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Evolution of the Cannabinoid and Terpene Content during the Growth of *Cannabis sativa* Plants from Different Chemotypes

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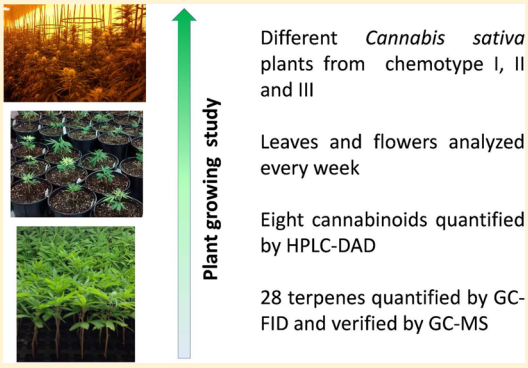
Supporting Information

ABSTRACT: The evolution of major cannabinoids and terpenes during the growth of *Cannabis sativa* plants was studied. In this work, seven different plants were selected: three each from chemotypes I and III and one from chemotype II. Fifty clones of each mother plant were grown indoors under controlled conditions. Every week, three plants from each variety were cut and dried, and the leaves and flowers were analyzed separately. Eight major cannabinoids were analyzed via HPLC-DAD, and 28 terpenes were quantified using GC-FID and verified via GC-MS. The chemotypes of the plants, as defined by the tetrahydrocannabinolic acid/cannabidiolic acid (THCA/CBDA) ratio, were clear from the beginning and stable during growth. The concentrations of the major cannabinoids and terpenes were determined, and different patterns were found among the chemotypes. In particular, the plants from chemotypes II and III needed more time to reach peak production of THCA, CBDA, and monoterpenes. Differences in the cannabigerolic acid development among the different chemotypes and between monoterpene and sesquiterpene evolution patterns were also observed. Plants of different chemotypes were clearly differentiated by their terpene content, and characteristic terpenes of each chemotype were identified.



Cannabis sativa is the most frequently used illicit plant worldwide but is also a highly promising medicinal plant, and its effectiveness for treating various medical conditions has been well documented. For example, it can be used as an appetite-stimulating agent for treating anorexia, cancer, or human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS),[1−3] its antiemetic effects can be beneficial for cancer chemotherapy patients,[4−6] and it provides chronic neuropathic pain relief for cancer, HIV/AIDS, and other types of chronic pain, such as fibromyalgia and rheumatoid arthritis[7−9] and multiple sclerosis.[10−12] Moreover, there are important emerging clinical applications for cannabis, such as in the treatments of several cancers,[13−16] epilepsy,[17,18] Alzheimer's disease,[19,20] Huntington's disease,[21,22] diabetes,[23,24] and Tourette's syndrome.[25,26]

There are at least 554 identified compounds in *C. sativa* L. plants, among them 113 phytocannabinoids[27,28] and 120 terpenes.[29] Cannabinoids are biosynthesized as prenylated aromatic carboxylic acids, and almost no neutral cannabinoid can be found in fresh plants.[30] However, they may convert to their neutral homologues by spontaneous decarboxylation in the presence of light or heat. Moreover, cannabinoids can be oxidized, as in the case of tetrahydrocannabinol (THC), which can be transformed to cannabinol (CBN).[31] The first cannabinoid in the biosynthetic pathway is cannabigerolic acid (CBGA). It is sequentially transformed into tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA), each by a particular synthase (Figure 1).[32] Moreover, monoterpenes and sesquiterpenes are derived from different addition reactions of geranyl ($C_{10}H_{16}$) and farnesyl ($C_{15}H_{24}$) units, respectively.[33]

The two major cannabinoids and those best known for their therapeutic potentials are THC and CBD, i.e., the neutral homologues of THCA and CBDA, respectively. THC is the main psychoactive agent of cannabis and has anti-inflammatory, analgesic, appetite-stimulant, and antiemetic properties.[34] In contrast, CBD can modulate the euphoric effects of THC and has antipsychotic, neuroprotective, anticancer, antidiabetic, and other positive effects, such as the ability to reduce tobacco addiction.[35−39] Moreover, cannabigerol (CBG) and cannabichromene (CBC) seem to be promising compounds for different medical applications. Although they have not been extensively studied, CBG has promising potential for the treatment of glaucoma,[40] inflammatory bowel disease,[41] and prostate carcinoma.[42] CBC has analgesic effects,[43] the potential to stimulate the growth of brain cells,[44] and the ability to normalize gastrointestinal hypermotility.[45]

Terpenes are responsible for the plant's aroma; in addition, they possess specific medical effects and may act synergistically

