Tarun Belwal
Naveen Chandra Belwal *Editors*

Revolutionizing the Potential of Hemp and Its Products in Changing the Global Economy

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Contents

Chapter 1
Hemp (*Cannabis sativa* L.)-Taxonomy, Distribution and Uses

Hari Prasad Devkota

Abstract *Cannabis sativa* L. (Family: Cannabaceae) is reported as one of the oldest cultivated crops for various purposes such as food, medicine and fiber. It is believed to be originated in central Asia around northwest Himalayas and has spread around the world. It is documented to be used as medicine in India and China for almost six thousand years. However, due to the presence of psychoactive tetrahydrocannabinol (THC) compounds such as (-)-*trans*-$\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC) and (-)-*trans*-$\triangle^8$-tetrahydrocannabinol ($\triangle^8$-THC), its cultivation and use is restricted/regulated in many countries. On the other hand, cannabidiol (CBD) oil is gaining a lot of attention in recent years for various medicinal purposes such as the treatment of chronic pain and opioid dependence. Apart from the highly disputed medicinal purposes, hemp seeds are used as food and nutritional products in various cultures around the world. Similarly, the oil obtained from the seeds is used as edible oil and other purposes. One of the main industrial use is the production of high-quality fiber from the stem bark used in textiles, clothing, papers, building materials and biofuel. This chapter covers the history, taxonomy, distribution and current uses and future potentials of hemp as sustainable agricultural crop.

Keywords *Cannabis sativa* · Hemp · Tetrahydrocannabinol · Cannabidiol · Fiber · CBD oil

1.1 Introduction

Cannabis sativa L. (Family: Cannabaceae) (Fig. 1.1) is reported as one of the oldest cultivated crops for various purposes such as food, medicine and fiber (Hillig 2005; Żuk-Gołaszewska and Gołaszewski 2018). It is an annual herb of about 2 m in height and propagated by seeds (Fig. 1.2). It is believed to be originated in central Asia around northwest Himalayas and has spread around the world. It has been reported

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Fig. 1.1 Photographs of *Cannabis sativa* plant (Photos by Basu Dev Neupane, Nepal)

Fig. 1.2 Photographs of fruits and seeds of *Cannabis sativa* (Photos by Basu Dev Neupane and Prakash Poudel, Nepal)

to be used in India and China for almost six thousand years as medicine (Hillig 2005; Liu et al. 2017). It was later introduced to Europe around 3500 years ago and to Africa around 2000 years ago by nomadic tribes and traders from Central Asia (Hillig 2005). It is highly regarded as an important medicinal plant in many countries (Watanabe et al. 2005).

Due to wide spread use of various parts of the plant, it had become one of the significant crops in the ancient times. Leaves are used as traditional medicines in many Asian countries for the treatment of diarrhea, dysentery, wounds, etc. However, due to the presence of psychoactive tetrahydrocannabinol (THC) compounds such as (-)-*trans*-$\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC) and (-)-*trans*-$\triangle^8$-tetrahydrocannabinol ($\triangle^8$-THC) (Fig. 1.3), its cultivation and use are restricted/regulated in many countries. Some varieties of *C. sativa* having low content of THC are cultivated to obtain cannabidiol (CBD) oil from leaves and flowers rich in (-)-*trans*-cannabidiol, hemp seed oil from the seeds and fiber from the stem bark. Such low THC-content and high

