from six strains have been assessed and we are still analyzing the remaining strains in our *Cannabis* research lab for extended chemical profiles and genetic study.

OBJECTIVES

At this point we set the objective as:

1. To compare chemical profiles from strains acquired from different sources;
2. To compare the chemical profiles with as reported on retail *Cannabis*;
3. To determine if the chemotype is parallel to genotype pattern.

MATERIALS AND METHODS

Sampling: *Cannabis* samples for 28 strains were acquired from recreational and medical dispensaries in Colorado, California and Washington. All samples were dried, pulverized, labeled and kept at 4°C for chemical analysis. We have assessed six strains so far: OG Kush, Banana Kush, Tahoe OG, Hash Plant, Purple Kush and Flo.

Sample extraction:

1. 0.01 mg of sample was extracted in 1 ml of acetone (HPLC grade, Fisher Chemical, USA), 1 ml of pentane (99+%, extra pure, ACROS Organics, USA) and 0.01 acetic acid, glacial (Fisher Scientific, USA).
2. All samples were soaked in a wrist shaker for 2 hours before centrifuged at high speed for 5 minutes.
3. The supernatants were collected and evaporated using flow of nitrogen gas. The evaporated extracts were reconstituted with 1 ml of ethanol (Molecular biology grade, Fisher Bioreagents, USA).

Chemical analysis:

1. A 7890A GCMS system with 5975C Triple-Axis Detector (Agilent Technologies) was used to determine delta 9-tetrahydrocannabivarin (Δ9-THCV), Cannabichromene (CBC), Cannabidiol (CBD), delta 8-tetrahydrocannabinol (Δ8-THC), delta 9- tetrahydrocannabinol (Δ9-THC), Cannabigerol (CBG) and Cannabinol (CBN).
2. Chromatographic separation was carried out on a 15m (length) x 0.25 mm (ID) with 0.25um (film) column (Rxi®-35Sil MS , Restek, USA). A 1 µl extract was injected using the splitless mode. The carrier gas helium was used with the initial oven temperature set at 150°C. The temperature was held for 0.9 min, and was raised to a final temperature of 350°C at the rate of 15°C/min. The total run time of a sample is 15.6 min.
3. Chromatograms were recorded using a MSD ChemStation E.02.00.493 (Agilent Technologies, USA). Each analyte was identified through the retention time and the most abundant ion, second, and third ions were used for the confirmation. The NIST/EPA/NIH Mass spectral library was mainly referred to confirm the type of analyte and its molecular structure.

Genetic analysis:

1. DNA was extracted using a modified CTAB extraction protocol with 0.035-0.100 grams of dried flower tissue per extraction. Flower material was ground using liquid nitrogen and micro-pestle in a 2.0 µL centrifuge tube. Cell lysis with ~800 µL of 2 X CTAB extraction buffer was followed by separation and precipitation of total genomic DNA with chloroform and 95% ethanol, respectively. The DNA was then centrifuged to form a pellet which was then washed with 80% ethanol, dried down and then suspended in 1 X TE Buffer.
2. The Cannabis draft genome from "Purple Kush" (Genbank accession AGQN00000000.1) was scanned for microsatellite repeat regions using MSATCOMMANDER-1.0.8-beta. Thirty microsatellites with 3-6 nucleotide repeat units were selected for primer design and optimization. One of the primers in each pair was tagged with a 5' universal sequence (M13, CAGT or T7) so that a matching sequence with a fluorochrome tag could be incorporated via PCR.
3. Microsatellite loci were amplified in 12 µL reactions using 1.0 µL DNA (10-20 ng/ µL), 0.6 µL fluorescent tag (5 µM; FAM, VIC, or PET), 0.6 µL non-tagged primer (5 µM), 0.6 µL tagged primer (0.50 µM), 0.7 µL dNTP mix (2.5mM), 2.4 µL GoTaq Flexi Buffer (Promega, Madison, WI, USA), 0.06 µL GoFlexi taq polymerase (Promega, Madson, WI, USA), 0.06 µL BSA (Bovine Serum Albumin 100X), 0.5-6.0 µL MgCl or $MgSO_4$, and 0.48-4.98 µL dH_2O. Amplified products were combined into multiplexes and diluted with water. The multiplexed PCR products were mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems, Foster City, CA, USA) before electrophoresis on a 3730 Genetic Analyzer (Applied Biosystems) at Arizona State University. Fragments were sized using GENEIOUS 8.1.8 (Biomatters Ltd). Ten of the thirty primer pairs produced consistent peaks within the predicted size range and were used for the genetic analyses herein.

