

AGRICULTURAL PRODUCTION OF CANNABIS AIMING TO INCREASE CANNABIDIOL (CBD) CONTENT FOR MEDICINAL PURPOSES – A REVIEW

A PRODUÇÃO AGRÍCOLA DA CANNABIS VISANDO O AUMENTO DO TEOR DE CANNABIDIOL (CBD) PARA FIM MEDICINAL – REVISÃO

PRODUCCIÓN AGRÍCOLA DE CANNABIS CON EL OBJETIVO DE AUMENTAR EL CONTENIDO DE CANNABIDIOL (CBD) CON FINES MEDICINALES – UNA REVISIÓN



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ABSTRACT

Cannabis sativa Linnaeus is a herbaceous plant from the Cannabaceae family. Its female inflorescences are rich in glandular trichomes, which contain secondary metabolites with medicinal properties, such as cannabidiol (CBD). The lack of information on the cultivation management of *Cannabis* for biomedical purposes hinders the development of high-quality and standardized production. A literature search was conducted for studies involving interventions in *Cannabis* cultivation aimed at improving CBD content for medicinal purposes. A systematic search was performed in the CAPES Periodicals Portal > Collection > Subject >, using the search term "*Cannabis* and Crop Production," filtering for open-access, original, peer-reviewed articles in English. Scientific articles containing cultivation practices for *Cannabis* chemotype III relevant to the objective were included. The following were excluded: articles on agricultural production of *Cannabis* for non-medicinal purposes, review articles, non-scientific studies, THC > 0.3%, without CBD improvement, without instrumental analysis of CBD content, and *Cannabis* Chemotypes I and II. The search resulted in 9 eligible articles, with the following interventions: manipulation of phosphorus concentrations; sowing density and nitrogen fertilization; application of auxin antagonists under in vitro conditions; cultivar selection; optimized agronomic practices in a controlled cultivation environment; strategic manipulation of the light spectrum; topping; treatment with 6-benzylaminopurine (BAP); cultivar selection and water management; genetically uniform material under different environmental conditions. For greater success in increasing CBD without increasing THC, choosing a Chemotype III strain for medicinal purposes is paramount.

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Keywords: *Cannabis*. Crop Production. Cannabidiol.

RESUMO

A *Cannabis sativa* Linnaeus é uma planta herbácea, da família Cannabaceae. Suas inflorescências femininas são ricas em tricomas glandulares, que possuem metabólitos secundários com propriedades medicinais, como o canabidiol (CBD). A carência de informações sobre o manejo do cultivo da *Cannabis* para fins biomédicos dificulta o desenvolvimento de uma produção de qualidade e padronizada. Buscou-se na literatura estudos com intervenções no cultivo da *Cannabis* visando a melhoria do teor de CBD para fim medicinal. Pesquisou-se sistematicamente no Portal de Periódicos da CAPES > Acervo > Assunto >, com o termo de busca *Cannabis* and Crop production, com filtro em artigos de acesso aberto, originais, em inglês, revisado por pares. Incluiu-se artigos científicos contendo práticas de cultivo da *Cannabis*, quimiotipo III em pertinência ao objetivo. Foram excluídos artigos de produção agrícola da *Cannabis* sem fim medicinal, artigo de revisão, estudos não científicos, THC > 0,3%, sem melhoramento de CBD, sem análise instrumental do teor de CBD e *Cannabis* Quimiotipo I e II. A busca resultou em 09 artigos elegíveis, com as seguintes intervenções: manipulação das concentrações de fósforo; densidade de semeadura e a fertilização com nitrogênio; aplicação do antagonista de auxina em condições *in vitro*; seleção de cultivares, práticas agrônomicas otimizadas em ambiente de cultivo controlado; manipulação estratégica do espectro de luz; topping; tratamento com 6-benzilaminopurina (BAP); seleção de cultivares e manejo hídrico; material geneticamente uniforme em diferentes condições ambientais. Para maior êxito no aumento do CBD sem o aumento do THC é primordial a escolha da cepa Quimiotipo III para fim medicinal.

Palavras-chave: *Cannabis*. Produção Agrícola. Canabidiol.

RESUMEN

Cannabis sativa linnaeus es una planta herbácea de la familia cannabaceae. Sus inflorescencias femeninas son ricas en tricomas glandulares, que contienen metabolitos secundarios con propiedades medicinales, como el cannabidiol (cbd). La falta de información sobre el manejo del cultivo de *cannabis* con fines biomédicos dificulta el desarrollo de una producción estandarizada y de alta calidad. Se realizó una búsqueda bibliográfica de estudios que incluyeran intervenciones en el cultivo de *cannabis* destinadas a mejorar el contenido de cbd con fines medicinales. Se realizó una búsqueda sistemática en el portal de publicaciones periódicas de capes > colección > tema >, utilizando el término de búsqueda "*cannabis* y producción de cultivos", filtrando por artículos originales, de acceso abierto y revisados por pares en inglés. Se incluyeron artículos científicos que contenían prácticas de cultivo para el quimiotipo iii de cannabis relevantes para el objetivo. Se excluyeron los siguientes: artículos sobre producción agrícola de cannabis con fines no medicinales, artículos de revisión, estudios no científicos, thc > 0,3%, sin mejora del cbd, sin análisis instrumental del contenido de cbd, y los quimiotipos de cannabis i y ii. La búsqueda arrojó 9 artículos elegibles, con las siguientes intervenciones: manipulación de las concentraciones de fósforo; densidad de siembra y fertilización nitrogenada; aplicación de antagonistas de auxinas en condiciones in vitro; selección de cultivares; prácticas agronómicas optimizadas en un entorno de cultivo controlado; manipulación estratégica del espectro lumínico; poda apical; tratamiento con 6-bencilaminopurina (bap); selección de cultivares y gestión hídrica; material genéticamente uniforme en diferentes condiciones ambientales. Para lograr un mayor éxito en el aumento del CBD sin aumentar el THC, es fundamental elegir una cepa de quimiotipo III para fines medicinales.