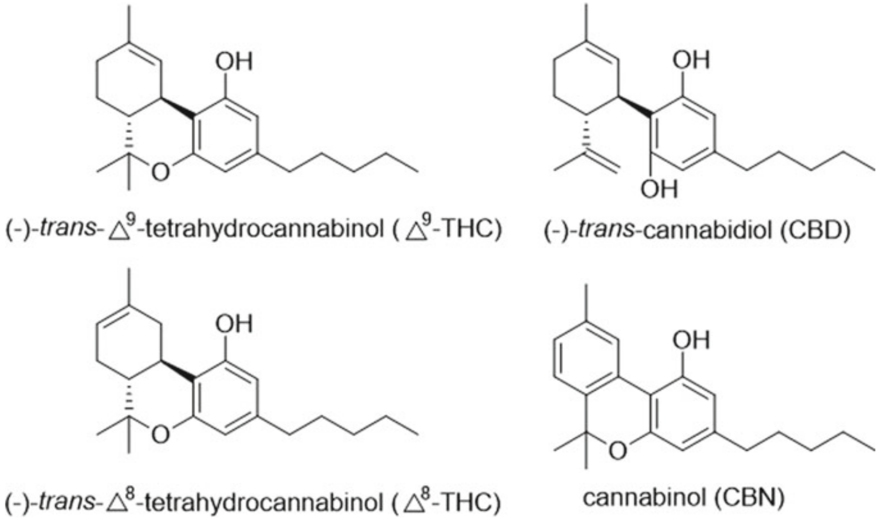

Fig. 1.3 Chemical structures of main compounds of *Cannabis sativa*

CBD content varieties usually cultivated for production of fiber are commonly known as hemp and those with high THC contents are known as marijuana (Hazekamp 2018). CBD oil is gaining a lot of attention in recent years for various medicinal purposes chronic pain and opioid dependence, however researchers have been cautious about their standardization and regulation as the contents in marketed products vary from one product to another (Hazekamp 2018; VanDolah et al. 2019). The Controlled Substances Act (CSA) of 1971 had restricted the cultivation and sell of *C. sativa* in USA. However, the Agricultural Act of 2014 allowed higher education institutions and State Department of Agriculture for the growing or cultivation of industrial hemp, i.e. *C. sativa* plants parts with no more than 0.3% of Δ^9-THC content on dry weight basis, for the research purposes (VanDolah et al. 2019). Cultivation of hemp is restricted by law in many other countries and few regions/countries have their own regulation for the content of THC. For example, in Canada, it is allowed to contain up to 3% of THC. The European Union allows up to 0.2% and Switzerland allows up to 1% of THC content (Hazekamp 2018).

Small (2015) has reported an interesting summary of *C. sativa* use, domestication and cultivation by humans. Along with human utilization, it has been evolved as one of the most useful and controversial plant species (Small 2015; Cerino et al. 2021). Hundreds of vernacular names have been used for *C. sativa* such as weed, hemp, marijuana, *Ganja*, *Bhang*, etc. As explained earlier, hemp is the name commonly used for the cultivar grown for obtaining the fiber having low contents of THC and marijuana is the common name for the cultivar used for euphoric and therapeutic properties having high contents of THC (Small 2015; Cerino et al. 2021). Hempseed is the common name for the seeds used to obtain oil for various purposes.

Apart from the highly disputed medicinal purposes, hemp seeds are used as food and nutritional products in various cultures around the world (Manandhar 2002; Cerino et al. 2021). Similarly, the oil obtained from the seeds is used as edible oil and for other purposes. One of the main industrial use is the production of high-quality fiber from the stem bark used in textiles, clothing, papers, building materials and biofuel (Adesina et al. 2020).

1.2 Taxonomy

The taxonomy of *C. sativa* is disputed as some authors consider it as a monotypic and others as polytypic one. Carl Linnaeus first named *Cannabis sativa* L. as monotypic genus. Later, other species were assigned such as *C. indica* was assigned for a specimen collected from India and *C. ruderalis* was assigned for a specimen from Russia (Pollio 2016). After 1970s, there have been many publications which assign *Cannabis* as monospecific or polyspecific genus (Pollio 2016; Cerino et al. 2021). While some authors argued that *C. indica* to be a variety of *C. sativa*, Schultes et al. described it as a separate species (Schultes et al. 1974). Later in 1976, based on the fruit morphology analysis and contents of THC, Small and Cronquist proposed that *Cannabis* is actually a monospecific genus with two subspecies i.e. (i) *C. sativa* subsp. *indica* and (ii) *C. sativa* subsp. *sativa* and four varieties i.e. (i) *C. sativa* L. subsp. *sativa* var. *sativa*, (ii) *C. sativa* L. subsp. *sativa* var. *spontanea* Vavilov; (iii) *C. sativa* L. subsp. *indica* Small and Cronquist var. *indica* (Lam) Wehmer and (iv) *C. sativa* L. subsp. *indica* Small and Cronquist var. *kafiristanica* (Vavilov) Small and Cronquist (Small and Cronquist 1976). Later in 2005, Hillig proposed that *Cannabis* is a polytypic genus with three species *C. sativa*, *C. indica* and *C. ruderalis* and seven putative taxa based on genetic analysis of the 157 samples collected from various geographical regions around the world (Hillig 2005).

However, many authors have also commonly mentioned two varieties of *C. sativa* i.e. *C. sativa* var. *sativa* commonly known as industrial cannabis/industrial hemp and *C. sativa* var. *indica* commonly known as medicinal cannabis or medicinal marijuana (Żuk-Gołaszewska and Gołaszewski 2018). Some other publications also refer them as subspecies not varieties i.e. *C. sativa* subsp. *sativa* (hemp) and *C. sativa* subsp. *indica* (marijuana) (Lim et al. 2021). Thus, the taxonomy of *C. sativa* has been always a topic of discussion among researchers due to its high market value and regulatory factors.

1.3 Global Dispersal, Distribution and Cultivation

Although the indigenous range is believed to be Central Asia, the northwest Himalayas and possibly China (Hillig 2005), *C. sativa* is widespread all around the world specially in temperate zones. It is cultivated and naturalized in most temperate and tropical areas of the world between 200 and 2700 m (Watanabe et al. 2005). Worldwide distribution map of *C. sativa* is presented in Fig. 1.4. (GBIF 2021).

For at least 6000 years, *C. sativa* has been transported and cultivated in various parts of the world by humans and thus is widespread out of its original range (Small 2015; Liu et al. 2017) which is also supported by many archaeological discoveries. In historic times, it was cultivated mainly as the source of fiber, oil, paper and clothing. It was cultivated widely in Europe in between sixteenth and eighteenth century (Żuk-Gołaszewska and Gołaszewski 2018). Both wild and cultivated varieties grow in open and sunny areas however few wild ones were also found to be growing in shaded places (Small and Cronquist 1976; Small 2015). Several factors such as phenotypic plasticity and resistance to insect damage may have resulted into widespread distribution of *C. sativa* (Small 2015). However at current times, due to strict regulation on the cultivation and use, the permitted verieties for cultivation, their cultivation condition and utilization depend upon these countries' legal frameworks.

