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Review

Cannabis sativa research trends, challenges, and new-age perspectives

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SUMMARY

***Cannabis sativa* L. has been one of the oldest medicinal plants cultivated for 10,000 years for several agricultural and industrial applications. However, the plant became controversial owing to some psychoactive components that have adverse effects on human health. In this review, we analyzed the trends in cannabis research for the past two centuries. We discussed the historical transitions of cannabis from the category of herbal medicine to an illicit drug and back to a medicinal product post-legalization. In addition, we address the new-age application of immuno-suppressive and anti-inflammatory extracts for the treatment of COVID-19 inflammation. We further address the influence of the legal aspects of cannabis cultivation for medicinal, pharmaceutical, and biotechnological research. We reviewed the up-to-date cannabis genomic resources and advanced technologies for their potential application in genomic-based cannabis improvement. Overall, this review discusses the diverse aspects of cannabis research developments ranging from traditional use as herbal medicine to the latest potential in COVID-19, legal practices with updated patent status, and current state of art genetic and genomic tools reshaping cannabis biotechnology in modern age agriculture and pharmaceutical industry.**

INTRODUCTION

Cannabis sativa L. is one of the earliest known cultivated plants since agricultural farming started around 10,000 years ago (Schultes et al., 1974). It is a multi-purpose crop plant with diverse agricultural and industrial applications ranging from the production of paper, wood, and fiber, to potential use in the medicinal and pharmaceutical industries. The first-ever report to reveal the prospects of *C. sativa* L. as a medicinal plant was already published in 1843 and described the use of plant extracts to treat patients suffering from tetanus, hydrophobia, and cholera (O'Shaughnessy, 1843). However, the first chemical constituent identified was oxy-cannabis (1869) (Bolas and Francis, 1869), isolated cannabinoid (1896), and fully identified in 1940 was cannabidiol (CBD) (Jacob and Todd, 1940) followed by tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964; Santavý, 1964) and cannabigerol (CBG) in 1964, and cannabichromene (CBC) in 1966 (Gaoni and Mechoulam, 1966). Identification of THC later led to an understanding of the endocannabinoid system followed by the discovery of the first cannabinoid receptor (CB1) in 1988 (Devane et al., 1988; Russo, 2016). CB1 receptor acts as a homeostatic regulator of neurotransmitters for pain relief mechanisms, but the same mode of action was responsible for intoxicating effects from cannabinoids' excessive use. Thus, the understanding of mode of action of CB1 receptor raised concerns about the adverse effects of cannabis use. Consequently, the plant was removed from the medicinal category and recategorized exclusively to the category of drug-type plants.

Cultivation and use of cannabis plants for recreational, medical, and industrial use were strictly banned and severely limited the scientific research in the field. Owing to strict legal regulations, the plant remained unexplored for its incredible potential in drug discovery for an extended period until it was legalized for medical use first in California and later in many countries around the globe. Extensive research followed legalization to explore the chemodiversity of cannabinoids for potential clinical value. In total, more than one thousand compounds—278 cannabinoids, 174 terpenes, 221 terpenoids, 19 flavonoids, 63 flavonoid glycosides, 46 polyphenols, 92 steroids—have been identified (ElSohly and Slade, 2005; Gould, 2015; Radwan et al., 2017). Nearly 278 of these compounds are cannabinoids and classified as phytocannabinoids (plant-based) to distinguish them from endocannabinoids (non-plant). Cannabimimetic drugs binding to CB1-receptors in the endocannabinoid system can also be found in algae, bryophytes, and monilophytes

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(Carvalho, 2017; Kumar et al., 2019). The major cannabinoids in *cannabis* include THC, CBD, and CBC, their precursor CBG and cannabinol (CBN) (Flores-Sanchez and Verpoorte, 2008). To date, 10 CBN-type, 17 CBG-type, 8 CBD-type, and 18 THC-type cannabinoids have been isolated (Gaoni and Mechoulam, 1964). Cannabigerolic acid (CBGA), a CBG-type cannabinoid, is the central precursor for the biosynthesis of psychoactive THC, non-psychoactive CBD, and CBC (ElSohly and Slade, 2005; Gould, 2015; Radwan et al., 2017).

Cannabinoid biosynthesis in plants occurs in specialized biosynthetic organs called glandular trichomes (Happyana et al., 2013) on female flowers and leaves. Several studies use metabolic profiling of trichomes to demonstrate variation in trichome size, density, and relative concentration of cannabinoids (Happyana et al., 2013; Small and Naraine, 2016). However, the genetic mechanisms underlying the developmental changes in trichomes and consecutive cannabinoid content are still unknown. Apart from natural and chemical biosynthesis methods (Bovens et al., 2009), heterologous biosynthesis of cannabinoids has also been reported (Luo et al., 2019). However, the considerable amount of side products is still one of the major bottlenecks (Luo et al., 2019; Thomas et al., 2020) in cannabinoid production.

This review highlights the latest research developments and challenges in cannabis plant sciences, the role of trichomes as biosynthetic sites, with a special focus on plant biology. In addition, we discuss the existing legal practices with patent information for the *C. sativa* L. We also discuss the new potential use of cannabinoids for COVID-19 treatment. Finally, we address the available genomic and transcriptomic resources and discuss their potential toward the genetic improvement of cannabis. Overall, we provide the first in-depth review of diverse aspects of *C. sativa* L. from traditional medicinal use to genomics insights and research perspective to broad industrial applications.

