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Original Article

Monitoring of Cannabis Cultivar Technological Maturity by Trichome Morphology Analysis and HPLC Phytocannabinoid Content

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ABSTRACT

Objectives: This study aims at determining technological maturity (ten, seven, two days prior and at harvest day) through analyzing morphological changes of trichomes and phytocannabinoid content in samples from cultivated medical cannabis at three plot spots (southeast - SE, central - CN and northwest - NW) in greenhouse (with 3600 m^2). **Materials and Methods:** Soil-grown medical cannabis T-492 plant flowers were sampled within a ten days' timeline from three plot spots of 3600 m^2 greenhouse. Morphology analysis was performed using Zeiss Stemi 508 stereomicroscope and phytocannabinoids content assessment was performed using German pharmacopoeial method for assay of cannabinoids. **Results:** HPLC analysis revealed that total THC (%) in the samples was declining from SE (13.14%), all the way through CN (12.38%) to NW (9.14%) spot. Regarding timeline, total THC (%) was the highest in the southeast location, starting with the highest levels on the tenth day before harvest (SE_{-10}- 13.14%) and then decreasing on the seventh day prior to harvest (SE_{-7} -11.78%), on the second day prior harvest (SE_{-2}-11.56%) andat the harvest day (SE_h- 10.00%). The presence of translucent-milky, yellow-orange and dark brown trichome heads was observed. An increment of the number of yellow-orange and dark brown colored capitate-stalked trichome heads was connected with cannabinoid content declining. **Conclusion:** This research gives directions for future conceptualization of correlational studies of cannabinoid content and changes in trichomes and purple coloration (probably from anthocyanins) in different Cannabis cultivars.

Keywords: Cannabinoid potency, Cultivation, Flowers, Harvest, Hemp, Liquid chromatography, Trichome morphology.

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INTRODUCTION

Following the regulatory requirements for medical cannabis quality control, Republic of North Macedonia is one of the countries that legalized growth of cannabis for medicinal purposes in 2016. 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 certificates mostly contain information for flowering time and chemical composition expressed as percentage of major phytocannabinoids or as relative ratio between THC and CBD, scarcely stating varietal purity. Every cannabis grower aims to produce potent herbal substances complying with the cannabinoid content specification of seed material. Particularly time of harvesting is specific for each cultivar or species, therefore growers who are growing different strains or even the same strain, cannot harvest different cultivars at the same harvest period. There is lack of scientific data regarding cultivation, production, technological maturity and harvest of 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 empirical information.[1]

Despite lack of scientific data, state-of-the-art knowledge on indoor cannabis production is mainly obtained from so-called 'grey' resources.[2-5] Information from different growers' experiences regarding the harvesting of cannabis flowers points out the changes in trichome head coloration (clear, cloudy, and amber or clear, cloudy, and dark brown/amber), pistil and leaves coloration[6,7] monitored by the magnifying glass or portable microscope. However, most utilized method for technical maturity assessment is HPLC method for analysis of phytocannabinoids content given in the German Pharmacopoeia as monograph for *Cannabis flos* and the Union method established

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by the European Commission for the quantitative determination of Δ9-THC content in hemp varieties.[8-10] These methods are accurate and give detailed information regarding cannabinoid content but also are time consuming and require more resources. Novel research points out the utilization of ATR-MIR (attenuated total reflectance mid-infrared) spectroscopy for quantification of the main critical parameters in cannabis flower and extract samples, showing high potential for utilizing this technique for technological maturity assessment.[11] Understanding that there is a lack of scientific data for different cannabis strains and cultivars investigating information from growers' experience, the main objective of this study is to monitor the technological maturity by observing and finding connection between phytocannabinoid content with changes in capitate-stalked trichome head coloration by stereomicroscope analysis and phytocannabinoid content determination in different time points.

MATERIALS AND METHODS

Plant material

Medical cannabis T-492 strain was soil-grown from seed in pots in the greenhouse with a surface area 3600 m^2, under controlled conditions (including temperature, moisture, and 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%). Three sampling plot spots of the greenhouse were chosen: southeast spot (SE), central spot (CN) and northwest spot (NW). Prior analysis, sampling of Cannabis inflorescence was done from four plants per spot. Sampling was performed ten days prior harvest (SE_{-10}, CN_{-10}, NW_{-10}), seven days prior harvest (SE_{-7}, CN_{-7}, NW_{-7}), two days prior harvest (SE_{-2}, CN_{-2}, NW_{-2}) and at the harvest day (SE_h, CN_h, NW_h).