Palabras clave: *Cannabis*. Producción de Cultivos. Cannabidiol.

1 INTRODUCTION

1.1 BOTANICAL CHARACTERISTICS OF CANNABIS

Cannabis sativa Linnaeus is a herbaceous plant in the Cannabaceae family. Its female inflorescences are rich in glandular trichomes, which have important secondary metabolites with medicinal properties, such as cannabidiol (CBD) (Bonini *et al.*, 2018). The classification of *Cannabis* can be carried out based on the relationship of the main phytocannabinoids in its constitution. Analyzing its phenotype, that is, chemical profile and the predominance in its composition of one of its secondary metabolites, characterizing it as a chemical fingerprint called chemotypes. *Cannabis* with a high ratio of Δ -9-Tetrahydrocannabinol (THC) to Cannabidiol (CBD) (>1) classifies as chemotype I, THC:CBD intermediate ratios (usually 0.5-2.0) are classified as chemotype II, and plants that have a low THC to CBD ratio (<1) classifies as chemotype III (Sampaio *et al.*, 2024).

1.2 CANNABIDIOL PHYTOCANNABINOID OF MEDICINAL INTEREST

CBD is the main non-psychoactive component of *Cannabis sativa* L. and for this reason there is interest in its therapeutic potential in various disease states investigated in animal models and in humans (Black *et al.*, 2019). It has anxiolytic, antidepressant, antipsychotic, anticonvulsant, anti-nausea, antioxidant, anti-inflammatory, antiarthritic and antineoplastic action (Agarwal; Burke; Maddux, 2019; Shannon *et al.*, 2019).

1.3 ASPECTS OF LEGISLATION

Regulatory bodies in North America and Europe classify Cannabis by the proportion of THC and CBD phytocannabinoids present in the plant, as a drug/marijuana (drug type/marijuana) or as fiber/hemp (fiber type/hemp). In North America, varieties with $\text{THC} \leq 0.3\%$ contents are classified as hemp (UNODC, 2019) and allowed in the production of fiber, food supplements, and cosmetics (MEAD, 2017), while $\text{THC} > 0.3\%$ are classified as marijuana and subject to greater control due to the psychoactive properties of THC (UNODC, 2019). In Europe, the requirements are greater, in which the classification for hemp needs to have THC levels lower than 0.2% (Cherney; Small, 2016). Unlike the US and Europe that regulated hemp for fiber production, Brazilian regulation does not differentiate the chemical profile between hemp and marijuana (Carvalho *et al.*, 2020).

1.4 THE INFLUENCE OF CANNABIS CULTIVATION FOR MEDICINAL PURPOSES AND BIOMARKERS

Botanical knowledge is essential for the correct management of the plant (Figueiredo;

Policarpo; Veríssimo, 2017). The amount of phytocannabinoids present in the *Cannabis* plant is extremely influenced by the gene pool and the environmental conditions of humidity, temperature, radiation, soil nutrients, and parasites (Citti *et al.*, 2018; Bonini *et al.*, 2018; Trancoso *et al.*, 2022) exposes that scattered rainfall, low humidity, and sunny weather produce a plant rich in phytocannabinoid content. Many varieties of *Cannabis* have been cultivated over time and the detailed knowledge of its phytocannabinoid profile plays an essential role in the correct selection of a plant. (Citti *et al.*, 2018) and their concentrations and interactions can determine medicinal effects and adverse side effects (Berman *et al.*, 2018; Trancoso *et al.*, 2022). Although there is a great diversity of chemical constituents among *Cannabis* strains and the number of phytocannabinoids is large, most studies profiling *Cannabis* phytocannabinoids report only the main phytocannabinoids in the extract, among which THC, CBD are its acidic precursors and inactive CBDA/THCA and CBN is a possible sleep inducer (Santos, 2021), and phytocannabinoid study because it is a product of THC degradation, in which they also represent markers that indicate quality in the extraction process of phytocannabinoids of interest to the *Cannabis plant* (Carvalho *et al.*, 2020; Berman *et al.*, 2018). The standardized production of secondary metabolites, in the class of herbal medicines, is limited because the active components are not perfectly uniform (Trancoso *et al.*, 2022). The study of abiotic factors together with management techniques can help in the development of management protocols that favor greater productivity, quality, and stability in the production of phytocannabinoids, and it is essential to know the biosynthesis process and the formation of these compounds (Glivar *et al.*, 2020; Trancoso *et al.*, 2022). Most agronomic research on hemp conducted in the field setting has focused mainly on fiber production and yield (Trancoso *et al.*, 2022). Hemp, even though it is used for the production of phytocannabinoids, is traditionally used for selective cultivation for fiber or seed production, instead of flower production and secondary metabolites (Trancoso *et al.*, 2022). The lack of basic information on the management of *cannabis* cultivation for biomedical purposes hinders the development of quality and standardized production. Insignificant studies have focused on the production of phytocannabinoids. Evaluating the effects of external factors and management techniques on phytocannabinoid production is essential to understand the agronomic treatments and methodologies that can be applied to suit the harvest and final products (De Prato *et al.*, 2022; Trancoso *et al.*, 2022). Several books have been published on techniques for managing cannabis crops, but the techniques described are based on common knowledge constructs, where there is a lack of systematic studies that prove their efficiency and effectiveness. Producers have access to horticulture guides based on the internet and online information, but much of this information is not based

