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Nonterpenoid Chemical Diversity of Cannabis Phenotypes Predicts Differentiated Aroma Characteristics

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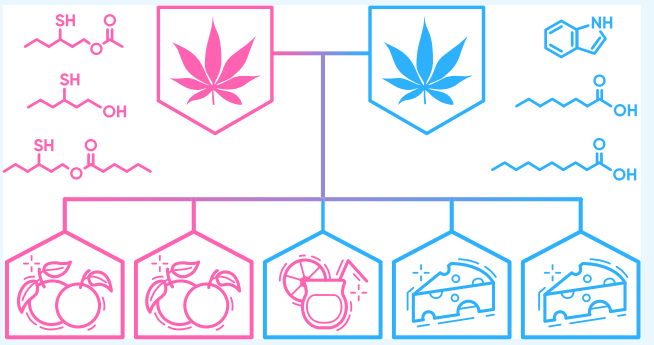
ABSTRACT: The recent increase in legality of *Cannabis Sativa L.* has led to interest in developing new varieties with unique aromatic or effect-driven traits. Selectively breeding plants for the genetic stability and consistency of their secondary metabolite profiles is one application of phenotyping. While this horticultural process is used extensively in the *cannabis* industry, few studies exist examining the chemical data that may differentiate phenotypes aromatically. To gain insight into the diversity of secondary metabolite profiles between progeny, we analyzed five ice water hash rosin extracts created from five different phenotypes of the same crossing using comprehensive 2-dimensional gas chromatography coupled to time-of-flight mass spectrometry, flame ionization detection, and sulfur chemiluminescence detection. These results were then correlated to results from a human sensory panel, which revealed specific low-concentration compounds that strongly influence sensory perception. We found aroma differences between certain phenotypes that are driven by key minor, nonterpenoid compounds, including the newly reported 3-mercaptohexyl hexanoate. We further report the identification of octanoic and decanoic acids, which are implicated in the production of cheese-like aromas in *cannabis*. These results establish that even genetically similar phenotypes can possess diverse and distinct aromas arising not from the dominant terpenes, but rather from key minor volatile compounds. Moreover, our study underscores the value of detailed chemical analyses in enhancing *cannabis* selective breeding practices, offering insights into the chemical basis of aroma and sensory differences.



INTRODUCTION

The cultivation of *Cannabis sativa L.* has rapidly evolved over the past few decades, resulting in varieties with highly diverse morphological, chemical, and sensory characteristics.[1−7] Apart from the medicinal use of cannabis and the need for pharmaceutical-grade cultivars (i.e., genetic stability and reproducibility), there is a desire to influence *cannabis* offspring to produce unique secondary metabolite profiles.[8,9] *Cannabis*' vast genetic diversity enables a spectrum of aromas ranging from sweet, savory, or prototypical.[4,8,10−13] In particular, many cultivators aim to produce varieties that express unique aromatic and flavor characteristics that can significantly impact consumer preferences.[4]

Volatile organic compounds (VOCs) make up part of *cannabis's* secondary metabolite profile and are responsible for the aroma it produces.[7,14] These compounds are found in different plants, fruits, and vegetables.[15−20] In *cannabis*, the aromatic compounds discussed often include monoterpenes, sesquiterpenes, and their respective terpenoids. However, recent studies show that low-concentration compounds such as volatile sulfur compounds and recently discovered flavorants (nonterpenoid-derived VOCs) are the primary source of the unique and diverse aromas produced by *cannabis*.[10,21] While these compounds are known in the context of aroma identification, they have rarely been studied in the framework of selective breeding. For instance, minimal studies exist comparing the secondary metabolite profiles of *cannabis* progeny.[22] This chemical information could potentially shed light on the chemodiversity of siblings and how they inherit the aroma characteristics of their parents, as well as yield information about phenotypic distribution.[23]

Selective breeding can be utilized in a variety of different ways depending on the application (e.g., hash oil, aroma distillation, and fiber production).[8,24,25] One of the key techniques used when selectively breeding *cannabis* is

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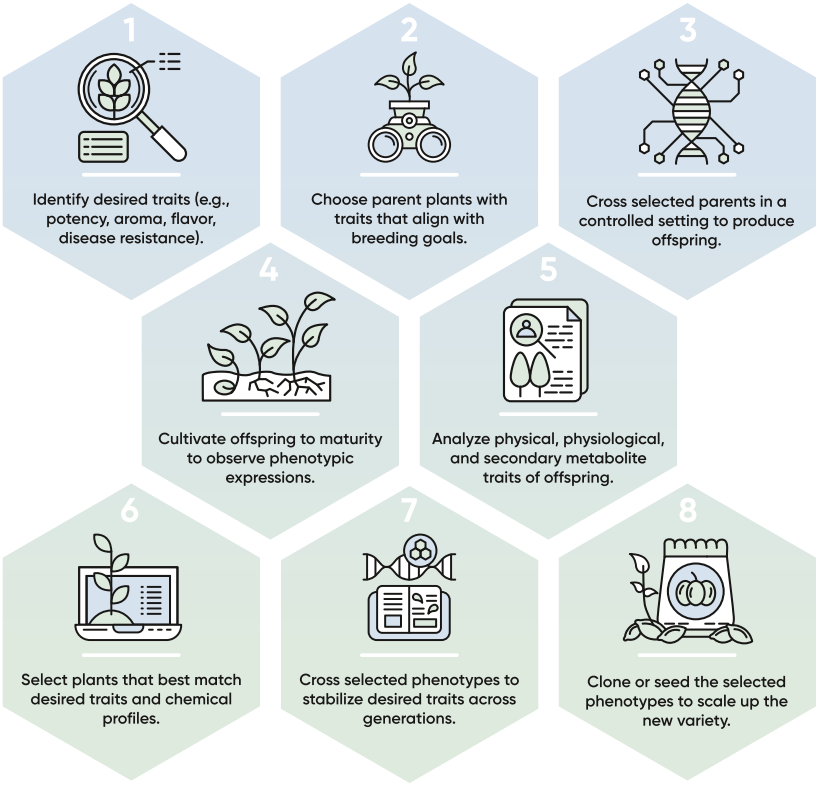

