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Tracking technical maturity of Cannabis cultivar by trichome morphology analysis and HPLC phytocannabinoid content

Определување на техничка зрелост кај култивиран канабис според морфологијата на трихомите и содржината на канабиноидите

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Абстракт

Анегдоталните податоци во врска со берба на цвет од канабис ја потенцираат значајноста на следење на промените на обојување на главата на трихомите, обојување на толчниците и листовите следени преку лупа или преносен стереомикроскоп. Оваа студија има за цел определување на техничка зрелост преку анализа на содржина на фитоканабиноиди и морфолошки промени на трихоми во култивиран медицинки канабис (Т-492 сорта), за време на финалниот период на зреење.

ХПЛЦ анализата покажа дека вкупниот ТХЦ (%) во примероците од три точки во оранжерија (3600 m^2) од југоисточна точка (SS_{-10}13.14%), централна (CS_{-10} 12.38%) и северозападна точка (NS_{-10} 9.14%) опаѓа. Во однос на временската линија на земање на примероци во југоисточната точка, вкупниот ТХЦ (%) беше највисок почнувајќи од SS_{-10} (13.14%), и потоа опаѓа на SS_{-7} (11.78%), SS_{-2} (11.56%) и SS_h 10.0% на ден на берба.

Највисок процент на транслуцентно-млечно обоени глави на трихоми е забележано кај примерок SS_{-7} (56.25%), % на жолто-портокалови трихоми SS_{-2} (62.22%) и највисок процент на темно кафеави трихоми на ден на берба NS_h (47.22%). Зголемување на процентот на жолто-портокалови и темно кафеави глави на трихоми индицира опаѓање на канабиноидна содржина. Ова истражување дава насоки за идно концептуализирање на студии на корелирање на канабиноидна содржина и промени во трихоми и обојување со антоцијани во различни култивари на канабис.

Abstract

Anecdotal information regarding harvesting of cannabis buds points out the changes of trichome head, pistil and leaves coloration [1,2] monitored by magnifying glass or portable stereomicroscope and many growers use these information as their harvest guide. This study aims at determining technical maturity through analyzing phytocannabinoid content and morphological changes of trichomes in cultivated medical Cannabis (strain T-492), during final maturing period.

HPLC analysis revealed that total THC (%) in the samples from 3 plot spots in greenhouse (3600 m^2) from southeast (SS_{-10}13.14%), central (CS_{-10} 12.38%) and northwest (NS_{-10} 9.14%) spot is declining. Regarding time line of sampling in southeast spot, total THC (%) was the highest starting at SS_{-10} (13.14%) and then decreasing at SS_{-7} (11.78%), SS_{-2} (11.56%) and SS_h 10.0% at harvest.

Highest percentage of translucent-milky trichome heads is observed in SS_{-7} (56.25%), of yellow-orange trichome heads is observed in SS_{-2} (62.22%) and highest percentage of dark brown trichome heads is observed in NS_h (47.22%), on harvest day. Increase in yellow-orange and dark brown colored capitate-stalked trichome heads percentage indicates decrease in cannabinoid content.

This research leads directions for future conceptualization of correlational studies of cannabinoid content and changes in trichomes and anthocyanin coloration in different *Cannabis* cultivars.

