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Challenges and opportunities for therapeutic use of medical cannabis

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Genet. Mol. Res. 23 (1): gmr19168
Received June 26, 2023
Accepted December 14, 2023
Published February 14, 2024
DOI http://dx.doi.org/10.4238/gmr19168

ABSTRACT. Research has advanced and pre and post-harvest management strategies for *Cannabis sativa* have been proposed, aiming to improve cannabis production and efficiently attain industrial scale cannabinoid production. In general, studies have focused on genetic modification and cultivation methods designed to increase the content of the substance of interest (in general, cannabidiol; CBD) and obtain varieties with high productivity and resistance to pests. For the cannabis industry, propagating vigorous and uniform plants remains a challenge, as it is a dioecious crop and therefore depends on cross-fertilization for seed production. In this context, this review, through searches in international databases, examined aspects of the *C. sativa* plant associated with its genotypic plasticity and chemical variability, as well as strategies and perspectives for achieving success in industrial scale production of phytocannabinoids. The methods and techniques that have been used include micropropagation to generate exclusively female individuals; development of semi-dwarf cannabis cultivars, which have a high leaf density and low Δ^9-Tetrahidrocanabinol (THC) levels; and

alterations in the spectrum of LED lights to stimulate photoreceptors and maximize the yield and quality of cannabis, with reduced operational costs. Polyploidization has been used to develop new strains of cannabis with significant increases in CBD concentration in the terpene profile and in the buds of tetraploid clones, without increased THC content. Genetically modified microorganisms have been developed for large-scale production of both natural (phytocannabinoids) and non-natural cannabinoids. Parameters such as plant variety and density, light intensity, and fertilization affect biomass and cannabinoid yields. Considering the complexity of the endocannabinoid system and the specificities of the diseases that medicinal cannabis could be used to treat, obtaining a pharmaceutical product that meets this demand remains a great challenge.

Key words: Cannabis; Cannabinoids; Phytocannabinoids; Breeding cannabis; Therapeutic use

INTRODUCTION

Cannabis sativa has great potential for industrial and medicinal use. In ancient societies, its medicinal role was already highlighted, with use for various diseases and, currently, although its medicinal use is not regulated in many countries, research has shown its therapeutic potential, corroborating historical records (Zuardi, 2006; Pisanti and Bifulco, 2017; Bonini et al., 2018; de Souza et al., 2022). According to Lucas and Walsh (2017), patients have replaced prescribed medications (opioids, benzodiazepines, and antidepressants) with the use of cannabis.

Cannabinoids (mainly cannabidiol – CBD – and Δ^9 -tetrahidrocanabinol – THC), secondary cannabinoids (such as cannabigerolic acid – CBGA) and other compounds (such as terpenes), through different preparations, either synthetic (cannabinoids) or natural (phytocannabinoids), interact with the endocannabinoid system (ES) modulating pathological processes through antimicrobial, anti-viral, antioxidant, anti-inflammatory and neuroprotective properties (Janecki et al., 2022; Martins et al., 2022). This benefits health as it can bring well-being to human life and even to animals (Samara et al., 1988; Verrico et al., 2020), with some evidence supporting the use of cannabinoids for spasticity and chronic pain (Whiting et al., 2015).

Although cannabis, due to its medicinal potential, has been seen as the hope of many people who suffer from a chronic illness, given the complexity of the endocannabinoid system, developing a pharmaceutical product that meets the specificities of many of these diseases, such as those that affect the nervous system and cancer (Legare et al. 2022), is quite complex. In this sense, many researchers, mainly in the area of plant breeding, pharmacology and biotechnology, have made efforts and created strategies to developf a product that meets the diverse health demands of the world population. Thus, the objective of this review was to briefly present the plant of the genus *Cannabis* and its chemical variability attributed to the quantitative and

qualitative differences that may exist in the composition of bioactive metabolites of different genotypes, and the strategies that have been used in order to efficiently meet the scale industrial use of cannabinoids. We also show in this work the perspectives for obtaining products that are accessible and of quality that can serve a large number of sick people who are waiting for advances in the current therapeutic approaches.