Standards, solvents and reagents

Cannabidiol (CBD) CRM solution with concentration of 1 mg/mL in methanol (purity 98.66%), cannabinol (CBN) CRM solution with concentration of 1 mg/mL in methanol (purity 99.50%), (-)-Δ9-tetrahydrocannabinol (Δ9-THC) CRM solution with concentration of 1 mg/mL in methanol (purity 99.39%), Δ9-tetrahydrocannabinolic acid A (Δ9-THCA-A) CRM solution with concentration of 1 mg/mL in acetonitrile (purity 96.99%) and cannabidiolic acid (CBDA) CRM solution with concentration of 1 mg/mL in acetonitrile (purity 97.88%) were purchased from Cerilliant Corporation (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.

Trichome morphology analysis

Zeiss Stemi 508 stereomicroscope connected to licensed software ZEN 2.6 (blue edition) was used for accessing trichomes morphology. Instrument and software settings were set as follows: camera adapter zoom 0.5x, objective 1x, zoom up to 5x, reflector BF, light adaptation was manual. Photos were taken and saved in CZI and TIF format for further analysis and processing. Trichome morphology analysis was performed on photos of brachtaeal capitate-stalked trichomes. Capitate-stalked trichomes (CST) were classified in three groups by different head coloration: translucent-milky (CST_{t-m}), yellow-orange (CST_{y-o}) and dark brown (CST_{db}) trichome heads. Capitate-stalked trichome head coloration relative ratio of abundance was calculated as a proportion of colored CST of total CST at the sampling time points.

HPLC method for cannabinoid content assessment

For determination of cannabinoid content, German Pharmacopoieal 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 Infinity Lab Poroshell 120 EC-C_{18} chromatographic column (150 mm x 3 mm ID, 2.7 µm, Agilent Technologies, USA). Mobile phase consisted of aqueous solution of *o*-phosphoric acid (8.64 g/L) as solvent A and acetonitrile as solvent B. Change of solvent gradient was as follows: 0 – 16 min from 36% to 18% A linear gradient, 16 – 17 min 18% to 36% A linear gradient and from 17 to 30 min re-equilibration of column with flow rate 0.7 mL/min. Column compartment temperature was maintained at 40°C throughout analysis and DAD measurements were carried out at 225 nm wavelength for neutral cannabinoids and 306 nm wavelength for acidic cannabinoid forms. For accurate calculation of cannabinoid content, loss on drying for each sample was determined according to German pharmacopoeial monograph for cannabis flower.[8] Sample preparation was performed as instructed in German Pharmacopoeial method for assay of cannabinoids. For that purpose, 500 mg fresh, and not decarboxylated cannabis flower was used. The final concentration of plant material was 1 mg/mL. Calibration curves for each cannabinoid are presented in Table 1.

RESULTS

Trichome head coloration analysis

Collected samples from three plot spots, during final maturation period, were visually analyzed by using stereomicroscope Zeiss Stemi 508. Cannabis flower and brachtaeal capitate-sessile glandular, capitate-stalked glandular and non-glandular unicellular, smooth mechanical trichomes were analyzed, focusing on the morphology of the brachtaeal capitate-stalked glandular trichomes, for further processing. Literature data classifies glands according to the color of trichome heads and cannabinoid content, corresponding to their secretory phases,

Table 1: **Calibration curve equations for five cannabinoids 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

Table 2: **Capitate-stalked trichome head coloration (presence of total counted capitate-stalked colored trichomes per location and time point).**