1. Introduction

Republic of North Macedonia is one of the countries that legalized growth of Cannabis for medicinal purposes in 2016, following the regulatory requirements for Cannabis quality control. The growth of the Cannabis industry is attributed to fast change of regulations for medicinal and/or recreational use of the plant. Regulatory novelties worldwide require export-import of certified Cannabis seed material. Seed certificate mostly contains information total flowering time and chemical composition expressed as percentage of major phytocannabinoids or as relative ratio between THC:CBD, scarcely stating varietal purity. Every cannabis grower aims to produce high potent herbal substance that complies to the cannabinoid content specification of seed material. Knowing that this plant is highly divergent, it's harvest is particularly time specific for each cultivar or species, therefore growers who are growing different strains cannot harvest them at the same time. There is lack of scientific data regarding cultivation, production, technical maturity and harvest for different strains, due to the historic prohibition of cannabis (*Cannabis sativa* L.) which stunted scientific research and left growers to rely on guides and online resources mostly based on anecdotal information [3]. Despite lack of scientific data, state-of-the-art knowledge on indoor cannabis production is mainly obtained from so-called 'grey' resources [4–7]. Anecdotal information regarding harvesting of cannabis buds points out the changes of trichome head, pistil and leaves coloration [1,2] monitored by magnifying glass or portable stereomicroscope and many growers use these information as their harvest guide. Official methods utilized for technical maturity assessment are HPLC methods for analysis of phytocannabinoid content such as monograph of cannabis flos of the German Pharmacopoeia and the Union method established by the European Commission for the quantitative determination of the Δ9 -THC content in hemp varieties [8–10]. These methods are accurate and give detailed information regarding cannabinoid content but can be time consuming and require more resources. Novel research points out the utilization of ATR-MIR spectroscopy for quantification of the main critical parameters in Cannabis flower and extract samples, showing high potential for utilizing this technique for technical maturity assessment [11]. Understanding there is lack of scientific data for different Cannabis strains and cultivars investigating the anecdotal information, the main objective of this study is to monitor technical maturity by correlating phytocannabinoid content with changes in trichome head coloration by stereomicroscope analysis and phytocannabinoid content in different time points.

2. Materials and methods

2.1 Plant material

Marijuana-type T-492 strain was soil-grown from seed in greenhouse with surface area 3600 m^2 under controlled conditions (temperature, moisture, light). Seed certification of this strain states that T-492 is Δ9-THC predominant strain with total Δ9-THC content up to 20% and very low levels of total CBD up to 0.03%. For sampling three sampling plot spots of the greenhouse were chosen: southeast spot (SS), central spot (CS) and northwest spot (NS). Prior analysis, sampling of Cannabis inflorescence was done from 4 plants per spot. Sampling was performed 10 days prior harvest (SS_{-10}, CS_{-10}, NS_{-10}), 7 days prior harvest (SS_{-7}, CS_{-7}, NS_{-7}), 2 days prior harvest (SS_{-2}, CS_{-2}, NS_{-2}) and harvest day (SS_h, CS_h, NS_h).

2.2 Standards, solvents and reagents

Cannabidiol CRM solution concentration 1 mg/mL in methanol (CAS: 13956-29-1, purity 98.66%), cannabinol CRM solution concentration 1mg/mL in methanol (CAS: 521-35-7, purity 99.50%), (-)-Δ9 -tetrahydrocannabinol CRM solution concentration 1mg/mL in methanol (CAS: 1972-08-3, purity 99.39%), Δ9 -tetrahydrocannabinolic acid A CRM solution concentration 1 mg/mL in acetonitrile (CAS: 23978-85-0, purity 96.99%) and cannabidiolic acid CRM solution concentration 1 mg/mL in acetonitrile (CAS: 1244-58-2, purity 97.88%) were purchased from Cerilliant Corporation (USA). 85% o-phosphoric acid and acetonitrile HPLC grade were purchased from Carlo Erba. Ethanol 96% PhEur grade was purchased from Alkaloid AD Skopje.

2.3 Trichome morphology analysis

Zeiss Stemi 508 stereomicroscope connected to licensed software ZEN 2.6 (blue edition) was used for accessing thrichomes morphology. Instrument and software settings were set as follows: camera adapter zoom 0.5x, objective 1x, zoom 5x, reflector BF, light adaptation was manual. Files were saved as CZI and TIF format for further analysis and processing. Trichome morphology analysis was performed mostly on brachtaeal capitate-stalked trichomes due to lower density of trichomes, better visualization and literature data pointing out highest cannabinoid content in stalked trichomes [12–14]. Capitate-stalked trichomes were classified in three groups by different coloration: translucent-milky, yellow-orange and dark brown trichome heads. Trichome count was highly dependent on quality of the taken images with stereomicroscope and only trichomes with good distinction in color were counted. Total count and percentage of total count for each capitate-stalked trichome head coloration of all sampled spots is presented in Table 1 in results section.