CANNABIS RECORDS IN BIBLIOGRAPHIC DATABASES

Cannabis-related publications were searched in four major scientific literature and citation databases of biomedical and life-sciences journals: the EuropePMC (https://europepmc.org/) by EMBL-EBI (Data S1), Elsevier's Scopus (https://www.scopus.com/) (Data S2), PubMed Central at NCBI (NCBI PMC: https://www.ncbi.nlm.nih.gov/pmc/) (Data S3), and Web of Science (WoS: https://www.webofscience.com/wos/) of Clarivate Analytics. The search criteria—"cannabis OR marijuana OR hemp OR cannabinoids OR cannabidiol OR cannabinol" were used to examine available research articles. Nearly 80,979, 64,637, 43,182, 28,759 cannabis-related research articles were found in EuropePMC, Scopus, WoS, and NCBI PMC, respectively. The sheer difference in the number of articles could be attributed to the years for which the Cannabis records are present in the databases. Europe PMC currently holds cannabis records for 239 years since the oldest publication in 1783. Whereas, Scopus has data for 194 years (since 1828), NCBI PMC 182 years (since 1840), and WoS only 77 years (since 1945) (Figure 1A). Despite cannabis records for only 77 years WoS records exceed NCBI PMC, owing to the data acquisition policy similar to Scopus, wherein all the cited references for a publication are pulled and listed in the database. Another major reason for the different records in the archives could be owing to the source repositories and partner journals. Although NCBI PMC has only 6.9 million articles from over 10,656 journals by April 2021, Scopus has more than 77.8 million records from nearly 23,500 journals, and WoS comprises over 171 million records including journals, books, and proceedings. However, EuropePMC acquires data from multiple bibliographic repositories such as PubMed, MEDLINE (MED), PMC, AGRICOLA (AGR: AGRICultural OnLine Access), and Chinese Biological Abstracts (CBA) (Figure 1D). It includes more than 45,6 million documents including articles, books, preprints, patents, conference papers, and microPublications. Cannabis citation metadata was publicly available for bulk download from EuropePMC, Scopus, and NCBI PMC from 6586, 8647, 3864 journals, respectively (Figure 1B). Among the article identifiers such as DOI, PMCID, and PMID, DOI was found for 85.62% records of EuropePMC, 85.44% of Scopus, and 91.9% of NCBI PMC. Since DOI was the only common identifier, it was used for the comparison of three datasets (Figure 1C). Cannabis records in EuropePMC comprised nearly 76.73% of NCBI PMC and 49.75% of Scopus data (Figure 1C). Hence, metadata from EuropePMC was selected for downstream bibliometric analysis. Majority of Cannabis records in EuropePMC were from MEDLINE (94.94%), followed by 4.29% from PMC, only 0.75% from Agricola (AGR), and 0.02% from CBA (Figure 1D). The distribution of source databases indicates the most explored field in Cannabis research for the last 239 years.

TRENDS OF CANNABIS RESEARCH FROM 1783 TO 2021

C. sativa L. originated in central Asia and later spread to Europe during its cultivation for diverse applications. Archaeological evidence of early medical use was found in fossil records dating back to 315–392AD (Zias et al., 1993). There is a consensus that the plant has been used as traditional medicine