Time point	SE_{-7}	SE_{-2}	SE_{-h}	CN_{-7}	CN_{-2}	CN_{-h}	NW_{-7}	NW_{-2}	NW_{-h}
CST_{t-m} (%)	56.25	22.22	20.27	42.99	27.77	11.11	37.08	22.99	27.08
CST_{y-o} (%)	43.75	62.22	43.92	48.60	38.89	72.22	46.07	43.83	25.69
CST_{db} (%)	/	15.56	35.81	8.41	33.33	16.67	16.85	32.19	47.22

in three groups: translucent (mature), yellow (aged) and brown (senescent) glands.[12] In this study three distinct physiological maturation stages of CST (capitate-stalked trichomes) were monitored: CST_{t-m} (translucent-milky), CST_{y-o} (yellow-orange) and CST_{db} (dark brown) trichome heads (Figure 1-A, B, C). The results regarding capitate-stalked trichome head coloration (% of total counted capitate-stalked colored trichomes per location and time point) are presented in Table 2. Physiological maturing classification of CST head coloration was assessed based on literature data as mentioned above.[12,13]

CST_{t-m} had highest occurrence seven days before harvest, at all sampled positions SE_{-7} (56.25%), CN_{-7} (42.99%), NW_{-7} (37.08%) and their presence was declining towards harvest day. Seven days prior to harvest, these CST_{t-m} were predominant only in the flowers at SE position, while at CN and NW, CST_{y-o} were prevalent with 48.60% and 46.07%, respectively. Two days before harvest, at all samples from SE, CN and NW, CST_{y-o} were with principal presence, with 62.22%, 38.39% and 43.83%, respectively. Approaching towards end point, two days before harvest predominance started turning from CST_{t-m} to CST_{y-o} at positions SE_{-2} (62.22%), CN_{-2} (38.89%) and NW_{-2} (43.83%). At the harvest day, this situation was changed only at NW where CST_{db} (47.22%) occurred to be with major presence while at SE and CN positions remained with predominant presence of CST_{y-o}, with 43.92% and 72.22%, respectively.

Cannabinoid content analysis

Changes in cannabinoid (CBDA, CBD, CBN, (-)-Δ9-THC and Δ9-THCA A) content of the analyzed samples from three spots - SE, CN and NW during the sampling period, are given in Table 3. All analyzed samples had levels of total CBDA below limit of quantification, throughout sampling time points. Additionally, CBD was not detected, therefore total CBD was insignificant.

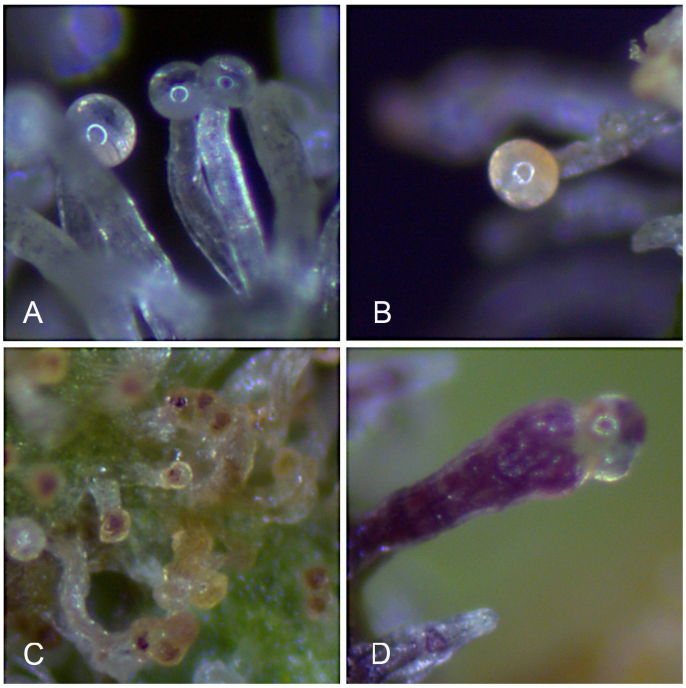

Figure 1: A: CST_{t-m} (capitate stalked translucent-milky trichome heads), B: CST_{y-o} (capitate stalked yellow-orange trichome heads), C: CST_{db} (capitate stalked dark brown trichome heads) and D: purple coloration (probably from anthocyanins) in trichomes.