on scientific research (Caplan; Dixon: Zheng, 2017; Trancoso *et al.*, 2022). In this sense, studies with interventions in the cultivation of the *Cannabis plant* aimed at improving the CBD content for medicinal purposes were searched in the literature.

2 METHODOLOGY

This is a study based on a systematic review of the literature, conducted according to the characteristics and recommendations of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The results were organized and described in a narrative way, highlighting the main findings of the included studies, with the support of the PICO strategy.

2.1 ELIGIBILITY CRITERIA

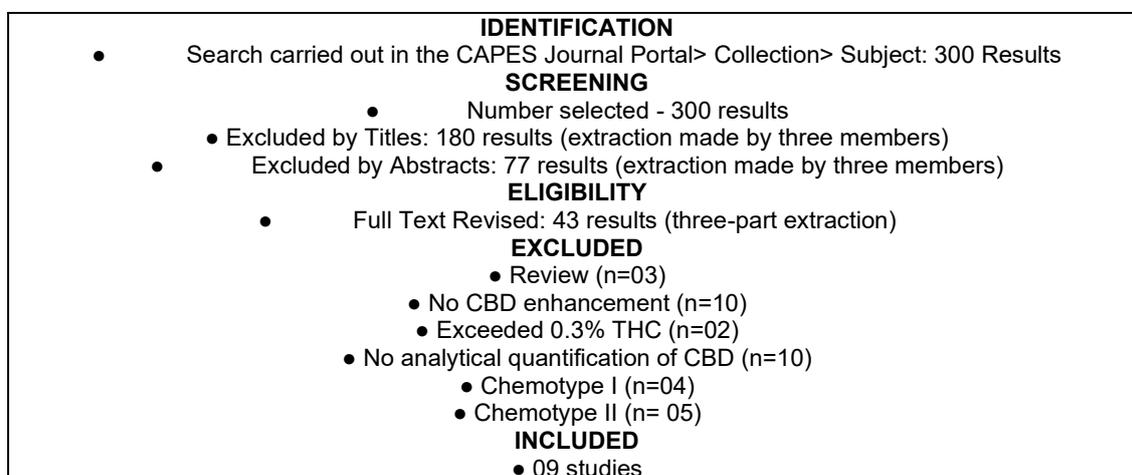
We systematically searched the CAPES Journal Portal> Collection> Subject>, with the search term *Cannabis* and Crop production, with a filter on open-access, original, English, peer-reviewed articles. Scientific articles containing cultivation practices of *Cannabis*, chemotype III in pertinence to the objective, were included. Articles on agricultural production of *Cannabis* without medicinal purpose, review article, non-scientific studies, THC > 0.3%, without CBD improvement, without instrumental analysis of CBD content and *Cannabis* Chemotype I and II were excluded.

3 RESULTS AND DISCUSSIONS

The screening and selection of studies are presented in the flowTable in Table 1.

Table 1

PRISMA FlowTable for Screening and Selection of Studies



Source: Prepared by the authors

The results of the 09 included studies are described below, presenting scientific studies with presentation of methodology, study design and statistical analysis.

POPULATION: The study by Cockson *et al.* (2020) used strains of *Cannabis sativa* BaOx (high CBD content), grown from rooted cuttings. The total cultivation period from taking the cuttings to harvesting was approximately 134 days (from July 24 to December 5, 2019). The study was carried out in Raleigh, North Carolina, in the United States of America (USA).

INTERVENTION: Six doses of Phosphorus (P), a fertilizer vital for plant development and the production of secondary metabolites, ranging from 3.75, 7.50, 11.25, 15.0, 22.50 to 30.0 mg/L, were tested using a modified Hoagland solution. The cultivation was carried out in a glass greenhouse with average temperatures of 25.8 (day) and 21.3 °C (night). The Critical Night Length (NLC) was reached in the pre-flowering stage consisting of 9 to 10 hours of darkness with 14 to 15 hours of light. The plants for treatment were arranged in a randomized block design (DBC) divided between three benches. At the flowering stage, flower buds (n = 5) were harvested for cannabinoid analysis in Ultra High Performance Liquid Chromatography equipment coupled to Tandem Mass Spectrometry. (UHPLC/MS/MS) Triple Quadrupole. For statistical analysis to compare the differences between concentrations, the F test (GLM PROC) and the LSD test with Tukey-Kramer adjustment were used, where the significance level was defined as ($p \leq 0.05$).