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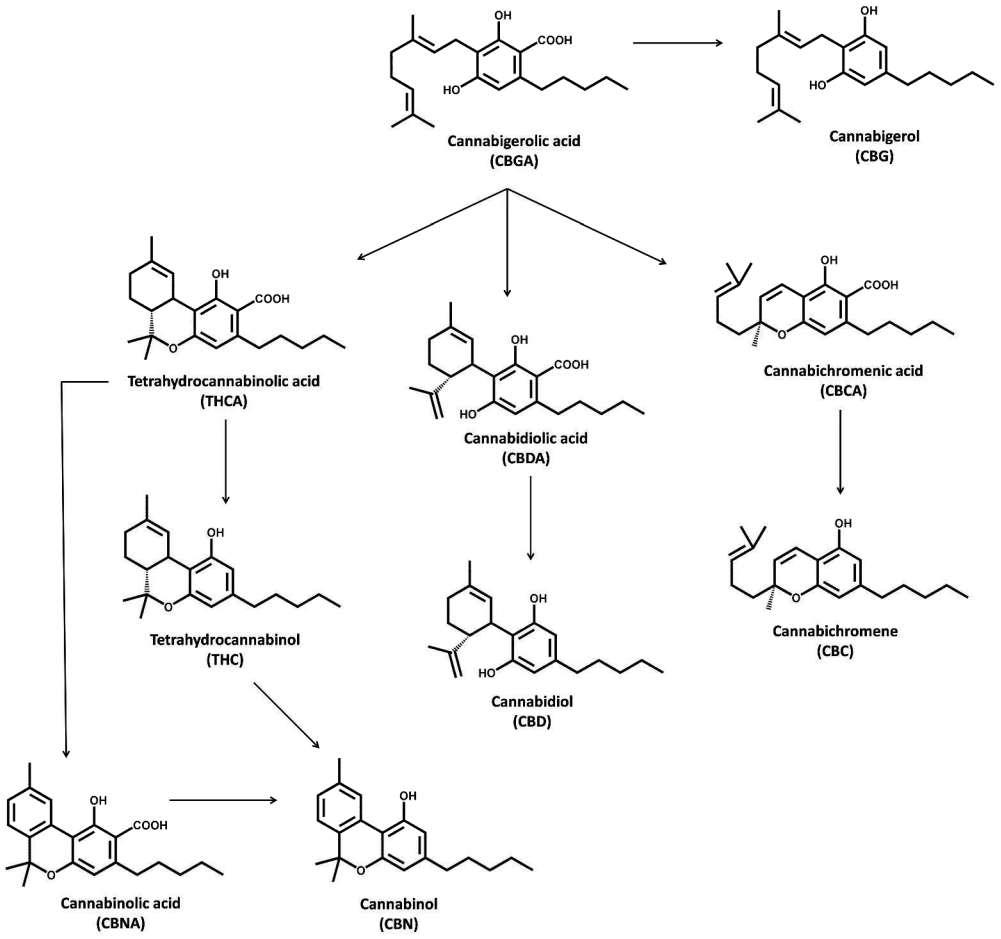

Figure 1. Structures and biosynthetic pathway of the studied cannabinoids.

with cannabinoids. In fact, there are several promising applications based on the combined use of cannabinoids and terpenes, such as new acne therapies utilizing CBD with the monoterpenes limonene, linalool, and pinene; new antiseptic agents with CBG and pinene; treatment of social anxiety disorder using CBD with limonene and linalool; and treatment of sleeping disorders by adding caryophyllene, linalool, and myrcene to 1:1 CBD/THC extracts.[46,47]

Based on the major cannabinoid concentrations, five different chemotypes of cannabis are recognized. Drug-type plants that have a high THCA/CBDA ratio ($\gg$1.0) are classified as chemotype I; plants that exhibit an intermediate ratio (usually 0.5−2.0) are classified as chemotype II; typical fiber-type plants that have a low THCA/CBDA ratio ($\ll$1.0) are classified as chemotype III; chemotype IV plants are fiber-type plants that contain CBGA as the main cannabinoid; and chemotype V plants are also fiber-type plants, but contain almost no cannabinoids.[48]

Genetic analyses have demonstrated that the chemotype is determined by the presence at the B locus of two codominant alleles, B_D and B_T, which are responsible for the CBDA and THCA occurrences in the plant. Thus, plants with B_T/B_T alleles are chemotype I plants, plants with B_D/B_D alleles are of chemotype III, and chemotype II plants have two different alleles, B_D/B_T.[49,50] Nonfunctional alleles, called B_0, can also exist at this locus. These alleles are unable to convert CBGA; hence, these plants are CBGA-predominant (chemotype IV).[51]

The increasing use of cannabis as a medicine and the growing interest in the medicinal effects of nonpsychotropic cannabinoids and terpenes have led to a requirement for the large-scale production of pure compounds and plants with different cannabinoid and terpene content. Thus, CBD-enhanced plants with more than 15% CBD and less than 1% THC have recently been produced.[52] However, to optimize the production for each compound, a better understanding of their development during growth is necessary. Some results regarding this topic have been published, but these applied a forensic perspective to differentiate drug-type plants from nondrug types in the early stages of growth.[48,53] Moreover, the terpene content was not analyzed, and only fiber-type and high-THC plants were studied.