2.4 Instrumentation and Chromatographic conditions

For determination of cannabinoid content DAB Pharmacopoeial method for assay of cannabinoids was 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, Agilent Technologies, USA). Mobile phase consisted of aqueous solution of orthophosphoric acid (8.64 g/L) as solvent A and acetonitrile isocratic grade as solvent B. Change of solvent gradient was as follows: 0 – 16 minute from 36% to 18% A linear gradient, 16 – 17 minute 18% to 36% A linear gradient and from 17 to 30 minute re-equilibration of column with 0.7 mL/min flow rate. Column compartment temperature was maintained at 40°C throughout analysis and DAD measurements were carried out at 225 nm wavelength neutral cannabinoids and 306 nm wavelength for acidic cannabinoid forms For accurate calculation of cannabinoid content loss on drying percentage for each sample was determined according to DAB monograph for Cannabis flower [8].

2.4 Sample preparation

Sample preparation was performed as instructed in DAB Pharmacopoeial method for assay of cannabinoids. for Analysis was performed on 500 mg fresh, not decarboxylated cannabis flower. Final concentration of plant material was 1 mg/mL.

3. Results

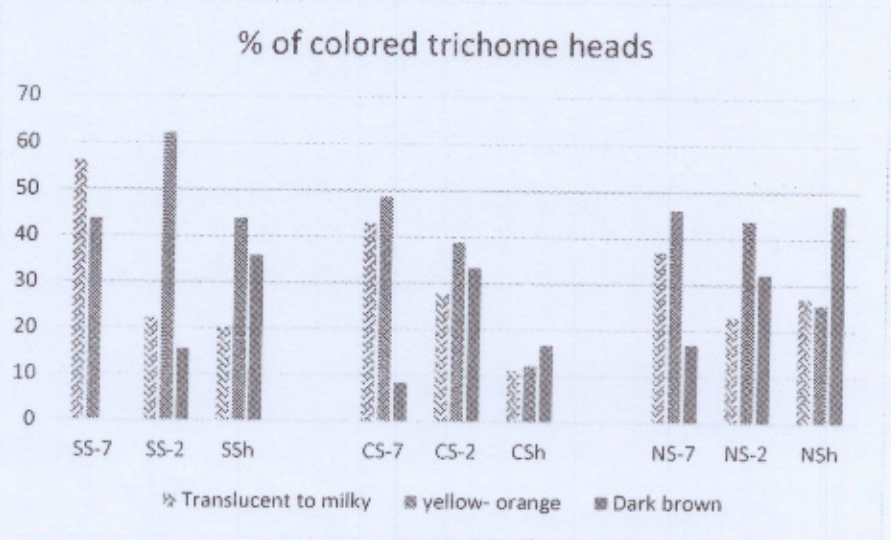
3.1 Trichome head coloration analysis

Three different stadiums of morphological changes in trichome head coloration are presented in Table. 1 and Fig. 1. Highest percentage of translucent-milky trichome heads is observed in SS_{-7}, CS_{-7} and NS_{-7} with 56.25%, 42.99% and 37.08% respectfully. Percentage drop of translucent-milky trichomes is noted in all tree sample plot spots from SS_{-7} (56.25%) to SS_h (20.28%), CS_{-7} (42.99%) to CS_h (11.11%) and NS_{-7} (37.08%) to NS_h (27.08%). Highest percentage of yellow-orange trichome heads is observed in SS_{-2}, CS_{-7}, NS_{-7} with 62.22%, 48.60% and 46.07% respectively. There is no significant difference in percentage of yellow-orange trichome heads in SS_{-7} (43.75%) to SS_h (43.92%), significant increase of percentage is noted from CS_{-7} (48.60%) to CS_h (72.22%), and decrease of percentage in NS_{-7} (46.07%) to NS_h (25.69%). Highest percentage of dark brown trichome heads is observed in NS_h, SSh and CS_{-2} with 47.22%, 35.81% and 33.33% respectively. Dark brown trichomes are not detected in SS_{-7}. Increase in percentage of dark brown trichomes is noted in SS (from 15.56% to 35.81%) and NS (from 16.85% to 47.72%) samples, whereas CS varies in % of dark brown trichomes in sampling timeline (CS_{-7} 8.41%, CS_{-2} 33.33%, CS_h 16.67%).

Table 1 Trichome head coloration count and % of total counted colored trichomes.