CONTROL GROUP: The study compared the results obtained at six P concentrations, the lowest being the one that served as a point of comparison within the experiment.

OUTCOME: In the Flowering Stage, plants with higher P concentrations (22.5 and 30.0 mg/L) were more branched and had larger diameters, when compared to the lowest P concentration of 3.75 mg/L ($p \leq 0.001$). The highest floral fresh weight was also observed at the concentration of P 22.50 mg/L, compared to the concentration of 3.75 and 7.50 mg/L, ($p \leq 0.001$), this fact occurred due to the increase in the branching of the *Cannabis plant* at the highest concentration of P, in which it produces inflorescences at each node, increasing the floral biomass of the plant. Also at this stage, there was a significant difference ($p \leq 0.001$) in the production of the cannabinoids CBDA, CBGA, THCA, total THC, and total CBD when compared to the lowest concentrations of P in relation to the highest concentrations. Their production reached a plateau in the P concentration at 11.25 mg/L P and, after this rate was reached, an increase in the P concentration did not produce statistically higher or lower levels of cannabinoids. At this P concentration, the analysis of total CBD resulted in 140.09 mg/g ± 10.49 .

POPULATION: The study by Poniatowska *et al.* (2022) used strains of *Cannabis sativa* L Futura 75, KC Dora and Tygra (fibrous and monoecious hemp) germinated through

sowing in the open field. The *cannabis* was harvested by hand at the beginning of seed ripening and occurred in September of each year. The study was conducted in Pytkowo, Wielkopolska, Poland, during the years 2014 to 2016.

INTERVENTION: Use selected cultivars, with determined factors of sowing density and fertilization with nitrogen (N), a fertilizer vital for plant development and the production of secondary metabolites. Densities of 60 seeds/m² and density of 180 seeds/m² were tested. The nitrogen fertilization rates tested were: 0, 30, 60 and 90 kg/ha. The average air temperature during the vegetation period of the study ranged from 8.3 °C to 22.2 °C. The samples of the inflorescences used for the chemical analysis were obtained by choosing 10 plants randomly selected from each replicate in all tested combinations. The experimental design was the randomized complete block (DBC) method of three factors (Cultivar; Sowing density and N fertilization), split-plots, with four replications. The cannabinoids were analyzed by gas chromatograph with flame ionization detector (GC-FID). Statistical analysis of the results recorded was performed by analysis of variance (ANOVA) in a split-split-plot design.

CONTROL GROUP: In the study, the control group refers to fertilization with 0 kg N/ha nitrogen to evaluate the effect of nitrogen.

OUTCOME: Regarding sowing density, only Tygra obtained an increase in density from 60 to 180 seeds/m², significantly increasing the yield of CBD by 0.97 g/m², which corresponds to 8.8%. At the highest doses of N, the yield of CBD increased significantly compared to the control (zero N dose). For the cultivars, Futura 75 and KC Dora, the highest yield of CBD was obtained with 90 kg N/ha. And for Tygra, the highest yield (12.68 g/m²) was expected with 44.5 kg N/ha. The result of the work regarding the improvement of CBD production demonstrated that the optimization of productivity depends crucially on nitrogen fertilization and, to a lesser extent, on the cultivar and the interaction between nitrogen and sowing density.

POPULATION: The study by Senkyřík *et al.* (2023) used two strains of *Cannabis sativa* L., the cultivar USO-31, hemp of Ukrainian origin, germinated *in vitro* through seeds and the cultivar Tatanka Pure CBD, hybrid and rich in cannabidiol (CBD) germinated *in vitro* from rooted cuttings. The study was carried out in the Czech Republic, by Palacký University and the Institute for Research and Culture located in Olomouc.

CONTROL GROUP: Medium containing the treatment with the auxin antagonist, α -(2-oxo-2-phenylethyl)-1H-indole-3-acetic (PEO-IAA) and the medium without PEO-IAA.

INTERVENTION: Application of the auxin antagonist, PEO-IAA under *in vitro* conditions. This intervention sought to increase the rate of sprout multiplication *in vitro*, since auxin is an essential phytohormone that regulates apical dominance, by interrupting this

apical dominance with PEO-IAA, it was hoped to improve the multiplication of plant material and investigate the influence of this antagonist on the relative gene expression (GER) of cannabinoid genes and cannabinoid content. The conditions of the *in vitro* experiment were the same for both cultivars. The nodal segments were cultured in a 100mL erlenmeyer flask containing five to six nodal segments grown under standardized conditions in a phytotron at 22 °C, 40% relative humidity and photoperiod of 16/8 ($36 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 4 weeks. After the cultivation period, the biomass was harvested, frozen in liquid nitrogen and then used for Relative Gene Expression (GER) analysis, and the phytochemical analysis of the cannabinoids, the latter being carried out in the UHPLC-MS/MS equipment. The *in vitro* experiment was carried out for each cultivar in three or four biological replications. Statistical analysis was determined by the *t-test* method to compare samples with PEO-IAA and controls in terms of cannabinoid content. To investigate the relationship between gene expression and cannabinoid concentration, regression analysis was used.