Therefore, the main aim of this work is to study the time evolution of the cannabinoid and terpene content during the entire growth period of the plant, i.e., from the rooting phase until the end of the flowering stage. In this work, the contents of seven cannabis varieties with regard to three different chemotypes (three each from chemotypes I and III and one from chemotype II) are examined.

RESULTS AND DISCUSSION

Figure 2 shows the evolutions of the major cannabinoids, i.e., THCA, CBDA, and CBGA, in the leaves and flowers during the growth of plants from chemotypes I, II, and III. Several conclusions can be drawn from this figure. The chemotypes of the plants, as defined by the THCA/CBDA ratio, were clear from the beginning and stable during growth. This pattern was previously observed in other studies[48,53] and is important for forensic purposes because it does not make sense to wait for plant

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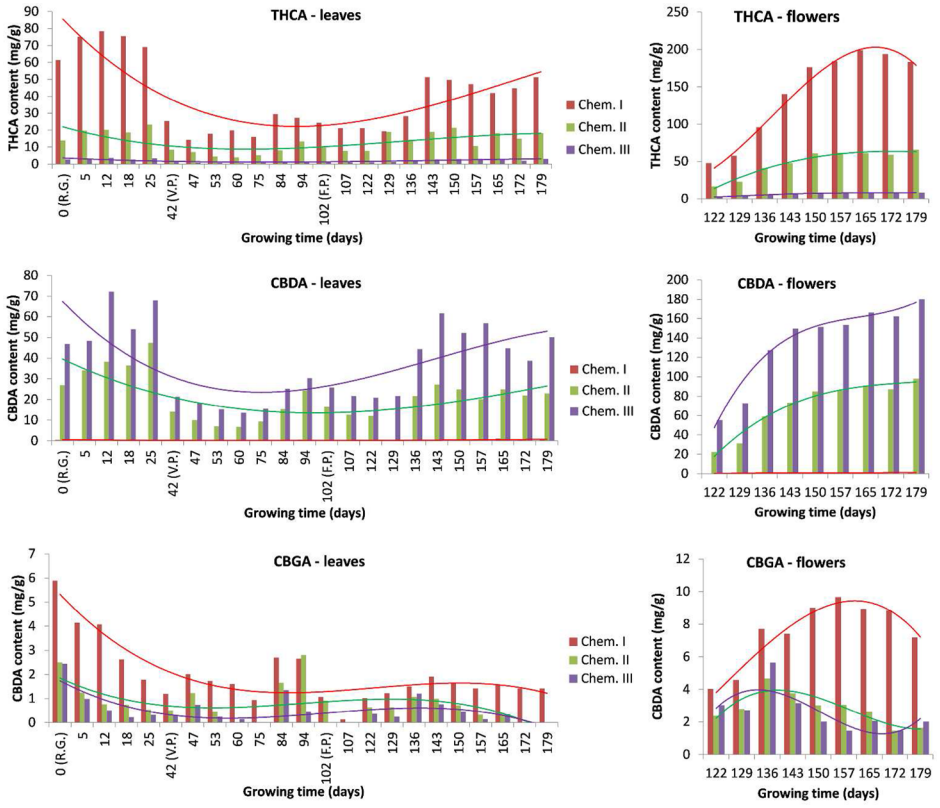

Figure 2. Evolution of the THCA, CBDA, and CBGA content in leaves and flowers during the growth of plants from chemotypes I, II, and III. These values are the averages of all studied plants in each chemotype, with each one measured in triplicate. R.G. refers to the root-growing phase, V.P. to the vegetative phase, and F.P. to the flowering phase.

flowering to identify whether a plant is of a drug type. However, the THCA and CBDA contents in the leaves exhibited the same time evolution for every chemotype. First, the concentrations clearly decreased during the first weeks of the vegetative phase while the plant was growing. Mother plants are well-developed plants that are kept in the vegetative phase. However, as is the case in this study, they are usually kept smaller than plants that are passing to the flowering phase; thus, the cannabinoid content is more concentrated. Therefore, the decrease in the cannabinoid content during plant growth was the expected pattern. In contrast, during the last 2 weeks of the vegetative phase (days 84 and 94), the concentrations of THCA and CBDA increased slightly although the plants did not grow; thus, accumulation of the cannabinoids was observed. Subsequently, a decrease was observed during the first weeks of flowering when the plants were transplanted from 2 L pots to 10 L pots, in which the plants showed further growth. Finally, when trichomes started to develop, an increase was observed not only in the flowers but also in the leaves.

An important observation from Figure 2 is that the maximum concentrations of THCA and CBDA in the flowers were attained at different stages or maturation, depending on the chemotype. For plants of chemotype I, peaks were observed in the ninth week of the flowering phase (day 165), followed by a decrease during the onset of senescence, whereas for chemotype II and III plants, the concentrations continued to increase until conclusion of the study.

