Suman Chandra · Hemant Lata
Mahmoud A. ElSohly *Editors*

Cannabis sativa L. - Botany and Biotechnology

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This book is dedicated to the many patients who are to benefit from the knowledge we have accumulated on cannabis and its beneficial constituents for the treatment of so many disease conditions

Foreword

Although cannabis preparations had been used over millennia for their psychoactivity, as well as for their therapeutic properties, their chemistry and biology were not well known until the last few decades. Indeed the major psychoactive cannabis constituent, Δ^9-tetrahydrocannabinol (THC), was isolated in a pure form, and its structure was elucidated, only in the early 1960s. This is in sharp contrast with the thorough knowledge on morphine and cocaine, the two other major illicit drugs, which already had been isolated during the nineteenth century. However, since the 1960s, a large number of investigations have been devoted to the phytocannabinoid and endocannabinoid fields.

From a somewhat pedantic viewpoint, one can note a gradual development of three major phases of cannabinoid research. The first phase engulfed the phytocannabinoids—their botany, chemistry and biological actions. The second phase developed after the identification of the specific cannabinoid receptors (CB1 and CB2), the endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), which bind to these receptors and the enzymes which form and metabolize these compounds. The third research phase, which is only now slowly developing, addresses a large number of endogenous anandamide-type fatty acid-ethanol amides and fatty acid-amino acids which have a wide spectrum of biological activities.

The gradual research advances in each of these phases—or should we call them independent branches of cannabinoid science—strongly depend on the extensive data published in the others. Thus, researchers learned about the therapeutic potential of blocked anandamide metabolism by studying the various uses of medical cannabis. They also noted that the biological activity of cannabinoids may be affected by constituents that do not show any activity (the entourage effect), an effect originally seen with endocannabinoids. Indeed patients prefer to use ‘medical marijuana’ rather than pure compounds!

This outstanding book edited by Chandra, Lata and ElSohly devotes most of its chapters on the botanical aspects of cannabinoid science. The data presented in some of them have been difficult to summarize so far due to the widely dispersed literature on many of topics presented and the editors and authors should be congratulated for reviewing topics such as comparisons between sativa and indica strains of cannabis, morpho-anatomy of cannabis or micropropagation of cannabis - to name a few. However the editors have also included chapters on the chemistry, analytical aspects, biosynthesis and pharmacology of cannabis. Thus the reader can have an overall view of cannabinoid science.

Over the last few years growing of cannabis has become a major agricultural industry in numerous countries. Unfortunately detailed knowledge of the various aspects of cannabis agriculture seems to be beyond the field experience of many of the growers and we continue to see medical cannabis sold without details as regards contents or even different extracts or mixtures sold under the same commercial name.

While the agricultural, chemical and pharmacological aspects of cannabis are well understood and developed - as witnessed by this book - we sorely miss clinical trials in most medical areas in which cannabis is used. Thus, there are many anecdotal reports on the treatment of various cancers; unfortunately well designed human trials have not been published on any type of cancer. It is unbelievable that neither government agencies nor private foundations have gone ahead or encouraged clinical trials - but this is a fact! Hence for the above reasons many physicians stay away from recommending this drug to patients.

Hopefully this book may encourage growers to work with agricultural specialists and analytical chemists to make possible the supply of standardized medical cannabis to patients.

I sincerely believe that this book will be of considerable importance not only in summarizing present-day knowledge but also in advancing medical use of cannabis.

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Preface

Plant-based drugs face unusual challenges during their journey from farm to pharmaceuticals. In the case of cannabis, a considerable additional complexity is derived from regulatory concerns, depending on the countries of production and marketing. Cannabis is one of the oldest plants cultivated for the purpose of food, medicinal and ritual use or as intoxicant drug for millennia. In the last few decades, cannabis has gained a lot of interest and popularity in the general public as well as in research community, not only because of its abuse potential but also because of its new emerging therapeutic potential to treat a variety of new disease conditions. Since the discovery of its principal psychoactive compound Δ^9-Tetrahydrocannabinol (Δ^9-THC) by Prof. Raphael Mechoulam and Yechiel Gaoni in 1964, cannabis research, by and large had been revolving around Δ^9-THC and its derivatives. However, in recent years, cannabidiol (CBD), a non-psychoactive compound in cannabis is drawing a lot of attention due to its therapeutic potential in childhood epilepsy and other disorders. The methods of drug delivery, however, are a challenging issue in cannabis based drugs.