The plant of the *Cannabis* genus

The medicinal value of cannabis is related to the production of secondary metabolites called phytocannabinoids. It is known that there are more than 150 phytocannabinoids in cannabis plants (Citti et al., 2019); Δ^9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) are the two most abundant in most genotypes (Lynch et al., 2016). In live plants and fresh plant material, they exist in the form of phytocannabinoid acid. As plant material ages or undergoes heating, acidic molecules lose a carboxyl portion. Decarboxylation results in the conversion of phytocannabinoid acids into their neutral forms; thus, THCA converts to THC and CBDA converts to CBD (Doorenbos et al., 1971; Hillig and Mahlberg, 2004). Furthermore, when THC is not stored properly and goes through an aging process, it undergoes oxidation and is substantially transformed into cannabinol (CBN) (McPartland and Small, 2020).

The taxonomic classification of the *Cannabis* genus has been a matter of debate in recent decades, as some authors consider the genus as monotypic (composed of one species) (van Bakel et al., 2011; McPartland, 2018) while other authors consider the genus as polytypic (composed of two or three species) (Anderson, 1980; Hillig and Mahlberg, 2004). The genotypes have considerable phytochemical diversity, particularly in phytocannabinoid and terpenoid profiles (Hillig and Mahlberg, 2004), and extensive morphological diversity (Lynch et al., 2016).

In addition to the genotype, other factors influence phytocannabinoid production, such as the stage of maturity at harvest, the part or parts of the plant used, and the cultivation conditions (Trancoso et al., 2022), as well as drying, storage and the methods used to process and formulate the material (Potter, 2014). Thus, the plant material is extremely heterogeneous and, consequently, the proportions of the active ingredients are affected by diverse factors. Maintaining the concentration and profile of cannabis-derived active principles is a challenge, as it involves the use of raw cannabis as a raw material. However, this is essential to obtain an efficient pharmaceutical product, which maintains the stability of the treatment and does not bring relevant side effects, as illustrated in Figure 1.

Cannabis spp. are predominantly annual dioecious species, with male plants (with staminate flowers) and female plants (with pistillate flowers); however, they can also display monoecious (with female and male flowers on the same plant) and hermaphrodite (with female and male organs in the same flower) plants (Small, 2015). Crops with exclusively female plants are preferred for phytocannabinoid production. These compounds are mainly concentrated products obtained from unfertilized female flowers (Russo, 2011), because are densely covered by trichomes, where most

phytocannabinoids are biosynthesized and stored (Potter, 2014). Male plants produce much smaller amounts of phytocannabinoids (Welling et al., 2021).