Here, CBGA will be discussed. This compound is the first cannabinoid biosynthesized in the plant, and from this compound, THCA, CBDA, and CBCA are synthesized, each by a particular synthase.[32] The CBGA evolution in the leaves was similar to those of THCA and CBDA until the flowering phase started, in which there was a noticeable increase in the THCA and CBDA content but not the CBGA content. As is evident from Figure 2, the concentration of CBGA remained constant in chemotype I plants, whereas it decreased in plants from the other two chemotypes. This difference was more pronounced in the flowers, in which an increase in the CBGA content was observed in chemotype I plants until the onset of senescence, whereas a slight decrease was observed in chemotype II and III plants. This observation was confirmed by statistical data obtained from cross-correlations, in which the correlation coefficients of CBGA with THCA and CBDA during the growth of the plants were found to be 0.789 and −0.114, respectively. In conclusion, the relationship between the CBGA biosynthesis rate and the THCA synthesis rate, controlled by the B_T alleles, was somehow stable during plant growth. In contrast, this relationship was unstable for CBDA synthesis, which was controlled by the B_D alleles.

The minor cannabinoid CBN, derived from THC, was not detected in the plants, indicating that THC did not suffer any measurable degradation. THC, CBD, and CBG, i.e., the neutral compounds of the aforementioned cannabinoids, are not included in Figure 2 because they were found in low concentrations. However, their development was different from those of their acidic cannabinoid homologues. During root growth and the vegetative phase, their content was small (below the limit of quantification), followed by sharp increases in the leaves and flowers during the last 6 weeks of the flowering phase because of decarboxylation of the acidic cannabinoids. A small amount of CBC was found in the plants, and this amount was higher in chemotype I plants. Complete data regarding the

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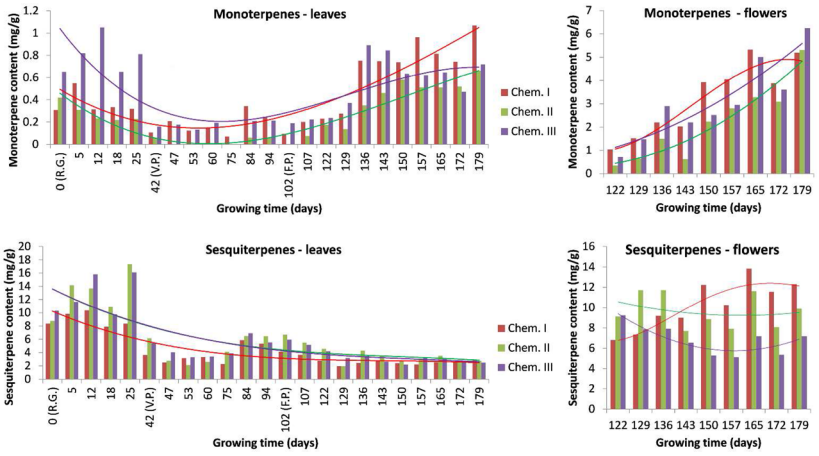

Figure 3. Evolution of total monoterpene and sesquiterpene content in leaves and flowers during the growth of plants from chemotypes I, II, and III. These values are the averages of all studied plants in each chemotype, with each one measured in triplicate. R.G. refers to the root-growing phase, V.P. to the vegetative phase, and F.P. to the flowering phase.