The purpose of "*Cannabis sativa* L. Botany and Biotechnology" is to present in a single volume the comprehensive knowledge and experiences of renowned researchers and scientists in the field of cannabis research. Each chapter is independently written by experts in their field of endeavor ranging from cannabis plant, species debate, its therapeutic potentials, constituents and their biosynthesis, use of modern biotechnology in conservation, propagation and enhancement of cannabis production to contaminants of concern in cannabis for the quality control of biomass product.

The subject, whether genus *Cannabis* contains single species (*Cannabis sativa* L.) with several subspecies and/or varieties, or several distinct species, has been a matter of debate for a long time. The book begins with an introductory chapter on classification of *Cannabis* in relation to agricultural, biotechnological, medical and recreational utilization (Chap. 1, Ernest Small) and history of cannabis as medicine with a special note on nineteenth century Irish physicians and correlations of their observations to modern research (Chap. 2, Ethan Russo) followed by *Cannabis* botany and horticulture (Chap. 3, Chandra et al.), *Cannabis sativa* and *Cannabis* *indica* versus "Sativa" and "Indica"—a nomenclature debate (Chap. 4, John M. McPartland), morpho-anatomy of marijuana for its identification (Chap. 5, Raman et al.), and chemical and morphological phenotypes in *Cannabis* (Chap. 6, Grassi and Mc Partland). In the next two chapters the discussion is focused on the constituents of cannabis with special focus on cannabinoids, modern methods of cannabinoids analysis (Chap. 7, Radwan et al.) and their biosynthesis (Chap. 8, Sirikantaramas and Taura).

The plant cannabis and its crude preparations have been used as natural therapeutic agents since ancient times. Its early therapeutic properties have been referenced back in 2900 BC, where the Chinese emperor Fu-Hsi references marijuana as a popular medicine. The next group of chapters is focused on the pharmacological and therapeutic potential of phytocannabinoids (Chap. 9, Cascio et al.), cannabinoid CB2 receptor mechanism (Chap. 10, Onaivi et al.), therapeutic properties of cannabidiol, a compound of interest these days (Chap. 11, Brian Thomas) and allergenicity to *Cannabis* (Chap. 12, Ajay P. Nayak et al.).

Biotechnology plays an important role in propagation, conservation and improvement of medicinal plants. *In vitro* propagation provides a means of robust multiplication of disease free, chemically consistent batches of desirable plant material which is a basic demand of the pharmaceutical industry. On the other hand, *in vitro* propagation also opens the door for alterations and modifications in chemical constituents of plants by using genetic engineering. Chapters 13–21 provide an in-depth discussion on *in vitro* propagation efforts, genetic and metabolic engineering, manipulation of beneficial secondary metabolites through induction of polyploidy, endophytes and physical and chemical elicitation in *Cannabis* plants. Chapter 13 (Lata et al.) summarizes the state-of-the-art research being done in the field of cannabis micropropagation, while in Chap. 14 (Wahby et al.) and Chap. 16 (Feeney and Punja) focus is laid on different gene-transfer technologies using hairy root cultures of *C. sativa*. Chapter 15 (Onofri and Mandolino), Chap. 17 (Mansouri and Bagheri), Chap. 18 (Karlov et al.) and Chap. 19 (Punja et al.) highlight the genomics and molecular markers, induction of polyploidy and its effects, classical and molecular cytogenetics and genetic diversity associated to *Cannabis*, respectively. Chapter 20 (Kusari et al.) describes cannabis endophytes and their application in breeding and physiological fitness, whereas Chap. 21 (Gorelick and Bernstein) is focused on chemical and physical elicitation for enhanced cannabinoid production.