Highest total Δ9-THC content is determined ten days prior harvest at diagonally terminal spots SE_{-10} (13.14%) and NW_{-10} (9.14%). Lowest total THC content is determined at harvest day SE_h (10.00%) and NW_h (8.40%). HPLC analysis revealed that total THC (%) is declining from SE (13.14%), all the way through CN (12.38%) to NW (9.14%) spot. Total THC content linearly declines in these two spots (SE and NW) from ten days prior harvest SE_{-10} (13.14%) and NW_{-10} (9.14%), seven days prior harvest SE_{-7} (11.78%) and NW_{-7} (9.04%), two days prior harvest SE_{-2} (11.56%) and NW_{-2} (8.67%) to harvest day SE_h (10.00%) and

NW_h (8.40%). Total THC content changes marks inconsistencies at CN, throughout the timeline of sampling.

DISCUSSION

The morphology of trichomes and cannabinoid profile are dependent on genetic and environmental factors.[14] On female cannabis flowers, three types of glandular trichomes have been described based upon their surface morphology: bulbous, sessile, and stalked.[15] Bulbous trichomes are the smallest in size and produce limited specialized metabolites.[16] 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.[17] 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] Our study complies to this literature data regarding trichomes classification, with difficulties in distinguishing bulbous from sessile trichomes due to zooming limitations of stereomicroscope.

Two photon laser scanning fluorescence microscopy was used to analyze the excited intrinsic fluorescence of metabolites and noted that stalked trichomes have the highest cannabinoid content by monitoring the strong blue shifted fluorescence.[13] 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,[13] such as peppermint (*Mentha* x *piperita*)[19] and lavender (*Lavandula pinnata*).[20]

Research from 2004 year states that cannabis glands can be classified according to their secretory phases from the color of their contents. The most active glands in secretion (mature) are translucent at appearance, whereas aged glands are yellow and senescent glands are brown. These led 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 (Δ9-THC and total cannabinoids), 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] In our research, only bracteal stalked glandular trichomes were analysed, due to lower presence and abundance of these trichomes, better visualization, as the most of all-literature data pointing out highest cannabinoid content in capitate-stalked trichomes.[12,13,17] Changes in in total THC in SE, CN and NW (during the final maturing period) and relative abundance of capitate stalked trichomes (CST) colored heads are graphically presented in Figures 2 and 3.

Our data analysis showed a connection between changes in cannabinoid content and capitate-stalked trichome head coloration which can be seen in samples from SE and NW spots.

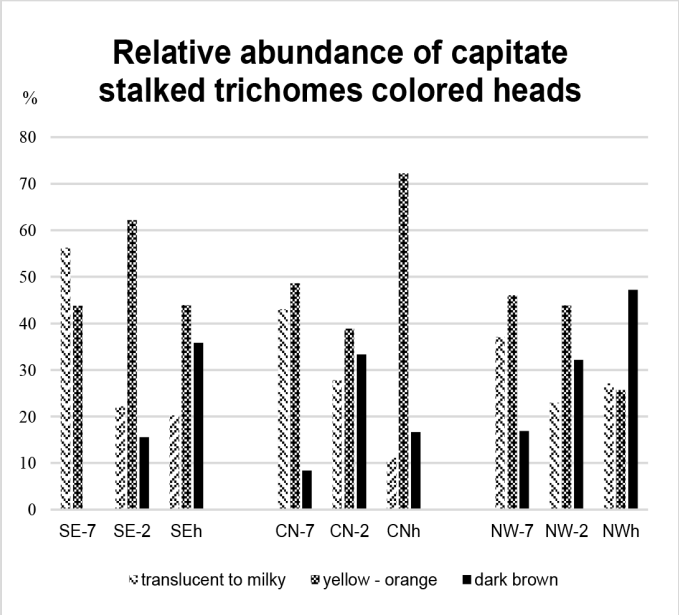

Figure 2: Graphical representation of relative abundance of capitate stalked trichomes (CST) colored heads.

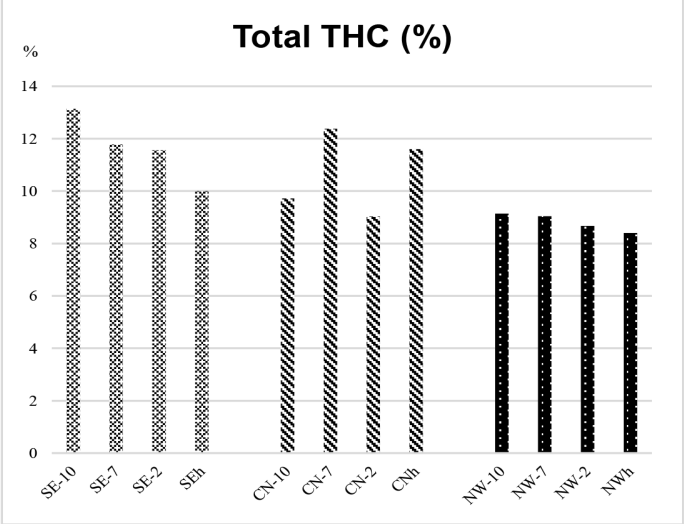

Figure 3: Changes in total THC in SE, CN and NW (during the final maturing period).