According to Adesina et al. (2020), it is now legal to cultivate hemp in 46 states in USA and it is cultivated for scientific or commercial purposes in at least 46 states. Various researchers are now focusing their studies to understand the optimal cultivation conditions for cultivation of hemp to obtain high quality fiber and products for food, nutritional and other purposes.

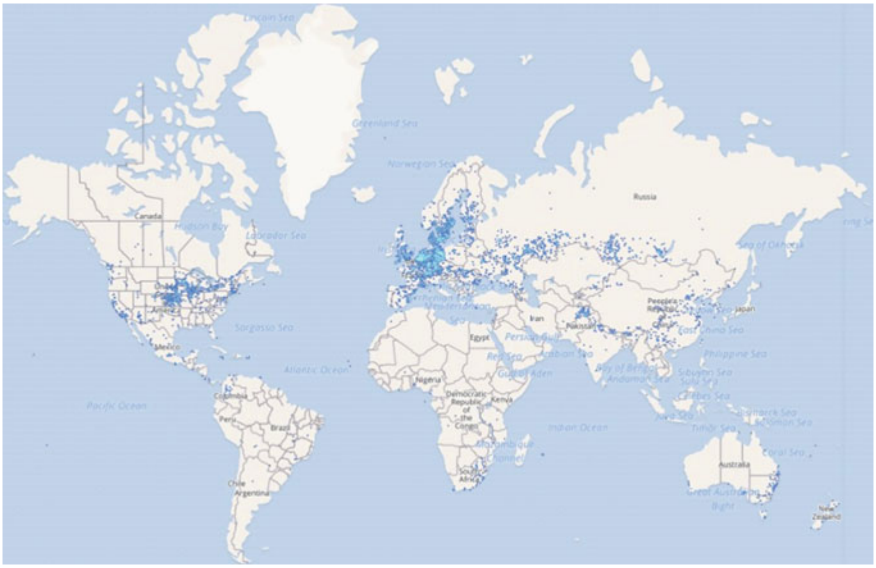

Fig. 1.4 Global distribution of *Cannabis sativa* (GBIF 2021)

1.4 Uses

Hemp has been reported to be used in China and India for almost six thousand years as medicine (Hillig 2005; Liu et al. 2017) and its various uses has made it one of the significant crops in the ancient times. Various plant parts of hemp are used for diverse purposes from food and medicine to fibers which are discussed in detail in following subsections.

1.4.1 Use as Traditional Medicine, Food, Cosmetics and Others

Cannabis sativa has long history of being used as traditional medicine in various countries. Mainly seeds were used in traditional medicines in China by mixing with other herbs to treat constipation (Liu et al. 2017). In Ayurveda, the dried leaves, known as *Vijaya*, are used in many traditional formulations. It is locally known as *Ganja* or *Bhang* in Nepal and the seeds are roasted and used to make pickle. People also chew roasted seeds. Paste of leaves is applied in cuts and wounds. Juice of the leaves is reported to be used in the treatment of diarrhea and dysentery. Leaves are mixed with animal feed to treat diarrhea and dysentery in animals. Leaves are also used as anthelmintic (Manandhar 2002). Leaf juice is used to stop bleeding from cuts and the paste of inflorescence is used to get relief from stomach pain and diarrhea. Resinous exudations of the stem, young leaves and flower buds is used to treat headache, cough, asthma and pain (Watanabe et al. 2005). In Japan, leaves were traditionally used for their pain-relieving properties, however, its use is prohibited now under the law. The seeds known as *Masinin* are used in the treatment of constipation and cough. Seeds are also one of the important ingredients included in a traditional Kampo formulation *Mashiningan* and a famous spice known as *Sichi-mi-togarashi* (Mitsuhasi et al. 1988). Hemp seeds are also used widely in China as food where roasted ones are still sold in markets. They are used as snack and by making porridge along with the edible seed oil (Liu et al. 2017).

Hemp seeds contain about 28–35% oil depending upon various factors such as plant variety, environmental factors, cultivation conditions, etc. (Rezvankhah et al. 2019). Hempseed oil is rich in polyunsaturated fatty acids (70–80%) and have unique ratio of 3:1 of omega-6 and omega 3-fatty acids (Smeriglio et al. 2016; Rezvankhah et al. 2019). Due to being rich in α-linolenic acid and γ-linolenic acid, hempseed oil has received great attention for various applications from foods to cosmetics (Cerino et al. 2021). The oil is also rich in tocopherols having the total tocopherol contents of 832.61 and 927.67 mg/kg for oils obtained by Soxhlet extraction and microwave assisted extraction (MAE), respectively (Rezvankhah et al. 2019). Hempseed oil is also rich in various antioxidant polyphenolic compounds. The methanol extract of the cold-pressed oil obtained from the Finola cultivar of *C. sativa* contained various antioxidant compounds such as phenolic acids (gallic acid, protocatechuic acid, vanillic acid, chlorogenic acid, etc.) and flavonoids belonging to different subgroups including flavonols (e.g. quercetin and kaempferol glycosides), flavanones (eriodictoyl, naringenin, naringin, etc.), isoflavones (diazein, genistein), flavone (apigenin) and flavanols (catechin, epicatechin) (Smeriglio et al. 2016).

