Macedonian pharmaceutical bulletin, 68 (Suppl 1) 575 - 576 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.275
Short communication

Individual variability of the cannabinoids' content in outdoor cultivated Bubba Kush x OG Kush Cannabis strain

Andrea Belazelkoska, Ana Stoilkovska, Veronika Stoilkovska Gjorgievska*, Ana Trajkovska, Ivana Cvetkovikj Karanfilova, Marija Karapandzova, Svetlana Kulevanova, Gjose Stefkov

Institute of pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000, Skopje, R. North Macedonia

Introduction

Cannabis sativa and/or cannabis-based products have been legalized for medical use in 41 countries (23 in Europe) between 2012–2021 (Stefkov et al., 2022). Having in mind that this industry is exponentially growing, many different cultivation practices such as indoor, outdoor and greenhouse cultivation, have been established. Outdoor cultivation is the traditional and low cost method for growing hemp, while indoor cultivation is much more employed for growing medicinal cannabis. There is a great variety of different strains and hybrids of cannabis, created for better yield of different cannabinoids and terepenes. Such strain is Bubba Kush x OG Kush, a hybrid of parents Bubba Kush and OG Kush, mostly Indica variety, with total THC up to 17% and very low total CBD (under 0.1%) which has flowering time of 9 weeks. Higher total THC content makes it suitable for obtaining rich medicinal cannabis plants for utilization for medicinal purposes such as THC rich extracts preparation. The aim of this study is to assess individual plant variability of cannabinoid content in group of cultivated plants of the same strain Bubba Kush x OG Kush.

Materials and methods

Reagents

Cannabidiol CRM solution (purity 98.66%), cannabinol CRM solution (purity 99.50%), (-)-△9-tetrahydrocannabinol CRM solution (purity 99.39%), △9-tetrahydrocannabinolic acid A CRM solution (purity 96.99%) and cannabidiolic acid CRM solution (purity 97.88%) with concentration of 1 mg/mL were purchased from Cayman Chemicals (USA). 85% o-phosphoric acid and acetonitrile HPLC grade were purchased from Carlo Erba. Ethanol 96% Ph.Eur. grade was purchased from Alkaloid AD Skopje.

Plant material

Seeds from cannabis strain Bubba Kush x OG Kush were generously donated by licenced cannabis company. For plant nutrition BIOBIZZ liquid nutrition mixture of 20:20:20 N:P:K with supplementary Mg and BIOBIZZ root antistress liquid nutrition mixture 10:52:10 N:P:K with supplementary Mg were used. 15 seeeds in total were used in this experiment (M1-(1-15)). 5 seeds were placed between a dampened cotton ball with distilled water and covered with a dampened cotton ball in a Petri dish sealed with parafilm. Only 9 seeds were imbibed in a period of 3-5 days. Seedling stage lasted 3 weeks, vegetative growth for 11 weeks and flowering stage 7 weeks. At 7th week of vegetative growth plants were transplanted at outdoor experimental plot, regularly treated with water but nutrition was limited since the effect of natural conditions for growing plants was considered in this experiment. Plants (plant codes M1-1 to M1-9) were havested at 7 weeks in the flowering stage which is prior floral technical maturity (9 weeks) due to presence of a caterpillar pest. M1-6 plant died in early vegetative stage due to broken main stem.

HPLC determination of phytocannabinoids

For determination of cannabinoid content, DAB Pharmacopoeial method for assay of cannabinoids was

*vsgjorgievska@ff.ukim.edu.mk

applied. The chromatographic analyses were carried out using Agilent 1200 Model HPLC equipped with DAD G1315D, quaternary pump G1311A, column thermostat G1316A and thermostatted autosampler G1329A (Agilent Technologies, USA). Separation was achieved using InfinityLab Poroshell 120 EC-C18 chromatographic column (150 mm x 3 mm ID, 2.7 μm) at 40°C, gradient method with flow rate 0.7mL/min, DAD measurements were carried out at 225 nm wavelength for neutral cannabinoids (CBD, CBN and Δ9 - THC) and 306 nm wavelength for acidic cannabinoid forms (CBDA and Δ9-THCA). Sample preparation (triplicates) and loss on drying for each sample was determined according to DAB monograph for Cannabis flower (German Pharmacopoeia 2020th ed. 2020). Prior sample preparation flowers were trimmed, pulverised and sieved through sieve No. 710.

