Industrial Crops & Products 112 (2018) 363–367

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

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

Anežka Janatová[a], Adéla Fraňková[b], Pavel Tlustoš[c], Karel Hamouz[a], Matěj Božik[b], Pavel Klouček[b,*]

[a] Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Crop Science, Kamycka 129, 16500, Prague, Czech Republic
[b] Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products, Kamycka 129, 16500, Prague, Czech Republic
[c] Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Agroenvironmental Chemistry and Plant Nutrition, Kamycka 129, 16500, Prague, Czech Republic

ARTICLE INFO

Keywords:
Medical cannabis
Genotypes
Cultivation
Delta-9-tetrahydrocannabinol
Cannabidiol
Yield
Hemp

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

* Corresponding author.
E-mail addresses: kloucek@af.czu.cz, pavel.kloucek@gmail.com (P. Klouček).

https://doi.org/10.1016/j.indcrop.2017.12.006
Received 15 August 2017; Received in revised form 27 October 2017; Accepted 4 December 2017

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

Table 1
Description of individual genotypes.

Genotype	Breeder	Parents[a]	Sativa: Indica*	Flowering[a]	Indication[a]
Conspiracy Kush (CSAJ001)	TGA Subcool Seed	Obama Kush × Space Gueen	30:70 %	8 weeks	stress, insomnia, pain, depression
Nurse Jackie (CSAJ002)	TGA Subcool Seed	Medicine Woman × Jack the Ripper	85:15 %	8–9 weeks	intraocular pressure, stress, insomnia, headache, depression, inflammatory
Jilli Bean (CSAJ003)	TGA Subcool Seed	Unknown Orange Skunk × Romulan × Cindy 99 BCGA	60:40 %	8 weeks	nausea, stress, pain, depression
Nordle (CSAJ004)	Mr Nice Seedbank	Afgan × Skunk	20:80 %	8–10 weeks	stress, insomnia, pain, spasmolytic pain, inflammatory, depression
Jack Cleaner 2 (CSAJ005)	TGA Subcool Seed	Pluton × Purple Haze × Lambsbread × NL × Jack Herer × Jack The Ripper Male	80:20 %	8–10 weeks	intraocular pressure, pain, depression, stress,
Jack Skellington (CSAJ006)	TGA Subcool Seed	Cindy 99 x G–13 x Jacks Cleaner × Space Queen	70:30 %	8 weeks	stress, pain, depression, nausea, anorexia
National Health Service (CSAJ007)	Mr Nice Seedbank	Northern Lights 5/Haze × Skunk	Mostly sativa	8–10 weeks	homeostatic effect

[a] http://www.tgagenetics.com/; https://www.leafly.com/; http://en.seedfinder.eu/.

Table 2
Grow scheme applied in the cannabis cultivation experiment.

Week	Light[a] (day/night)	Grow[a] (ml/l)	Bloom (ml/l)	Micro (ml/l)	Enzymes (ml/l)	Bloom Booster (ml/l)	Root Stimulator (ml/l)	Final Solution (ml/l)
1.–3.	18/6	–	–	–	–	–	0.5	–
4.–5.	18/6	0.5	0.2	0.2	0.5	–	0.5	–
6.–7.	18/6	1	0.5	0.5	0.5	–	0.5	–
8.–9.	12/12	0.5	1	0.5	0.5	0.5	–	–
10.–13.	12/12	–	1.5	0.5	0.5	0.5	–	–
14.–15.	12/12	–	–	–	–	–	–	1

[a] Fertilization and a light regime were performed according to the manufacturers' recommendations (https://www.advancedhydro.com/).

content over six cycles of individual genotypes, the ANOVA was used at a significance level of $p < 0.05$ (ANOVA Statistica 12, StatSoft12, CZ) with a Tukey post hoc test.

3. Results

Seven genotypes were grown in a 2 m^2 grow-room. Their average yields for 6 growing cycles are shown in Table 3, mean Δ^9-THC content in Table 4 and average CBD contents in Table 5. Some properties of the genotype National Health Service were not suitable for indoor cultivation and therefore this genotype was excluded after the 2nd cycle (see below).

3.1. Yields

Total yields per one cycle from all genotypes ranged from 138.59 to 231.08 g/m^2. The results of the second cycle shown in Table 3 are not included in this range, because nearly 13 % of the dried inflorescence were seeds due to hermaphrodite plants of National Health Service genotype. The lowest yields were recorded in the 5th cycle, while the highest yields were recorded in the 4th cycle. The total yields per cycle were not statistically evaluated, because the values are simple sums of the real weights.