Decrease in total THC content from ten days prior harvest to harvest day was consistent in the analyzed samples from SE and NW, but there were variations in analyzed samples from CN. Unlike the results from HPLC analysis, the changes in coloration of bracteal capitate-stalked trichomes followed the timeline in the observed samples from all three spots (SE, CN and NW) of the cultivation plot. Total Δ9-THC in SE_{-7} to SE_h declined from 11.78% to 10.00%, notifying change in trichome heads from SE_{-7} to SE_h with decreasing of CST_{t-m} from SE_{-7} (56.25%) to SE_h (20.27%) and increasing of sum of CST_{y-o} and CST_{db} from SE_{-7} (43.75%) to SE_h (79.73%).

NW spot had smaller drop in total Δ9-THC content from 9.04% to 8.40% with change in trichome head coloration from NW_{-7}

Table 3: Cannabinoid content in SE, CN and NW spot in four different sampling time points.

(m/m %)	Southeast spot				Central spot				Northwest spot			
Components	SE_{-10}	SE_{-7}	SE_{-2}	SE_{h}	CN_{-10}	CN_{-7}	CN_{-2}	CN_{h}	NW_{-10}	NW_{-7}	NW_{-2}	NW_{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

*Total CBD - represents sum of content of CBD and CBDA x 0.877, expressed as CBD.
** Total THC - represents sum of content of Δ9-THC and Δ9-THCA x 0.877, expressed as Δ9-THC.

to NW_{h} with decreasing of CST_{t-m} from NW_{-7} (37.08%) to NW_{h} (27.08%) and increase in sum of CST_{y-o} and CST_{db} from NW_{-7} (62.92%) to NW_{h} (72.92%).

Central spot cannabinoid content changes marks inconsistencies throughout the timeline of sampling, with macroscopic studies stating higher bracteal representation in CN_{-10} and CN_{-2}, connecting this with decrease in total Δ9-THC content in CN_{-2}. Total Δ9-THC content in CN_{-7} is 13.95%, declining to 10.06% in CN_{-2} and 13.07% at the harvest day. Change in trichome head coloration was observed from CN_{-7} to CN_{h}, with decrease of CST_{t-m} from CN_{-7} (42.99%), CN_{-2} (27.77%) and CN_{h} (11.11%) and increase in total presence of CST_{y-o} and CST_{db} from CN_{-7} (57.01%), CN_{-2} (72.22%) to CN_{h} (88.89%). The key difference connecting the lower cannabinoid content in CN_{-2} (9.03%) and trichome coloration is in the changes in ratio of CST_{y-o} and CST_{db}. In CN_{-2} presence of CST_{y-o} is 38.89% and CST_{db} is 33.33%, whereas in CN_{-7} and CNh presence of CST_{y-o} is 48.60%, 72.22% and CST_{db} is 8.41%, 16.67%, respectively. CN_{-2} has higher presence of CST_{db} than CN_{-7} and $CN_{h,}$ indicating the loss in cannabinoid content.

SE spot has the highest cannabinoid content and NW spot the lowest cannabinoid content, pointing out that even in uniform greenhouse conditions, cannabinoid content varies depending on 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 must be analyzed to check the 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.