Figure 1. Schematic showing a general process used during cannabis phenohunting.

colloquially known as "phenohunting"—the process of propagating two varietals with desirable traits, as shown in Figure 1. Breeders will often produce hundreds of different progeny from a single cross, followed by conducting analysis on their morphological, growth, and secondary metabolite characteristics, specific to their final application.[3,23] After selection of the phenotypes that best exhibit the desired traits, these chosen phenotypes are further propagated through cloning or self-pollination to ensure genetic stability and consistency in their lineage.

While some of these characteristics can be monitored visually or without sophisticated equipment, the secondary metabolite profile, which includes the compounds responsible for the psychoactive properties of *cannabis* and aroma, is more difficult to ascertain without analytical chemistry instrumentation.[8] In fact, the majority of phenohunting aimed at developing new aromas and flavors relies heavily on sensory analysis of the offspring.[26,27] Nonetheless, utilizing chemical analysis for the offspring is becoming more common.[11,28] However, these analyses, which are typically conducted at regulated analytical *cannabis* testing laboratories, usually only test for certain dominant terpenes such as D-limonene, β-myrcene, β-caryophyllene, humulene, or terpinolene, among others.[14] As demonstrated in our previous study, terpenes often have minimal influence on the unique aroma of *cannabis* products.[10] Instead, minor nonterpenoid compounds often drive aroma differences; therefore a limited chemical analysis may serve only to confuse or deceive the cultivator in applying incomplete chemical data to their selection processes.[10] Furthermore, there are minimal studies investigating the volatile chemical profiles of different *cannabis* phenotypes, resulting in a limited understanding of their potential chemical diversity and the phenotypic distribution related to aroma characteristics.

Here, we expand upon our previous work by investigating the chemical diversity of genetically similar *cannabis*. To understand how the VOC profiles of phenotypes vary and relate to their aromatic properties, we conducted and compared chemical and sensory analyses of five different offspring from the same crossing. Cannabis and its extracts constitute a highly complex matrix, potentially encompassing hundreds of VOCs. Given this complexity, we utilized two-dimensional gas chromatography coupled with time-of-flight mass spectrometry, flame ionization detection, and sulfur detection (GC × GC−ToF−MS/FID/SCD). Sensory analysis was then used to validate the chemical compositions and correlate the specific aroma attributes. We found tropical volatile sulfur compounds, henceforth referred to as tropicannasulfur compounds (TCSCs), to have a large impact on the aroma of each variety. In particular, we identified a new TCSC, 3-mercaptohexyl hexanoate, as yet another key component in producing these pungent citrus aromas. We also identified octanoic and decanoic acids, two fatty acids that are implicated in the production of savory or cheese-like aromas. Sensory studies were then used to relate the chemical compositions to specific aroma attributes and user preference, revealing a strong relationship between tropical aromas produced by the volatile sulfur compounds and preference. We last discuss how these key minor compounds may provide chemotaxonomic utility in differentiating cannabis on an aromatic level whereas terpenes do not. These findings demonstrate the effectiveness of detailed chemical analysis alongside sensory analysis in phenotyping, providing enhanced chemical differentiation among the samples. Furthermore, these results reveal that cannabis from the same genetic lineage

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can produce distinct aromas, which can be attributed to minor compounds present in low concentrations.

EXPERIMENTAL SECTION

Sample Procurement and Preparation. Ice hash rosin *cannabis* samples of Starburst 36, phenotypes #1, 23, 38, 39, and 40 (referred to as SB-1, SB-23, SB-38, SB-39, and SB-40, respectively) were procured from 710 Laboratories. Samples were stored in a negative 8 °C freezer until measured to retain their volatile chemical profiles. Samples, each weighing approximately 100 mg, were weighed into a 20 mL scintillation vial, followed by addition of two mL of hexanes. The resulting solution was vortexed for 5 min to fully dissolve the matrix. The resulting solution was then transferred into a two mL sample vial by using a 0.25 μL filtered syringe. All samples were handled, stored, and prepared in a similar manner to minimize differences resulting from sample stability. The initial chemical analysis of all samples was performed within 24 h of each other. Sensory analysis data were collected by all participants on the same day. Each sample was collected in triplicate.

Analytical Standards. Several different reference materials were acquired for the purpose of compound quantitation and confirmation, as described in our previous work.[10] A thirty-five-compound terpene analytical standard (LGC Standards) was used to quantify the major components in the samples. A custom 17-compound flavorant standard (FLV-1) was supplied from LGC Standards prepared in triacetin that was further diluted in methanol. Multiple custom flavorant standards were then created in-house using analytical grade standards when available. Standard materials were purchased from different sources, including Sigma-Aldrich, Vigon International, and Penta International. Prenylthiol (Penta International, 95% in 1% triacetin) was prepared in methanol. 3-Mercaptohexanol, 3-mercaptohexyl acetate, 3-mercaptohexyl butyrate, and 3-mercaptohexyl hexanoate (Excellentia, >97%) were prepared in hexanes. Senecioates were synthesized in-house (>97%) as previously reported and prepared in hexanes.[10] Table S1 shows the complete list of standards used and their calibration statistics. Five or six-point calibration curves were used to quantify the compounds. Figures S7−S9 show mass spectra of newly identified flavorant analytes in select varieties along with NIST v17 mass spectral database data. Additionally, each analyte reported was structurally validated by confirming similar elution times and mass spectra of the standards.