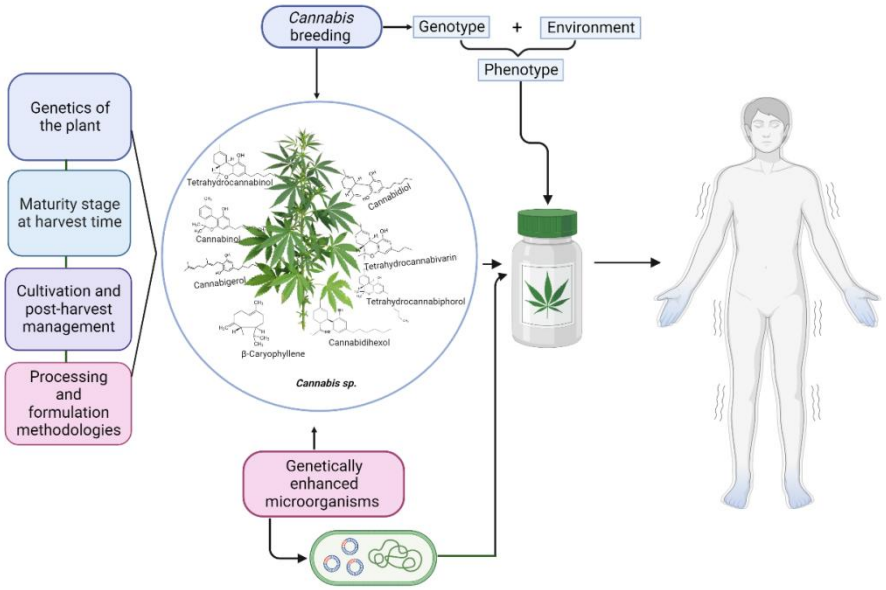

Figure 1. Factors influencing *Cannabis sativa* and its therapeutic potential. Diagram showing the heterogeneity of cannabis plant products under the influence of factors such as plant genetics, the part or parts of the plant used, the state of maturity at harvest, the growing, drying and storage conditions, and the methods used to process and formulate the material. Keeping the concentration and profile of the active ingredients consistent is essential and technologies such as plant breeding (considering the influence of genetic and environmental factors) and construction of genetically modified microorganisms have been applied to obtain a pharmaceutical product. (Created with BioRender.com.)

Chemical variability in the *Cannabis* genus

Genetic variability in germplasm collections or banks is an important source of variation in plant secondary metabolites, influencing metabolite amounts and types produced. This is the case for cannabis plants, where significant quantitative and qualitative differences can exist in the composition of bioactive secondary metabolites between different genotypes (Gorelick and Bernstein, 2017). Cannabis plants are an economically important source of fiber, nutritious seeds, psychoactive drugs, and compounds of medicinal interest, although much remains to be learned about these plants from the genetic point of view (Weiblen et al., 2015).

Cannabis can be classified based on the chemical phenotype (chemotype), that is, the qualitative relationship of the main cannabinoids in (Hillig and Mahlberg, 2004). Chemotype refers to a chemically distinct variety with differences in the composition of secondary metabolites, which is also known as a chemical fingerprint (Hazekamp and Fischedick, 2012). There are five recognized chemotypes. Three groups are based on the ratio of CBD and THC (neutral and acidic forms) (de Meijer et al., 2003; Lewis et al., 2018). Type I plants have a high THC:CBD ratio (>1), plants with an intermediate THC:CBD ratio (generally 0.5 – 2.0) are classified as chemotype II, and plants that have a low THC:CBD ratio (<1) are classified as chemotype III (de Meijer et al., 2003; Aizpurua-

Olaizola et al., 2016; Lewis et al., 2018). Chemotype IV includes plants with a greater predominance of cannabigerol (CBG), which is the main phytocannabinoid in this group, and chemotype V plants contain almost no cannabinoids (de Meijer et al., 2003; Aizpurua-Olaizola et al., 2016).

Plants with a high THC:CBD ratio are called marijuana, while those with a low THC:CBD ratio are called hemp (van Bakel et al., 2011). According to the United States Department of Agriculture, marijuana contains 3 to 15% of Δ^9-THC based on dry matter weight, while hemp has less than 1%. According to Sawler et al. (2015), the genetic differences between hemp and marijuana are not only limited to the genes involved in THC production because the genetic differences between the two are distributed throughout the genome and not restricted to loci involved in phytocannabinoid production. In other words, in general terms, the differences are quantitative in nature and characterized by a polygenic inheritance with a pronounced environmental effect. The terms "hemp" and "marijuana" are also related to the utility of the plant; hemp is generally the plant intended for fiber and seed production, whereas marijuana consists in plants intended for psychotropic use (Small, 2015). However, this designation has changed due to the medicinal use of compounds produced by both marijuana and hemp.

Quantitative components such as total amount of dry biomass, proportion of floral tissue and total phytocannabinoid content are polygenic factors that are not related to specific metabolic pathways and are greatly affected by the environment. On the other hand, the composition of cannabinoids strictly depends on the metabolic pathways for converting common precursors into specific end products (de Meijer and Hammond, 2005). Although genetics exerts a greater influence on the production of phytocannabinoids, there is evidence demonstrating that environmental factors have a direct influence on the modulation of the amount of phytocannabinoids (de Meijer et al., 2003).