The average yield per plant over all cycles was 21.02 ± 3.33 g. Genotype Nurse Jackie showed the most profitable (24.74 ± 6.11 g/plant) during the observed cycles, while the lowest yields were recorded for genotype Jack Skellington (15.41 ± 4.02 g/plant). However, there were no statistically significant differences among genotypes.

3.2. Δ^9-THC content

Regarding the cycles, average content of Δ^9-THC ranged from 15.69 ± 2.6 % (w/w) to 19.31 ± 2.47 % over six individual cycles.

Table 3
Average yields of dried inflorescences (g/plant) of individual genotypes over six cycles.

Genotype	1st cycle[a]	2nd cycle[b]	3th cycle	4th cycle	5th cycle	6th cycle	Total/plant
	Mean ± SD[c]	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Conspiracy Kush	15.64 ± 2.62	20.02 ± 5.25	18.12 ± 8.13	23.97 ± 7.20	12.60 ± 1.98	19.40 ± 3.08	18.07 ± 3.90
Nurse Jackie	20.61 ± 2.57	29.46 ± 6.85	33.36 ± 9.32	23.78 ± 1.65	16.50 ± 6.50	22.41 ± 0.94	24.74 ± 6.11
Jilli Bean	17.05 ± 5.60	24.14 ± 7.96	26.68 ± 7.56	30.98 ± 1.68	16.31 ± 2.55	21.34 ± 0.83	23.03 ± 5.68
Nordle	14.35 ± 2.83	19.76 ± 1.65	24.94 ± 2.03	29.34 ± 4.65	11.71 ± 3.93	18.66 ± 4.57	20.02 ± 6.54
Jack Cleaner 2	19.29 ± 3.75	21.47 ± 1.48	27.43 ± 6.23	27.36 ± 5.51	18.75 ± 2.03	23.58 ± 1.27	22.86 ± 3.83
Jack Skellington	13.11 ± 3.73	12.66 ± 5.79	16.13 ± 5.19	18.62 ± 10.5	16.52 ± 7.25	23.61 ± 5.83	15.41 ± 4.02
National Health Service	18.56 ± 3.97	27.50 ± 7.50					23.03 ± 6.32
Total/m[b]	177.91[d]	232.51	220.00	231.08	138.59	193.51	21.02 ± 3.33

[a] In the 1st cycle, the plants were grown from seeds and, due to the selection of the male plants, they were left for a longer period of vegetation.
[b] In the 2nd cycle, plants of the National Health Service genotype experienced hermaphroditism and subsequent pollination of other plants.
[c] results are expressed in g/plant.
[d] the total yield values are sums of the real yields of all genotypes divided by total cultivation area.

Table 4
Average Δ^9-THC content in dried inflorescence (%/w/w) of individual genotypes over six cycles.

Genotype	1st cycle	2nd cycle	3th cycle	4th cycle	5th cycle	6th cycle	Average/genotype
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Conspiracy Kush	21.07 ± 3.39	17.85 ± 2.53	13.86 ± 2.00	18.30 ± 2.30	18.16 ± 2.76	19.32 ± 2.51	18.09 ± 2.38^{bc}
Nurse Jackie	18.55 ± 1.7	17.35 ± 2.31	18.52 ± 1.01	14.96 ± 1.98	16.73 ± 2.35	18.36 ± 1.71	17.41 ± 1.41^{bcd}
Jilli Bean	16.39 ± 2.1	16.44 ± 1.36	14.68 ± 1.91	18.38 ± 2.87	17.78 ± 1.50	16.76 ± 1.00	16.74 ± 1.28^{cd}
Nordle	16.46 ± 3.06	16.15 ± 0.96	11.99 ± 0.57	16.03 ± 1.13	16.75 ± 0.55	18.01 ± 0.46	15.90 ± 2.04^{d}
Jack Cleaner 2	22.15 ± 0.53	21.74 ± 0.52	18.26 ± 0.40	19.93 ± 0.42	20.09 ± 0.67	22.43 ± 1.12	20.77 ± 1.62^{a}
Jack Skellington	22.07 ± 1.79	20.21 ± 1.45	16.81 ± 1.25	15.23 ± 1.15	23.56 ± 0.7	18.82 ± 0.92	19.45 ± 3.15^{a}
National Health Service	18.51 ± 1.65	19.83 ± 1.46					19.17 ± 0.93^{ab}
Average/cycle	19.31 ± 2.47^{a}	18.51 ± 2.11^{cd}	15.69 ± 2.6^{e}	17.14 ± 2.01^{bcde}	18.85 ± 2.62^{bc}	18.95 ± 1.91^{ab}	

Different letters as superscripts in the same column (average/genotype) or row (average/cycle) indicate significant difference ($P < 0.05$).