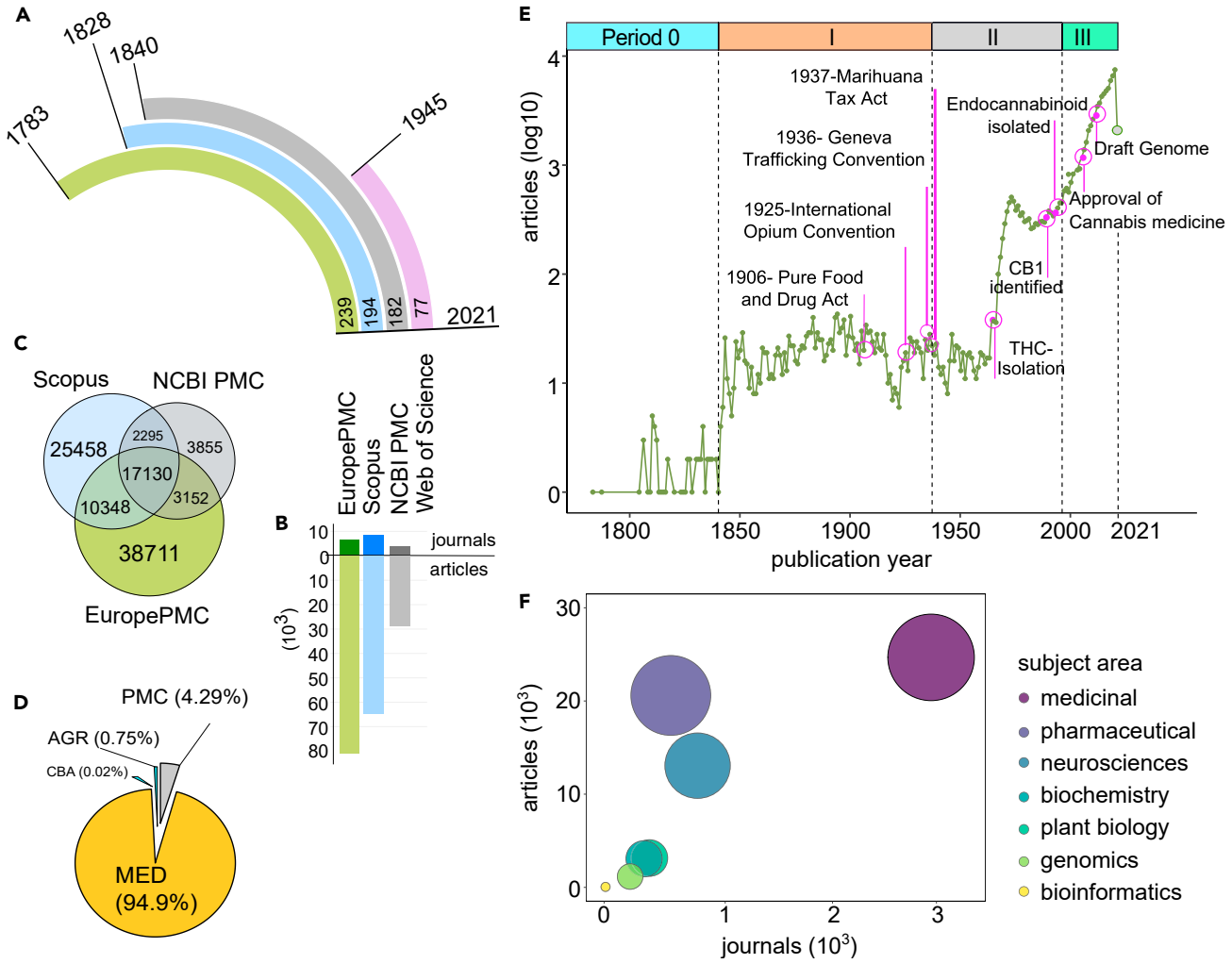

Figure 1. Cananbis Research Trend

(A) Timeline of Cannabis records. Timeline and years from the earliest to the latest cannabis records in the literature databases of life science and biomedical journals are depicted with color coded radial bar for EuropePMC (green), Scopus (blue), NCBI PMC (gray), and Web of Science (lilac). The publication year of the first available Cannabis related document in each database is marked by a verticle line at top of corresponding bar. The number of years from the oldest available article to the most recent publication in 2021 are indicated at the base of each bar.

(B) Cannabis documents in the literature archives. The bar plot shows number of documents in the selected databases with Y axis divided into two sections to show the journals (upper section) and articles (lower section).

(C) Common citations in databases. EuropePMC, Scopus and NCBI PMC records were compared using DOI identifiers and number of shared and unique documents between three sets are shown as venn plot. The size of circle corresponds to number of articles with DOI in each database.

(D) Cannabis literature source in EuropePMC. Color coded pie chart depicts the source repositories of Cannabis articles in EuropePMC. The contribution (percentage of articles) from MEDLINE (MED), AGRICOLA (AGR), PubMed Central (PMC) and Chinese Biological Abstracts (CBA) are shown by orange, gray, turquoise, and cyan color.

(E) Cannabis Research trends. The overall pattern of Cannabis research from 1783 to 2021 is depicted by the trend line (log scaled) with each dot representing the number of articles (Y axis) per year (X axis). The research periods categorized as zero (1783–1840), I (1840–1937), II (1937–1996) and III (1996–2021) are separated by vertical dotted lines. The three major achievements (I) discovery of cannabinoids, ii) cannabinoid receptors and iii) endocannabinoids that pushed the interest in Cannabis are marked in pink color. The number of articles in 2021 are still growing and therefore depicted as hollow enlarged dot.

(F) Cannabis research subjects. Bubble plot depicts the most and least explored scientific subject areas with size corresponding to the number of articles (Y axis) and journals (Y axis) in each category. Major proportion of Cannabis research was related to medical and medicinal sciences followed by pharmacology and pharmaceuticals, neurosciences and psychology, biochemistry and biotechnology, genetics and genomic sciences, agriculture and plant biology, and bioinformatics.

(Bridgeman and Abazia, 2017). Based on the research during the past two centuries, we divide the scientific era into four periods (Figure 1E). The period zero (1783–1840) marked the first-ever mention of Cannabis in the category of medicinal plants in the years 1783 (Laurentius Crellius and Huntero, 1783) and 1787 (Wright et al., 1787). There were only 52 articles and 38 reviews in the next five decades (source: EuropePMC). Most reports mentioned the botanical aspects of hemp, the quality of fiber, and few observations about its use in traditional medicines. The first period (1840–1937) started with the detailed evidence-based report of chemical properties and medicinal potential of *Cannabis indica* (hemp) by William O'Shaughnessy (O'Shaughnessy, 1843) followed by an array of medicinal reports in 1923 articles and 183 reviews in the next 96 years.

Scientific endeavors to experiment, observe, and understand the diverse medicinal applications of cannabis were still in the early stages. However, 1900s witnessed a series of legal regulation in the direction of the criminalization of cannabis. Cannabis was starting to be categorized into the list of narcotic drugs and Poisons Rules including the Pure Food and Drug Act (1906) pushed for stricter measures for cannabis distribution. Later International opium Convention (1925) called for measures to regulate Indian hemp. Exports unless exclusively for medical or scientific purposes or European hemp (for fiber) were banned. Uniform State Narcotic Drug Act (1925), Geneva Trafficking Conventions (1936) resulted in criminalizing the cultivation, possession, manufacture, and distribution of cannabis derivatives. Marihuana Tax Act (1937) levied heavy taxes on the possession and selling of cannabis, excluding medical, and industrial use. As a consequence, the cultivation and procurement of cannabis for research purpose became increasingly difficult and severely limited the research of medicinal cannabis during this era (Figure 1E: Period I). During the second period (1937–1996), cannabis research suffered major restrictions owing to legal regulations in the first two decades until the identification of the first cannabinoid—cannabidiolic acid in 1954 (Hanuš et al., 1975; Krejčí and Šantavý, 1955; Krejčí et al., 1958; Santavý, 1964), isolation of the most psychoactive component of cannabis, the THC in 1964 (Mechoulam et al., 1964). Isolation of the THC, discovery of CB1 (Devane et al., 1988), and CB2 (Munro et al., 1993) receptors, followed by the Compassionate Investigational New Drug program (1978) paved the way for decriminalization laws. The discovery of endocannabinoid and the role of cannabis in the medicinal field have been reviewed in (Hanus, 2009; Kabelik and Santavy, 1955) As a consequence a steep surge was observed in the number of cannabis-related articles from 445 articles and 25 reviews during 1937–1964 to nearly 8,888 articles and 773 reviews during 1964–1996 (Figure 1E: Period II), although with a short period of decline between 1973 and 1982. Finally, the third period (1996-till date) began with the historical Compassionate Use Act of 1996 in California approving medical cannabis. Post-legalization (1996 onwards), cannabis has been extensively explored for its diverse potential in the pharmaceutical and medicinal industries. During the third period, cannabis research witnessed an unprecedented growth with nearly 67,777 articles, 13,202 reviews, and 493 preprints (source: EuropePMC), of which 97.01% articles were published in the last two decades since 2000 (Figure 1E:period III. Approval of the first cannabis-based inhaler spray in 2005 (Perras, 2005; Pain, 2015) and the first draft of the cannabis genome in 2011 (van Bakel et al., 2011) in this era were the two major accomplishments that exponentially accelerated the research development.