Hempseed oil is used for various purposes from as cooking oil to an ingredient in cosmetic formulations, detergents, soaps, lighting, lubricants, etc. Recent studies are also focused to explore its potential as a source of biofuel (CAB International 2021; GBIF Secretariat 2021; Cerino et al. 2021).

1.4.2 As Therapeutic Agent in Modern Medicine

Cannabis sativa has multipurpose application in medical and pharmaceutical field. Hundreds of compounds have been reported from this plant including cannabinoids, phenolic compounds and terpenes (Andre et al. 2016). Psychoactive tetrahydrocannabinol (THC) compounds such as (-)-*trans*-$\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC) and (-)-*trans*-$\triangle^8$-tetrahydrocannabinol ($\triangle^8$-THC) have received widespread attention due to their both therapeutic potential and narcotic properties. More than 100 cannabinoids are reported which include both psychoactive ones and non-psychoactive such as cannabidiol (CBD) derivatives. The content of THC and CBD derivatives is reported to be inversely proportional (Small 2015). Small and Cronquist (1976) had proposed 0.3% content of THC as a differentiating factor for *C. sativa* subsp. *sativa* (<0.3% THC) and *C. sativa* subsp. *indica* (>0.3% THC) for dry inflorescence or young infructescence. However, other than genetic factors, their contents also vary depending upon the plant part, flowering stage of the plant, cultivation conditions, etc.

Hundreds of preclinical and clinical studies have been published regarding the therapeutic benefits of *C. sativa*-based products for pain management, multiple sclerosis, injury, cancer, diabetes, mental health condition, etc. various systematic reviews have also been published. Pratt et al. (2019) analyzed the published systematic reviews on medical benefits of *Cannabis* products and concluded that the outcomes of these reviews were not sufficient to draw a conclusion on their therapeutic benefit. Similarly, Lim et al. (2021) systematic review of clinical and preclinical research articles about the potential therapeutic benefits of hemp products in various conditions such as dependence, anxiety and constipation and reported that the finding in these studies were not sufficient to provide clinical evidence. Based on the analysis of systematic reviews of randomized trials, Riera et al. (2022) reported that cannabinoids were not therapeutically effective in pain management, but had some benefits in chemotherapy induced nausea and vomiting.

Non-psychotic compounds such as cannabidiol are receiving increasing attention in recent years for their medicinal purposes in management of opioid dependence, pain, cancer, anxiety, etc. The most commonly available formulation is CBD oil (also known as hemp oil) in which the extracts of flowers or leaves of industrial hemp are dissolved in edible oils (Hazekamp 2018; VanDolah et al. 2019). In general, the content of THC in CBD oils should be below 0.3% and the content of CBD about 12–18% (VanDolah et al. 2019). But, the analysis of various marketed CBD oils samples has shown very high content of THC and researchers have been recommending strong guidelines for the standardization and regulation of these products (Hazekamp 2018; VanDolah et al. 2019). Various natural and synthetic derivatives of CBD are being investigated in pre-clinical and clinical studies to understand their mechanism of action, therapeutic potentials, adverse effects and safety profiles (Morales et al. 2017; Nelson et al. 2020).

1.4.3 As Source for Fiber for Various Purposes

Use as fiber is considered to be one of the oldest uses of hemp plant and many archaeological discoveries from China support its use in textile even before the introduction of cotton (Liu et al. 2017). The long and strong fiber in hemp was traditionally used for rope, sails, tarpaulin, canvas bags, clothes and sacs (Watanabe et al. 2005). In recent years, the growing interest in renewable biomass has resulted into the great emphasis on industrial hemp, a fast-growing herbaceous plant, as a potential source of biomass. Fibers obtained from hemp are also used as substitute of glass fibers and to produce bioplastics (Andre et al. 2016). Hemp fiber was also used traditionally to make paper (Liu et al. 2017). Petit et al. (2020) reported that the quality of hemp fiber was affected by environmental factors such as temperature, cultivation conditions and water availability along with genetic factors. Proper understanding of these factors may help in optimal cultivation condition of hemp for obtaining high quality multi-purpose fiber.

1.5 Conclusion

Hemp (*C. sativa*) is one of the oldest cultivated plants and has been used by humans for various purposes from medicinal to food and fiber. Long history of utilization and cultivation by humans has resulted in various verieties of this plant with varying contents of THC and CBD. Strict regulation on the cultivation and use of the products obtained from the plant specially the cultivars with high THC content has resulted in extensive research on the cultivars with low THC content to be used as a source of fiber, CBD oil and hempseed oil along with other non-therapeutic purposes. Further research is necessary to understand the therapeutic benefits of hemp-base products in human health. However, it has high market demand for food and nutraceutical properties of hempseed oil and the wide application of fibers from stems in textiles, fabrics, composites and biofuels. Evidence based regulatory practices, innovations in plant cultivation and product formulations, standardization of the products and commercialization can contribute to global economy and sustainable development.