The lowest levels of Δ^9-THC were measured in the 3rd cycle, which differed significantly ($p < 0.001$) from the 1st, 2nd, 5th, and 6th cycles. The first cycle, where the highest Δ^9-THC values were measured, was statistically different ($p \leq 0.009$) from the 3rd as well as from the 4th cycle. Generally, we can report that except the 3rd cycle, the content of Δ^9-THC appear to be stable.

Regarding the genotypes, average Δ^9-THC levels ranged from 15.9 ± 2.04 % to 20.77 ± 1.62 %. The lowest contents were measured in the Nordle genotype, which was significantly different ($p \leq 0.013$) from the Conspiracy Kush, Jack Cleaner 2, Jack Skellington and the National Health Service. The highest values were found in the Jack Cleaner 2 and Jack Skellington genotypes, which were statistically distinct ($p \leq 0.029$) from all of the genotypes except National Health Service, for which only two cycles were evaluated (Table 4).

3.3. CBD content

Average CBD levels in the plants, regardless of the genotypes, ranged from 0.45 ± 0.1 % to 0.57 ± 0.08 % (w/w) over the six individual cycles (Table 5). The lowest CBD contents were measured in the 3rd cycle, which is consistent with the measured Δ^9-THC contents. We can say that except the 3rd cycle ($p \leq 0.045$), the levels of CBD appear to be stable.

The CBD content of the genotypes averaged from all growing cycles ranged from 0.45 ± 0.06 % to 0.6 ± 0.06 %. The lowest contents were measured in genotype Nordle, which was significantly different ($p < 0.001$) from the genotypes Nurse Jackie, Jack Cleaner 2 and Jack Skellington. This is also consistent with the amounts of Δ^9-THC. The highest CBD values were measured in the Jack Cleaner 2, Jack Skellington and Nurse Jackie genotypes, which were statistically different ($p \leq 0.031$) from the genotypes Conspiracy Kush and Jilly Bean. Again, it is in line with the Δ^9-THC values.

4. Discussion

In the treatment of cannabis, it remains a question of which genotypes from many cultures are suitable for different diagnoses, even with regard to the economics of their cultivation. From relevant sources, information on so-called technical hemp grown in outdoor conditions is available in order to achieve the highest yields of quality fibre or seed. There is information on cannabis grown in indoor conditions for the production of female inflorescence with a high content of active substances, especially in forensic science studies. However, these studies can not be fully exploited for therapeutic use because the plant material comes from confiscated illegal production (Huizer and Poortman-van der Meer, 1995; Vanhove et al., 2012; Toonen et al., 2006; Decorte, 2010). In these cases, many factors are not known to affect the yields and cannabinoids content and experiments can not be repeated within the relevance of verifying the results.

Each genotype has its specific cropping characteristics and requirements, such as the height of growth or the length of the growing season. For these reasons, the genotype National Health Service had to be excluded from the experiment after the 2nd growth cycle, as it significantly exceeded the growth rate of other genotypes. This resulted in the second cycle of its inflorescences burning under the fluorescent lamps, the plants were in stress and began to create male hermaphrodites beside the female flowers. The pollination of other genotypes in the plant was also observed, which affected the yields in the 2nd cycle. Surprisingly, this problem in the 2nd cycle did not have a detrimental effect on the content of Δ^9-THC and CBD, as could be expected. However, it is possible that the effects of hermaphroditism and pollination appeared in the 3rd cycle, where the Δ^9-THC and CBD contents were the lowest (See Tables 4 and 5). Also, with the discontinuation of the National Health Service, the density was changed from 3rd to 6th cycles and from 10.5 to 9 plants/m^2, which, however, did not have any significant effect on Δ^9-THC and CBD content and yield/m^2.

The cannabis plant reacts to many factors such as temperature, humidity, stress, etc., but the most important factor is light (Adams,

Table 5
Average CBD content in dried inflorescence (%/w/w) of individual genotypes over six cycles.