OUTCOME: The Tatanka Pure CBD cultivar showed a statistically significant increase ($p < 0.05$) in the concentration of the cannabinoid CBDA (approximately 1.47 times compared to the control). This was the only statistically significant increase found. The study suggests that the use of PEO-IAA is an appropriate condition for the formation of multiple shoots and that its presence in the culture medium probably does not significantly disrupt the gene expression of key genes related to the biosynthetic pathway of the main cannabinoids, nor does it reduce cannabinoid concentrations. However, the study recognizes the need for additional research into other genotypes and monitoring of plants after transfer to *ex vitro* conditions and the flowering stage to gain more valuable information.

POPULATION: The García-Tejero *et al.* (2020) used new varieties of hemp (*Cannabis Sativa* L.). Three CBD chemotype varieties (Sara, Pilar, Theresa) and two CBG chemotype varieties (Aida and Juani). The form of planting used was through rooted apical cuttings. The trial was carried out during two consecutive seasons (2014 and 2015), with the growing period from May to October. The study was carried out in the Guadalquivir River basin in southwestern Spain belonging to the Andalusian Institute for Agricultural Training and Development and Research (IFAPA, in Spanish). Country Spain.

CONTROL GROUP: The study did not use a control group, but a comparative study between the varieties and densities of plants tested.

INTERVENTION: Combination of cultivar selection, optimized agronomic practices, highlighting plant density (PD) and a controlled cultivation environment. The plants were grown in polyethylene-lined tunnel structures. The environment inside these tunnels had an average temperature between 23 and 35 °C and relative humidity around 65%. Three

densities were tested: PD1 (9,777 plants \cdot ha $^{-1}$), PD2 (7,333 plants \cdot ha $^{-1}$) and PD3 (5,866 plants \cdot ha $^{-1}$). In the 2014 season (Sara, Pilar, Aida) a randomized block design was performed, considering four replicates per variety. The plant density used was PD1. In 2015 (Sara, Theresa, Aida, Juani) the design and replicates per variety were the same as in the previous year, except that three different densities were tested. Harvest was defined by weekly observations of the change in stigma color from white/yellow to orange/brown, harvested when approximately 80% of the shoots reached this maturity. Cannabinoid analysis was performed by gas chromatography. In 2014, the statistical analyses of the data were submitted to one-way analysis of variance (ANOVA), with Tukey's test for separation of means ($p < 0.05$). In 2015, the statistical analysis of the subjects underwent a two-way ANOVA, evaluating the effects of the cultivar, PD and their interactions. A Levene test was previously applied to verify the homogeneity of the variances.

OUTCOME: The Sara cultivar stood out as the most effective strain in terms of CBD cannabinoids in the 2014 and 2015 seasons. It is distinguished by its relevant volume and pronounced lateral growth, with a high capacity to develop inflorescences. The highest CBD yields were obtained with Sara under PD1 and PD3, (many plants in PD1 or higher individual development in PD3). Sara reached dry weight values of flowers and leaves, DW(F+L) between 3,478 and 3,307 kg \cdot ha $^{-1}$, surpassing the values of Pilar and Theresa. CBD yields have reached up to 350 kg \cdot ha $^{-1}$. This value is significantly higher (almost four times higher) than that reported in indoor conditions controlled by other studies (about 84 kg \cdot ha $^{-1}$ in six annual production cycles). In short, the combination of new hemp cultivars genetically selected for CBD (especially Sara), propagated by clones, grown in macrotunnels in a Mediterranean semi-arid environment and with plant density optimization, resulted in a substantial and cost-effective increase in CBD production.

POPULATION: The study Kotiranta *et al.* (2024) used the cultivar called FINOLA, a hemp genotype of *the C. sativa* dominant in cannabidiol (CBD). Its seeds were sown in peat, grown in different pot sizes during the growing season. The study was conducted from April to September. The work was carried out at the Department of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, Helsinki, Finland.

CONTROL GROUP: Treatment of opposite lights High and Low, R:FR Ratio (Red to Far Red) and another treatment used the original white LED spectrum (control), in which it excludes BlueL, UV-A and UV-B light, in which they were used in the comparative treatment.