Comprehensive Two-Dimensional Gas Chromatography. GC × GC analysis was performed using the INSIGHT reverse fill flush flow modulator (SepSolve Analytical). This was coupled with an Agilent 7890B GC equipped with a BPX5 (20 m × 0.18 mm ID × 0.18 μm film thickness) first dimension column and Mega Wax HT (4.8 m × 0.32 mm ID × 0.25 μm film thickness) second dimension column and BenchTOF Select mass spectrometer (Markes International). ToF-MS was used to identify the compounds. Quantification of all nonsulfur-containing analytes was performed using an FID. Sulfur-containing analytes were quantified using SCD. Sample introduction was done using direct injection with an Agilent 7693 Injector Tower (G4513A). The syringe was washed three times with isopropyl alcohol and hexanes before and after injection. The injection volume used was 5 μL. The inlet split flow and temperature were 20:1 and 280 °C, respectively. The TOF-MS ion source was held at 280 °C and a transfer line temperature of 260 °C. Mass spectral data were acquired at 60 Hz with a scan range of 40−350 m/z with a solvent delay of 6 min.

The GC × GC configuration includes two columns: apolar to polar setup. The GC oven ramp rates were programmed as follows: the temperature was initially set to 45 °C and held for 3 min. The temperature was then ramped at 3 °C per minute to 98 °C, followed by a 6 °C per minute ramp rate to 140 °C, followed by an 8.5 °C ramp rate to 170 °C followed by a 2 °C ramp rate to 190 °C, followed last by a 15 °C ramp to 260 °C, and held for 13 min. The modulation period set for the flow modulator was 6.00 s. Data was collected, integrated, and analyzed using the ChromSpace software platform (Sepsolve Analytical). Integration, statistical analysis, and data transformations were done using Terplytics and Python 3. Figures showing GC × GC chromatograms have been realigned to account for the void time (2.5 s) in the second dimension. Analyte concentrations can be found in part S13.

Sensory Analysis and Panel Methodology. A preliminary sensory panel was performed to select external reference materials for the questionnaire. These reference materials were samples that had been previously analyzed both analytically and via a sensory panel. They were utilized to help train panelists on specific aroma properties of the rosin-type samples, drawing on previously reported data.[10] The samples "Grape Pie × Do-Si-Do" (exotic score 87.4/100) and "GMO" (exotic score 1.7/100) were used as references for aromatic traits such as sweetness so that panelists could quantify these properties numerically. Eleven participants were recruited. All the participants were at least 21 years of age and consented to the protocol outlined below. Participants were given five blind-coded 2 mL sample containers, containing approximately 500 mg of sample, with the lids closed. The samples were removed from their storage and brought to room temperature. A stirring utensil was provided for each sample for participant use. The participants were tested individually with as much time as was needed.

Product Evaluation Questions. The survey participants used a form containing a variety of question types for each sample. The questionnaire included a check all that apply descriptor list with thirty-five terms. Questions for ranking citrus, sweetness, and creamy (0−10 scale), an overall intensity of aroma ranking (0−10 scale), and an overall preference ranking (0−10 scale). The 35 terms were chosen to encompass the scope of aromas present in the samples. A free response portion allowed the panelists to describe more specific terms not included in the thirty-five-term lexicon.

RESULTS

Cannabis phenotypes resulting from the same cross offer a unique opportunity to study genetically similar cannabis and understand its chemical and organoleptic differences. We have previously shown that cannabis produces a wide range of low-concentration compounds beyond terpenes, referred to as flavorants, that significantly modulate sensory perception.[10] We further found that terpene profiles can remain remarkably similar between samples with significantly different aroma qualities. However, in that study, each variety was genetically distinct, having different lineages from one another or produced by different cultivators. Our interest was in understanding how even the most genetically similar varieties (e.g., phenotypes of the same crossbreeding), cultivated and processed similarly, may differ both aromatically and chemically.

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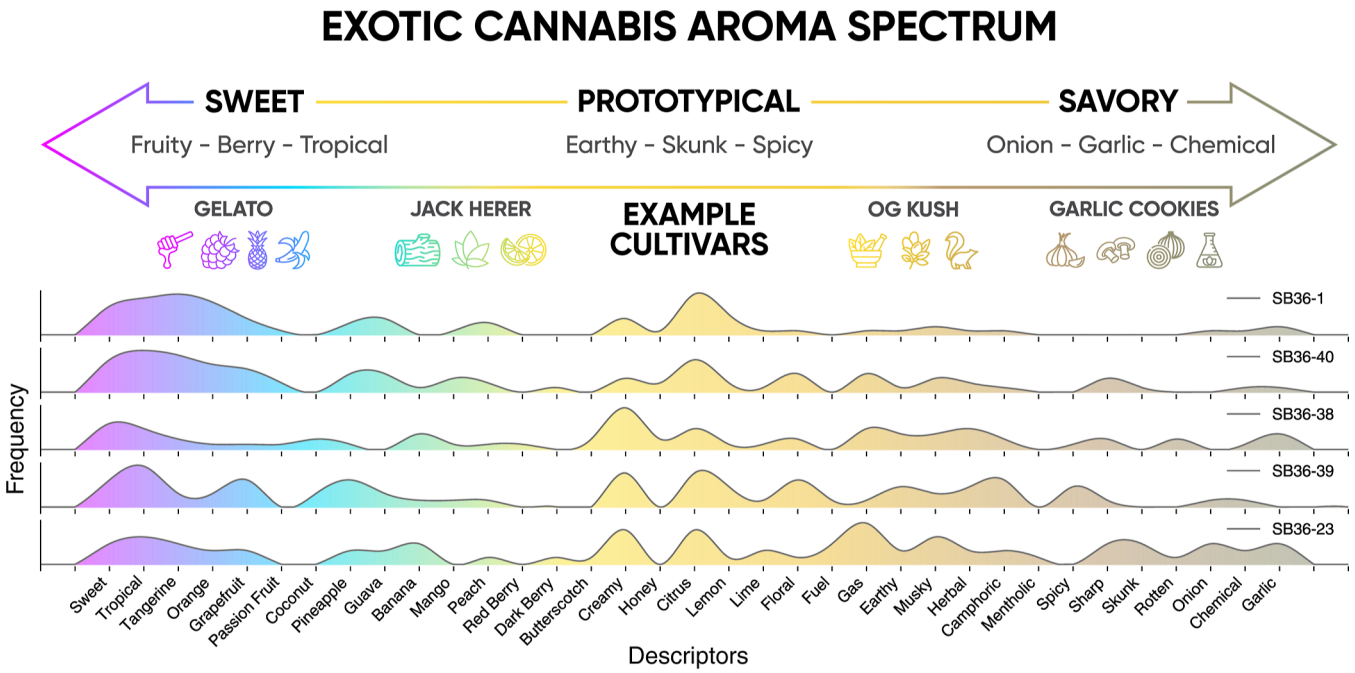