Genotype	1st cycle	2nd cycle	3th cycle	4th cycle	5th cycle	6th cycle	Average/genotype
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Conspiracy Kush	0.55 ± 0.06	0.45 ± 0.08	0.40 ± 0.02	0.52 ± 0.05	0.48 ± 0.04	0.54 ± 0.05	0.49 ± 0.06^{d}
Nurse Jackie	0.59 ± 0.03	0.50 ± 0.02	0.64 ± 0.36	0.52 ± 0.06	0.51 ± 0.06	0.59 ± 0.07	0.56 ± 0.06^{ac}
Jilli Bean	0.46 ± 0.03	0.45 ± 0.02	0.40 ± 0.02	0.60 ± 0.22	0.46 ± 0.03	0.47 ± 1.00	0.47 ± 0.07^{d}
Nordle	0.46 ± 0.06	0.42 ± 0.03	0.35 ± 0.01	0.48 ± 0.02	0.48 ± 0.02	0.52 ± 0.01	0.45 ± 0.06^{d}
Jack Cleaner 2	0.58 ± 0.03	0.60 ± 0.03	0.49 ± 0.02	0.67 ± 0.04	0.59 ± 0.03	0.65 ± 0.06	0.60 ± 0.06^{a}
Jack Skellington	0.61 ± 0.05	0.52 ± 0.03	0.44 ± 0.01	0.49 ± 0.01	0.64 ± 0.01	0.67 ± 0.02	0.56 ± 0.09^{ab}
National Health Service	0.51 ± 0.05	0.51 ± 0.05					0.51 ± 0.00^{bcd}
Average/cycle	0.54 ± 0.06^{bc}	0.49 ± 0.06^{bcd}	0.45 ± 0.1^{d}	0.55 ± 0.07^{ab}	0.53 ± 0.07^{cd}	0.57 ± 0.08^{a}	

Different letters as superscripts in the same column (average/genotype) or row (average/cycle) indicate significant difference ($p < 0.0$).

2012). In addition, the intensity of light and plant density have a greater impact than the genotype on the yields and on the other hand, the genotype has a greater influence on the cannabinoid content (Vanhove et al., 2011; Toonen et al., 2006). The results show that there were no statistically significant differences between genotypes in the yields. In contrast, Δ^9-THC and CBD contained statistically significant differences among genotypes. From the results (See Tables 4 and 5) and the description of the varieties (Table 1), it can be seen that the differences can be attributed to the fact that the hybrid is predominantly ssp. *sativa* or ssp. *indica*. Higher levels of cannabinoids were found in *C. sativa* ssp. *sativa* than in *C. sativa* ssp. *indica*.

In Vanhove et al. (2011), four genotypes (Super Skunk, Northern Light #5 x Haze, White Widow and Big Bud) were tested in one experiment cycle. Vanhove et al. cultivated plants in four different cultivation modifications. 400 W and 600 W fluorescents were used at a density of 16 and 20 plants/m^2. At a density of 16 plants/m^2 and 400 W/m^2, similar yields were achieved in g/m^2 as in our study, but the yields of Vanhove et al. in g/plant were lower, logically due to the higher plant density per m^2 in their study (Vanhove et al., 2011). The Huizer survey estimated cannabis yields of 22 g/plant (Huizer and Poortman-van der Meer, 1995). Although this research was based on confiscated immature plants, resulting in inaccurate estimates of yields (Vanhove et al., 2012), this estimate is consistent with the average yield in g/plant in our study. Toonen et al. (2006) took into account the growth phase in the time of seizure and an average yield of 28.1 g/plant at a density of 15 plants/m^2 and a light of 510 W/m^2 was found.

It is still to be remembered that plants grown in an indoor environment are much more demanding not only for stable cultivation conditions, but also for suitable form of reproduction than plants grown outdoor. In our case, this was the probable cause of the lower yields and the highest Δ^9-THC content of the 1st cycle of plants grown from regular seeds. These plants were further cloned for the 2nd cycle and from that cycle on, each plant was cloned into the next cycle. As six of the genotypes appear to be relatively stable, it had no significant effect over six cycles of plant re-cloning on the yields or cannabinoids content.

A significantly used genotype of MC in the Netherlands is Bedrocan (Bedrocan BV), which has an average Δ^9-THC content of 18 % (15.5–21.0 %) and CBD content up to 1 % (Hazekamp, 2006). The same chemotype is required by the National Agency for Medical Cannabis in the Czech Republic and will probably be demanded in other countries as well. All genotypes evaluated in this study are Δ^9-THC and CBD content compatible with this range.