The trends of cannabis study in the diverse array of research articles and journals indicate the core interests of the scientific community. To further investigate the most researched field, the journals of cannabis articles were categorized into scientific and social areas. The journals related to social, law, and policy-based studies were merged into the subject category of social research. Although the majority of broad scientific subjects were grouped into the following seven major categories: (i) medicinal (all medical and medicinal subjects), (ii) pharmaceutical-comprised of pharmacology, pharmaceuticals, drug, toxicology, and chemical studies, (iii) neurosciences-comprised of neurological, brain-related, psychiatry, psychology, and cognitive studies, (iv) biochemistry-included biotechnology, microbiology, immunology, virology, and biochemistry, (v) genomics-grouped genetical and genomic studies, (vi) plant biology-included plant sciences, agricultural, botanical aspects, plant-pathogen and environment studies, and lastly, (vii) bioinformatics (includes data analytics). Journals that could not be classified into either of the aforementioned categories or social research categories were excluded from downstream evaluation. The Scientific subject areas (74.47% of journals) were further compared for the corresponding number of articles and journals (Figure 1F). A distinct pattern was observed for the Clinical aspects of cannabis which remained a major focus since the very beginning with nearly 94.76% published articles including 64.51% articles in medicinal subject areas, 19.55% in pharmaceutical sciences, and 10.70% in neurosciences. In contrast, plant biology and agricultural sciences comprised only 2.62% of articles, followed by 0.71% genomics,

and 0.07% bioinformatics-based cannabis research. Genomics and bioinformatics are relatively new subjects growing at a fast pace since the release of the first Cannabis draft genome in 2011 together. Recent advances in sequencing technologies have further propelled genomic and transcriptomic studies with the purpose of dissecting the regulatory networks. The growth of genomic data in public space has met with the fast-paced development of bioinformatics tools for data analysis. In addition, ongoing developments of machine-learning (ML) and artificial intelligence (AI)-based genomic tools will facilitate genetic-level understanding of cannabis metabolism for the selective breeding of genetically modified cannabis with improved metabolic traits.

CANNABIS SATIVA L. PHYSIOLOGY AND LEGAL STATUS

Physiological, morphological, and developmental aspects of Cannabis are key in understanding the plant growth patterns and chemical profiles. However, plant growth and function are substantially influenced by abiotic factors and nutrient availability. Although botanical aspects (Farag and Kayser, 2017), plant architecture, and florogenesis of female *C. sativa* plants (Spitzer-Rimon et al., 2019) with detailed trichome morphogenesis (Hammond and Mahlberg, 1977) provided crucial insight into plant biology. However, it also became increasingly important to determine the effect of abiotic factors on Cannabis growth and chemical yield, especially for large-scale commercial breeding programs. Hence, in-depth analysis of the effect of soil fertilization, salinity, temperature, and light conditions, as well as nutrient and water-use efficiency is key in establishing industrial-scale systems for the cultivation of hemp and marijuana varieties. The first available records about the mineral nutrition of hemp plants were published by Tibeau et al., in 1936 (Tibeau, 1936). Later in 1944, Clarence H Nelson published the effect of varying soil temperature on hemp growth (Nelson, 1944). The first publication with a detailed response of greenhouse cultivated cannabis to nitrogen (N), phosphorus (P), and potassium (K) was published in 1977 (Coffman and Gentner, 1977). Furthermore, two parallel reports by HMG et al., in 1995 discussed the impact of nitrogen fertilization on sex expression in hemp (van der Werf and van den Berg, 1995), and the effect of temperature on leaf and canopy formation (van der Werf et al., 1995). Importantly, most physiological studies in the second and third period (Figure 1A) were published for hemp with a focus on photosynthetic response and biomass yield with varying conditions such as temperature, water availability, nitrogen, and mineral nutrition (Amaducci et al., 2002; Aubin et al., 2015; Finnan and Burke, 2013; Papastylianou et al., 2018; Tang et al., 2017, 2018). However, the first study to assess the chemical response of hemp plants was published in 1997 (Bócsa et al., 1997).

Since the physiological response of drug-type medical cannabis plants may differ from hemp plants owing to the distinct genetic and chemical differences. Hence, a clear understanding of optimum factors for medical cannabis is inevitable for the efficient cultivation of plants with desired chemical composition. Among the first few studies that addressed medical cannabis, photosynthetic response to photon flux densities, temperature, and CO2 conditions were published by Chandra et al., in 2008 and 2011 (Chandra et al., 2008, 2011). Bernstein and the group further addressed the growth and chemical response of medical cannabis to mineral nutrition especially N, P, and K (Saloner and Bernstein, 2020; Shiponi and Bernstein, 2021; Saloner et al., 2019; Bernstein et al., 2019). Saloner and Bernstein (Saloner and Bernstein, 2020) reported optimum N concentration at 160 mg L^{-1}, N with lower levels showed several symptoms inducing necrosis and growth retardation while the higher levels impacted in reducing concentrations of THCA and CBDA. Shiponi and Bernstein (Shiponi and Bernstein, 2021) showed a negative association of cannabinoid concentrations and yield with increasing P supply. Saloner et al. further (Saloner et al., 2019) determined genotype-dependent effect of K nutrition on medical cannabis reporting 240 ppm K detrimental for the genotype Royal Medic and stimulant for Desert Queen genotype while 15 ppm K was insufficient for both genotypes. Further in 2019 Bernstein et al. (Bernstein et al., 2019) discussed the combined effect of NPK nutrition upon cannabinoid concentration. In addition to soil nutrients, heavy metal uptake potential of hemp varieties has also been thoroughly investigated by multiple reports in past years (Citterio et al., 2003; Ferrarini et al., 2021; van Ginneken et al., 2007; Hoseini et al., 2012; Sakizadeh et al., 2016; Shi and Cai, 2010; Rheay et al., 2021; Vandenhove and Van Hees, 2005). Industrial hemp varieties of *C. sativa* have also been shown to grow in soils contaminated with heavy metals (Citterio et al., 2003; van Ginneken et al., 2007; Hoseini et al., 2012; Sakizadeh et al., 2016; Shi and Cai, 2010; Vandenhove and Van Hees, 2005) and reported for heavy metal accumulation. Several field projects have assessed the phytoremediation potential of hemp plants for the reclamation of contaminated and radioactive soils (reviewed in detail (Ferrarini et al., 2021; Rheay et al., 2021)).