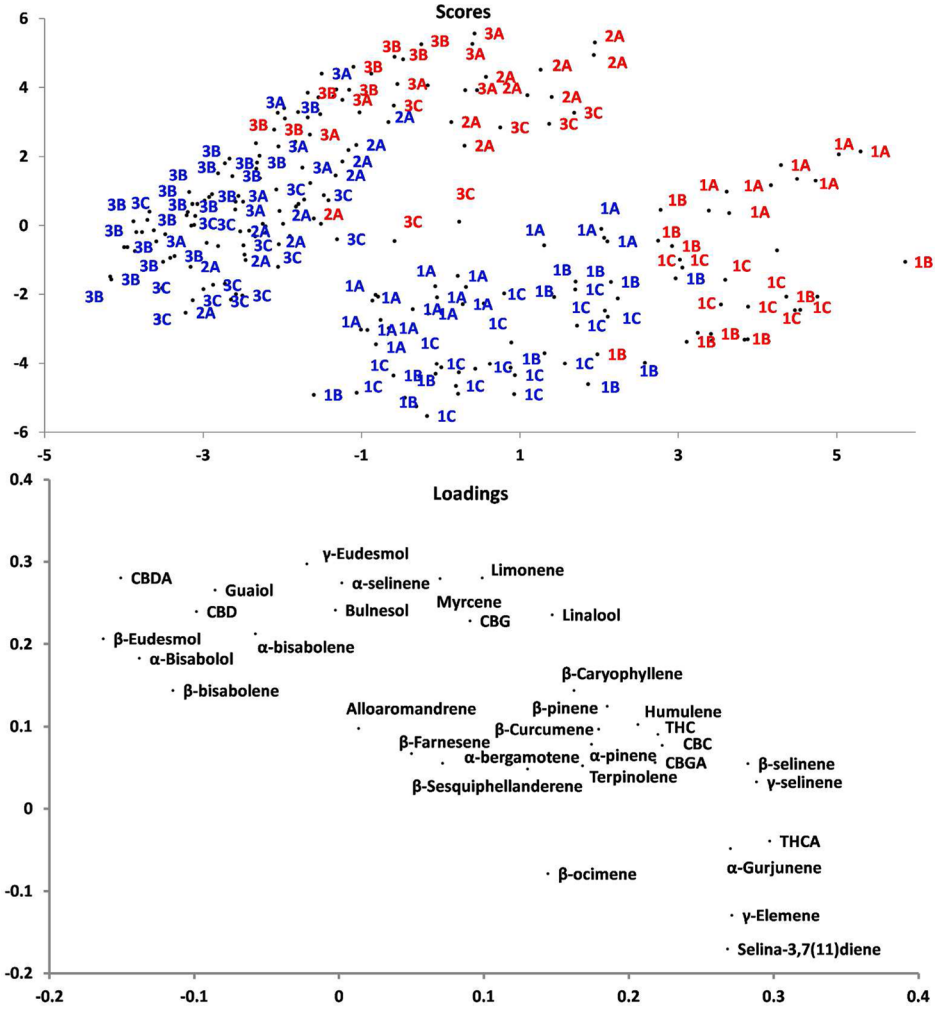

Figure 4. Score and loadings (PC1 vs PC2) obtained via PCA according to the concentrations of all analyzed compounds during the growth of the plants. 1 refers to chemotype I plants, 2 refers to chemotype II plants, and 3 refers to chemotype III plants, whereas A, B, and C denote the different plants of each chemotype. Leaves are colored blue, and flowers are colored red.

evolutions of all studied cannabinoids and terpenes in each plant are available in Tables 1−36 in the Supporting Information.

Figure 3 shows the development of the total monoterpene and sesquiterpene content in the leaves and the flowers of the three different chemotypes. The total monoterpene and sesquiterpene content values were calculated by summing all analyzed terpenes of each kind: eight monoterpenes and 20 sesquiterpenes. The same evolution patterns found for THCA and CBDA in the

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leaves were observed for monoterpenes, i.e., a clear decrease during the first weeks of the vegetative phase, a small increase in the last 2 weeks of the vegetative phase, and a slight decrease during the first weeks of the flowering phase followed by a clear increase. The maximum concentration in the flowers was also chemotype-dependent, and as for THCA and CBDA, this maximum for the total monoterpenes was found in the ninth week of the flowering phase, while for chemotype II and III plants, the concentrations continued to increase until the end of the experiments.

In contrast, sesquiterpenes exhibited a different evolution in both plant matrices. In the leaves, the pattern was similar until the first weeks of the flowering phase, but after that, the content remained stable. In the flowers, the amount of sesquiterpenes did not change significantly during flowering. All terpenes are derived from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The condensation of one DMAPP and two IPP molecules leads to the formation of farnesyl diphosphate (FPP), i.e., the precursor of sesquiterpenes, whereas the condensation of one DMAPP and one IPP molecule leads to the formation of geranyl diphosphate (GPP), i.e., the precursor of monoterpenes. Following the formation of FPP and GPP, sesquiterpenes and monoterpenes are generated by the actions of many specialized terpene synthases (TPSs).[54] However, the expression of these TPSs can differ among the plant tissues and different stages of plant development, thereby resulting in differences in terpene content.[54] Thus, monoterpene synthase expressions were more abundant during this phase, leading to an increase in the monoterpene content during the flowering phase.

To obtain a broader view of the formation of cannabinoids and terpenes, principal component analysis (PCA) and partial least squared regression (PLS) were performed, taking account all of the experimental data (x (224 samples × 36 variables), y (224 samples × 1 time)). Although the PCA model requires more than four PCs to explain the original data structure, up to 60% of the variance is explained by the first three PCs. As observed in Figure 4 in the PC1−PC2 projection, there was a clear distinction between chemotype I plants from the rest (two clusters) and between the leaves and the flowers in each cluster (leaves in blue and flowers in red). The chemotype II plant was closer to the chemotype III plants than the chemotype I plants, most likely because of its higher CBDA content. From the loading projection, the cannabinoids and terpenes of each class of samples were identified as those that are similar to CBDA and THCA. Thus, the higher CBGA and CBC content can be attributed to chemotype I plants. Moreover, terpenes, such as β-eudesmol, γ-eudesmol, guaiol, α-bisabolene, α-bisabolol, or eucalyptol, were much more pronounced in chemotype III plants, whereas γ-selinene, β-selinene, α-gurjunene, γ-elemene, selina-3,7(11)diene, and β-curcumene were characteristic of the chemotype I plants. This chemotype-dependent terpene distribution was also observed in the correlation analysis of the data. As indicated in Table 1, terpenes that were more pronounced in chemotype III plants had higher correlation coefficients with CBDA than with THCA. In contrast, the characteristic terpenes of chemotype I had high correlation coefficients with THCA and negative coefficients with CBDA.

As shown in the 3D score projection (Figure 5), a closer view of the chemotype I plants revealed a fine distinction in the A, B, and C varieties of the plants. Although the leaves and flowers were clearly distinguished by their colors, the class features of each plant were shared in both the leaves and the flowers. Similar results were found for chemotype III plants (data not shown).