5. Conclusions

This study shows that within seven tested genotypes the best quantitative parameters are shown by Nurse Jackie and Jilly Bean. The content of biologically active substances was relatively stable by all evaluated genotypes. Only the National Health Services genotype is not suitable for indoor cultivation. While the situation of decriminalization of medical cannabis is changing dramatically across the globe, and, at the same time, the number of patients suffering from lifestyle diseases is increasing, it is necessary to describe the available genotypes urgentlyto make them available for particular patients.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

This work was supported by CULS CIGA Project number: 20162030

References

Adams, P., 2012. Weedology/Marihuana – Vše o pěstování konopí. Positive Publishers.
Casano, S., Grassi, G., Martini, V., Michelozzi, M., 2011. Variations in terpene profiles of different strains of *Cannabis sativa* L. Acta Hortic. 925, 115–122. http://dx.doi.org/10.17660/ActaHortic.2011.925.15.
Decorte, T., 2010. The case for small-scale domestic cannabis cultivation. Int. J. Drug Policy 21, 271–275. http://dx.doi.org/10.1016/j.drugpo.2010.01.009.
Devane, W.A., Dysarz, F., Johnson, M.R., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharm. 34, 605–613.
Dolin B., (2001). National Drug Policy: The Netherlands.
ElSohly, M.A., Slade, D., 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci. 78, 539–548. http://dx.doi.org/10.1016/j.lfs.2005.09.011.
Hanuš, L.O., Mechoulam, R., 2005. Cannabinoid Chemistry: an Overview, In: Cannabinoids as Therapeutics. Birkhäuser Basel, pp. 23–46. http://dx.doi.org/10.1007/3-7643-7358-X_2.
Hanuš, L.O., 2009. Pharmacological and therapeutic secrets of plant and brain (endo) cannabinoids. Med. Res. Rev. http://dx.doi.org/10.1002/med.20135.
Hazekamp, A., Fischedick, J.T., 2012. Cannabis – from cultivar to chemovar. Drug Test. Anal. 4, 660–667. http://dx.doi.org/10.1002/dta.407.
Hazekamp, A., 2006. An evaluation of the quality of medicinal grade cannabis in the Netherlands. Cannabinoids 1, 1–9.
Huizer, H., Poortman-van der Meer, A., 1995. Report on the Yield of Indoor Cannabis Plants. Forensic Laboratory of the Department of Justice.
Mechoulam, R., 2012. Cannabis – A valuable drug that deserves better treatment. Mayo Clin. Proc. 87, 107–109. http://dx.doi.org/10.1016/j.mayocp.2011.12.002.
Michka Cervantes, J., Conrad, C., Jansen, A., Mechoulam, R., Guzman, M., Richard, D., et al., 2015. Medical Cannabis from Marijuana to Synthetic Cannabinoids. Mama Éditions, Paris.
Morgan, C.J., Freeman, T.P., Schafer, G.L., Curran, H.V., 2010. Cannabidiol attenuates the appetitive effects of (9-Tetrahydrocannabinol in humans smoking their chosen cannabis. Neuropsychopharmacology 35, 1879–1885. http://dx.doi.org/10.1038/npp.2010.58.
National Conference of State Legislatures, 2017. State Medical Marijuana Laws. http://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx (accessed 07.12.17).
Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products. United Nations publication, Vienna.
Russo, E., Guy, G.W., 2005. A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med. Hypotheses. http://dx.doi.org/10.1016/j.mehy.2005.08.026.
Russo, E.B., 2011. Taming THC: Potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br. J. Pharmacol. http://dx.doi.org/10.1111/bph.2011.163.
Snoeijer, W., 2001. A checklist of some cannabaceae cultivars. part 1: cannabis. div. pharmacogn. Leiden/Amsterdam cent. Drug Res.
Toonen, M., Ribot, S., Thissen, J., 2006. Yield of illicit indoor cannabis cultivation in The Netherlands. J. Forensic Sci. 51, 1050–1054. http://dx.doi.org/10.1111/j.1556-4029.2006.00228.x.
United Nations, 2016. The International Drug Control Conventions Schedules of the Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol, as at 18 May 2016.
Vanhove, W., Van Damme, P., Meert, N., 2011. Factors determining yield and quality of illicit indoor cannabis (Cannabis spp.) production. Forensic Sci. Int. 212, 158–163. http://dx.doi.org/10.1016/j.forsciint.2011.06.006.
Vanhove, W., Surmont, T., Van Damme, P., De Ruyver, B., 2012. Yield and turnover of illicit indoor cannabis (Cannabis spp.) plantations in Belgium. Forensic Sci. Int. 220, 265–270. http://dx.doi.org/10.1016/j.forsciint.2012.03.013.
Wilkinson, J.D., Whalley, B.J., Baker, D., Pryce, G., Constanti, A., Gibbons, S., Williamson, E.M., 2003. Medicinal cannabis: is Δ 9 – tetrahydrocannabinol necessary for all its effects? J. Pharm. Pharmacol 55, 1687–1694. http://dx.doi.org/10.1211/0022357022304.