Quality of biomass is a key parameter for the safety and efficacy of any phytopharmaceutical compound. Like any other agricultural crop, cannabis biomass can be contaminated by several factors such as heavy metal, microbes, pesticide, etc. These contaminants may be passed on by previous crop or from a pesticide or herbicide spray drift from adjacent field or plants may be grown in a contaminated soil. For the quality and efficacy of cannabis biomass product, the concluding chapter (Chap. 22, McPartland and McKernan) of this book discusses contaminants of concern in cannabis.

It has been a pleasure to edit this book, primarily due to the splendid cooperation of contributors, strict adherence to time schedules and the richness of the material

provided by them. We express our gratitude and heartfelt thanks to each author for their generous contribution of time and effort. We also wish to thank Dr. Christina Eckey, Dr. Jutta Lindenborn and Ms. Abirami Purushothaman at Springer Heidelberg, for their patience and generous assistance. Suman Chandra and Hemant Lata in particular are thankful to their parents and kids Rishi and Riddhi for their love and support. Mahmoud A. ElSohly is grateful to his cannabis working group for their support and dedication in studying different aspects of this great plant.

Oxford, MS, USA

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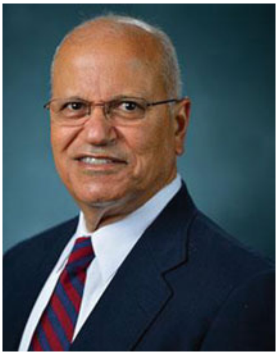
About the Editors

Suman Chandra received a M.Phil. degree in Environmental Plant Physiology and a Ph.D. degree in Plant Physiology from The High Altitude Plant Physiology Research Center, HNB Garhwal University, India. He worked at The High Altitude Plant Physiology Research Center and GB Pant Institute of Himalayan Environment and Development, India, from 1992 to 2000. Dr. Chandra joined the University of Mississippi, MS, USA in 2001 and is currently working as a Senior Research Scientist at The National Center for Natural Product Research, Research Institute of Pharmaceutical Sciences (RIPS), School of Pharmacy. His research interests include conservation, cultivation and improvement of medicinal plants, and effects of climatic changes on physiology and secondary metabolites of medicinally important plants. Dr. Chandra has more than 50 publications, 100 meeting presentations, and an edited book to his credit.

Hemant Lata received her Ph.D. (Plant Physiology) from High Altitude Plant Physiology Research Center, HNB Garhwal University, India; MS (Biotechnology) from Guru Nanak Dev University, India and BS (Botany Hons.) from University of Delhi, India. She obtained her postdoctoral training from Laboratory of Cellular and Molecular Cytogenetics, Department of Botany, University of Delhi. Dr. Lata joined the University of Mississippi in year 2000 as postdoctoral research associate and has been promoted to the research faculty position in December 2003. She is currently working as a Senior Research Scientist at the National Center for Natural Products Research (NCNPR), School of Pharmacy. She has developed and published micropropagation protocols for several highly valuable medicinal plants. For last 10 years Dr. Lata has been working on screening, micropropagation, conservation and multiplication of different varieties of *Cannabis sativa*. She has more than 60 publications and an edited book entitled "Biotechnology for Medicinal Plants–Micropropagation and Improvement, Springer-Verlag Berlin Heidelberg" to her credit.