INTERVENTION: Strategic manipulation of the light spectrum, focusing on two main areas: the Red to Far Red ratio (long wavelength) (R:FR) and the short wavelength radiation (Blue, UV-A and UV-B). The light factor is considered one of the main factors for the successful

cultivation of *Cannabis* indoors, with the photoperiod, light quality and intensity being significant factors. The plants were grown on benches (blocks) inside a research greenhouse compartment, closed environment, blackout curtains were implanted on all walls and roof continuously during the experiments to exclude external radiation and maintain photoperiods. The ambient temperature (Day 24 °C and Night 22 °C), relative humidity (Day 60% and Night: 50%) and CO₂ concentration in the air was 386 ± 14 ppm when the lights were on, were automatically controlled by the greenhouse control system. In addition, the light (photoperiod, quality and intensity) was precisely controlled using LED luminaires. The study was divided into two separate experiments, Experiment A used a HIGH R:FR white LED spectrum (Ratio 11) and used the same white LED spectrum as the HIGH R:FR treatment, but with the addition of LOW R:FR far red LED fixtures (Ratio 1) The plants were under the light treatments (HIGH R: FR and LOW R:FR) for 48 days until harvest 70 days after sowing (DAS). Experiment B included the treatments: Control, Blue, UV-A and UV-B in which 78 DAS were applied to the plants for 50 days until harvest. The experiments were organized in a randomized complete block design with three replicated blocks. Fifteen plants were randomly chosen from each light treatment, five per repetition plot for analysis of the cannabinoid constitution, which were performed through the high-performance liquid chromatography (HPLC) equipment, coupled to the Diode Array Detector (DAD). For the statistical analysis, the mixed-effects ANOVA model was used, in which the light treatment was defined as a fixed factor and the block of replicas as a random effect.

Outcome: The HIGH R:FR treatment (ratio of 11) resulted in higher total yield of inflorescences per plant, increased branching, demonstrated by the higher number of internodes, and the plants were thicker, avoiding the shade avoidance syndrome induced by LOW R:FR (ratio of 1), which causes stem elongation and decreased yield. The HIGH R:FR treatment increased CBD concentrations compared to the LOW R:FR treatment. Experiment B did not impact the total yield of the inflorescence, and the concentration of CBD.

POPULATION: The study by Folina *et al.* (2020) used two monoecious industrial hemp cultivars originating in France, Fedora 23 and Futura 75. The sowing of the cultivars was carried out through a standard grain seeder. The field experiment was carried out in Farsala, Central Greece. Sowing on May 11 and harvesting on August 14, 2019.

INTERVENTION: The main agronomic intervention adopted was *topping*. Stressful conditions such as environmental factors and pruning can increase the synthesis and accumulation of cannabinoids. Topping was performed by cutting the main bud of the *Cannabis* plants 40 cm from the top of the plants, carried out at 40 days after sowing in the open field. Climatic data were recorded daily, average temperature for the month of May

18.4°C, June 24.8°C, July 25.5°C, August 25.7°C The experimental design used was split-plot, containing three replications. The factors tested were main plots containing the two European hemp cultivars Fedora 23 and Futura 75 and subplots with *the topping and non-topping* treatments. Plant sampling was carried out at harvest time, on August 14, 2019, when the trichomes of the inflorescence changed from white to milky white. Samples of 10 plants were randomly selected from the middle rows of the plots, and cut above ground level. Five samples of harvested plants were subjected to the measurement of the CBD content in fresh Cannabis inflorescences using the GemmaCert equipment (GemmaCert Ltd., Israel) and after the fresh inflorescences were dried in an oven at 60 °C for 48 hours and weighed to determine the dry matter of the inflorescence. The experimental data were submitted to statistical analysis according to the split-plot design. The differences between the means were separated using the Least Significant Difference (LSD) test. All comparisons were made at the significance level of 5%.

CONTROL GROUP: The *non-topping group* functioned as the control group, being compared with the group that received the intervention (*topping*).

OUTCOME: The application with the *topping* treatment exerted a significant and positive effect on both the cannabinoid content and the yield attributes of the flowers ($p < 0.05$). In the cultivars tested, the CBD content (%) was significantly higher in the *toppings* than in the *non-toppings*, with Futura 75 being covered: 2.32% vs. uncovered: 1.9% and Fedora 23 covered: 2.2% vs. uncovered: 1.7%. In the *toppings*, the values of total dry matter (DM) productivity of the inflorescences were significantly higher (206.3 g/plant in Futura 75 and 156.3 g/plant in Fedora 23) than in the *non-topping* plants (179.7 and 131.8 g/plant in Futura 75 and Fedora 23). The number of inflorescences was higher in topping, which is attributed to the high number of secondary shoots that develop after removal of the main shoot (pruning).

POPULATION: The study by Mishchenko *et al.* (2022) used seeds of the USO 31 variety, monoecious industrial hemp (*Cannabis sativa* L.) of the Central European type. The experiment was carried out in the field in a region that presented a temperate/continental climate with a tendency to be warmer and drier. The growing season analyzed runs from April (early spring) to September (late summer and early fall), harvesting occurs at maturation (BBCH 89) is the thrashing phase, which represents biological maturation. The field research, which lasted from 2016–2019, was conducted in northeastern Ukraine.

INTERVENTION: Use of exogenous growth regulators (phytohormones and vitamins), and one of the objectives of the study was to evaluate the result of exogenous growth regulators on cannabinoid content. To evaluate the feasibility of epigenetic effects under the

influence of growth regulators, mature seeds were collected from treated plants. These seeds were then sown again in the fourth year (2019) for the offspring to be analyzed for their cannabinoid content. The climatic conditions fluctuated during the period of the experiment, thus being able to broadly evaluate the content of cannabinoid compounds. The substance 6-benzylaminopurine (BAP), a phytohormone of the cytokine group has been identified as the most effective growth regulator for increasing CBD. The content of cannabinoid compounds was determined by a gas chromatograph. Statistical analysis used the least significant difference test (LSD) and Student's t-test, with statistical significance determined at the level of $p < 0.05$.