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Chapter 2
Hemp Varieties: Genetic and Chemical Diversity

Varsha Mishra, Khashti Dasila, Mithilesh Singh, and Deepika Tripathi

Abstract *Cannabis sativa* (hemp) as multifunctional crop have traditional application as fiber, food, paper, textile and pharmaceutical potential as inflorescences and seed as sources of exciting bioactive secondary metabolites. The Genus *Cannabis* is the only producer of phytocannabinoids. Extensive studied have been made to describe the origin history, geographical ranges and genetic identity of the *Cannabis* species but it remains obscured to date. Various high through put genetic marker have been studied to assess the genetic diversity in hemp varieties. Studies also indicated that domestication origin affects the genetic groups of hemp which further consequences on the chemical diversity of the cannabis. Chemotaxonomy using chemical markers also played a crucial role in differencing and allocating the *Cannabis* taxa. Cannabinoids ratio and terpene composition are the major marker to play an important role in studying chemical diversity of *Cannabis* sp. *Cannabis* genus is the only source of phytocannabinoids the dominant chemical class. Other than cannabinoids terpene and non-cannabinoid phenolic compounds also contribute in the chemical diversity of the species. The vast array of phytochemicals presents in the genus have potential application in pharmaceutical industries. However, due to its legalization status very limited study on its chemical and genetic diversity have been done. Therefore, the species needs attention to explore its commercial value.

Keywords *Cannabis sativa* · Hemp · Phytocannabinoids · Genetic diversity · Chemotaxonomy

2.1 Introduction

Cannabis is an erect, annual, dioecious and economically important aromatic medicinal herb belonging to the cannabaceae family (Pellati et al. 2018). The origination of *cannabis* was believed to be from Central Asia about ~500 BC (Farag and Kayser

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2017). Plants belongs to this genus are well grown in wet land sites and near water bodies, where the concentration of nitrogen are found high (Small 2015). *Cannabis* is highly used and described genus in Ayurveda to provide various pharmacological bioactive compounds and benefits. Now a days, people take interest in this multi-purpose plant due to the presence of high content of various nutrients along with bioactive therapeutic compounds having analgesic, anti-spasmodic, anti-tumour, anti-inflammatory, anti-oxidant, antineoplastic**,** neuro-protective, immunosuppressive, anti-nociceptive, antiepileptic, and anti-depressant properties (Carchman et al. 1976; Ameri et al. 1999; Callaway 2004; Gomes et al. 2008; Appendino et al. 2011) Cannabinoids, the major fundamental phytoconstituent of this genus which are chemically a unique class of terpenophenolic compounds having pharmaceutical potential such as anti0inflammatory, anti-cancer, antimicrobial, anti-arthritic, neuro antioxidative, etc. (De Petrocellis et al., 2011). Apart from this, hemp is also used by the mankind as natural fibres in the textile industry and as seed oil in the cosmetic production (Gautam et al. 2013; Clarke and Merlin 2013; Russo et al. 2008; Farag and Kayser 2017). Hemp use as a suitable eco-friendly option for phyto-remediation and bio fuel production has also been reported (Kumar et al. 2017).

Hemp is a highly variable species in plant system and it has been a matter of debate that whether the genus *Cannabis* having one species or more than one species (Chandra et al. 2017). According to Hazekamp and Fischedick (2012), *Cannabis* is a monotypic genus and consists a single species namely *Cannabis sativa* (described by Leonard Fuchs in the sixteenth century). Approximately 700 different varieties/cultivars of *Cannabis* have been identified and distinguished by the plant breeders and recreational users due to the results of centuries of breeding and selection. However, it is unclear whether or not these cultivars reflect any relevant differences in chemical composition.

2.2 Varieties of Hemp

With the course of time, different varieties of hemp have been evolved as the result of plant breeding and selection programme. Till so far, no in-dept study on the monospecific or/and polyspecific character of genus *Cannabis* has been made. According to the (United Nations Office on Drugs and Crime (UNODC) hemp is divided into three different categories like (a) fiber hemp (b) oil seed hemp and (c) drug hemp (Farag and Kayser 2017). Similarly, Schultes et al. (1974) also differentiated this genus into three species such as *C. sativa* L.*,* *C. indica* L.*,* and *C. ruderalis*. However, many reports are available on *Cannabis* is monotypic genus that consist only a single species *C. sativa* (Beutler and Dermarderosian 1978; Hoffmann 1961). Small and Cronquist (1976) divided the monotypic species *C. sativa* into the subspecies 'sativa' and 'indica' each with two different variants i.e., domesticated (*C. sativa* subsp. *sativa* var. *sativa* and *C. sativa* subsp. *indica* var. *indica*) and wild varieties (*C. sativa* subsp. *sativa* var. *spontama* and *C. sativa* subsp. *indica* var. *kafiristanica*). In last