Cannabis cultivars are classified into drug-type (marijuana) fiber-type (hemp) and neutral (zero cannabinoid) plants with distinct cannabinoid constitutions. Drug-type cultivars with THC/CBD ratio ≥10 are classified as chemotype I, while those with THC/CBD ratio ranging from 0.2 to 10 are grouped as chemotype II. In contrast, fiber-type cultivars with THC/CBD ratio <0.2 are categorized as chemotype III. Chemotype IV also has low THC contents but with the potent percentage of CBG. Furthermore, the chemotypes producing very little to almost zero cannabinoid compounds (neutral) are grouped as chemotype V -was first described by Mandolino et al. (Cascini et al., 2012; Hartsel et al., 2016; Mandolino and Carboni, 2004). Apart from cannabinoid (THC, CBD) content, drug and fiber-type plants have significant genetic variation. Sawler et al., 2015 described that marijuana is genetically inclined toward "*sativa*" and hemp have a similarity with the "*indica*" type (Sawler et al., 2015). Moreover, each plant type has unique applications differentiating them from each other. For example, the fiber-type "hemp" plant has mostly food and industrial applications, including production dietary products, hemp oil, seeds, and fiber, while the "marijuana" drug-type plant is used exclusively for medicinal and recreational purposes.

Despite such a huge genetic and application diversity, both types of cannabis plants were categorized as "Scheduled 1 drug" according to the "Controlled Substances Act" in 1970 (Drug Enforcement Administration, 1970). These restrictions had a serious impact on the research preventing the scientific community to study the potential of diverse yielding traits for hemp. However, after 44 years in 2014 the "agricultural act section 7606" was implemented which distinguish hemp from marijuana (Drug Enforcement Administration, 1970). Approval of law opened the window for scientific community to conduct research and cultivate hemp. Since then, 33 US states and more than 47 countries around the world have been growing hemp for research and industrial use (Schluttenhofer and Yuan, 2017). On the other hand, Marijuana research and legalization have been expanding at a comparatively slower rate and till now only 16 countries have legalized medicinal cannabis (Aguilar et al., 2018). Furthermore, a detailed study would be desirable to understand the gene function, the genetic composition, and the underlying mechanisms regulating the diversity of cannabinoids in both major varieties. Availability of the regeneration protocol (Lata et al., 2016) and transformation (Schachtsiek et al., 2018) studies could be utilized for the expression studies to unravel the mystery of these mechanisms, especially in trichomes.

TRICHOMES AND CANNABINOID BIOSYNTHESIS

Glandular trichomes are the primary site for cannabinoid biosynthesis and accumulation (Lanyon et al., 1981) in *C. sativa*. The biosynthesis of cannabinoids (Andre et al., 2016; Degenhardt et al., 2017) starts from the plastidial localized methylerythritol 4-phosphate (MEP) pathway resulting in the formation of geranyl pyrophosphate (GPP) (Marks et al., 2009) and the fatty acid pathway leading to the production of olivetolic acid (OA) (Raharjo et al., 2004). GPP and OA in the presence of olivetolic acid cyclase (OLS) (Gagne et al., 2012; Stout et al., 2012) and an aromatic prenyltransferase catalyze the reaction to form the cannabigerolic acid (CBGA) (Gagne et al., 2012; Fellermeier and Zenk, 1998; Taura et al., 2009), which is the central precursor for cannabinoids biosynthesis. van Bakel et al., 2011 analyzed the transcriptomic and genomic data and described the exclusive presence of the THCAS and CBDAS in the drug and hemp-type plant, respectively (van Bakel et al., 2011). It is suggested that the activation of respective enzymes from the central precursor CBGA is responsible for regulating the THC and CBD concentration for each chemotype. However, the precise regulatory mechanism is still unknown.

Besides biosynthesis, understanding the trichome physiology is also vital to elucidate the trafficking and localization of metabolites. For cannabinoid biosynthesis, there exist three major reactions: (i) biosynthesis of monoterpene precursor (GPP) via MEP and fatty acid intermediate (OA) from polyketide pathway, (ii) prenylation of the precursors, and (iii) cyclization. The MEP pathway in plastid prenylation is localized in the chloroplast membrane, where the C-prenylated CBGA synthase is membrane-bound. The integration of the enzyme in the membrane seems essential, and the folding pattern is crucial for its functioning. Therefore, simple cloning and functional expression of this enzyme in a heterologous host such as yeast to generate the desired cannabinoids is challenging. Terpenoid cyclization reactions are the most complex reactions found in nature and the biotransformation from CBGA to THCA by the THCA synthase is assumed to occur in the cytosol. This hypothetical model is under ongoing debate and it might be likely that biocatalysis occurs in the extracellular oil container under a non-aqueous environment (Lange et al., 2015). In 1992, Mahlberg and Kim postulated that THCA synthase is located in the outer membrane of the head cells or even attached on the outer membrane surface extending into the essential oil (Mahlberg and Kim, 1992). In recent studies, LC-MS/MS was used to detect a functional active THCA and CBGA

synthase in the exudates from glandular trichomes of cannabis (Rodziewicz et al., 2019). Zirpel et al., described the need for an excellent understanding of protein chemistry and folding of these enzymes to produce the cannabinoid using a heterologous host (Zirpel et al., 2018). Detailed knowledge of genetic regulatory mechanisms underlying cannabinoid biosynthesis is a future challenge. Identification of regulatory elements such as transcription factors (TFs) and microRNAs (miRNAs) could be utilized to understand the mechanistic insights of trichomes initiation, development, and densities. An in-depth understanding could be applied toward the breeding of genetically improved cannabis varieties with enhanced cannabinoids concentration in trichomes.