Figure 2. Density plot showing the intensity and frequency of descriptors used for each phenotype chosen by the sensory panel, the top spectrum depicts the sweetest phenotype, and the bottom spectrum depicts the least sweet phenotype.

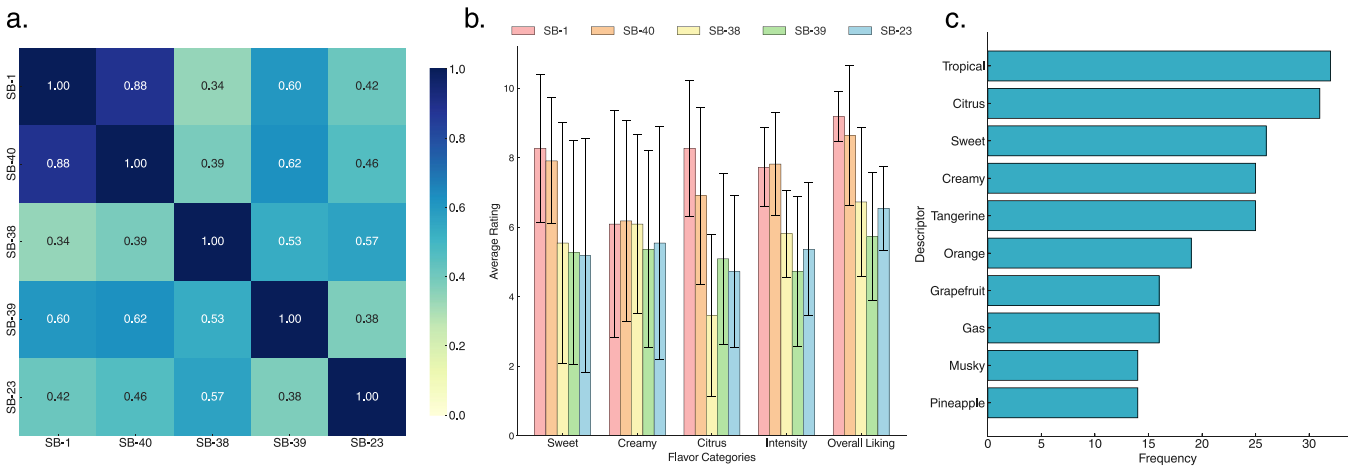

Figure 3. (A) Pearson correlation matrix of tabulated sensory descriptor data for each phenotype. (B) Bar graph showing average ranking for each sensory category for each phenotype. (C) Bar graph showing the top 10 descriptors and their respective frequencies.

Ice hash rosin samples of five phenotypes of cultivar Starburst 36 were analyzed. Starburst 36 was chosen for the following reasons: first, it is known to possess a pungent, tropical, fruity, and sweet aroma profile highlighting a complex underlying chemistry producing these odors.[29] Second, the five different phenotypes of Starburst 36 procured were all cultivated and processed using identical methods and environments, thereby eliminating these confounding variables. Lastly, these products were supplied in the form of ice hash rosin, which allows for easier low analyte analysis compared with cannabis flower or other extracts.[10]

Sensory Analysis. We initially conducted a sensory analysis to quantify the aromatic properties of each phenotype. An aroma lexicon and sensory questionnaire was developed utilizing varied ice hash rosins as reference materials (see Experimental Section). The 11-member panel individually assigned aroma descriptors to each phenotype from the terms found in Figure 2. Each panelist then rated on a scale of 0−10 (0 representing low, 10 high, etc.) how creamy, citrus, and sweet the samples smelled. These traits were chosen as they are considered characteristic aroma and flavor attributes of Starburst 36.[29]

Figure 2 depicts the classification scheme previously utilized in helping differentiate *cannabis* aromatically.[10] This scheme simplifies the complex aromas of *cannabis* into three predominant categories: sweet exotic, prototypical, and savory exotic. Below are the distributions and frequencies of individual aroma descriptors for each phenotype along the spectrum of exotic aroma descriptors, as ranked by the sensory panel. The phenotypes are ordered from sweetest (SB-1, 8.3(±2.1)) to least sweet (SB-23, 5.2(±3.4)) from top to bottom. The differences between aroma descriptor frequencies of the five phenotypes confirm that our panel can identify nuanced differences in aroma despite having some similar