Table 1. Correlation Coefficients between the Characteristic Terpenes of Chemotype I and III Plants and THCA and CBDA Obtained via a Cross-Correlation Analysis

chemotype I	THCA	CBDA	chemotype III	THCA	CBDA
γ-selinene	0.921	−0.188	β-eudesmol	−0.160	0.564
β-selinene	0.920	−0.128	γ-eudesmol	0.129	0.517
α-gurgujene	0.858	−0.346	guaiol	−0.109	0.487
γ-elemene	0.790	−0.323	α-bisabolene	−0.151	0.452
selina-3,7(11)-diene	0.704	−0.404	α-bisabolol	−0.293	0.369
β-curcumene	0.702	−0.091	eucalyptol	−0.387	0.365

The development process of the plants was characterized via the PLS analysis, and this characterization was used as a framework to interpret the patterns of the different cannabinoids and terpenes. As was the case in the PCA analysis, the first regression was performed with all of the data, and although the final model with five PCs was able to correlate satisfactorily the main variation pattern with time and to select the most significant variables, the uncertainty of the model was too high to provide a robust interpretation. Therefore, more-restrictive models were built by taking into account only each different type of chemotype. For the chemotype I plants, the final model required five PCs, but the first three explained up to 65% of the variance in x and 88% in y; in the case of chemotype III, the PCs explained 52% of the variance in x and 82% of that in y. The projection of the samples in the PC1−PC2−PC3 space showed the distinction of the three types of plants, as observed before in the PCA model, with a slightly clearer definition of the leaves and the flowers as a consequence of the different maturation processes. The robustness of each PLS model was estimated via a cross-validation procedure and statistical features.[55] Among these features, the regression coefficients of each variable with the growth time were obtained. In Figure 6, the values of the regression coefficients obtained for chemotypes I and III are plotted, including both the significant and nonsignificant coefficients.

From the regression coefficients, it can be observed that neutral cannabinoids exhibited generally higher positive values than acidic cannabinoids, which was in agreement with the increasing concentrations due to decarboxylation as long as the plants were growing. However, these values were clearly influenced by the high level of acidic cannabinoid content in the leaves of the mother plant. In the case of terpenes, clear differences between monoterpenes and sesquiterpenes were found. The monoterpenes exhibited positive coefficients, whereas the sesquiterpenes had mostly negative coefficients, with the clear exception of β-curcumene.

From these results, it is possible to select not only the most acceptable type of plants to produce the target blend of cannabinoids and terpenes but also the growth time needed to fulfill these requirements. In addition, important clues about the biosynthesis rate of CBGA in the different chemotypes and the ratios of this particular cannabinoid with CBDA and THCA were obtained. Finally, it is important to note the relations found with the terpenes because of the synergic effects with cannabinoids and the suitability of this combination for certain therapies.

EXPERIMENTAL SECTION

General Experimental Procedures. The analysis of cannabinoids was performed in an HPLC system that consisted of an Agilent 1100 series chromatograph equipped with a quaternary pump, an

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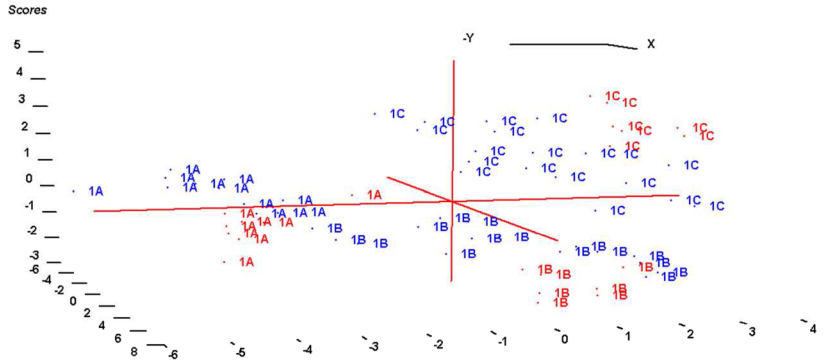

Figure 5. Score loadings (PC1 vs PC2) obtained via PCA according to the concentrations of all analyzed compounds during the growth of chemotype I plants. One refers to chemotype I plants; A, B, and C are the different plants of each chemotype. Leaves are colored blue, and flowers are colored red.