CONTROL GROUP: Control group without treatment for comparison purposes with plants that received treatment with exogenous growth regulators.

Outcome: Treatment with the cytokinin BAP was the most efficient growth regulator to increase CBD content. The concentration of CBD in the plants treated with BAP (0.2654%) is significantly higher than the average value found in the untreated group (control) (0.1271%). From the point of view of epigenetic effects, a promising direction to increase the level of non-psychoactive cannabinoids is the treatment of vegetative plants with cytokinin BAP, and the concentration of CBD in the offspring of plants treated with this regulator resulted in the content of (0.2540%) significantly higher than the average value found in the untreated group, control (0.1323%). Under the influence of BAP, the highest content of cannabinoid compounds (including CBD) manifested steadily during each of the three processing years (2016–2018). These changes were inherited by at least one generation of descendants.

POPULATION: The study by Campbell *et al.* (2019) used a diverse set of 13 industrial hemp cultivars (*Cannabis sativa* L.), these strains are not considered CBD-rich, originating in Europe and Asia. The cultivars CS 45; Eletta Campana 45y; Tiborszallasi 46 are dioecious and the other cultivars are monoecious, Carmaleonte 45; Diana, 45; Monoica 46 ; Phedora 17; Féline, 32; Férimon 12; Futura 75; Santhica 27; Bialobrzeskieskie 52; USO 31 Ukraine. The field study that resulted in the analysis of CBD was in Colorado, United States, in Fort Collins, which we are analyzing in this study. The duration between planting and harvesting was measured as days to Duration of Maturity (TMD). The mean days to maturity was 116 days for the limited irrigation treatment ("dry") and 117 days for the fully irrigated treatment ("wet").

INTERVENTION: Use several cultivars (genotypes) for planting in two types of irrigation treatment to test germplasm imported from Europe and Asia. The study evaluates how genetic effects between cultivars, environmental effects, and genotype × environment (GEI) interactions influence traits such as cannabinoid content such as THC and CBD. Water

availability was an important environmental factor manipulated in the experiment. In Fort Collins, two irrigation treatments were used, through an overhead linear sprinkler irrigation system, being "Limited irrigation" (dry) and "Fully irrigated" (wet), the cultivars were replicated four times in the experiment. In Fort Collins, a Latinized row and column design was used. The cannabinoid profiles and quantification were analyzed by UHPLC/ coupled to a photodiode array detector (PDA), using 10 cm upper than the female flowers, three random plants were collected per plot at maturity. Variance (ANOVA) was used to define the effects appropriate to genotype, environment and GEI by linear regression.

CONTROL GROUP: The study compares the performance of the cultivars with each other and under different water management conditions

OUTCOME: The study resulted in average total CBD content: 2.24% under full irrigation conditions ("wet") and 1.43% under limited irrigation conditions ("dry"). The relationship between maturity (phenology) and Cannabidiol (CBD) production is of a positive and significant correlation ($p \leq 0.001$). This means that, generally speaking, hemp cultivars that have late flowering (i.e., take more days to reach maturity) tend to produce higher levels of total CBD. The number of days to maturity (DTM) was significantly correlated with the total CBD content. Pearson's correlation coefficient (r) between TMD and total CBD was 0.68, indicating a positive relationship. The genotype \times environment (GEI) relationship was not significant for the increase in CBD. The percentage variance of the trait explained by the $G \times E$ interaction was only 1.7%. This suggests that the cultivars, in general, maintained their relative classifications of CBD production in the different environments tested (limited irrigation and complete irrigation), demonstrating stability in relation to this interaction. Genotype was by far the most important factor, explaining 82.7% of the variation in CBD content ($p \leq 0.001$). To achieve a specific CBD content, cultivar selection is the most important decision. Although the interaction (GEI) was not significant, the regression model detected significant effects of the environment ($p \leq 0.001$) for total CBD. However, the magnitude of this effect was small, with only 6% of the variation attributed to environmental effects. The TMD had a negative correlation with grain yield ($r = -0.53$), indicating that, in general, cultivars that took longer to mature produced fewer grains. In summary, the lack of significance of the Genotype \times Environment (GEI) interaction for total CBD indicates that the observed differences in CBD content between cultivars are predominantly determined by plant-specific genetics rather than by how the cultivar uniquely reacts to a specific environment (such as irrigation).

POPULATION: The study by Giupponi *et al.* (2020) used *Cannabis sativa* L strain . Kompolti is a dioecious industrial hemp variety developed in Hungary. The cuttings were

taken from the parental pistillate plant. The study was conducted in Italy, during the 2018 growing season, in two locations in the Piedmont region of northern Italy.