DEVELOPMENTS IN CANNABIS GENOMICS

Drug- and fiber-type plants differ in biosynthesis, concentration, and composition of metabolites (Finnan and Burke, 2013). To determine the genetic variations regulating plant-specific differences, it is essential to compare the genomes. Advanced sequencing technologies combined with continuously improving bioinformatics tools have allowed rapid sequencing and analysis of multiple genomes and transcriptomes. The very first draft genome of *C. sativa* was released in 2011 by Bakel et al. (van Bakel et al., 2011). They sequenced marijuana cultivar Purple Kush by using Illumina short reads and assembled them in combination with 454 reads. They also sequenced fiber-type hemp cultivar Finola for a genome-level comparison. In addition to whole genome, the first complete mitochondrial reference genome was also obtained in 2016 from the cannabis hemp variety Carmagnola (White et al., 2016). Later in July 2016, two complete chloroplast genomes of marijuana (THC dominant) African variety Yoruba Nigerian and Korean hemp non-drug variety (low THC) Cheungsam (Oh et al., 2015) were sequenced and used to determine the phylogenetic position of *C. sativa* relative to other members in the order Rosales. Furthermore, in September 2016 released complete chloroplast genomes of two *Cannabis* hemp varieties, the Carmagnola (Italian) and Dagestani (Russian), to determine their genetic distance compared with the closest cannabaceae chloroplast of *Humulus lupulus* variety Saazer (Vergara et al., 2016).

Increasingly growing support for open-data policy by multiple industries is improving transparency in cannabis agriculture. In 2016, the industrial lead in cannabis research from Courtagen Life Sciences and Phylos Bioscience independently generated the genomes of hybrid marijuana strain (THC dominant) Chemdog91 (by Illumina GAII) and marijuana strain (CBD dominant) Cannatonic (using PacBio), respectively. Phylos Bioscience also released genomic data of 850 *Cannabis* strains as a part of "Open Cannabis Project" for plant breeding programs. With an objective to explore Cannabis population genetics, Phylos Bioscience developed three-dimensional interactive map of nearly 1000 cannabis strains (https://phylos.bio/galaxy/). In 2017, the genome of hybrid marijuana cultivar Pineapple Banana Bubba Kush (PacBio) was released as part of Cannabis Genomic Research Initiative. In 2018, Grassa et al. generated the first chromosome-level assembly for the genome of CBDRx, a high CBD cultivar of *C. sativa* by using advanced long-read Oxford Nanopore Technology (ONT) and PacBio Single-Molecule Real-Time (SMRT) sequencing (Grassa et al., 2018). Later in 2019, Laverty et al., improved the initial draft assemblies (van Bakel et al., 2011) of drug-type Purple Kush and hemp-type Finola to chromosome-level by using ultra-long PacBio reads (Laverty et al., 2019). In addition to genomes of high CBD and THC marijuana and hemp cultivars, a medicinal *Cannabis* strain with a balanced THC/CBD ratio was sequenced by Shivraj et al. (Braich et al., 2020).

Until 2020, nearly all Cannabis genomes had been obtained from the hemp and marijuana cultivars, selectively bred for generations. However, cultivars lose genetic diversity owing to domestication and successive plant breeding for selected traits. In contrast, the wild-type genomes exhibit relatively high heterozygosity and genetic diversity, which might provide unique evolutionary insights into the cannabis genome. Therefore, in 2020, Gao et al. sequenced the first samples of *C. sativa* wild-type "Jamaican Lion" variety growing in the geographically isolated Himalayan region in Tibet. Because these wild-type plants retained the ancestral genetic make-up, therefore, the data generated from this study was used as a tool to determine the inheritance patterns and evolutionary inference of cannabis (Gao, 2020).

The published genomes of high THC, high CBD marijuana cultivars, and hemp varieties, exhibited inconsistent chromosomal nomenclature, arrangement, and varying degree of gaps. Therefore, by end of 2020, Shivraj Braich et al. generated a relatively complete draft genome assembly for Cannbio-2, the medicinal cannabis strain with a balanced THC/CBD ratio (Braich et al., 2020). To present date, only 13 *Cannabis* genomes are publicly available at National Center for Biotechnological Information (NCBI). Of which 3 assemblies are at chromosome-level, 7 at contig-level, and one at scaffold-level. However, by March 2021, the

1000 Cannabis Genomes Project comprises of genomic data of nearly 1000 samples from multiple cannabis strains. These datasets were the first genome data released on Google Cloud Big Query database.

Continuously expanding the list of cannabis genomes needs collaborative efforts toward curating the information. Therefore, academic and industry experts in diverse fields formed the International Cannabis Research Consortium (ICRC) during the annual PAG meeting in 2020. Despite several cannabis genome assemblies, the selection of single standard reference genome is still a huge challenge for the scientific community, especially plant breeders. Therefore, ICRC proposed CBDRX Cs10 assembly as the most complete reference for use in cannabis genome research (Grassa et al., 2021). Additionally, a member genomics company, NRGene, generated an integrated Cannabis, and Hemp Genomic Database (CannaGENE) optimized and curated for the genomics-based breeding of cannabis varieties. Finally, in 2021, the first-ever open-access and comprehensive database of cannabis genome CannabisGDB (https://gdb.supercann.net/) were released (Cai et al., 2021) with integrated bioinformatic tools for the analysis of datasets.