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Chapter 1
Classification of *Cannabis sativa* L. in Relation to Agricultural, Biotechnological, Medical and Recreational Utilization

Ernest Small

Abstract *Cannabis sativa* has been utilized for millennia, primarily as a source of a stem fiber (both the plant and the fiber termed "hemp") and a resinous intoxicant (the plant and its drug preparations commonly termed "marijuana"), and secondarily as a source of edible seeds. In domesticating the species for these divergent purposes, humans have altered the morphology, chemistry, distribution and ecology of cultivated forms by comparison with related wild plants. Wild-growing plants appear to be either escapes from domesticated forms or the results of thousands of years of widespread genetic exchange with domesticated plants, making it impossible to determine if unaltered primeval or ancestral populations still exist. There are conflicting botanical classifications of *Cannabis*, including splitting it into several alleged species. The different approaches to classifying and naming plants such as *Cannabis*, with interbreeding domesticated and wild forms, are examined. It is recommended that *Cannabis sativa* be recognized as a single species, within which there is a high-THC subspecies with both domesticated and ruderal varieties, and similarly a low-THC subspecies with both domesticated and ruderal varieties. Alternative approaches to the classification of *Cannabis* that do not utilize scientific nomenclature are noted.

1.1 Introduction

The process of "classification" refers to defining and naming new groups, as well as assignment of entities to established groups. Virtually everything in the universe can be classified in some manner, indeed often in multiple ways (i.e. by different criteria and by various methods of organization). The classification of living (and once-living) organisms is an especially complicated and sophisticated exercise

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because every individual in the world is historically related by evolutionary lineage to every other individual, sometimes by multiple pathways. While alternative biological classifications of *Cannabis* are the primary focus of this chapter, it should be kept in mind that other classificatory aspects are also important (note the following discussion of legal, pharmacological and cannabinoid phenotype classifications). As will be discussed, biological taxonomists are fond of the phrase "natural classification," suggesting that ideal classifications necessarily reflect a fundamental structure and organization in nature, perhaps exemplified by the clarity of the periodic table of the elements. However, perception and modeling of nature's organization are human activities, involving both theoretical and pragmatic aspects, as well as artistry. In general, the merit of a classification depends substantially on its utility for one or more purposes, and this simple dictum applies even to biological classifications of organisms like *Cannabis*, as will be presented.

The word "cannabis" is used in various ways. In its broadest sense, it refers to the cannabis plant (*Cannabis sativa*), especially its psychoactive chemicals (employed particularly as recreational and medicinal drugs), fiber products (such as textiles, plastics and dozens of construction materials), edible seed products (now in over a hundred processed foods), and all associated considerations. In short, cannabis is a generic term referring to all aspects of the plant, especially its products and how they are used. Biologists and editors conventionally italicize scientific names, such as *Homo sapiens*. Italicised, *Cannabis* refers to the biological genus name of the plant (of which only one species is commonly recognized, *C. sativa* L.). Non-italicised, "cannabis" is a generic abstraction, widely used as a noun and adjective, and commonly (often loosely) used both for cannabis plants and/or any or all of the intoxicant preparations made from them. However, as noted in this paragraph, in its most comprehensive sense "cannabis" also includes non-intoxicant preparations.

1.2 Legal Classification

Cannabis is widely classified as a "narcotic," a term which is most often used as an arbitrary juridical category (compare pharmacological usage in the next section). A narcotic is frequently defined as a substance or preparation that is associated with severe penalties because of real or alleged dangerous (usually addictive) properties. Because cannabis has been considered to be a leading drug of abuse it has been seriously criminalized since the Second World War in Western countries, and almost all research and economic development—both drug and non-drug aspects—were suppressed for most of the twentieth century. After the Second World War, *C. sativa* became the leading illicitly cultivated black market crop in the Western World, law enforcement dedicating huge efforts to eradicating the plants wherever they were discovered. Most scientific investigations authorized in Western countries were either forensic studies to aid law enforcement, or medical and social research specifically intended to document and reduce harmful effects. Criminalization of cannabis has been associated with enormous law enforcement costs and social upheaval, and currently many jurisdictions are reclassifying cannabis to a less punitive status. There is widespread legalized medical usage, although medical cannabis remains highly contentious. Most of the Western World still prohibits the recreational use of marijuana, but legalization has occurred in Uruguay and several U.S. states, and is expected in others areas, particularly in the Americas. De facto legality of recreational marijuana has been the case in the Netherlands for decades, although not officially accepted. In democratic countries, there has been a general softening of penalties, or at least of prosecution, coinciding with increasing public tolerance of illicit usage. Nevertheless, in some countries, particularly in Asia, capital punishment is possible.