Although the morphology of CST was primarily analyzed, stereomicroscope observations also revealed purple coloration in many of the samples, probably coming from anthocyanins (Figure 1-D). In some of the samples the coloration was weak, barely identifiable, hence in others the coloration was intense. Intensive purple coloration (probably coming from anthocyanins) was noted at the base and stalk of capitate-stalked trichomes and epidermal tissue purple 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.[21] Due to the ionic nature of the molecular structure, the color of anthocyanins is influenced by pH value. In an acidic environment, at pH=1, anthocyanins are found in the form of flavylium cation which makes them very soluble in water[22,23] and also this is the form responsible for the production of red and purple colors. When pH is between 2 and 4, quinoidal blue species is found, while at pH between 5 and 6, carbinol pseudobase and a chalcone appear, which are colorless compounds.[23,24] It is also known that anthocyanin synthesis can be induced by environmental stress.[25] Anthocyanins accumulate in plants upon exposure to drought, salt, stress, UV stress, high light and high temperature[26-28] and are, therefore, considered nature's "Swiss army knife" of plant responses to stress.[25,29,30] The main roles attributed to anthocyanins in mediating responses to stress are linked with their antioxidant,[31,32] light-screening[25,27,33,35] and photoprotective properties.[36,37] Several senescence related physiological changes have been linked to increased susceptibility to photoinhibition, such as reduced capacity to repair photosystem II reaction centers,[38] chlorophyll degradation[39] which led to greater light sensitivity, resulting in photodamage at relatively modest irradiances.[38] Imbalance on the photosynthetic apparatus caused by differential rates of decline during senescence increases vulnerability to photoinhibition.[40] Several studies have found[41-43] 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.[37] There is no literature data to our knowledge that tests the connection between changes in cannabinoid content and anthocyanin coloration in trichomes.

CONCLUSION

There is great potential for use of trichome morphology analysis, especially trichome head coloration, in determination of technological maturity of cannabis plants, but yet it remains unexplored topic. Our study noted decrease in total THC content from ten days prior harvest to harvest day which was consistent in the analyzed samples from SE and NW, but variability was noted in analyzed samples from CN. Unlike the results from HPLC analysis, the changes in coloration of bracteal capitate-stalked trichomes followed the timeline in the observed samples from all three spots (SE, CN and NW) of the cultivation plot. This confirms the connection 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 trichomes head coloration. On the other hand, there is no literature data to our knowledge that tests the correlation between changes in cannabinoid content and purple coloration (probably from anthocyanins) in trichomes, giving this research field a very exciting and unique direction for future experimentally designed studies.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ABBREVIATIONS

HPLC: High pressure liquid chromatography; **ATR-MIR:** Attenuated total reflection mid-infrared spectroscopy; **CBD:** Cannabidiol; **CBDA:** Cannabidiolic acid; **CBN:** Cannabinol; **(-)-Δ9-THC:** (-)-Δ9 –tetrahydrocannabinol; **Δ9 –THCA:** Δ9 -tetrahydrocannabinolic acid A; **BLQ:** Below limit of quantification; **ND:** Not detected; **LOD:** Loss on drying; **SE:** Southeast spot; **CN:** Central spot; **NW:** Northwest spot; **SE_{-10}, CN_{-10}, NW_{-10}:** Collected samples ten days prior harvest; **SE_{-7}, CN_{-7}, NW_{-7}:** Collected samples seven days prior harvest; **SE_{-2}, CN_{-2}, NW_{-2}:** Collected samples two days prior harvest, **SEh, CNh, NWh:** Collected samples at harvest day; **CST:** Capitate stalked trichomes; **CSTt-m:** Capitate stalked translucent-milky trichome heads; **CSTy-o:** Capitate stalked yellow-orange trichome heads; **CSTdb:** Capitate stalked dark brown trichome heads.

SUMMARY

- In this study German pharmacopoeial method for HPLC analysis of cannabinoids potency in medical cannabis flowers was successfully applied.
- Bracteal stalked glandular trichomes were analysed by stereomicroscope analysis, due to lower presence and abundance of these trichomes and better visualization.
- Three distinct physiological maturation stages of CST (capitate-stalked trichomes) were monitored: CST_{t-m} (translucent-milky), CST_{y-o} (yellow-orange) and CST_{db} (dark brown) trichome heads.
- Increment of the number of yellow-orange and dark brown colored capitate-stalked trichome heads can be correlated with declining of the cannabinoid content.
- Steromicroscope observations also revealed purple coloration in many of the samples, probably arising from the presence of anthocyanins.
- There is no literature data that tests the correlation between changes in cannabinoid content and purple coloration (probably due to the anthocyanins) in trichomes.

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