Overall, the genomic data of diverse cannabis genotypes are the untapped reservoirs of genetic information which could be applied toward pan-genomic understanding of cannabis evolution and determining the effect of genetic variations upon the pathways, development, and concentration of cannabis derivatives. Detailed genetic atlas would facilitate the designing and further breeding of *cannabis* varieties for preferred metabolic yields.

DEVELOPMENTS IN CANNABIS TRANSCRIPTOMICS

The availability of several high-quality cannabis genomes made it easier to apply the transcriptome sequencing to elucidate detailed expression dynamics in time-, tissue-, stage-, and chemotype-dependent manner. Furthermore, the differential expression analysis provides in-depth insight into co-related gene networks. In 2011, Bakel et al. sequenced and compared the transcriptomes of marijuana variety Purple Kush (PK) and hemp cultivars Finola (FN) and USO-31. Gene expression analysis revealed preferential expression of cannabinoid and precursor pathway-associated genes in marijuana (PK). Expression of THCA synthase in the PK and cannabidiolic acid synthase in FN was found to be consistent with the exclusive production of psychoactive THC in marijuana. In a recent study, transcriptomics of hemp-type plants was analyzed to determine the expression profile of the fiber-type plant at the various developmental stages (Guerriero et al., 2017). Similarly, the transcriptome of marijuana flowers at different stages was captured and sequenced and found the gene expression pattern consistent with the cannabinoid contents (van Bakel et al., 2011).

As glandular trichomes are the central reservoir for cannabinoids (Lanyon et al., 1981; Turner et al., 1981), therefore, the trichome transcriptome could yield valuable insight to determine the variation in cannabinoid biosynthesis, composition, and concentration between the drug and fiber-type plants. Importantly, the identification of the differentially expressed genes could unravel the underlying molecular mechanisms of natural genetic and metabolic variation. The gene expression in trichomes of female plant strain Cannobio-2 was compared with genome-wide transcriptomics of female floral tissues at different stages of development as well as other tissues including female and male flowers, leaves, and roots (Braich et al., 2019). The extensive-expression atlas was applied toward the identification of genes expressed preferentially in various tissues at different developmental stages. Interestingly, the majority of genes involved in terpenoid and cannabinoids synthesis were significantly overexpressed in trichomes. In 2021, Grassa et al. used genomic, and expression associated expression of THCAS and CBDAS with THC:CBD ratio by Quantitative trait Loci (QTL) analysis of Cannabis cultivars (Grassa et al., 2021).

Datasets from similar genomics, transcriptomics, microbiome, and metagenomics studies of various cannabis strains are currently accessible from the Sequence Read Archive (SRA) repository at NCBI. In the past 3 years, there has been unprecedented growth in Cannabis *genome and* transcriptome studies and corresponding SRA entries. To date, there are over 4571 BioSamples from multiple studies related to *Cannabis* of which 2871 public BioSamples are exclusively for *C. sativa* with *2546* DNA and 325 RNA-Seq datasets in SRA. The SRA data for transcriptomics and metagenomics have reportedly procured from various tissues including seeds (3), flowers (116), leaves (138), shoot (13) stem (175), root (76), and trichomes (62), while genomic data lacks tissue-specific information. In-depth transcriptomic studies will be required in the future to improve the understanding of regulatory genetic networks.

PATENTS FOR *CANNABIS SATIVA* L.

One of the fundamental aspects of patents, especially in medical science or biotechnology, is to involve industrial partners in investing in research and development (Cook-Deegan and Niehaus, 2014). Cannabis-related patents have been issued by the US-patent office since 1942. More than 1,500 applications have been filed only in the US patent office. Among them, approximately 500 applications got patent protection rights (Weed, 2017) and most of them were from the last decade. The exponential increase in the number of patents shows the future potential for the growing cannabis industry. Here, we analyzed the patents spatiotemporally and categorized them into four main classes: (i) patents related to cannabinoids as constituents, (ii) pharmaceutical applications, (iii) endocannabinoid pharmacology, and (iv) genome and gene related. Among the suggested four categories, the patents related to the pharmaceutical application were the most significant category with 73 patents registered. These are further sub-grouped into the (i) preparation of the drugs, (ii) treatment, (iii) delivery technology, and (iv) detection method each with 14, 33, 13, and 13 patents, respectively. Endocannabinoids-related patents comprised of the CB1/2 receptor (26), TRPV1 (6), and GPR119 (4) reviewed in (Gerra et al., 2010). The category of cannabinoids consists of (i) cannabinoid isolation, (ii) extraction, and (iii) synthesis or biosynthesis-related patents each with 6, 6, and 12 patents granted, respectively. For the division of the sequences, 15 patents are from enzyme inhibition followed by the gene and the protein each with two patents. Most of the patents are from the US (49.6%) followed by the GB (11%) and the other European countries Figure 2 (Flores-Sanchez and Ramos-Valdivia, 2017). In addition, 25 patents for fiber/textile, 10 for foodstuff, 5 for the paper industry, 3 for architecture, 1 for biofuel, and 3 for plant breeding have been registered. Also, four patents each in the category of oil, extracts, and cosmetics each with four have been filed.

However, we have to keep in mind that a certain cannabinoid invention can be referred into more than one patent category. For instance, cannabinoids are highly hydrophobic by nature and thus they have low bioavailability in the human body. As a result, a new class of cannabinoid-glycosides has been created, whose representatives are produced through enzymatic glycosylation. This novel strategy led to increased aqueous solubility of the target cannabinoids and resulted in four patents (WO2017053574, US20190153461, US20190078168, and WO2020239784). Recently a new method of producing one or more cannabosides by feeding an insect a cannabinoid was patented (WO2021146687). These new classes of cannabinoid glycosides generated vast structural diversity and have greatly improved water solubility, enabling new pharmaceutical formulations, and multiple administration routes (oral, parenteral, or transdermal). The discovery of the genes encoding glycosyltransferases may belong to different categories of the cannabinoid patent family, that is, genes, enzymes, delivery technology, etc.