Quantitative and qualitative aspects of phytocannabinoid production are often confused. Qualitative traits are controlled by one or two major genes, and quantitative traits are controlled by several genes, each responsible for a small effect. The THC:CBD ratio is a qualitative characteristic, and the THC + CBD yield is quantitative (Hillig and Mahlberg, 2004); the qualitative determination of THC:CBD can be performed early in the cycle and is stable throughout the plant cycle (Hillig and Mahlberg, 2004; Pacifico et al., 2008; De Backer et al., 2012; Aizpurua-Olaizola et al., 2016).

According to de Meijer et al. (2003), the genetic inheritance of CBD and THC is controlled by a codominant monogenic mechanism. It was postulated that a single locus called B, with two B_D and B_T alleles, encodes the enzymes CBD and THC synthetase, respectively. According to this model, the development of a genuine CBD plant has B_D/B_D genotype at the B locus, while a genuine THC plant has B_T/B_T genotype, and plants with CBD and THC ratios are heterozygous B_D/B_T (de Meijer and Hammond, 2005). According to de Meijer et al. (2003), there is a hypothesis that the enzymes CBD-acid synthetase and THC-acid synthase are "isoforms" of the same enzyme and encoded at a single locus by two alleles called B_D and B_T, respectively.

Although most chemotypes have a predominance of phytocannabinoids with a pentyl chain, there are also genotypes with a higher proportion of compounds with a propyl chain, such as tetrahydrocannabivarin (THCV). THCV has anticonvulsant potential and can suppress carrageenan-induced hyperalgesia and inflammation (Russo, 2011). One study

described a genotype originating in South Africa with THCV proportions above 80% (de Meijer and Hammond, 2016).

Given the importance of these various components, an alternative would be their biochemical characterization, focused on the chemotype as a key-parameter; however, the biochemical screening of other compounds, and the identification of those more prevalent in cannabis species should be also considered (Russo, 2018). Aizpurua-olaizola et al. (2016) observed that plants could be classified into different chemotype groups based on CBD and THC concentrations were clearly differentiated by their terpene content, with the characteristic terpenes of each chemotype being identified. This can be interesting for the development of research that evaluates the synergistic effect (entourage) between phytocannabinoids and terpenes or with other plant components (McPartland and Russo, 2001; Russo, 2011; Russo, 2018; Ferber et al., 2020).

It is increasingly clear that components other than THC and CBD, such as terpenes, flavonoids and cannabinoids present in lower concentrations, are involved in the physiological effect of cannabis-derived substance (Hazekamp and Fischedick, 2012; Namdar et al., 2019; Silva Sofras and Desimone, 2022). Such synergy would be apparent under conditions where the activity of a chemical component at a lower concentration complements the main one, decreases adverse effects, or contributes to the stability of a preparation or therapeutic efficacy (Lewis et al., 2018; Russo, 2018; Koltai and Namdar, 2020). For example, β-myrcene, humulene, and linalool are associated with specific strains and can produce sedative effects (Hazekamp and Fischedick, 2012).

With the progress of science concerning plant genetics of the cannabis plant and phytocannabinoid biosynthesis, genotypes with high concentrations of certain phytocannabinoids were developed, which are often found at lower concentrations, such as THCV, cannabichromene (CBC), CBG, cannabidivarin (CBDV), cannabigerivarin (CBGV), and cannabichromevarin (CBCV) (de Meijer and Hammond, 2016; Russo, 2018; Citti et al., 2019). Phytocannabinoids at lower concentrations in the plant and with therapeutic potential have already been reported elsewhere, including Δ^9-tetrahydrocannabutol (THCB), Δ^9- tetrahydrocannabiphorol (THCP), and cannabidibutol (CBDB) (Citti et al., 2019). *In vitro* studies have indicated that CB1 cannabinoid receptors have an affinity for THCP thirty times greater than their THC counterpart (Citti et al., 2019). In addition to the list of phytocannabinoids, cannabidihexol (CBDH) and Δ^9-tetrahydrocannabihexol (THCH) have been shown to have an analgesic potential when administered at low doses (Linciano et al., 2020). Phytocannabinoids such as CBDH and THCH have been shown to have analgesic potential when administered in low doses (Linciano et al., 2020). Furthermore, (-)-Δ8-trans-tetrahydrocannabinols (Δ8-THC), cannabicyclol (CBLs), cannabielsoin (CBE), cannabinodiol (CBND), cannabitriol (TCC) were mentioned in the literature (Goncalves et al., 2019; Gulck and Moller, 2020; Sommano et al., 2022). Therefore, it would not be surprising to see in the near future cannabis varieties rich in other phytocannabinoids generally present in lower concentration in this or that species.