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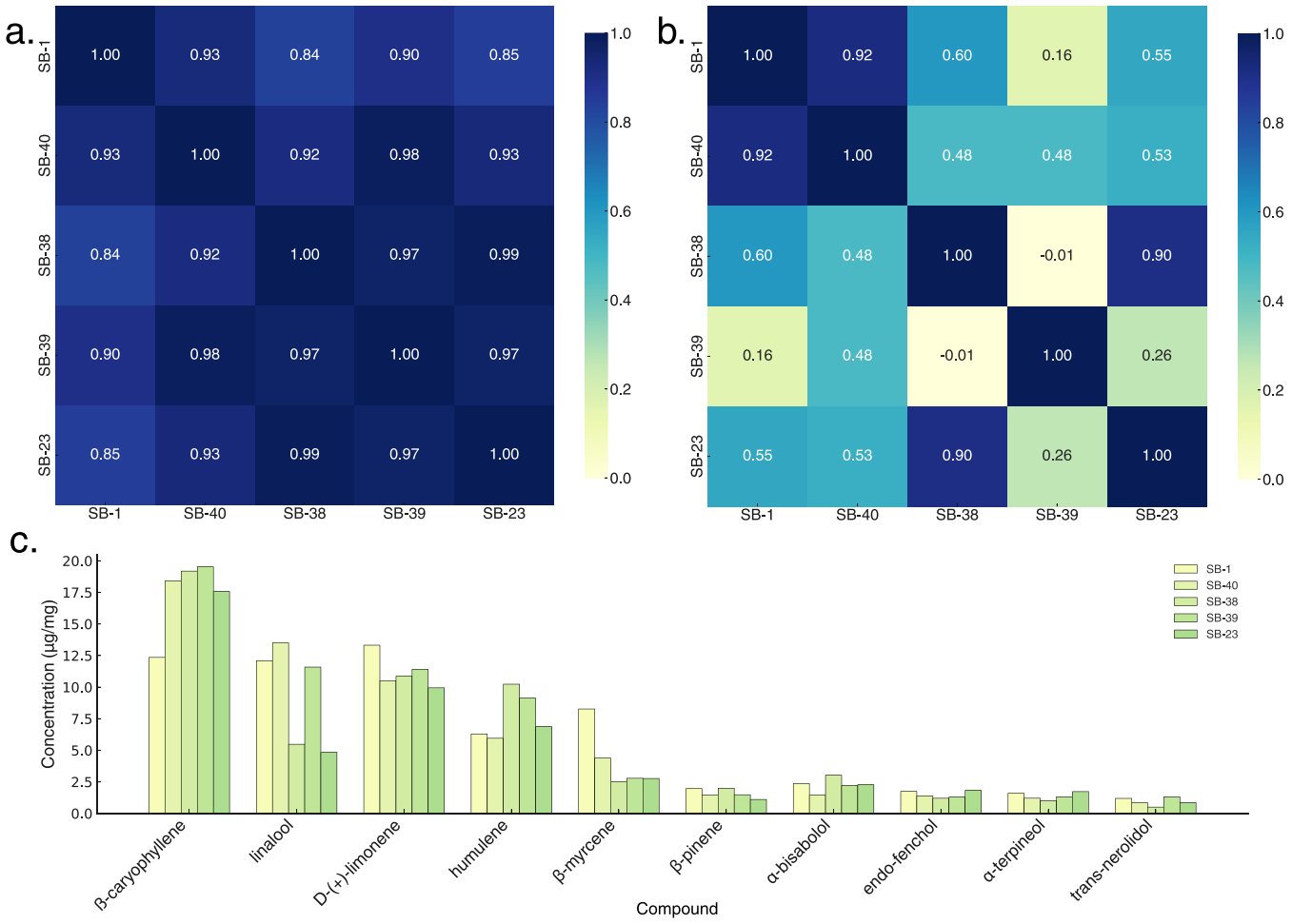

Figure 4. (A) Pearson correlation matrix showing correlation values for terpenoids and sesquiterpenoids analytes. (B) Pearson correlation matrix showing correlation values for flavorant analytes. (C) Bar graph showing differences in the top 10 terpenoids.

aromatic attributes, consistent with other sensory studies.[4,22] A notable finding from these data highlights the similarities between SB-1 and SB-40, primarily characterized by descriptors from the sweet and prototypical side of the aroma spectrum. While each phenotype had some contribution of "tangerine", "orange", or "grapefruit", these samples had a greater amount than the others. These data suggest that of the five samples, SB-1 and SB-40 appear to have more similar aromas to one another, while the others were more divergent. Conversely, the remaining samples had more contributions within the prototypical or savory descriptors, suggesting greater aromatic similarities between them.

The predominant descriptors used for all samples reveal a key point: many of the terms used to describe the aromas are terms not typical of terpene or terpenoid aroma descriptions, as shown in Figure 3c. While citrus has often been associated with limonene in the context of *cannabis'* aroma, we have previously shown a new class of TCSCs, have a larger influence on this scent.[10] The array of sensory descriptors used by the panelists again conforms to descriptors that would be considered sweet exotic or savory exotic rather than prototypical. This suggests that minor, nonterpenoid compounds have a large influence on the aromatic qualities of the samples.

To determine how similar the sensory data of the phenotypes were to one another, we calculated Pearson correlation coefficients for each sample, shown in Figure 3a. We observe a strong correlation between the perceived aroma of cultivars SB-1 and SB-40 (r = 0.88), confirming the similar aromas between these samples. Additionally, these samples were identified to be the most similar in aroma intensity (7.73(±1.13) and 7.82(±1.47), respectively) and overall liking (9.18(±0.71) and 8.64(±2.01), respectively), as shown in Figure 3b. These high preference and intensity scores suggest that the strong citrus aromas produced by SB-1 and SB-40 are highly desirable amongst the panelists.