INTERVENTION: Clones of the Kompolti variety were cultivated in two distinct ecological environments — mountain and plain, in order to evaluate the impact of this environmental condition on the chemical composition (cannabinoids) of the inflorescences. To ensure that the observed differences in secondary metabolites were due solely to altitude-related ecological conditions, the uniform clones were grown in pots with a uniform substrate. The material of the inflorescences of each clone was sampled five times to perform the analyses. The quantification of cannabinoids used the average of four biological samples and they were analyzed by HPLC/MS with a quaternary pump. To compare the two groups, the two-tailed Student's t-test was used. A p-value lower than 0.05 was statistically significant.

CONTROL GROUP: The study compared the results of plants grown in the plains with plants grown in the mountains.

OUTCOME: CBDA was the most abundant cannabinoid, with significantly higher concentrations in the mountain samples ($p < 0.01$) compared to the plain. Although the natural process of decarboxylation of CBDA into Cannabidiol (CBD) occurs under the action of heat and light, the geographic location of the crop (mountain versus plain) did not significantly influence the decarboxylation of CBDA in the production of CBD in the samples studied. The Kompolti strain has a predisposition to have prolonged flowering in late summer/early autumn, when average daily temperatures are moderately higher, it may have led to a partial conversion of CBDA to its neutral form (CBD) in both locations.

The included studies showed significant results in increasing CBD, with variations when compared between the cultivars and intervention of the same study. In Cockson *et al.* (2020) the treatment performed on the BaOx cultivar (high CBD content) at the intermediate dose of P caused a greater increase in the concentration of total CBD compared to the higher doses of P that produced a more branched plant, with higher floral weight. The highest nutritional dose is not always the best dose to promote increased synthesis of secondary metabolites (Troncoso *et al.* 2022). In the study by Poniatowska *et al.* (2022) the application of higher N doses resulted in a significant increase in CBD in the cultivars, Futura 75 and KC Dora. There are studies that show that an increase in the content of mineral macronutrients can produce an increase in the production of inflorescences and phytocannabinoids (Shiponi, S.; Bernstein, N., 2021). In the study by Senkyřík *et al.* (2023) with the application of the auxin antagonist PEO-IAA, there was a significant increase in CBDA in the CBD-rich cultivar, Tatanka Pure CBD this study was considered in the work because it is a phytocannabinoid precursor of CBD, which through decarboxylation in the extraction process can become rich

in CBD and the application of PEO-IAA in the culture medium presented an important result as it did not affect the relative gene expression (GER) of cannabinoid genes and in the Mishchenko *study et al.* (2022) cytokinin application BAP, resulted in a significant increase in CBD and its offspring as well (epigenetics). Both interventions alter the hormones auxin and cytokine respectively and thus stimulate the development of lateral shoots (Crispim Massuela, D. *et al.*, 2022). In the study by García-Tejero, *et al.* (2020) the cultivar Sara (rich in CBD) showed a significant increase in CBD in PD1 and PD3, and in the study by García-Tejero, *et al.*, (2019), with another cultivar, Ermes, also rich in CBD, there was also an increase in CBD in PD1 and PD3, corroborating the importance of choosing the CBD-rich genotype for agronomic practices in controlled environments. In the study Kotiranta *et al.* (2024) strategic manipulation of the LED light spectrum (red: far red) resulted in an increase of inflorescence and CBD in the ALTO R:FR treatment (ratio of 11) in Finola cultivar (rich in CBD). According to Lefsrud, M. G.; Kopsell, D. A.; Sams, C LED lamps make it possible to present specific wavelengths, facilitating research on the impact of wavelength on the production of secondary metabolites. In the study by Folina *et al.* (2020) the topping performed on the Fedora 23 and Futura 75 cultivars resulted in a significant increase in the increase of inflorescences and CBD. According to Verma, N.; Shukla, S., (2015) pruning is a common intervention in Cannabis to increase the production of inflorescences and induce hormonal changes that cause metabolic changes. In the study by Campbell *et al.* (2019) the selection of 13 cultivars and water management resulted in a significant increase in CBD in complete irrigation. The relationship between late maturity and CBD production was significant, this may have interfered with the different result of Caplan, D.; Dixon, M.; Zheng, Y. (2019), in which on the contrary water restriction increased CBD yield by stress. In the study by Giupponi *et al.* (2020) there was a significant increase in CBDA in cultivation in a mountainous region, this study was considered in the work to be a phytocannabinoid precursor of CBD, which through decarboxylation in the extraction process can become rich in CBD. According to Jin, D. *et al.* (2020) in their study deduced that plants grown in regions with higher altitude and temperature exhibited higher density and increased trichomes. The study was limited to the search for open access articles. In addition, there was no expansion of the databases consulted, which made it impossible to exhaust the search.

4 CONCLUSION

Our study showed that the lack of CBD analysis in 10 excluded studies and 10 studies without CBD improvement corroborates with the information that there is a lack of studies for improvements in CBD production. Increased inflorescence production is not always indicative

of increased CBD. The objective of planting must be well defined, as the production of seed and inflorescence can occur in opposite ways in the maturation of the plant. Cultivars of CBD-rich genotypes seem to show more significant results in increasing CBD when the interventions, so the choice of the CBD-rich genotype is a better way to produce CBD for medicinal purposes. For greater success in increasing CBD without increasing THC, it is essential to choose the Chemotype III strain for medicinal purposes.

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