1.3 Pharmacological Classification

The word "narcotic," often used to describe the psychological effects associated with marijuana, has been extensively and ambiguously employed in lay, legal and scientific circles. "Legally, cannabis has traditionally been classified with the opiate narcotics, and while they may share some euphorogenic and analgesic properties, they are otherwise quite distinct pharmacologically" (Le Dain 1972). Etymologically, based on "narcosis," a narcotic would be expected to be a substance promoting sleep, and indeed some use the term to characterize any drug which produces sleep, stupor or insensibility. Both THC and CBD, at least one of which dominates the cannabinoids of most biotypes of *C. sativa*, have sleep-inducing properties at some dosage, albeit CBD is stimulative at low and moderate dosages (Piomelli and Russo 2016) and is sedative only at quite elevated doses (Carlini and Cunha 1981; Pickens 1981). Moreover, the terpene myrcene is common in *C. sativa* (especially in marijuana strains with appreciable CBD) and is sedative (Russo 2011). Accordingly, the soporific property of cannabis provides some limited justification for referring to it as a narcotic, although it is by no means best known for its sedative properties. Nevertheless, the term narcotic is better known as characterizing an intoxicant than a sedative. Because "narcotic" is often used pejoratively, it is probably best avoided as descriptive of pharmacological effects. Although substances called narcotics are widely viewed as intrinsically evil, the world's leading controlled so-called narcotic crops have some legitimate, useful applications (Small 2004; Small and Catling 2009).

The pharmacological classification of cannabis is controversial. It has been characterized as a sedative-hypnotic-general-anesthetic like alcohol and nitrous oxide; a mixed stimulant-depressant; a mild hallucinogen, especially at higher doses; a "psychedelic," like LSD at very high doses; and as a separate category of psychic experience (Le Dain 1972). The following terms have been used to describe cannabis: psychedelic (mind-manifesting or consciousness-expanding), hallucinogenic (hallucination-producing), psychotomimetic (psychosis-imitating), illusinogenic (illusion-producing), and psychodysleptic (mind-disrupting); as noted in Le

Dain (1972, p. 396), all of these terms are problematical. None of them is completely satisfactory to denote the euphoric psychological effects of marijuana in general and THC in particular.

There is little dispute that cannabis is a "psychoactive" drug (one altering sensation, mood, consciousness or other psychological or behavioral functions). However, "psychoactive" is so broad it applies to a very wide variety of psychological states. "Psychotropic," meaning mind-altering, is also widely used, but both marijuana and hemp types of *Cannabis* can influence the mind by virtue of the properties of THC and CBD. "Hallucinogenic" is less appropriate since true hallucinations are rarely produced. Psychotomimetic (mood-altering) is perhaps the most appropriate pharmacological term, but is hardly definitive, since it could be applied to numerous preparations, including chocolate and caffeinated beverages. Although not a technical phrase, "mood enhancer" is sometimes applied to marijuana. Marijuana is an inebriant and euphoriant, but these are not well defined terms. Marijuana can loosely be described as an "intoxicant," but intoxication often has the technical meaning of toxicity (poisoning).