Conversely, the remaining three samples were more aromatically different from SB-1 and SB-40, with the highest correlation of r = 0.62 between SB-40 and SB-39. The two most different samples from SB-1 and SB-40 were SB-38 and SB-23 (Figure 3a). For instance, SB-38 has significantly more prototypical and savory descriptor terms compared to those of SB-1 and SB-40. This is consistent with SB-38 having a modest sweetness ranking (5.54(±3.47)). We also note that terms related to cheese were used in the open-response form for both SB-38 and SB-23, indicating that certain panelists detected unique aromas from these relative to the others. These results show that while genetically similar, phenotypes can possess significantly different aromas that may alter human preference.

Chemical Analysis. To understand the chemical diversity of the phenotypes and identify their differences, we analyzed each sample's volatile profiles to determine their chemical

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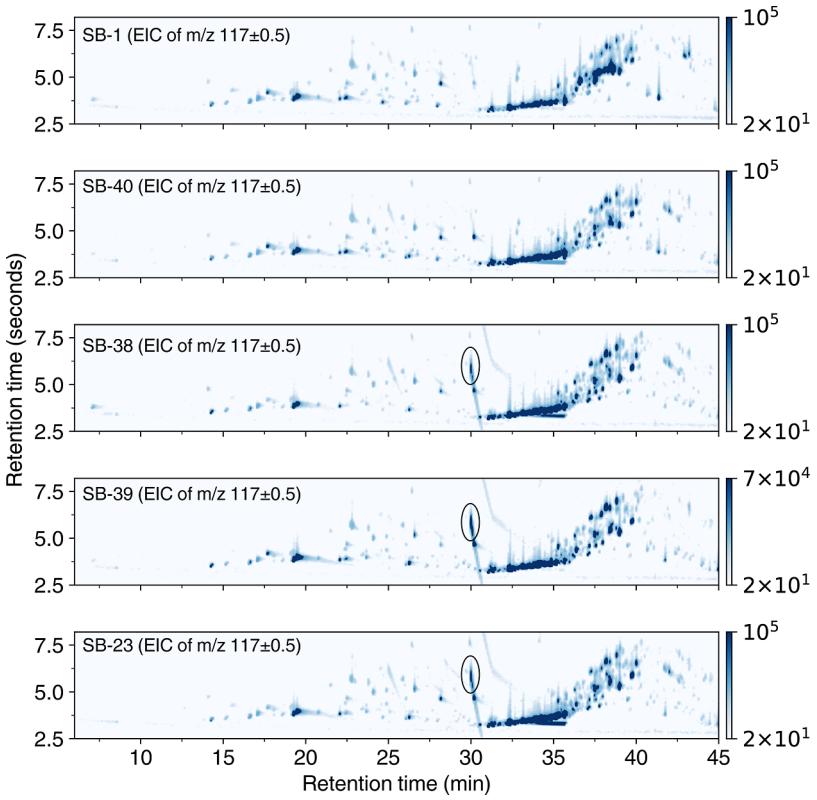

Figure 5. Extracted ion chromatograms of all five phenotypes showing m/z = 117 ± 0.5. The circle denotes the compound, indole.

similarities or distinctions using GC × GC−TOF−MS/FID/SCD, a more advanced technique than traditional gas chromatography. Given that the sensory panel determined differences among certain samples, our focus shifted to identifying specific compounds that might contribute to these variations.

Terpene Analysis. *Cannabis* is known to produce a variety of terpenes, predominantly monoterpenes, sesquiterpenes, and their oxygenated derivatives.[7] As these compounds are the highest concentration of volatile constituents of the essential oil of cannabis, we began our investigation with these compounds.

The terpene profiles of the phenotypes appeared similar, as shown in the abbreviated terpene profile of each phenotype in Figure 4c. β-Caryophyllene emerged as the dominant terpene in four out of five samples, followed by either D-(+)-limonene or linalool as the second most prevalent. β-Caryophyllene, a sesquiterpene, is characterized by a spicy, earthy, and clove-like aroma. These aroma characteristics contrast with the citrus and sweet aromas described for these phenotypes, suggesting that their aroma impact is minimal in these cases. While D-(+)-limonene and linalool might contribute to citrus or floral notes, they are not typically associated with tropical citrus flavors. This is not unexpected: D-(+)-limonene has been identified as the dominant terpene in varieties such as "GMO" and "OG", which are known for having minimal citrus notes.[10] Consequently, the variation in aroma profiles observed among these phenotypes indicates that it is likely compounds present in lower concentrations, rather than high concentration compounds, that are essential in determining their distinct sensory characteristics.

To validate the similarities between the analyzed terpene profiles, we calculated Pearson correlation coefficients using chemical data (Figure 4a). Unlike the sensory analysis where we only saw strong similarities between specific pairs (e.g., SB-1 and SB-40), the terpene analysis shows strong similarities between all samples. For instance, the two samples that possessed the most citrus and tropical aromas, SB-1 and SB-40, have similar terpene profiles (r = 0.93). We likewise found SB-38 and SB-40 to also have a strong chemical correlation (r = 0.92), in spite of a weak sensory correlation (r = 0.39). Furthermore, even the two phenotypes that were perceived to be the most aromatically different, SB-1 and SB-38, have a strong chemical correlation (r = 0.84). This supports the hypothesis that the major terpenoids do not necessarily correspond to the unique and differentiating aromatic qualities of *cannabis*, even when considering genetically similar phenotypes.

Flavorant Analysis. After determining that minimal variation is present in the overall terpene profiles of the five phenotypes, we analyzed strictly the nonterpenoid compounds, referred to as flavorants. This compound class contains compounds in low concentration with diverse chemical functionality, such as esters, volatile sulfur compounds, heterocycles, ketones, and more, that can strongly impact the aroma of *cannabis* even when found in small concentrations.[10]

Figure 4b shows the correlation table of this subset of compounds, revealing clear distinctions among certain phenotypes. Interestingly, we observe a strong relationship between flavorants in SB-1 and SB-40 (r = 0.92), which mimics the sensory data closely. A lower correlation is observed between these two samples and the remaining three phenotypes, aligning with sensory data. These results are highly suggestive that the minor flavorants in the phenotypes may help explain the aromatic differences observed in our sensory experiments.