Time point	SS_{-7}	SS_{-2}	SS_h	CS_{-7}	CS_{-2}	CS_h	NS_{-7}	NS_{-2}	NS_h
Total tichome count	32	45	148	107	144	36	178	87	144
Translucent-milky trichomes (%)	56.25%	22.22%	20.27%	42.99%	27.77%	11.11%	37.08%	22.99%	27.08%
Yellow-orange trichomes (%)	43.75%	62.22%	43.92%	48.60%	38.89%	72.22%	46.07%	43.83%	25.69%
Dark brown trichomes (%)	0%	15.56%	35.81%	8.41	33.33%	16.67%	16.85%	32.19%	47.222%

Fig. 1 Graphical representation of percentage of different trichome head colorations



3.2. Cannabinoid content analysis

Changes in cannabinoid content of the analyzed samples are given in Table 2 and graphically presented with Fig. 2 and calibration curves are presented in Table 3. Analyzed samples revealed levels of total CBDA are below limit of quantification in each analyzed sample throughout sampling time points and CBD is not detected, therefore total CBD is below limit of quantification. Highest total Δ9-THC content is observed in SS_{-10} (13.14%), CS_{-7} (12.38%) and NS_{-10} (9.04%). Total Δ9-THC in SS notes drop of 10.35% of total content from SS_{-10} to SS_{-7}, 12.02% from SS_{-10} to SS_{-2} and 23.90% from SS_{-10} to SS_h. Percentage drop of total Δ9-THC in SS_{-7} and SS_{-2} are similar, whereas they differ in Δ9-THCA and Δ9-THC content, notifying increase in Δ9-THC content from 0.22% to 0.33% and detection of CBN below limit of quantification. CS cannabinoid content changes marks inconsistencies throughout the timeline of sampling with increase of total Δ9-THC content for 21.49% from CS_{-10} to CS_{-7}, drop of 27.06% from CS_{-7} to CS_{-2} and again increase 22.16% from CS_{-2} to CS_{-7}. Northwest spot notes smallest difference in drop of total Δ9-THC between sampling time points, with 8.10 % relative percentage from NS_{-10} to NS_h.

Table 2 Cannabinoid content in Southeast spot, Central and northwest spot in different sampling time points: 10 days prior harvest, 7 days prior harvest, 2 days prior harvest and harvest day; CBDA (cannabidiolic acid), CBD (Cannabidiol), CBN (cannabinol), (-)-Δ9 –THC ((-)-Δ9 –tetrahydrocannabinol) and Δ9 –THCA A (Δ9 -tetrahydrocannabinolic acid A), BLQ (below limit of quantification), ND (not detected), LOD (loss on drying).

(m/m %)	Southeast spot				Central spot				Northwest spot			
Components	SS_{-10}	SS_{-7}	SS_{-2}	SS_H	CS_{-10}	CS_{-7}	CS_{-2}	CS_H	NS_{-10}	NS_{-7}	NS_{-2}	NS_H
CBDA	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
CBD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CBN	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
(-)-Δ9 –THC	0.23	0.22	0.33	0.16	0.15	0.14	0.20	0.14	0.15	0.12	0.22	0.11
Δ9 –THCA A	14.72	13.18	12.8	11.22	10.91	13.95	10.06	13.07	10.25	10.17	9.63	9.45
Total CBD	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Total (-)-Δ9 –THC	13.14	11.78	11.56	10.00	9.72	12.38	9.03	11.60	9.14	9.04	8.67	8.40
LOD	76.90	73.01	74.62	67.89	75.38	71.15	70.28	71.57	77.76	67.94	69.75	73.18

Fig. 2 Changes in total THC in SS, CS and NS (10 days prior harvest to harvest day).