1.4 Folk Classification: "Hemp" Versus "Marijuana"

"Folk taxonomy" refers to the spontaneous ways people have traditionally described, named and organized (or classified) objects, thoughts, events, or indeed any aspect of human experience. A folk taxonomy of a set of living things often is reminiscent or even identical to how professional biologists conceive and organize them, although the use of scientific (Latin) names adds sophistication to the exercise. It is important to understand that a vernacular name employed in popular culture (i.e. in folk taxonomy) may or may not be synonymous with the same common name employed by scientists, or with a particular scientific name. For example, to most people a "bug" is any small crawly animal, and this could include beetles, centipedes, cockroaches and spiders. An entomologist, however, is likely to confine the meaning of "bug" to a member of a particular lineage of insects, the Hemiptera ("true bugs"), which excludes beetles, centipedes, cockroaches and spiders. In this example, there is some overlap: bed bugs are "bugs" both in the sense of the average person and the entomologist. In the case of *Cannabis sativa*, the most popular folk taxonomy concerns the distinction between "hemp" and "marijuana"—terms which are applied both to populations of plants and to their economic products. As discussed later, the distinction between these two classes of plant has substantial scientific validity from a professional biological classification viewpoint, as well as reflecting popular folk classification.

The name "hemp" can be confusing. It usually refers to *C. sativa*, but the term has been applied to dozens of other species representing at least 22 genera other than *Cannabis*, often prominent fiber crops. Montgomery (1954) listed over 30 "hemp names." Especially confusing is the phrase "Indian hemp," which has been used both for intoxicating Asian drug varieties of *C. sativa* (so-called *C. indica*

Lamarck of India), for jute (*Corchorus capsularis* L., also called Bengal hemp, Calcutta hemp, and Madras Hemp), and for *Apocynum cannabinum* L. (also known as American hemp as well as by other names), which was used by North American Indians as a fiber plant.

Although "hemp" and "marijuana" have been occasionally interpreted as synonyms, the industries concerned with the non-intoxicating fiber and oilseed usages have been at pains to distance themselves from the drug aspects of *C. sativa* because of the stigma long attached to illicit drugs. Great efforts are made to point out that "hemp is not marijuana." The key phrase that has been used to distinguish plants authorized for non-euphoric drug uses (both fiber and oilseed) is "industrial hemp." "Industrial hemp" is now commonly employed to designate fiber and oilseed cultivars of *C. sativa* with very limited content of the intoxicating chemical THC. "Hemp" usually refers to *C. sativa* plants used for fiber, and also is the term employed for the fiber obtained from the stalk (i.e. the main stem). When hemp is grown for oilseed, it is distinguished as "oilseed hemp" or "hempseed."

1.5 Ancient Phytogeography

Cannabis sativa is widely regarded as indigenous to temperate, western or central Asia, but may trace to eastern Asia (Li 1974). However, no precise area has been identified where the species occurred before it began its association with humans. De Candolle (1885), the first authoritative student of the biogeography of crop plants, speculated that the ancestral area was the southern Caspian region. Other authors (e.g. Walter 1938; Sharma 1979) have suggested that the plant is native to Siberia, China or the Himalayas. Piomelli and Russo (2016) stated "*Cannabis* originated in Central Asia and perhaps the Himalayan foothills." Certainly, the plant is of Old World origin, and in pre-historical times could have naturally occupied many areas across the breadth of Asia, as evidenced by the present distribution of wild-growing (ruderal) plants, which are widespread in Asia.

Fossilized pollen grains of *C. sativa* that are preserved in sediments of lakes and bogs have some potential for discerning ancient distribution areas of the species. However, the grains of *C. sativa* and its close relative *Humulus lupulus* are quite difficult to distinguish (Fleming and Clarke 1998), and wild populations of both species frequently occur near streams and rivers, making it difficult to identify which species left pollen deposits in wetlands such as lakes and bogs where pollen is often preserved.

There are discernible areas in Eurasia where *C. sativa* has been selected for fiber or marijuana, but it is well known from the study of other crops that such areas may represent secondary centers—i.e., the species were transported from an original, often quite distant indigenous area (Harlan 1951). The "homeland" of an ancient crop like *C. sativa* is difficult to ascertain.