In view of the quantity and variety of secondary metabolites present in cannabis and the synergistic effects between these compounds, the main challenge regarding the medicinal use of these plants is finding a product with a chemical profile that corresponds to the pharmacological need for the treatment of the patient. With the development of

knowledge of the therapeutic effect of different compounds and their combinations, it is necessary to develop genotypes with varied chemical profiles, as show in Figure 2.

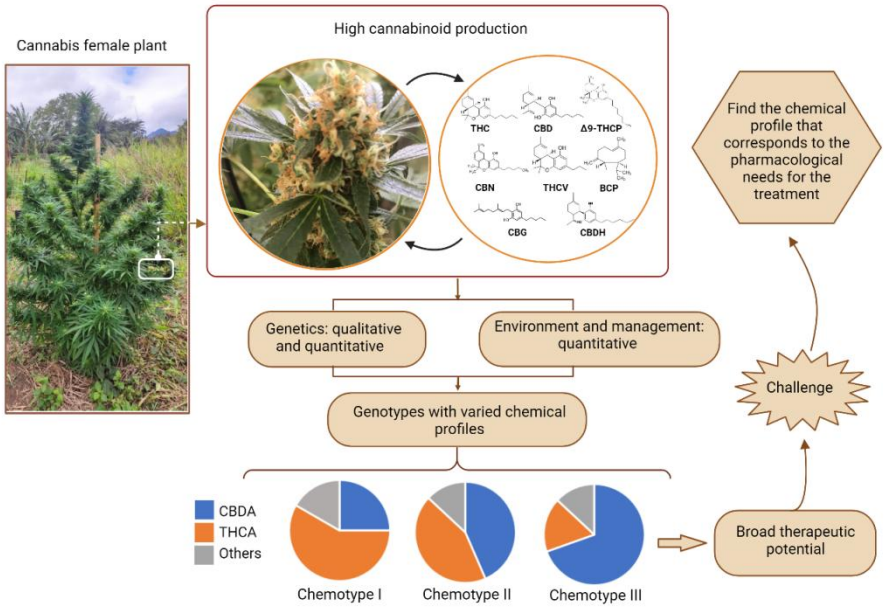

Figure 2. *Cannabis* and the development of genotypes with varied chemical profiles. Diagram showing a female cannabis plant in the reproductive stage, with emphasis on its flowers, the organ with the greatest cannabinoid production. The production and modulation of the amount of cannabinoids are influenced by qualitative (genetic) and quantitative factors (strongly affected by the environment) resulting in genotypes with varied chemical profiles, with three groups based on the ratio between CBD and THC, in the neutral and acidic forms (chemotypes I, II and III). It is a great challenge to find a product with a chemical profile that corresponds to the pharmacological need for the treatment of the patient. BCP, Beta-caryophyllene; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDH, cannabidihexol; CBG, canabigerol; CBN, cannabinol; THC, Δ^9 -tetrahidrocanabinol; THCA, Δ^9-tetrahydrocannabinolic acid; THCV, Δ^9- tetrahydrocannabivarin; THCP, Δ^9-tetrahydrocannabiphorol. (Created with BioRender.com).

Strategy of the pre- and post-harvest management of *Cannabis sativa*

For the cannabis industry, the propagation of vigorous and uniform plants remains a challenge as it is a dioecious crop and therefore relies on cross-fertilization for seed production (Chandra et al., 2020). Technical advances, including the use of different methods such as genetic modifications, cultivation methods that increase the content of the substance of interest (in general CBD) and obtaining plant varieties with cannabinoid profiles of high productivity and resistance to pests have contributed, mainly, to improve cannabis production and to efficiently meet the industrial scale of cannabinoids (Kojoma et al., 2002; Small, 2018; Schwabe and McGlaughlin, 2019; Backer et al., 2020; Slosse et al., 2021; Hurgobin et al., 2021).

