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Yield and cannabinoids contents in different cannabis (*Cannabis sativa* L.) genotypes for medical use

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ABSTRACT

In the last decades, there has been a significant increase in the number of lifestyle and auto-immune diseases, such as various cancers or multiple sclerosis. In countries where cannabis is decriminalized for medical purposes, it is most often prescribed for these diagnoses. Today, over 700 different cannabis genotypes are being bred, and it is very important to describe in detail their cultivation, potential yields, chemical profile and stability, to be recommended to a particular patient with a specific diagnosis. The aim of this study was to evaluate the inflorescence yields and the content of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) of seven traditional genotypes of cannabis – Conspiracy Kush, Nurse Jackie, Jilly Bean, Nordle, Jack Cleaner 2, Jack Skellington and National Health Services. The plants were grown under controlled climatic conditions during six growing cycles at a density of 9 plants/m^2. Dried inflorescences from each plant were homogenized and analyzed by gas chromatography with flame ionization detection. The average yield per plant was 21.02 ± 3.33 g and the highest yields showed genotype Nurse Jackie (24.74 ± 6.11 g). The lowest yields were shown by genotype Jack Skellington (15.41 ± 4.02 g). Average Δ^9-THC levels for each variety in all 6 growing cycles ranged from 15.69 ± 2.6 % to 19.31 ± 2.47 % (w/w). The lowest contents of Δ^9-THC were measured in the Nordle genotype and the highest values were found in the Jack Cleaner 2 and Jack Skellington genotypes. Average CBD levels in the plants ranged from 0.45 ± 0.1 % to 0.57 ± 0.08 % (w/w) over six individual cycles. This study shows that among genotypes studied, the best parameters – high yield and stable cannabinoids production – are shown by genotypes Nurse Jackie and Jilly Bean.

1. Introduction

Preserved records of cannabis use in medicine can be found in China and are nearly 5000 years old (Hanuš and Mechoulam, 2005). The healing properties of cannabis products have been recognized for millennia, but because of the psychoactive nature of the major active substance Δ^9-tetrahydrocannabinol (Δ^9-THC) and the fact that cannabis is the most commonly used illegal narcotic substance not only in Europe, but around the world, this substance has been criminalized for a long time in most countries in the world (Wilkinson et al., 2003; Hanuš, 2009). Because of this, the legal use in medicine remains controversial. This is due, among other things, to the United Nations Convention of 1961, where cannabis was also included in the list of the most dangerous substances, e.g. with morphine and its derivatives (United Nations, 2016).

From a phytochemical point of view, plants from the genera *Cannabis* contain a large number of compounds belonging to almost all chemical groups. Secondary metabolites in cannabis that were already identified are cannabinoids, terpenoids, flavonoids, steroids, alkaloids, lignans, etc (ElSohly and Slade, 2005). Studies have also demonstrated their synergistic effects (Russo, 2011; Mechoulam, 2012). The most well-known and most specific chemical group of secondary cannabis metabolites includes cannabinoids, especially the psychoactive Δ^9-THC and its CBD modifier (Mechoulam, 2012). The CBD alleviates the anxiety and psychotic conditions that Δ^9-THC can cause in some patients (Morgan et al., 2010) and, for example, in chronic neuropathic pain, their combination has a higher activity than Δ^9-THC alone (Russo and Guy, 2005). The ratio of these two active substances in cannabis

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products is very important in the treatment of patients. Based on the amount and ratio of Δ^9-THC: CBD, it should be possible to determine individual dosages for specific patients depending on the diagnosis and the stage of the disease (Mechoulam, 2012). For these two cannabinoids, Russo (2011) also described their synergism with some terpenoids in his study.

With the discovery of the first cannabinoid receptors bound by Δ^9-THC present in a rat brain, which was made in the late 1980s (Devane et al., 1988) and the enormous development of endocannabinoid system research, the global situation slowly changed. Currently, in some countries, the cultivation and use of medical cannabis (MC) is permitted by law.

In 1993, cannabis was decriminalized for treatment in Israel. Eight companies grow cannabis there with the authority of the Ministry of Health who provides many different genotypes of healing therapies. More than 23,000 patients are treated in Israel today, 13,000 more than in 2012 (Michka et al., 2015).

In the United States of America, 29 states and Washington DC are allowed to use cannabis for a number of health problems. The first US state, where MC was decriminalized in 1996, is California (National Conference of State Legislatures, 2017). In 2001, MC use was authorized in Canada, where patients are given relief from pain for severe illnesses and incurable patients (Michka et al., 2015).

Initiators of MC decriminalization in Europe were the United Kingdom and Switzerland, where high-quality cannabis, derivatives or standardized extract (Hazekamp, 2006) are administered to patients. In 2003, the cultivation and use of MC was authorized in the Netherlands, where the use of cannabis for recreational purposes was also regulated from the 1970's and much more moderately than in other countries in the world (Dolin, 2001). Growing cannabis and production of herbal products is provided in the Netherlands by only one company authorized by the Ministry of Health, Welfare and Sport, Bedrocan BV Medicinal Cannabis. Since 2013 the cultivation and prescription of MC is allowed also in the Czech Republic, partial decriminalization is currently going on in Germany, Macedonia and Poland.