The exponential enhancement of the patent number during recent years in the diverse areas of cannabinoid applications is indicative of the increased commercial interest in this class of natural compounds. The various pharmaceutical applications will continue to shape primarily the the path of the future invention cannabinoids.

CANNABIS IN COVID-19

C. sativa has been well-known for the anti-inflammatory properties reviewed in (Prakash et al., 2009). As lung inflammation is a critical malfunction in case of COVID-19. Therefore, the reduction of lung inflammation has been tested in the mice animal model. Interestingly, cannabinoids isolates such as CBD and THC has also been tested in human as well even long before the onset of global pandemic owing to the spread of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV2) infection (Petrosino et al., 2018; Pellati et al., 2018; Almogi-Hazan and Or, 2020). Immune responses during severe cases of COVID-19 trigger the inflammation of human lung tissue resulting in acute respiratory distress and failure. This immune response for the overproduction of the pro-inflammatory cytokines is known as a cytokine storm (Esposito et al., 2020). Respiratory distress from the COVID-19 induced lung inflammation is the leading cause of high mortality rate. Phyto-cannabinoids especially CBD have exhibited a remarkable anti-inflammatory effect through CB2 inhibitory activity and agonistic effect on the peroxisome proliferator-activated receptor γ (PPARγ) reviewed in (Malinowska et al., 2021). Additionally, CBD, CBN, and THC have also been shown to exhibit anti-viral effect against COVID-19 in cell-based assay with the same potency as the standard clinical references (remdesivir and lopinavir) (Raj et al., 2021). However, the complete antiviral mechanism of cannabinoids against SAR-CoV2 infection is still unknown. Therefore, detailed pharmacological research studies are urgently needed to explore the immunotherapy potential of cannabis against SARS-CoV2 infection.

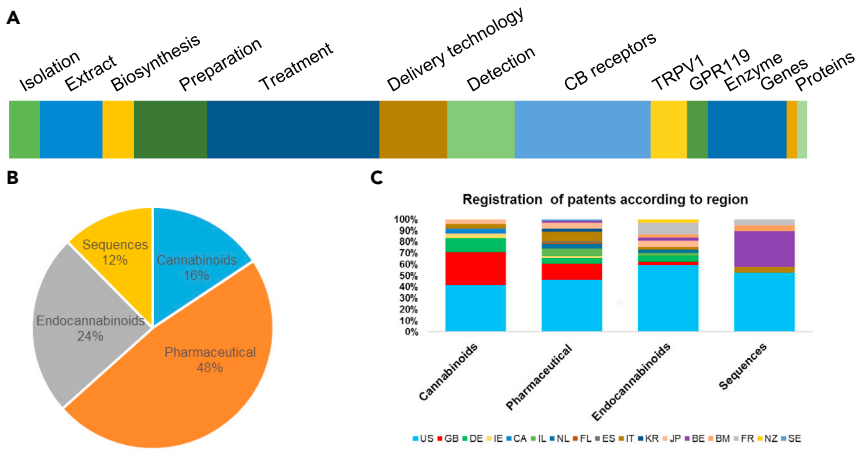

Figure 2. Cannabis patent development

(A) Number of patents registered for each category. Isolation, extraction and biosynthesis belong to the category of cannabinoid-related patents. Pharmaceutical-related patents were subcategorized into preparation, treatment, delivery technology and detection. CB receptor, TRPV1 and GPR119 were grouped into endocannabinoids. Three sub-groups, e.g., enzymes, genes and proteins are grouped for the category of sequences related patents.

(B) Distribution of the patents into four major categories.

(C) Region-wise division of the patents registered for each category. (All the information shown here are up to 2017)

CONCLUSION AND FUTURE PROSPECTIVE

Cannabis legalization fueled the scientific research in cannabinoid compounds for potential in medicinal, pharmaceutical, and neurological applications. However, with recent developments in sequencing technologies, there has been a paradigm shift in cannabis research toward the genetical genomics of fiber- and drug-type plants. Remarkable growth in genomic data combined with fast-paced development of artificial intelligence (AI)-based data analysis tools have made it possible to explore cannabis plant at the genetic and molecular levels. Integrated omics studies combining genomic and expression data with metabolite profiles are now beginning to understand the genetical regulation of the cannabinoid biosynthesis pathway. Especially, by unraveling the association between the expression of cannabinoid genes with THC:CBD ratio and cannabinoid content. The knowledge could be further applied to genetically modify cannabis with optimized pathways for preferred metabolite yield and composition. Advanced biotechnology methods could be further extended for recombinant production of cannabinoids in metabolically engineered hosts such as yeasts or bacteria. Currently, the recombinant production of THC in yeast is challenging owing to unstable THCA and CBGA expression and high amounts of side products. However, in the future, the combination of genetic technologies to obtain enhanced expression rates will lead to enhanced cannabinoid yields in an economically feasible manner. In addition, cannabinoids have been recently shown to exhibit anti-inflammatory and immunosuppressing effects against the COVID-19 immune response. However, further evidence-based clinical studies are needed to determine the efficacy and safe dosage of cannabis extracts for treatment or prevention of COVID-19. Pharmacological research coupled with rapidly evolving genome-based biotechnology will further facilitate exploring cannabis plants for tremendous potential in drug-discovery.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103391

AUTHOR CONTRIBUTION

T.H. and O.K. designed the concept, T.H. and G.J. performed a literature search and did bibliometric analysis. T.H. and G.J. wrote the sections "*History of cannabis research*, *Trends in past two centuries of cannabis research* and *Development in Cannabis Genomic and transcriptomes*". T.H. an N.V. wrote "*patent*" section. T.P. contributed in "*Cannabis in covid-19*" section. O.K. supervised the study and all the authors contributed to the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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