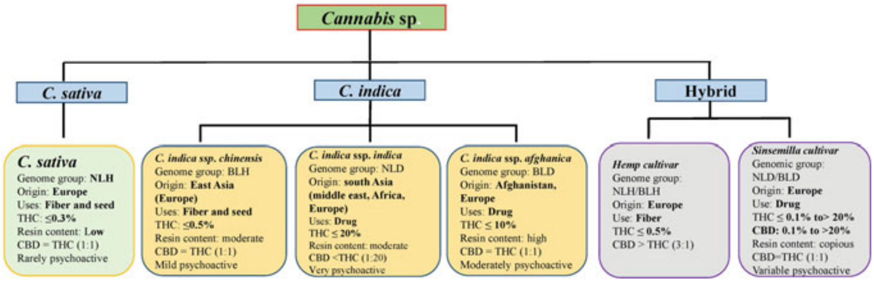

Fig. 2.1 Modern *Cannabis* taxonomy given by Clarke and Merlin (2016). Abbreviation: NLH = narrow leaflet hemp; BLH = broad leaflet hemp; NLD = narrow leaflet drug; BLD = broad leaflet drug; CBD = cannabidiol, and THC = delta-9-tetrahydrocannabinol

two decades, various new hybrid varieties have been also developed like 'Super-sativa', 'Bedrocan', 'Bedrobinol', and 'Bediol' etc. (Clarke and Watson 2002; de Meijer 2004; Flemming et al. 2007). Many *Cannabis* hybrid varieties and some pure strains have been now commercialized by many private farms and ~20 strains are well defined for the cultivation of Hemp. A large number of plant breeders cultivate fiber hemp variety with the target to reduce THC concentration (de Meijer 1995). During the origination process of plant, particularly Hemp opened the path to hybridization and leads the development of thousands of cultivars. Small (2015) stated that there is a serious taxonomic issue to classify the different strains of *Cannabis* and divide the *C. sativa* L. species into 3 subspecies or variants such as '*sativa*' (industrial cannabis/hemp having a limited amount of tetrahydrocannabinol or THC), '*indica*' (medicinal cannabis/marijuana producing principally THC), and '*ruderalis*' (known for wild hemp strains). Clarke and Merlin (2016) referred *C. ruderalis* as ancestor of two modern *Cannabis* sp. (*C. sativa* and *C. indica*), originated from central Asia. The recent taxonomy of *Cannabis* was given by Clarke and Merlin (2016) as presented in Fig. 2.1.

2.3 Geographical Distribution

Cannabis has usually a wide range of geographical and ecological distribution. It is grown worldwide except Antarctica, in a broad range of environment from sub-arctic to temperate and tropical from sea level to over 3000 m elevation (Clarke and Merlin 2013; Glanzman 2015). This genus is believed to have originated in the Northwest Himalayas and widely distributed in the range of Africa. Small and Cronquist (1976) reported that genus *Cannabis*, geographically distributed towards latitude 30°N (North) and 60°N (South) (Hillig 2005).

Clarke and Merlin (2013), reported that *Cannabis* is distributed worldwide by humans for multiple purposes. According to the authors, the putative ancestor of *Cannabis* is originated in Central Asia. It is hypothesized that *Cannabis* distributed

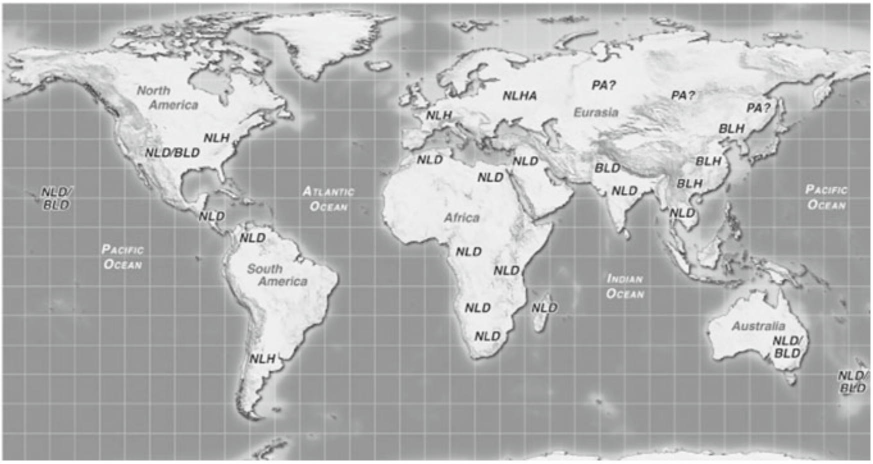

Fig. 2.2 Graphical distribution of *Cannabis* subspecies (Clarke and Merlin 2016)

into new geographical areas and evolved into 4 taxonomic groups along with gene pools as *Cannabis sativa* narrow leaflet hemp (NLH), *C. indica* broad leaflet hemp (BLH), *C. indica* narrow leaflet drug (NLD), *Cannabis indica* spp. *afghanica* broad leaflet drug (BLD). Based on the broad taxonomic groups the worldwide distribution of *Cannabis* is given in Fig. 2.2.

2.4 Genetic Diversity

Identification of functional gene variation and trait mapping is important step for understanding toward the evolutionary and functional aspects of *Cannabis*. Hemp possesses diploid genome (2n = 20) with difference in sizes as 818 Mb for female and 843 Mb for males (Sakamoto et al. 1998). Inspite of being restricted due to legalization stautus various authors attempted to study the hemp genetic for various traits like fiber quality, sex determination, sex expression, assessment of population diversity, and genetic relatedness between strains using various genetic tools etc. The genomic markers used for various trait mapping in hemp species are described in Table 2.1.

