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Short communication

Terpene profile in fresh flowers of outdoor cultivated commercial strains and wildtype of Cannabis

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Introduction

Components that give the characteristic aroma and flavor of cannabis strains and varieties comprise over 200 volatile terpenes and have been identified in various strains of cannabis (Lowe et al., 2021). The terpene profiling of cannabis plants gives opportunity for chemotype classification and also it is of great importance for the pharmacological effects. Although there are combinations of terpenes considered characteristic of specific chemotypes, the terpene profile is dependent upon many factors including: genetic and environmental factor, inflorescence age, cultivation and harvest conditions (Lowe et al., 2021). Determination of terpenoid profile in cannabis is important, especially when it can be used to correlate genotype with chemotype and phenotype. Therefore, the main objective of this study was to investigate the aroma profile of different cannabis cultivars and wildtype, cultivated under same conditions.

Materials and methods

Seed material

Seeds from 4 different cannabis strains (M1- Bubba Kush x OG Kush, M2 - NYC Diesel, M3 - Great White Shark and S1 - Charlotte Angels) were generously donated by licensed cannabis company. The wild growing cannabis varieties seeds were collected at 5 different locations (seed maturity growing stage) in R. N. Macedonia (W1 - Gorni and Dolni Podlog, near Kochani 2019, W2 - Dolni Balvan, near Kochani 2020, W3 - Prosenikovo and Saraj, near Strumica 2019, W4 - Bosilovo, near Strumica2019, W6 - Mojanci, near Kochani 2019).

Cultivation of plant material

Seeds were placed between a dampened cotton ball with distilled water in a Petri dish sealed with parafilm. The seeds imbibed in a period of 3-5 days. Seedling stage lasted for 3 weeks, vegetative and flowering stage were different for each strain/variety. Regular nutrition before 7th week of vegetative growth consisted of liquid solution of 20:20:20 N:P:K with supplementary Mg. At 7th week of vegetative growth plants were transplanted at outdoor experimental plot with limited nutrition with three courses of liquid solution of 20:20:20 N:P:K with supplementary Mg. Fresh flower samples (weigh variation of samples 0.8 - 1.2 g) from upper third of the plants were collected in headspace vials. The samples were stored at -20°C until further analysis.

HS/GC/MS analysis

Fresh collected flower samples in headspace vials were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. For separation HP-5ms capillary column (30m x 0.25 mm, 0.25 μm) was used with helium as carrier gas (1 mL/min). Analytical conditions were as follows: oven temperature at 0 min 60°C, rate 4.5°C/min 4.5 °C to 250°C (1 min hold time) with runtime 43 min. FID detector temperature was 270°C. Mass spectrometry conditions were as follows: ionization voltage 70 eV, ion source temperature 230°C, transfer line temperature 280°C

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and mass range from 50 - 550 Da. The MS was operated in scan mode. Headspace method conditions were as follows: incubation temperature 80˚C; incubation time 5 min; syringe temperature 85˚C and agitator speed 500 rpm.

Components were identified with comparison of their mass spectra with reference spectra from libraries such as NIST, Wiley and Adams (Adams, 2007) and were quantified using normalization method of peak areas with no correction factors.

Results and discussion

Total of 39 components were detected representing 97.55 - 99.58% of the volatile content, and only 9 components (myrcene, α-pinene, limonene, terpinolene, (E)-β-ocimene, β-pinene, trans-(E)-caryophyllene, linalool and α-humulene) were present with more than 1% in at least one analysed samples of commercial specimens.

High variability of relative presence of these 9 compounds was observed within the cultivated commercial strains. Myrcene was present from 23.72% in M3 to 68.57% in M1, α-pinene was present from 1.49% in M1 to 54.31% in M3, limonene from 3.81% in M3 to 36.45% in M1, terpinolene from 0.16% in M1 to 26.89% in S1), (E)-β-ocimene from 0.12% in M1 to 9.62% in S1, β-pinene from 1.82% in M1 to 9.21% in M3), trans-(E)-caryophyllene from 0.43% in M2 to 8.13% M1), linalool from 0.12% in M3 to 3.39% in M1) and α-humulene from 0.15% in M3 to 1.36% in M1. On the other hand, the terpenes present with less than 1% were: α-selinene, β-selinene, amorpha-4,7-(11)-diene, α-gurjunene, α-aromadendrene, sibirene, selina 3,7-(11)-diene, β-germacrene, γ-terpinene, Δ-2 carene, *o*-cymene, β-fellandrene, γ-elemene, (-)-endo-fenchol, β-farnesene, ocimene allo/neo-allo and camphene. Our results showed that the monoterpenoid fraction represent more than 80% of the components identified in the analysed cultivars.

Furthermore in the cultivated wildtypes, only 7 terpenes were detected with more than 1%: myrcene (26.09% in W4 - 64.02% in W1), α-pinene (14.85% in W2 - 54.90% in W4), terpinolene (1.95 - 15.69% in W1), (E)-β-ocimene (0.16 in W4 - 11.61% in W2), limonene (2.34% in W6 - 11.79% in W2), β-pinene (3.19% in W2 - 9.66% in W4), trans-(E)-caryophyllene (0.26% - 2.57% in W3).

Additionally, less terpenes (valencene, β-farnesene, γ-terpinene, Δ2-carene, o-cymene, linalool, (-)-endo-fenchol, ocimene allo/neo-allo) were identified in amount below 1% in the cultivated wildtype cannabis samples. Camphene and α-humulene were detected in all of the analysed wildtype cultivars.

From the obtained data we can observe great variability among the three most predominant terpenes in each of the commercial strains: M1 - myrcene (68.57%), limonene (16.69%), and trans-(E)-caryophyllene (4.84%), M2 - myrcene (46.23%), α-pinene (37.59%) and limonene (3.81%), M3 - α-pinene (54.31%), myrcene (23.72%) and β-pinene (7.67%), S1 - myrcene (42.14%), terpinolene (25.63%) and (E)-β-ocimene (9.62%).

On the other hand W2 and W6 from the cultivated wildtypes exhibit similarity patern of the three most predominant terpenes: myrcene (49.62%, 44.73%), α-pinene (20.89% and 34.02%) and (E)-β-ocimene (11.61% and 6.48%). Moreover, W1 and W3: myrcene (64.02%, 48.52%), α-pinene (24.17%, 22.78%), limonene (3.50%,11.653), respectively, indicating possible genetic similarity, while W4 sample is characterized by α-pinene (39.64%,), myrcene (26.09%) and limonene (11.79%).

Conclusion

Using HS GC-MS, the volatile profile of cultivated cannabis strains and wildtypes were determinated showing considerable variability of the terpenes presence. Additionally, the variability of these highly volatile aroma compounds, is the most probable reason responsible for the specific scent of each cannabis specimen. Finally, further genetic and chemotype research should be conducted in order to correlate terpene profile to genetic groups of cannabis strains/wildtype varieties.

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