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A dramatic difference between SB-1 and SB-40 and the remaining phenotypes was observed in the key heterocyclic compound indole. We previously reported this compound and its implication in producing unique scents in *cannabis*.[10] It possesses a complex aroma that can range from mothball- and chemical-like to floral depending on concentration. Indole was undetected in SB-1 and SB-40; however, it was identified in the remaining phenotypes, as shown in the ion extraction trace ($m/z = 117 \pm 0.5$) in Figure 5 with indole annotated in SB-23, SB-38, and SB-39. The clear difference between the two phenotype groups aligns with the sensory analysis, suggesting that indole may play a role in differentiating these samples aromatically. This also highlights how certain minor compounds may have a chemotaxonomic utility that correlates with detectable aroma differences in cannabis.

TCSC Analysis. The high frequency of citrus-related descriptors in the sensory data suggested TCSCs may be present, as they were previously implicated in producing strong citrus and tropical aromas in *cannabis*.[10] We identified the three previously reported compounds, 3-mercaptohexanol (3MH), 3-mercaptohexyl acetate (3MHA), and 3-mercaptohexyl butyrate (3MHB), shown in Figure 6, in the phenotypes. Despite being found in low concentrations (<0.01 μg/mg), their high aromatic potency can strongly impact the aroma of *cannabis*.

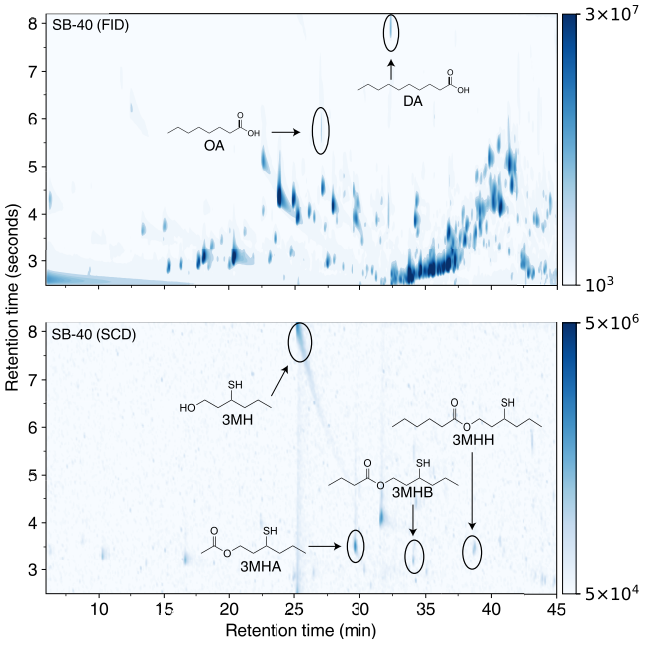

Figure 6. Top: Starburst-40 FID chromatogram, showing octanoic and decanoic acid peaks with structures. Bottom: Starburst-40 SCD chromatogram showing TCSCs annotated with structure.

In addition to the three previously reported TCSCs, we also identified 3-mercaptohexyl hexanoate (3MHH) in each sample. This compound, similar to other TCSCs, possesses an intense sulfuric and citrus-like aroma in low concentrations. 3MHH elutes among several sesquiterpenes, making detection without sulfur chemiluminescence difficult. Chemical validation via a chemical standard unequivocally confirmed the correct assignment. Figure 6 shows GC × GC−FID/SCD chromatograms of SB-40 with each of these important TCSCs annotated. These data further exemplify the utility of SCD in the analysis of key aromatic compounds in *cannabis*.

Citrus-related sensory descriptors used during the sensory analysis were analyzed using Pearson correlation values in relation to these compounds. For example, the term tangerine had a frequency of 25 between the five phenotypes. All four of the TCSC compounds—3-mercaptohexanol (3MH, $r = 0.96$), 3-mercaptohexyl acetate (3MHA, $r = 0.91$), 3-mercaptohexyl butyrate (3MHB, $r = 0.81$), and 3-mercaptohexyl hexanoate (3MHH, $r = 0.95$)—demonstrate strong correlations with the descriptor tangerine. In addition to the relationships between these compounds and specific descriptors, we found a very strong relationship between the overall citrus rank and concentrations of key TCSCs. For instance, we found that the relationship between 3MHH and the average citrus rank ($r = 0.91$) is particularly strong. 3MH likewise has a strong positive correlation with citrus rank ($r = 0.87$). 3MHA has a lower but still significant correlation ($r = 0.82$). These relationships further suggest that TCSCs produce the citrus and tropical-related aromas described above and can easily overpower the aromas produced by the more plentiful terpenes or other flavorants.

Fatty Acids and Cheese Aromas in *Cannabis*. An observation made during the analysis of our sensory data revealed that SB-38 and SB-23 possess aromas reminiscent of cheese. The open response section of our sensory panel contained descriptors such as “astringent”, “sharp”, and “cheese” for SB-23, while SB-38 elicited descriptions such as “cheese”, “stilton”, and “blue cheese”. Indeed, certain cannabis varieties have been described in this way previously, including the varieties “Cheese”, “Blueberry Cheesecake”, and “Donny Burger”.[32] Previous research has identified cheese as a unique, noteworthy, and rare aroma descriptor among cannabis cultivars.[22]

During the untargeted chemical analysis of the samples, we identified octanoic acid and decanoic acid in both SB-38 and SB-40. Figure 6 shows the GC × GC−FID chromatogram with these compounds annotated. The high polarity of these compounds resulted in poor peak shapes, thereby making it difficult to obtain an accurate quantitation. Nonetheless, their identification presents another unique chemical class that can impact the aroma of *cannabis* in a nonprototypical manner.