Thus, several strategies have been proposed, including, for example, methods such as controlled vegetative and micropropagation, used to ensure, when convenient, that the controlled material is female, generating exclusively female individuals (Trancoso et al.,

2022). According to Backer et al (2020), parameters such as plant variety and density, light intensity and fertilization should be considered in obtaining biomass and phytocannabinoid yields, and specificities such as pot size, type of light and duration of flowering period are predictors of THC production and accumulation. It is also noteworthy that changing the spectrum of LED lights can stimulate photoreceptors and maximize cannabis yield and quality, with reduced operating costs. In this sense, changing the phytocannabinoid profile of cannabis through the artificial light spectrum and also through genetic interactions that can act on the quality of artificial light is a promising strategy to customize the bioactive profile of each plant favoring patients and final products (Magagnini et al., 2018; Eaves et al., 2020; Danziger and Bernstein, 2021; Wei et al., 2021).

Parsons et al. (2019) used polyploidization to establish new *Cannabis* strains with diverse chemical profiles. It is a valuable tool in the genetic improvement of cultivated plants. Tetraploid *Cannabis sativa* strains, THC/CBD balanced drug type, were developed to evaluate the profile of secondary metabolites: THC, CBD or terpenes. The morphology of tetraploid clones was evaluated, observing an increase in the density of trichomes and stomata, and also in the size of fan leaves and sugar leaves, when compared to diploid clones. There was a significant increase in CBD concentrations in the terpene profile and in the buds of tetraploid clones without this increase in THC content.

Another addressed point is that factors such as the low abundance of many of the phytocannabinoids in the plant and their structural complexity are limiting for mass chemical synthesis. However, other plants can produce phytocannabinoid-like molecules such as perrottetinene extracted from *Radula perrottetii*, or anandamide and 2-arachidonoyl glycerol extracted from some bryophytes (Kumar et al., 2019). Considering the evolutionary heterogeneity of the cannabimimetic plants, we might consider the putative evolutionary convergence between plants and animals in regard of neurobiological role played by endocannabinoid system. The biochemical study of that alternative source of phytocannabinoids seems to be promising.

The replacement of the cultivation of the cannabis plant, either in greenhouses or in agricultural fields, by genetically modified microorganisms has been presented by researchers and companies with the objective, among others, of efficiently producing cannabinoids of pharmaceutical interest and also other potentially useful compounds, plus THC and CBD. Among these alternative organisms are, for example, *Komagataella phaffii* (Zirpel et al., 2017; Zirpel et al., 2018), a species of yeast *Saccharomyces cerevisiae* -(Luo et al., 2019)- the bacteria *Escherichia coli* and *Zymomonas mobilis* (used in the production of tequila), and a green alga, *Chlamydomonas reinhardtii* (Dolgin, 2019). Lastly, semi-dwarf cannabis cultivars, for example, have been developed that have a high leaf density and low THC level (Small, 2018).

Perspectives

Depending on local culture and regulations, around the world, *C. sativa* is traded and consumed for a variety of purposes. In this review, medical cannabis, advances, strategies, and perspective concerning the therapeutic potential were pointed out. Faced with an expanded endocannabinoid system, the specificities and complexity of diseases, obtaining a pharmaceutical product that serves the patient is a great challenge. In this sense, there are many investigations in different fields.

In the productive process of cannabis for medicinal purposes, breeding is an area that stands out because, taking into account the different needs, it acts by developing and selecting varieties to be produced on a large scale, and in this case, it can be promising, since the *C. sativa* plant has wide genetic variability. But, given this scenario or this challenge, it is important to have targeted breeding based on knowledge of the inheritance of the main secondary metabolites and intended pharmacological uses. Thus, different varieties must be obtained, given the differences required by the range of medicinal applications.