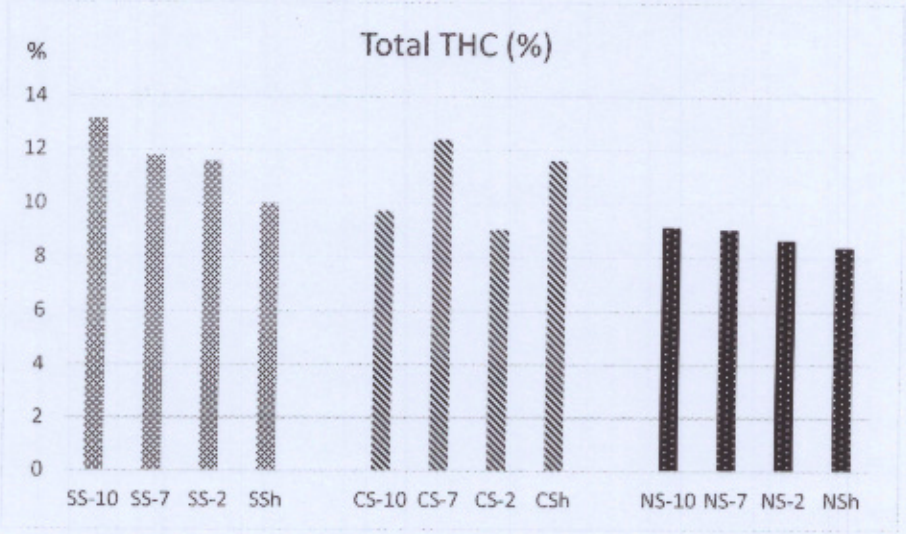


Table 3 Calibration curve equations and their respective R^2 values.

Component	Concentration range	Linear equation	R^2 value
CBDA	0.5 - 100 µg/mL	y= 11043x + 8.0762	0.9997
CBD	0.5 - 75 µg/mL	y= 38138x + 10.994	0.9996
CBN	0.1 - 10 µg/mL	y= 95429x + 1.7425	1.0000
Δ9-THC	0.5 - 75 µg/mL	y= 35199x + 3.1263	0.9998
Δ9-THCA-A	0.5 - 250 µg/mL	y= 13334x+12.01	0.9999

4. Discussion

The morphology of trichomes and cannabinoid profile are dependent on genetic and environmental factors [15]. On female cannabis flowers, three types of glandular trichomes have been described based upon their surface morphology: bulbous, sessile, and stalked [16]. Bulbous trichomes are the smallest in size and produce limited specialized metabolites [17]. Sessile trichomes of cannabis sit on the epidermis with a short stalk and have a globose head comprised of a multicellular disc of secretory cells and a subcuticular metabolite storage cavity [13]. By comparison, stalked trichomes of cannabis have a similarly shaped, slightly larger, globose head elevated several hundreds of microns above the epidermal surface by a multicellular stalk [12,18]. In our research, stalked glandular trichomes were chosen for stereomicroscope analysis based on literature data [12,14]. One research used two photon laser scanning fluorescence microscopy to analyze the excited intrinsic fluorescence of metabolites and noted that stalked trichomes have highest cannabinoid content by monitoring the strong blue shifted fluorescence [14]. The research also confirms that cannabis stalked glandular trichomes represent a terminal state of differentiation for floral sessile glandular trichomes, as opposed to the drastically different morphology and developmental trajectories of capitate and peltate glandular trichomes in other species [14].

Research from 2004 year states that glands can be classified according to their secretory phases from the color of their contents and glands most active in secretion (mature) are translucent at appearance, whereas aged glands are yellow and senescent glands are brown. They came to few conclusions: capitate-stalked glands contained more THC (and total cannabinoids) than capitate-sessile glands, glands at different positions on the leaf or bract can differ in cannabinoid content, THC, and total cannabinoid, quantity in both gland types can differ during the year, cannabinoid contents in glands decreased with aging of glands and cannabinoids occurred in the secretory cavity of the gland [12].

Our data analysis shows correlation between changes of cannabinoid content and capitate-stalked trichome head coloration which can be seen in samples from southeast and northwest spot. Cannabinoid content in SS_{-7} to SS_h is declining by 1.78% total Δ9-THC, from 11.78% to 10.00% total Δ9-THC, notifying change in trichome heads from SS_{-7} to SS_h with decrease in percentage of translucent-milky trichome heads from SS_{-7} (56.25%) to SS_h (20.27%) and increase in total % of yellow-orange and dark brown trichome from SS_{-7} (43.75%) to SS_{-7} (79.728%).

Northwest spot has smaller difference in cannabinoid content decline by 0.64% total Δ9-THC, from 9.04% to 8.4% total Δ9-THC with change in trichome head coloration from NS_{-7} to NS_h with decrease in percentage of translucent- milky trichome heads from NS_{-7} (37.08%) to NS_h (27.08%) respectfully and increase in total % of yellow-orange and dark brown trichome heads from NS_{-7} (62.92%) to NS_h (72.92%).