Both identified fatty acids are well-known to produce aromas that can be described as fatty, waxy, or cheesy.[30] In fact, they are found in varying concentrations across many dairy products or derivatives thereof, including cheese. Blue cheeses, such as Stilton or Roquefort, which are ripened with *Penicillium roqueforti* or *Penicillium glaucum* molds, tend to have higher concentrations of free fatty acids, including octanoic and decanoic acids. This is primarily due to lipolytic enzymes that break down milk fats into free fatty acids, contributing to the distinct sharp, pungent flavors and aromas of blue cheeses.[30,31] We additionally note that 2-heptanone is often found in *cannabis*, including the phenotypes measured in this study. This compound is also found in cheese and contributes to the odor and flavor of these products.[30]

Fatty acids have also previously been identified in the pollen, seeds, and inflorescence of cannabis and hemp.[33−38] Octanoic and decanoic acid are identified frequently in hempseed oil, which contains a multitude of fatty acids and lipids and is often used as a nutritional supplement and raw ingredient in cosmetics.[36,37] While certain herbicides used for invasive weed management contain fatty acids, these additives were not used

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during the growth of the samples analyzed, thereby eliminating this as a possible vector for these compounds.[39]

SB-38 is particularly interesting in the possible relationship between octanoic and decanoic acids and its aroma. This phenotype has a lower concentration of TCSCs relative to other phenotypes, which may allow the aromas of other minor components to be more detectable during sensory analysis. While SB-40 also had these acids present, it had minimal descriptors related to cheese. However, it had a much higher concentration of TCSCs, which may indicate that the odor priority of these compounds can dominate the aroma characteristics of a variety and thus make it difficult for consumers to detect less potent aromas such as cheese. We also note that "cheese" is a broad term that is highly subjective, as many cheeses exist with very different aromas and flavors that may make it difficult for consumers to agree upon. Analysis of other varieties described as cheese-like will help clarify how this aroma arises from a chemical perspective and how strongly octanoic and decanoic acids are implicated.

DISCUSSION

The diverse aromas produced by these phenotypes highlight how low-concentration compounds can impact the olfactory properties of *cannabis*. Further, it exemplifies how even genetic siblings can produce distinct chemical compounds that differentiate them in a detectable manner to consumers. While terpene profiles may remain similar between phenotypes, their underlying chemistry can vary drastically. This variation necessitates the need for in-depth chemical analysis to provide meaningful data for chemotyping methodologies when selectively breeding *cannabis*, especially when the aroma is a desired attribute. By investigation and identification of clear chemical distinctions between phenotypes that align with sensory evaluations, this approach could assist in finding relevant genetic markers for secondary metabolites. Moreover, the observation that indole and TCSC concentrations appear to be inversely related in this population prompts further questions about the underlying biochemical mechanisms. Understanding these mechanisms could offer valuable insight into how the *cannabis* plant regulates the production of these potent aroma compounds, potentially guiding future efforts in selective breeding.

Lastly, we note that in the *cannabis* industry, it is often assumed that higher terpene percentages indicate a greater intensity in aroma or desirable aromatic properties. As such, both customers and producers heavily focus on this quality metric. However, this relationship may falter in cases where nonterpenoid compounds with very high odor activity and low human detection limits are present. This is especially important when considering the growing chemical diversity of *cannabis* in modern varieties with pungent and complex aromas. Our findings add to the body of evidence suggesting that the pungency (and often quality) of a variety is closely associated with key minor compounds, which, despite their minimal impact on the overall aroma concentration, play a significant role. This underscores the importance of characterizing these compounds in understanding the aroma attributes of *cannabis*.

CONCLUSIONS

We analyzed the volatile chemical profile of ice hash rosin samples of five phenotypes of the same progeny that were cultivated and processed in a similar manner. Despite their genetic similarities, key minor aroma compounds beyond terpenes lead to differentiated sensory experiences, which was validated by a sensory panel. In particular, TCSCs, including the newly reported compound 3-mercaptohexyl hexanoate, in particular, provide defining characteristic aromas for these samples, imparting strong citrus and tropical scents. Two fatty acids, octanoic and decanoic acids, were implicated in the production of "cheese-like" nuances to certain varieties. Additionally, other key low-concentration compounds, such as indole, provide clear distinctions between certain phenotype aroma classes, indicating that chemotaxonomic classifications that correlate to the aroma characteristics may be possible based on these key chemical compounds. These results not only expand the growing volatile chemical list in cannabis but also highlight how detailed chemical analysis may aid in the breeding process by shedding light on key compounds that drive the unique aromas of each phenotype.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03225.

> Calibration data of analytes; tabulated sensory panel data; GC × GC analyte concentration table; free response sensory panel data; GC × GC chromatograms of all samples; and mass spectra of new analytes (PDF)

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Author Contributions

T.R.P. conducted GC × GC experiments, analyzed the results, and wrote the manuscript. I.W.H.O. conceived the study, analyzed the results, conducted GC × GC experiments, and wrote the manuscript. M.E.S. synthesized and characterized senecioates. M.V.H. provided guidance on sensory studies and sensory analysis. J.J.G. helped develop GC × GC methodology and edited the manuscript. B.G.M. and I.S. provided samples and guidance on extraction processes, and edited the manuscript. K.A.K. managed the project and edited the manuscript. M.F.Z.P. managed the project, provided guidance on synthesis experiments, and edited the manuscript. T.J.M. managed the project, provided guidance on sensory experiments, and wrote and edited the manuscript.

Notes

The authors declare the following competing financial interest(s): I.W.H.O., T.R.P., M.A.O., T.J.M., M.E.S., and K.A.K. have filed patents related to the results described herein.

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