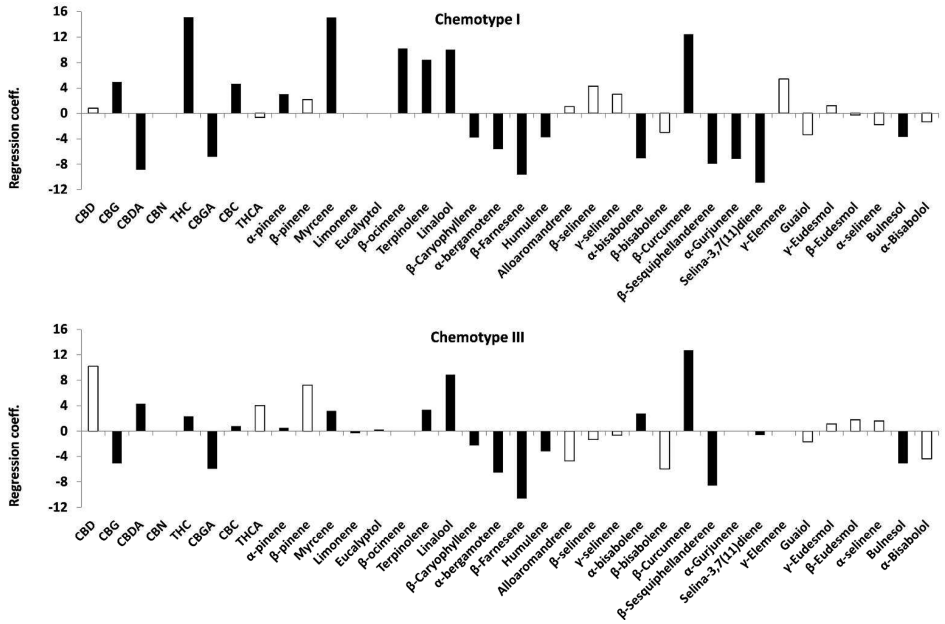

Figure 6. Regression coefficients between the growth time and all studied variables in chemotype I and III plants. Black filled bars are significant variables, and empty bars are nonsignificant ones.

autosampler, and a diode-array spectrophotometer. The analysis was performed according to the Lehmann method with some modifications.[56] Chromatographic separation was achieved using a Nucleosil C_8 column (3 μm, 125 mm × 4 mm i.d.) with a guard column (3 μm depth filter × 4 mm) (Macherey-Nagel, Oensingen, Switzerland) and a binary A/B gradient (solvent A was MeOH, and solvent B was H_2O with 0.1% of HOAc). The gradient program was as follows: the initial conditions were 50% A, which was then increased to 90% A over 20 min, maintained at 90% A over the next 1.5 min, decreased to 50% A over the next 0.5 min, and held at 50% A until 27 min for re-equilibration of the system prior to the next injection. A flow rate of 0.7 mL/min was used, the column was set at 40 °C, and the injection volume was 10 μL. Cannabinoids were quantified at a detection wavelength of 230 nm.

The quantification of cannabinoids was performed with an external calibration, using the average values of three sets of standards containing target compounds at concentrations ranging from 0.5 to 400 μg/mL in MeOH. Low- and high-calibration ranges were used in each set, i.e., 0.5−25 and 10−400 μg/mL, respectively. System fluctuations were corrected with the internal standard phenanthrene at 20 μg/mL, and quality control samples were injected every week along with the samples. The limit of detection and the limit of quantification for all compounds were 0.1 and 0.5 μg/mL, and the correlation coefficients (R^2) were ≥0.9974.

Terpene analysis was performed via GC-MS using an Agilent 6890 series instrument equipped with a 7683 autosampler, a DB5 column (0.25 μm, 30 m × 0.25 mm i.d.) from Agilent Technologies (Santa Clara, CA, USA), and a 5973 single quadrupole mass spectrometer. The transfer line temperature was set to 280 °C, the MS source to 230 °C, and the single quadrupole to 150 °C. The same oven gradient and injection and flow conditions used for the GC-FID were applied, but in this case, helium was used as the carrier gas instead of nitrogen. The analyzed mass range was 50−400 amu. The GC-MS was controlled using the Enhanced Chemstation MSD D.01.00 Build 75 software package (Agilent Technologies), and the NIST 11 library (Standard References Data Program of the National Institute of Standards and Technology, distributed by Agilent Technologies) was used for compound identification. The terpenes α-pinene, β-pinene, myrcene, limonene, eucalyptol, terpinolene, linalool, β-caryophyllene, humulene, and guaiol were identified by comparing their retention times and the obtained mass spectra with reference standards, whereas β-ocimene, α-bergamotene, β-farnesene, alloaromandrene, β-selinene, γ-selinene, α-bisabolene, β-bisabolene, β-curcumene, β-sesquiphellandrene, α-gurjunene, selina-3,7(11)diene, γ-elemene, γ-eudesmol, β-eudesmol, α-selinene, bulnesol, and α-bisabolol were identified using the NIST library.

Finally, an Agilent GC 6890 series equipped with a 7683 autosampler, a flame ionization detector, and a DB5 column (0.25 μm, 30 m × 0.25 mm i.d.) from Agilent Technologies was used for quantitative analysis of terpenes. The analysis was performed according to the Fischedick

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method with minor modifications.[57] The injector temperature was set to 230 °C, the injection volume was 4 μL, and a split ratio of 1:20 was used. A carrier gas (N_2) at a flow rate of 1.2 mL/min was used. The oven temperature program started at 60 °C with a ramp rate of 3 °C/min until 220 °C was reached, and then the temperature was increased to 300 °C with a ramp rate of 40 °C. The temperature was held for 10.67 min at 300 °C, resulting in a total run time of 66 min/sample. The FID detector temperature was set to 250 °C. A H_2 flow of 30 mL/min, synthetic air flow of 400 mL/min, and N_2 make up flow of 25 mL/min were used.