New cultivars and varieties (strains) have been made available by companies; however, one of the problems that also has to be solved is the difficult identification of varieties from phenotypic characteristics. In this sense, techniques such as fingerprinting, which allows the analytical characterization of the genome, guarantee the consistency and stability of the samples. On the other hand, the quantification of chemical compounds, mainly cannabinoids (THC and CBD) and terpenes, are commonly analyzed by chromatographic processes.

The induction of polyploidy in cannabis is a subject strongly discussed among plant breeders (Bagheri and Mansouri, 2015; Parsons et al., 2019; Kurtz et al., 2020), whose results depend on long years of research to demonstrate whether polyploidy would be advantageous for therapeutic purposes. On the other hand, although micropropagation is a good strategy to maintain the uniformity and high quality of the final product, it is a complex process and may be influenced by several factors. The perspective is that innovations coming from the computational area can optimize in vitro processes and solve many problems in cannabis tissue culture, such as low replicability and recalcitrance. In this sense, technological advances are increasingly present in the creation of different machine learning algorithms, in the use of nanoparticles and in the synthesis of novel plant growth regulators (PGRs) (Hesami et al., 2020; Monthony et al., 2021).

Another important point that contributes positively to agricultural production is that *C. sativa* has been seen as a green vaccine, also considered a climate-friendly crop that can lead the world towards sustainable development and well-being of the planet. It is a crop with a high capacity to capture carbon dioxide from atmospheric air and to reduce greenhouse gases and desertification. In addition, it collaborates in the biodiversity of species, attracting many pollinating insects due to a high emission of aromatic essences and terpenes from its inflorescences (Adesina et al., 2020; Sorrentino, 2021).

Other paths follow towards the production of cannabinoids from fermentation carried out by yeasts (Luo et al., 2019). Although this technology represents a cheaper and more sustainable option, further optimization of this process is needed to define the conditions for yeast cultivation and to obtain a large-scale production of cannabinoids that can compete with traditional plant-based sources. Additionally, considering that there is great difficulty in tracking and identifying large producers of cannabinoids, a THC biosensor was recently developed, based on the coupling of G-protein-coupled receptors (GPCRs) in yeast, which made it possible to measure the production yields of a library of Δ^9-tetrahydrocannabinol acid synthase (THCAS) mutants and this meant having a tool to increase yield and reduce screening cost, although, still needing improvements, mainly regarding the localization and coupling of GPCRs in yeast (Shaw et al., 2022).

The fact is that advances in medicinal cannabis have been gradual and range from cannabis breeding to efficiently meet the industrial scale of cannabinoids to therapeutic

interventions that seek to cure or minimize the losses caused by a disease. Therefore, it is concluded that it is not so simple to manipulate the plant *C. sativa* for medicinal purposes, nor to manipulate an expanded endocannabinoid system, considering the activity of endocannabinoids on different cannabinoid and non-cannabinoid receptors. Therefore, considerable multidisciplinary research, in the fields of agriculture and health, is needed so that more advances can be made.

AUTHOR'S CONTRIBUTIONS

Maria de Fátima dos Santos Sampaio: writing, reviewing and figures design. Ingrid Trancoso da Silva: writing, revising and designing figures. Messias Gonzaga Pereira and Norberto Cysne Coimbra: writing, revised and supervised. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Research Grant 2020/15050-7), Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (FAEPA) (Research Grant 374/2022), and Pro-Rectory of the University of São Paulo (USP) Research Grant (NAP-USP-NuPNE; process IaPq2012-156-USP-12.1.25440.01.6). M. de Fátima dos Santos Sampaio is a postdoctoral researcher supported by CNPq (PDJ grant 155489/2018-6). N.C. Coimbra is a researcher from CNPq (PQ1A-level grants 301905/2010-0 and 301341/2015-0; PQ2-level grant 302605/2021-5). Each organization had no role in the study design; the collection, analysis, and interpretation of the data; the writing of the report; or the decision to submit the paper for publication. All figures in the manuscript were created with BioRender.com.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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