The chief reason that there is uncertainty regarding the primeval location of *C. sativa* is that for at least the last 6000 years it has been transported widely, providing extensive opportunities for establishment outside of its original range (Abel 1980; Clarke and Merlin 2013). Since the present geographical range of wild-growing plants in Asia could be entirely or substantially the result of distribution by humans, it is not a reliable guide to the original indigenous area. Because the species has been spread and modified by humans for millennia, there does not seem to be a reliable means of accurately determining its original geographical range, or even whether a plant collected in nature represents a primeval wild type or has been modified by domestication (Schultes 1970). The seeds of some wild-growing populations in India are remarkably small, unlike those collected from any other area of the Old World. Such plants may represent an ecotype specialized for the stresses of montane habitats (small seeds require limited energy to produce, and annual plants like *C. sativa* would be at a disadvantage during occasional late-summer killing frosts if they were unable to produce at least a few small seeds). The genetic nature of these plants and their relationships to domesticated forms of *C. sativa* has not been determined.

Agriculture, which began as long ago as 13,000 B.P. in some places (Hancock 2012), is the foundation of civilization. Of the thousands of plant species that humans have used for various purposes, only a few dozen have been critical to the advancement of civilization, and *C. sativa* is one of these. Indeed, it is one of the most ancient of crops. The earliest archaeological evidence for human use of the plant has been speculated to be hemp strands in clay pots from tombs as old as 10,000 BCE (Kung 1959; Chang 1968), although this interpretation is doubtful. *Cannabis* may have been harvested by the Chinese 8500 years ago (Schultes and Hofmann 1980), but it should be kept in mind that harvesting could have been from wild-growing, not domesticated plants. *Cannabis* has certainly been deliberately grown for at least 6000 years (Fleming and Clarke 1998). As with many major crops that trace to very early times, the ancient history of *C. sativa* is poorly known because it was cultivated and used well before the appearance of writing.

As illustrated in Fig. 1.1, dating back at least a millennium in the Old World, there developed a remarkable north-south separation of *C. sativa* selections grown mostly for fiber and those cultivated particularly for intoxicating drug preparations. In Europe and northern Asia *C. sativa* was grown virtually exclusively for fiber, just occasionally for its edible seeds (also useful for lubricating and illumination oil). In southern Asia and Africa, the non-intoxicant uses of the stem fiber and oilseed were sometimes exploited, but the plants were particularly employed as drugs for recreational, cultural and spiritual purposes. As discussed later, strong selection for fiber in the north led to the evolution of races of *C. sativa* with characteristics maximizing fiber production. Conversely, strong selection in the southern Old World led to the evolution of races of *C. sativa* with characteristics maximizing the production of inebriating drug content. A side-effect of the north-south split is different photoperiodic adaptations to the different daylight regimes encountered in the two areas. Northern fiber-type races are particularly adapted to relatively early flowering to survive in the shorter growing seasons of the north.