Various issues regarding naming, breeding and quality control arises in hemp varieties cultivation. According to Lynch et al. (2016) and Schwabe et al. (2019) traditional classification did not determine the genetic relationship in drug-type and fibre-type *Cannabis* species i.e., '*indica*' and '*sativa*'. Both drug-type and fiber-type *C. sativa* plants are genetically different and have been used for breeding practices for various purposes (van Bakel et al. 2011; Sawler et al. 2015; Lynch et al. 2016; Vergara et al. 2021). Previous studies usually focused on *Cannabis* species having high CBD/low THC but sometime these are closely related to marijuana

Table 2.1 Genetic markers/tools used for the study genetic diversity studies in *Cannabis sativa* (Hamp)

S. no	*Cannabis* sp.	Genetic markers/tool used	Application	Reference
1	*Cannabis sativa*	Random amplified polymorphic DNA (RAPD)	Genetic variation between cultivars/accession	Faeti et al. 1996
2	*Cannabis sativa*	Amplified fragment length polymorphism (AFLP)	Genetic variation hemp and marijuana	Datwyler and Weiblen 2006
3	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	genetic relatedness in drug- type and hemp- type	Hakki et al. 2007
4	*Cannabis sativa*	Organelle DNA (chloroplast and mitochondria)	Differentiation between hemp and marijuana population	Gilmore et al. 2007
5	*Cannabis sativa*	Genomic libraries	Fiber quality	Van den Broeck et al. 2008
6	*Cannabis sativa*	Genomic libraries	Transcriptomes analyse	van Bakel et al. 2011; Laverty et al. 2019
7	*Cannabis sativa*	Amplified fragment length polymorphism (AFLP)	Population genetic diversity	Hu et al. 2012
8	*Cannabis sativa*	Single-nucleotide polymorphisms (SNPs)	Genetic diversity and population structure among hemp and marijuana	Sawler et al. 2015
9	*Cannabis sativa*	amplified fragment length polymorphism (AFLP)	Effect of Gene duplication and divergence on cannabinoid production	Weiblen et al. 2015
10	*Cannabis sativa*	amplified fragment length polymorphism (AFLP)	sex expression in monoecious and dioecious hemp	Faux et al. 2016
11	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	Genetic relationship between strains	Punja et al. 2017
12	*Cannabis* sp. (Hemp and marijuana)	Short tandem repeats (STR)	Characterization of drug vs. non-drug *Cannabis*	Dufresnes et al. 2017

(continued)

Table 2.1 (continued)

S. no	*Cannabis* sp.	Genetic markers/tool used	Application	Reference
13	*Cannabis sativa*	Genotyping-By-Sequencing (GBS)	Genetic diversity and population structure in Iranian cannabis germplasm	Soorni et al. 2017
14	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	Genetic relationship among strains	Punja et al. 2017
15	*Cannabis* sp.	Simple sequence repeat (SSR)	Genetic and population structure	Zhang et al. 2020
16	*Cannabis* sp.	Single-nucleotide polymorphisms (SNP)	Population structure, phylogenetic relationship, population genetics	Henry et al. 2020
17	*Cannabis sativa*	next generation sequencing and nanoHPLC mass spectrometry	Proteomic and metabolomic analysis	Jenkins and Orsburn 2020; Conneely et al. 2021

species (Grassa et al. 2021). However, both types have been practicing to bred for specific compound production like cannabinoids and terpenoids. *Cannabis* genetics evolving with focus on *C. sativa* marijuana-type as compared to CBD-type hemp (Lynch et al. 2016; Vergara et al. 2021, Johnson and Wallace 2021).

2.5 Chemical Diversity in *Cannabis Sativa*

Apart from being controversial crop for various issues related to taxonomic status, origin, morphological and ecological diversity, *Cannabis* exhibited extensive phytochemical diversity in particular reference to cannabinoid and terpenoid (Hillig and Mahlberg 2004). Phytocannabinoids are the dominant chemical class of genus *Cannabis*. Cannabinoids are terpenophenolic compounds which are chemically associated terpenes with its ring structure derived from C10 monoterpene subunit i.e., geranyl pyrophosphate. Geranyl pyrophosphate are the biogenetic origin of cannabinoids (Hanus et al. 2016). Two independent pathways namely cytosolic mevalonate and plastidial methylerythritol phosphate (MEP) are responsible for phytoterpene biosynthesis. MEP pathway is reported for the biosynthesis of the cannabinoid terpenoid moiety biosynthesis (Sirikantaramas et al. 2007; ElSohly et al. 2017). The cannabinoids accumulated in cannabis plant as cannabinoid acids and non-enzymatically decarboxylated into their neutral forms during storage (Small 2015).

Radwan et al. (2021) reviewed the phytochemistry, isolation, identification and structural elucidation of more than 500 constituents including cannabinoids and non-cannabinoids class of C. *sativa*. To date different secondary metabolites class of *C. sativa* were presented in Table 2.2. The chemical structures of cannabinoids and terpenoids compounds are presented in Figs. 2.3 and 2.4.