Central spot cannabinoid content changes marks inconsistencies throughout the timeline of sampling, with macroscopic studies throughout timeline stating higher brachteal representation in CS_{-10} and CS_{-2}, correlating this with decrease in total Δ9-THC content in CS_{-2}.

CS_{-7} total Δ9-THC is 13.95%, declining to 10.06% in CS_{-2} and 13.07% total Δ9-THC at harvest day. Change in trichome head coloration from CS_{-7} to CS_h, with decrease noted in percentage of translucent-milky trichome heads from CS_{-7} (42.99%), CS_{-2} (27.77%) and CS_h (11.11%) and increase in total percentage of yellow-orange and dark brown trichome heads from CS_{-7} (57.01%), CS_{-2} (72.22%) to CS_h (88.89%).

The key difference correlating the lower cannabinoid content in CS_{-2} (9.03%) and trichome coloration is in the changes in ratio of yellow-orange and dark brown trichome heads. In CS_{-2} total percentage of yellow-orange trichome heads is 38.89% and percentage of dark brown trichome heads is 33.33%, whereas in CS_{-7} and CS_h % of orange to light brown trichome heads is 42.99% and 72.22% respectively and percentage of dark brown trichome heads is 8.41% and 16.66% respecttively. CS_{-2} has higher percentage of dark brown trichomes than CS_{-7} and CS_h, indicating cannabinoid content loss.

Southeast spot has highest cannabinoid content and northwest spot lowest cannabinoid content, pointing out that even in uniform greenhouse conditions, cannabinoid content varies depending on geographical position in the greenhouse.

These results indicate that harvest of the plant material cultivated in greenhouse should be divided into areas of the greenhouse and not mixed for further processing, but analyzed to check for cannabinoid content similarity. If there are differences in cannabinoid content, regardless being same strain, the end product should be further processed and packed separately. There is no research data that points out the limits and ranges that can be used as decision rule whether to mix Cannabis flowers from same batch or separate them according to differences in cannabinoid content for further processing of the material.

Steromicroscope analysis also revealed anthocyanin coloration in many of the samples. In some of the samples anthocyanin coloration was weak, barely identifiable, hence in others the coloration was intense. Intensive anthocyanin coloration was noted at the base and stalk of capitate-stalked trichome and epidermal tissue anthocyanin coloration in all of the samples. As it is known, some strains such as the strain "Purple God", produce anthocyanin pigmentation. In strains where such pigmentation is a characteristic feature, the pistils and surrounding bract tissues develop a red or purple pigmentation [19]. It is also known that anthocyanin synthesis can be induced by environmental stress [20]. Anthocyanins accumulate in plants upon exposure to drought, salt stress, UV stress, high light and high temperature [21–23] and are, therefore, considered nature's "Swiss army knife" of plant responses to stress [24–26]. The main roles attributed to anthocyanins in mediating responses to stress are linked with their antioxidant [27,28], light-screening [22,24,29–31] and photoprotective properties [32,33]. Several senescence-related physiological changes have been linked to increased susceptibility to photoinhibition, such as reduced capacity to repair PSII reaction centers [34], chlorophyll degradation [35] which led to greater light sensitivity, resulting in photodamage at relatively modest irradiances [34]. Imbalance on the photosynthetic apparatus caused by differential rates of decline during senescence increases vulnerability to photoinhibition [36]. Several studies have found [37–39] relationship between chlorophyll degradation and anthocyanin production. These studies revealed that anthocyanin accumulation begins shortly after the onset of chlorophyll decline, typically before any visible change in leaf color. This demonstrates a direct association between anthocyanin production and the period of increased vulnerability to photoinhibition during senescence, and provides further evidence that anthocyanins may perform a photoprotective role [32]. There is no literature data to our knowledge that tests the correlation

between changes in cannabinoid content and anthocyanin coloration in trichomes, giving this research field a very exciting and unique direction of future experimentally designed studies.

5. Conclusion

There is great potential for use of trichome morphology analysis, especially trichome head coloration, in determination of technical maturity of Cannabis plants, but yet it remains unexplored topic. Our study confirms the correlation in changes of cannabinoid content with change of color in capitate-stalked trichome heads, but only concerning the chosen strain. With scarcely any published articles regarding this topic, this research leads directions for future conceptualization of correlational studies of cannabinoid content and changes in trichome head coloration. On the other hand, there is no literature data to our knowledge that tests the correlation between changes in cannabinoid content and anthocyanin coloration in trichomes, giving this research field a very exciting and unique direction of future experimentally designed studies.