Statistical analysis:

1. A one-way ANOVA with Post Hoc was done to determine whether the groups have the same means. We assume that all the groups have Normal distribution, and that the variances for all the groups are similar. A Tukey HSD is carried out to determine which groups' means are significantly different and to tabulate a comparison table. Descriptive statistic was done using multiple correlation and regression.
2. A population differentiation test was done using Arlequin software to determine heterozygosity.

RESULTS

Comparison of chemical profiles from different strains and sources

There is no significant difference in the total percentage of compounds among test strains. However, there is a significant difference between individual compounds in all samples. Tukey's HSD suggested the mean of groups as similar and different in percentage, as in Table 1 (Banana Kush, Tahoe OG, Hash Plant, Purple Kush, Flo) and Table 2 for OG Kush.

Table 1

	Similar	Different
Δ9-THCV	CBC, CBD, Δ8-THC, CBG, CBN	Δ9-THC
CBC	CBD, Δ8-THC, CBG, CBN	Δ9-THC
CBD	Δ8-THC, CBG, CBN	Δ9-THC
Δ8-THC	CBG, CBN	Δ9-THC
Δ9-THC		CBG,CBN
CBG	CBN	

Table 2

	Similar	Different
Δ9-THCV	CBC, CBD, Δ8-THC, CBG, CBN	Δ9-THC
CBC	CBD, Δ8-THC, CBG, CBN	Δ9-THC
CBD	Δ8-THC, CBG, CBN	Δ9-THC, CBG
Δ8-THC		Δ9-THC, CBG, CBN
Δ9-THC		CBG,CBN
CBG	CBN	

Comparison of compound area % and % sativa as reported on retail labels

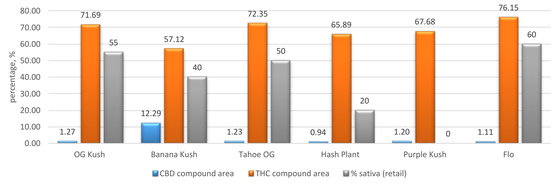

Table 3

	% CBD area	% THC area	% sativa (retail)
% CBD area	1		
% THC area	-0.6987	1	
% sativa(retail)	0.0431	0.2714	1
P-value	0.8568	0.2471	

Positive Pearson's correlations were found for both %CBD area and %THC area, but are not significant as P> .05.

Chemotype Vs Genotype pattern

	Chemotype	Genotype
Statistical analysis	1. One-way ANOVA with Post-Hoc to compare compounds vs locations 2. One-way ANOVA with Post-Hoc to compare between compounds	1. Population/Strain differentiation test
Output	1. Accept H_0 : All % of compounds are similar in all samples 2. Reject H_0 : Different compounds have different %	1. Observed heterozygosity (0.43164 ± 0.23585) is less than expected heterozygosity (0.61134 ± 0.17119) 2. P value for F_{ST} Strain structure analysis showed non-significant results
Conclusion	All test samples contained similar amounts of cannabinoids	All plant samples are related between strains and among strains

CONCLUSIONS

1. Seven compounds were identified using GCMS : Δ9-THCV, CBC, CBD, Δ8-THC, Δ9-THC, CBG and CBN.
2. All plant samples have similar amounts of cannabinoids, regardless of the type of strain.
3. Amount of each compound can be predicted from other compounds, except for Δ9-THC.
4. % sativa (retail) correlates with %CBD area and %THC area, but is not significant.
5. Cross – breeding had occurred among strains and between strains that leads to low heterozygosity.
6. Chemical profile pattern is in line with genetic profile pattern as both show no significant differences.

FUTURE STUDY AND SUGGESTION

1. To screen more samples to further confirm the chemical profiles, heterozygosity and correlation study on retail label.
2. To compare retail strains with the non-hybrid strain of *Cannabis* plant.

Suggestion

- A regulation on cross-breeding activity should be introduced
- A guideline on the strain composition should be imposed

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Cannabis sativa