Current hemp taxonomy research identifies *Cannabis sativa* L., and differentiates it into subspecies *C. sativa* spp. *sativa*, *C. sativa* ssp. *indica*, and *C. sativa* ssp. *Ruderalis* (Casano et al., 2011). Today, over 700 different cannabis genotypes are being bred (Snoeijer, 2001) and are still breeding new ones. For genotypes that have been on the market for a long time, it can be assumed that the main active ingredient content is more stable than genotypes that have been bred recently. Many of them have common ancestors, but their chemical composition differs considerably and their biological activity has not yet been fully explored (Hazekamp and Fischedick, 2012). The question then remains, which of the many genotypes should be available for use in medicine. Therefore, since Czech University of Life Sciences Prague was the first institution with approval for experimental growing of MC in the Czech Republic, we have decided to describe stability of the yield and chemical composition of seven well-known genotypes, which could be utilized for medicinal purposes.

2. Material and methods

2.1. Genotypes

Genotypes (Table 1) were selected from traditional breeders based on proclaimed amounts of cannabinoids. The requirement was genotypes with an average content of 19 % Δ^9-THC and up to 1 % CBD which corresponds to chemotypes used in most countries where healing therapies are decriminalized.

2.2. Cultivation

Seven genotypes of cannabis (*Cannabis sativa* L.) were cultivated in the grow-room in total area of 2 m^2. The plants were cultivated in a mix of peat and zeolite (Grandpa's Soil, Vojtěch Karban, CZ). Fertilization and a light regime were performed according to the Advanced Hydroponics (Netherland) grow scheme (Table 2). The pH was maintained in the range of 5.8–6.2 by 40 % nitric acid. Room temperature was maintained between 22 and 30 °C and humidity between 40 and 70% with an ultrasonic humidifier (Cronwel Eletronics, United Kingdom) depending on the growing phase (Adams, 2012). The grow-room was equipped with ventilator 1000 m^3/h (TORIN, Switzerland). Lamp height in each of the subplots was continuously adjusted so that the lamps remained at 0.6 m above the canopy level. For the vegetative phase, 400 W Master HPI-T Plus (Philips, Netherland) lamps were used with a blue spectrum of absorbance (correlation chromatics temperature 4500 K) and for the generative phase, XTREME OUTPUT 400 W (GIB-lighting, Germany) lamps were used with a red spectrum of absorbance (correlation chromatics temperature 2000 K).

In the first cycle, the plants were cultivated from regular seeds. After the two months of long-day photoperiod (18/6 h), one stem from each plant was cut. Rooting the cuttings was stimulated by dipping them in nicotinic and naphthalene acetic acid (STIMULATOR AS-1, Lucie Nemcova, CZ). These cuttings were cultivated under the red light spectrum during a short-day photoperiod (12/12 h). After one week on the short-day photoperiod it was recognizable, which of the parental plants were female or male gender. The male plants were separated and the female plant cuttings were taken for the next cycle and then the female plants were exposed to a short-day photoperiod. After 46 days, the female flower buds consisted of dark green calyxes containing brown or orange pistils that formed a compact flowering mass. The plants were harvested and their inflorescences were dried in an oven at 30 °C. After the second cycle, each plant was cloned by means of stem cuttings for the next cycle. All genotypes were grown in three independent replicates (plants) over six consecutive cycles.

2.3. Quantification of Δ^9-THC and CBD

For the quantification of Δ^9-THC and CBD, gas chromatography with flame ionization detection method (GC-FID) recommended for analysis of cannabis by The United Nations Office on Drugs and Crime (*Recommended methods for the identification and analysis of cannabis and cannabis products*, 2009) was used and reference standards Δ^9-THC and CBD were purchased from Sigma Aldrich (CZ).

The flower buds from each plant were dried at 30 °C, homogenized with a laboratory blender and analyzed by GC-FID (Agilent 6890, Agilent Technologies, Palo Alto, CA). One hundred milligrams of dried plant material was extracted with 10 ml of internal standard solution (0.5 mg/ml tribenzylamine in 96 % ethanol) for 15 min in an ultrasonic bath. Five hundred microliters of the solution was transferred to a 2 ml GC vial. The opened vial was put into a block heater (150 °C) for 12 min where the solvent is evaporated and the acid form of Δ^9-THC is decarboxylated. The residue was dissolved in 1.5 ml of ethanol, the vial was shaken well and the resulting solution was then analyzed by GC-FID. Separation was achieved on a ZB-5 column (15 m × 0.25 mm × 0.25 μm). The carrier gas, nitrogen, had a constant flow of 1.1 ml/min. A 1.5 μl sample was injected with a split ratio of 20:1 at 280 °C. The detector parameters were set at a temperature of 300 °C, a hydrogen flow of 35 ml/min and an air flow of 350 ml/min. Oven temperature was programmed for 2 min at 200 °C, 10 °C/min 200–240 °C and 2 min at 240 °C. Samples from each plant were prepared and measured in triplicate. The quantitation was achieved by external calibration measuring set of Δ^9-THC (R^2 = 0.9962) and CBD (R^2 = 0.9991) calibration solutions (0.1–32% and 0.1–10% w/w, respectively) and the results were adjusted according to the internal standard response.

2.4. Statistics

In order to assess the stability of the yields and cannabinoids