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Conflict of interest

The authors declare no conflict of interest.

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УНИВЕРЗИТЕТ „СВ. КИРИЛ И МЕТОДИЈ“ ВО СКОПЈЕ
ШКОЛА ЗА ДОКТОРСКИ СТУДИИ
ФАРМАЦЕВТСКИ ФАКУЛТЕТ
Студиска програма: Трет циклус за докторски студии од областа фармација

РЕЦЕНЗИЈА

на семинарски труд од III семестар под наслов „ Tracking technical maturity of Cannabis cultivar by trichome morphology analysis and HPLC phytocannabinoid content“ или „Определување на техничка зрелост кај култивиран канабис според морфологијата на трихомите и содржината на канабиноидите“ на докторандот, магистер по фармација Вероника Стоилковска Ѓоргиевска

Со одлука на Советот на докторски студии на Фармацевтскиот факултет во Скопје, определена е рецензентска комисија за оцена на семинарскиот труд од трет циклус на докторски студии во состав: проф. д-р Ѓоше Стефков, проф. д-р Светлана Кулеванова и проф. д-р Марија Карапанџова. По прегледот на доставениот семинарски труд, Рецензентската комисија го доставува следниот

ИЗВЕШТАЈ

Семинарскиот труд под наслов **„Определување на техничка зрелост кај култивиран канабис според морфологијата на трихомите и содржината на канабиноидите** “ претставува самостојно изработен труд, структуриран во следните поглавја: вовед, цели, материјали и методи, резултати, дискусија, заклучок и прилози. Систематизацијата на деловите во наслови и поднаслови обезбедува соодветно следење на материјата која е обработена во трудот.

Во „**Воведот**”, докторандот дава мал осврт на историски значаен период на легализација на канабисот за медицинска употреба во Р.С. Македонија, значајноста на регулаторните барања и промена на истите. Потенцира дека постои недостаток на научни податоци за култивирање, производство, определување на техничка зрелост и берба на различни сорти од канабис, ставајќи акцент на техничката зрелост. Докторандот дава детален осврт на анегдоталните информации за определување на техничка зрелост и веќе постоечките научни методи кои рутински се користат за определување на техничка зрелост, но се долготрајни и поскапи. На крајот од воведот докторандот ја изложува главната цел која е следење на техничката зрелост на канабис преку содржина на канабиноиди поврзувајќи ја со промените во обојување на глава на трихоми со анализа со стереомикроскоп.

Во делот „**Материјали и методи**”, дадени се основните податоци за растителниот материјал кој е користен за анализа. Опишан е начинот на кој е изведено узорцирањето на примероци во текот на 4 временски точки при следење на техничката зрелост на испитуваната сорта. Приложени се експерименталните услови користени за стереомикроскопска анализа и објаснување како е направена класификацијата на трихоми според обојувањето на главата на трихомите (поделба на транслуцентно-млечни, жолто-портокалови и темно кафеави обоени глави од главичести трихоми со врат). Детално се наведени сите реагенси кои се користени за изведба на хроматографска анализа според метода за содржина на канабиноиди според Германската фармакопеја, детално објаснувајќи ги сите информации околу истата. Подготовката на примероци за анализа на HPLC е накратко посочена, посочувајќи дека методологијата за подготовка на примерок е методолошки опишана во фармакопејскиот пропис посочувајќи дека е анализирана 1 mg/mL концентрација на растителен материјал.

Во делот „**Резултати**”, на почетокот се презентирани резултатите од стереомикроскопскат анализа на цвет од канабис, потенцирајќи дека сите резултати се прикажани со табеларен приказ (Табела 1) и приказ со дијаграм (Слика 1) каде соодветно се воочуваат разликите. Докторандот изнесува интересен податок околу варијабилноста на процентите на различно обоени трихоми во различен временски период.