Because of the low variability in the response factors of similar molecular masses,[57] the quantification of all terpenes was performed using the average of three sets of standards containing γ-terpinene at concentrations ranging from 0.5 to 1000 μg/mL in EtOH. Low- and high-calibration ranges were prepared in each set, i.e., 0.5−50 and 25−1000 μg/mL, respectively. System fluctuations were corrected with the internal standard, i.e., 1-octanol, at 100 μg/mL, and quality control samples were injected every week along with the samples. The limit of detection and the limit of quantification for all compounds were 0.1 and 0.5 μg/mL, respectively, and the correlation coefficients (R^2) were ≥0.9998.

Plant Material. Approximately 50 clones of each standardized mother plant (three each from chemotypes I and III and one from chemotype II) were grown indoors under controlled conditions (20−28 °C and 40−70% humidity). Cannabis plants were grown in three cycles. In the beginning, they were cultivated in 25 mm × 25 mm slabs until the roots grew and then were transferred to 2 L pots. During these two steps, the plants were grown under an indoor vegetative light cycle of 18 h of light, first with a Philips Master TL-D 36 W and later with a Philips Master HPI-T Plus 400 W. Upon reaching an appropriate size, the plants were transferred to 10 L pots and exposed to a flowering light cycle of 12 h of light (Philips Master Green Power Plus 600 W) until harvest. The soil used was Subtract 144 from Ricoter (Aarberg, Switzerland), and the nutrient used was Plantactiv 18+12+18 Type A from Hauert (Grossaffoltern, Switzerland). Each week, some plants were cut, dried for 1 week at 20 °C and 45% humidity, and analyzed to determine their cannabinoid and terpene contents.

Extraction. Cannabis plant materials (0.1 g) were weighed in a glass vial, and after adding 1 mL of EtOH/$CHCl_3$ (9:1 ratio), the mixtures were sonicated for 15 min. The samples were subsequently filtered and diluted in EtOH at 1:100 for cannabinoid analysis and at 1:10 for terpene analysis. Internal standards were added in the dilution step. Phenanthrene was added to obtain a final concentration of 20 μg/mL for cannabinoids, and 1-octanol was added to obtain a final concentration of 100 μg/mL for terpenes. The samples were injected just after preparation.

Standards and Materials. Reference cannabinoids THCA, THC, CBD, and CBN were purchased from Lipomed (Arlesheim, Switzerland), and CBDA, CBGA, CBG, and CBC were purchased from Echo Pharmaceuticals BV (Weesp, The Netherlands). Reference terpenes α-pinene, β-pinene, myrcene, limonene, eucalyptol, terpinolene, linalool, β-caryophyllene, humulene, and guaiol, internal standards phenanthrene and 1-octanol, and ≥99% HOAc were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-quality MeOH, EtOH, $CHCl_3$, and H_2O were purchased from Carl Roth (Karlsruhe, Germany). Nitrogen, hydrogen, helium, and synthetic air of 99.999% purity were obtained from Carbagas (Lausanne, Switzerland).

Data Analysis and Statistics. On the basis of the terpene and cannabinoid concentrations, a multivariate data analysis was performed to identify the characteristic terpenes for each chemotype. PCA and PLS were accomplished with the statistical software package The Unscrambler (9.7 Camo Asa, Oslo, Norway) to identify specific patterns in the study design and to build regression models against the maturation time. PCA uses an orthogonal transformation to transform a number of possibly correlated variables into linearly uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the matrix data as possible, and the next principal component accounts for as much of the remaining variability as possible. Thus, the dimensionality of the data set can be reduced, and the underlying variables can be identified. Moreover, cross-correlation was used to evaluate how the compounds were correlated with each other during plant growth. PLS employs a similar strategy, but instead of finding the PCs that minimize the variance between the response and independent variables, the algorithm maximizes the correlation of each PC with the dependent variables (i.e., the time).

Concentration data [x (224 samples × 36 variables) and y (224 samples × 1 variable (time)], including categorical data, such as the type of plant, the phase of plant development, and the analysis of leaves or flowers, were uploaded in The Unscrambler. Because in most of the cases the concentrations of the cannabinoids and terpenes ranged from values lower than the detection limits at the early development stage, the distribution of the concentrations was not normal, and there were many values < LOD or missing values. Thus, the data were transformed to $\log(x + 0.005)$ to eliminate the values < LOD and to improve the normality of the distribution.

Initial PCA and PLS models were built with standardized x variables to give equal weight to all variables and make use of the leverage correction method as the internal validation procedure. Once the putative outliers were removed and a robust model was obtained, a final model was built making use of the cross-correlation as the internal validation procedure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00949.

Additional details (PDF)

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Notes

The authors declare no competing financial interest.

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