Table 2.2 Chemical diversity of *Cannabis sativa* (Radwan et al. 2021)

Chemical class	No. of compound reported	Analytical techniques used for identification
Total cannabinoids	*125*	Column chromatography, Thin Layer Chromatography (TLC), Vaccum Liquid Chromatography (VLC), Solid Phase extraction (SPE), Ultraviolet–visible infrared spectroscopy (UV IR), Nuclear Magnetic Resonance (NMR) spectroscopy (1D&2D), UV spectroscopy, Mass spectrometry (MS), semi preparative (Riverse phase- High performance Liquid Chromatography (RP-HPLC), Gas Chromatography Mass spectrometry (GCMS), X-ray analysis and High Resolution mass spectrometry (HRMS)
Δ9-THC type	25	
Δ8-THC type	5	
CBG type	16	
CBC type	9	
CBD type	10	
CBND type	2	
CBE type	5	
CBL type	3	
CBN type	11	
CBT type	9	
Miscellaneous types	30	
Total non-cannabinoids	*400*	
Non cannabinoid phenol	*76*	Silica gel column chromatography, TLC, VLC, 1H and 13C NMR and MS, X-ray crystallography, EIMS, flash chromatograph, HPLC, High resolution electrospray ionisation mass spectrometry (HR-ESIMS), and ESI–MS spectroscopy, GC–MS and RP-HPLC, HPLC–DAD-MS
Spiro-Indans	16	
Dihydrostilbenes	12	
Dihydrophenanthrenes	7	
Simple phenol	*7*	
Flavonoids	34	
Total Terpenes	*120*	Capillary gas chromatography, GC–MS analysis, GC-FID, Spectral data comparison
Monoterpenes	61	
Sesquiterpenes	51	
Diterpenes	2	
Triterpenes	2	
Miscellaneous terpenes	4	
Alkaloids	*2*	Silica gel chromatography, TLC and X-ray crystallography,

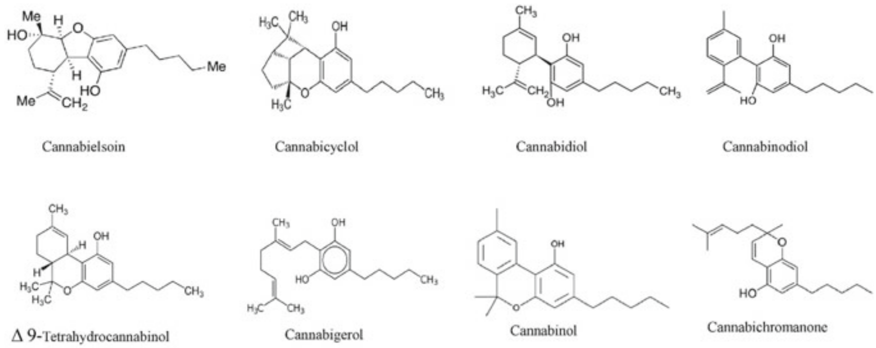

Fig. 2.3 Cannabinoids compounds in hemp

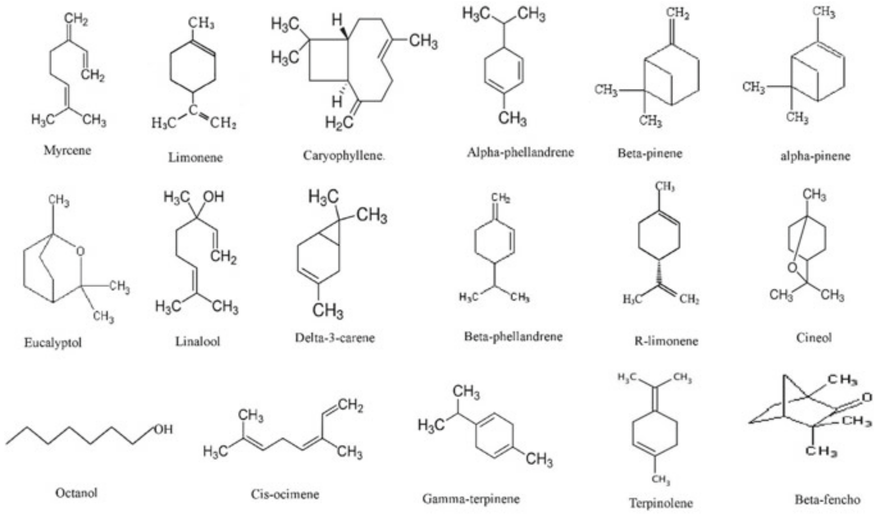

Fig. 2.4 Terpenoids compounds of hemp

2.6 Chemotaxonomic Classification of Hemp

Chemotaxonomy/chemosystematics is used to classify according to confirmable differences and similarities in their biochemical compositions. According to Small (1979a) amount of THC in *Cannabis* is essential for taxonomic characterization. Gas chromatography-flame ionization detection (GC-FID) is commonly used techniques to differentiate *indica* strains from *sativa* strains on the basis of THC content (Small and Beckstead 1973a, Small et al. 1975; Small and Cronquist 1976). Numerous phytochemical markers used for chemotaxonomic classification of *Cannabis* species/varieties are presented in Table 2.3. Based on the quantitative difference in the cannabinoids ratio of tetrahydrocannabinol acid (THC), cannabinol