Највисок процент на транслуцентно-млечно обоени глави на трихоми е забележано кај примерок SS_{-7} (56.25%), точка седум дена пред берба, процент на жолто-портокалови трихоми SS_{-2} (62.22%), точка два дена пред берба и највисок процент на темно кафеави трихоми на ден на берба NS_h (47.22%). Со цел определување на содржина на канабиноиди, приложен е табеларен приказ (Табела 3) калибрационите криви од пет канабиноиди (CBDA, CBD, CBN, Δ9-THC и Δ9-THCA) со коефициент на корелација за линеарност на одговорот поголем од 0.999 за сите анализирани канабиноиди во соодветен концентрациски ранг приложен табеларно во самиот труд. Пресметаните резултати за содржина се прикажани со табеларен приказ со детални информации за параметарот губиток со сушење и содржина на поедините компоненти. Со оваа анализа се покажува дека вкупниот Δ9-THC (%) во примероците од три точки во оранжерија (3600 m^2) од југоисточна точка (SS_{-10}13.14%), централна (CS_{-10} 12.38%) и северозападна точка (NS_{-10} 9.14%) опаѓа. Во однос на временската линија на земање на примероци во југоисточната точка, вкупниот Δ9-THC (%) бил највисок почнувајќи од SS_{-10} (13.14%), и потоа опаѓа на SS_{-7} (11.78%), SS_{-2} (11.56%) и SS_h (10.0%) на ден на берба.

Во делот „**Дискусија**", докторандот се осврнува на морфологијата на трихоми и научните податоци за типови на трихоми кои се застапени кај цвет од канабис. Го поткрепува изборот на трихоми за стереомикроскопска анализа со научни податоци и споделува научни трудови (кои се во многу мал број) во кои се следи проблематиката за определување на техничка зрелост преку проценка на морфолошките карактеристики на трихомите како обојување на глава на трихоми. Докторандот ја приложува поврзаноста на опаѓање на содржината на канабиноиди со промена на бојата на главата на трихоми од транслуцентно-млечна кон темно-кафеава боја. Притоа потенцира и дека географското позиционирање на растенијата во оранжеријата е значајно, бидејќи во ова истражување се забележува опаѓање на содржината од југоисточна кон северозападна страна на оранжеријата. Овде докторандот сугерира дека бербата на материјалот треба да биде поделена на зони од оранжеријата и следствено да се определува точната содржина на канабиноиди со цел правилно пакување и хомогеност на растителниот материјал од аспект на содржина на канабиноиди. Докторандот исто така се надоврзува и на забележување на појавата на обојување на антоцијани на самиот брактеален лист кој се анализира како дел од цветот или пак ги бои трихомите. Се надоврзува на литературни податоци за значењето на присутност на антоцијани во насока на стареење на растението.

Во **заклучните согледувања**, докторандот нагласува дека постои голем потенцијал за следење на морфологијата на трихоми како алатка за определување на техничката зрелост на канабис, но дека сеуште претставува недоволно истражена тематика. Докторандот образложува дека со оваа студија се коментира корелацијата меѓу промена на содржината на канабиноидите и промената на обојување на главата на трихомите, конкретно за испитуваната сорта на канабис. Ова истражување дава насоки за идно концептуализирање на студии на корелирање на канабиноидна содржина и промени во трихоми и обојување на антоцијани во различни култивари на канабис.

ЗАКЛУЧОК

По прегледот на семинарскиот труд под наслов „Определување на техничка зрелост кај култивиран канабис според морфологијата на трихомите и содржината на канабиноидите" на докторандот Вероника Стоилковска Ѓоргиевска, Рецензентската комисија констатира дека станува збор за значаен научен труд кој ја обработува проблематика за определување на техничката зрелост на култивиран канабис со корелацијата меѓу промена на канабиноидната содржина и промена на обојување на главата на трихомите .

ОЦЕНКА И ПРЕДЛОГ

Врз основа на горенаведеното, Рецензентската комисија позитивно го оценува доставениот семинарски труд под наслов „ Определување на техничка зрелост кај култивиран канабис според морфологијата на трихомите и содржината на канабиноидите“ на докторандот Вероника Стоилковска Ѓоргиевска и му предлага на Советот на докторски студии на Фармацевтскиот факултет при УКИМ во Скопје да го прифати и закаже негова презентација.

Рецензентска комисија:

Проф. д-р Ѓоше Стефков

Проф. д-р Светлана Кулеванова

Проф. д-р Марија Карапанџова