Results and discussion

Measurements were done on harvested flowers of 8 plants. HPLC analysis revealed that CBDA was below limit of quantification in all of the samples, except M1-4 (0.01%), M1-8 (0.03%) and M1-5 (0.08%). CBD content was quite uniform in all the plants (c.0.01%) thus total CBD (expressed as sum of CBDA (%) x 0.877 and CBD (%)) was below 0.1% in all of the samples. Unlike total CBD content, total THC content (sum of Δ9-THCA (%) x 0.877 and Δ9-THC (%)) varied throughout the samples: from minimal M1-1 (4.50%), M1-3 (6.96%), M1-2 (7.69%), M1-9 (7.88%), M1-7 (9.70%), M1-4 (13.29%), M1-8 (14.38%) to maximal M1-5 (17.38%), with predominant share of Δ9-THCA and Δ9-THC varying from 0.41 - 1.03%. The origin of THCA content variability can be of different nature such as: different individual plant accomodaton due to limited nutrition, the stability and uniformity of the genetic material or flower damage caused by caterpillar pest. Research by Bernstein et al. (2019) demonstrated that nutrient supplementation can modulate cannabinoid content in an organ- and location-specific manner, pointing out that relationship between cannabinoid content and nutritional supplementation is not very clear yet. Also insect plant damage influence on cannabinoid content was investigated by Park et al. (2022) who discovered that in 5 day treatment of plants with caterpillar larvae of tobacco hornworm *Manduca sexta* had significant reduction in CBGA, CBG and CBD content. Many strains are offered by different seed companies, and the degree of genetic similarity or difference among providers has not been quantified; therefore, it is generally expected and accepted that there is significant variation within a single strain among seed companies and even within seed lots (Adhikary et al., 2021).

Conclusion

Within this research eight individual plants Bubba Kush x OG Kush were successfully cultivated and cannabinoid content was determined. Quite large variability in THCA content was observed (4.50-17.38%) in individual plants most likely due to genetic non-uniformity of seed material and flower damage from caterpillar pest. Further genetic and phytochemical research is necessary to determine the origin of cannabinoid content variability of this strain.

Acknowledgement

Hereby we sincerely acknowledge “OAZA ALKALOIDI” and “GM LAJT MEDIKAL”, licenced cannabis companies in Republic of North Macedonia.

References

Adhikary, D., Kulkarni, M., El-Mezawy, A., Mobini, S., Elhiti, M., Gjuric, R., Ray, A., Polowick, P., Slaski, J.J., Jones, M.P., Bhowmik, P., 2021. Medical Cannabis and Industrial Hemp Tissue Culture: Present Status and Future Potential. Front. Plant Sci. 12, 627240. https://doi.org/10.3389/fpls.2021.627240

Bernstein, N., Gorelick, J., Zerahia, R., Koch, S., 2019. Impact of N, P, K, and Humic Acid Supplementation on the Chemical Profile of Medical Cannabis (Cannabis sativa L). Front Plant Sci. 10, 736. https://doi.org/10.3389/fpls.2019.00736

German Pharmacopoeia 2020th ed. (2020) Cannabisblüten. Bonn: Geschäftsstelle der Arzneibuch-Kommissionen, Bundesinstitut fur Arzneimittel und Miedizinprodukte.

Park, S.H., Pauli, C.S., Gostin, E.L., Staples, S.K., Seifried, D., Kinney, C., Vanden Heuvel, B.D., 2022. Effects of short-term environmental stresses on the onset of cannabinoid production in young immature flowers of industrial hemp (Cannabis sativa L.). J Cannabis Res. 4(1). https://doi.org/10.1186/s42238-021-00111-y

Stefkov, G., Cvetkovikj Karanfilova, I., Stoilkovska Gjorgievska, V., Trajkovska, A., Geskovski, N., Karapandzova, M., Kulevanova, S., 2022. Analytical Techniques for Phytocannabinoid Profiling of Cannabis and Cannabis-Based Products—AComprehensive Review. Molecules. 27, 975. https://doi.org/10.3390/molecules27030975