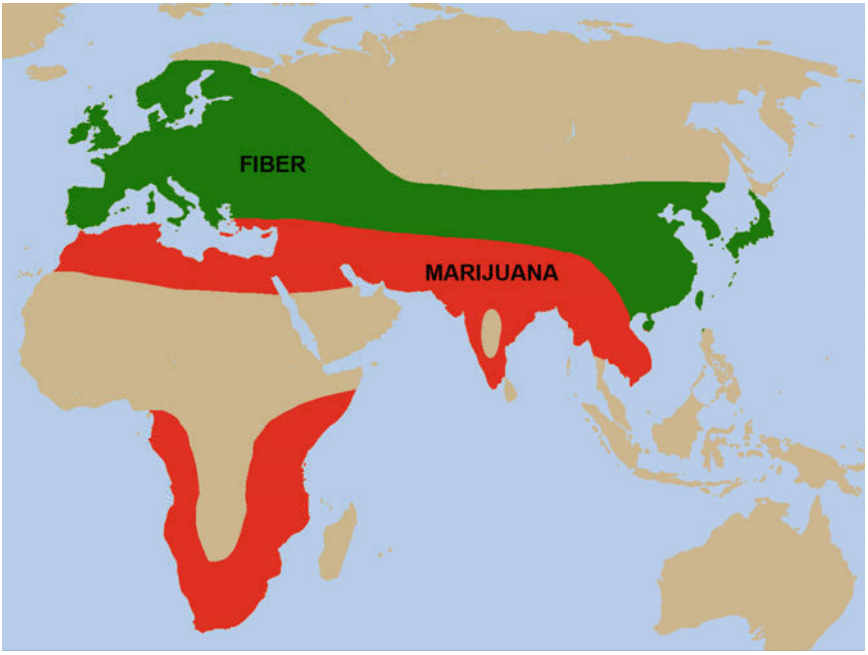

Fig. 1.1 Approximate pre-Columbian distribution of fiber *Cannabis sativa* (in *green*) and marijuana *Cannabis sativa* (in *red*)

1.6 Four Utilitarian Classes of *Cannabis*

The many different kinds of plant of *C. sativa* can be grouped into four basic utilitarian categories, including: (1) "wild" (weedy) plants that have escaped from cultivation and grow independently in nature; and three groups of cultivated plants that have been selected for distinctive economic products: (2) fiber from the main stalk (employed for textiles, cordage, and numerous recent applications); (3) Oilseed (oil-rich seed employed for human food, livestock feed, nutritional supplements, industrial oils, and occasionally as a biofuel); and (4) psychoactive drugs from the flowering parts (used mostly illicitly for recreation and more recently legally as medicinals). These groups are discussed sequentially, followed by an examination of their classification.

1.6.1 Wild Plants

Plants of *C. sativa* growing outside of cultivation are common in much of the world. These frequently possess distinctive adaptations, which are not present in one or more of the different categories of domesticated plants. As discussed in this section, on the basis of visually evident adaptations, most wild-growing plants are easily distinguished from domesticated plants, regardless of whether specialized for marijuana, fiber or oilseed.

1.6.1.1 "Ditchweed"

Ditchweed is a pejorative American (U.S.) term originally referring to wild-growing low-THC weedy plants common in the eastern U.S. and adjacent Canada, capable only of yielding low-quality marijuana. The term is often employed today in a more comprehensive but still pejorative sense to refer to both low-THC *plants* circulating in the illicit drug trade (regardless of whether obtained from wild plants), as well as low-THC *marijuana*. In Europe one encounters the term "Euroweed," and in the Netherlands one finds "Nederweed" ("Netherweed").

1.6.1.2 Primitive Versus Secondary (Ruderal) Wildness

The word "wild" can refer in a general way to plants or animals reproducing in nature without human care. However, the term is used in a more restricted sense to refer to individuals generated exclusively by nature, and never genetically altered by humans (all of their characteristics are "original" or "primitive"). Contrary to the latter precise usage, individuals are sometimes questionably termed wild although they are the result of substantial genetic alteration by humans, and have merely escaped from human care to live in the wilderness. Feral dogs exemplify this situation. A more ambiguous situation is often encountered: plants or animals genetically altered by humans escape from human care, and re-evolve characteristics more suited to wild existence (traits that are "secondary" by comparison). The Australian Dingo—a canine derived from ancient domesticated dogs, but which has acquired (or re-acquired) some wolf-like characteristics, illustrates this. "Wild" cannabis plants appear to belong to the latter situation. There do not seem to be genuinely wild plants that have not been changed genetically by humans. The world's so-called wild cannabis plants are likely extensively interbred with cultivated plants, and it appears the ancient wild ancestor of *C. sativa* that existed in pre-Neolithic times (i.e., prior to 10,000 B.C.) is no longer extant.

1.6.1.3 Adaptive Morphological and Anatomical Differences Between Wild and Domesticated *Cannabis sativa*

Cannabis sativa is a quite flexible species, capable of growing as a huge herb in hospitable circumstances, or as a dwarf in hostile environments (Fig. 1.2). Wild plants in excellent cultural conditions develop a central, very woody stalk bearing many branches (Fig. 1.2a), an architectural pattern that has been suppressed or

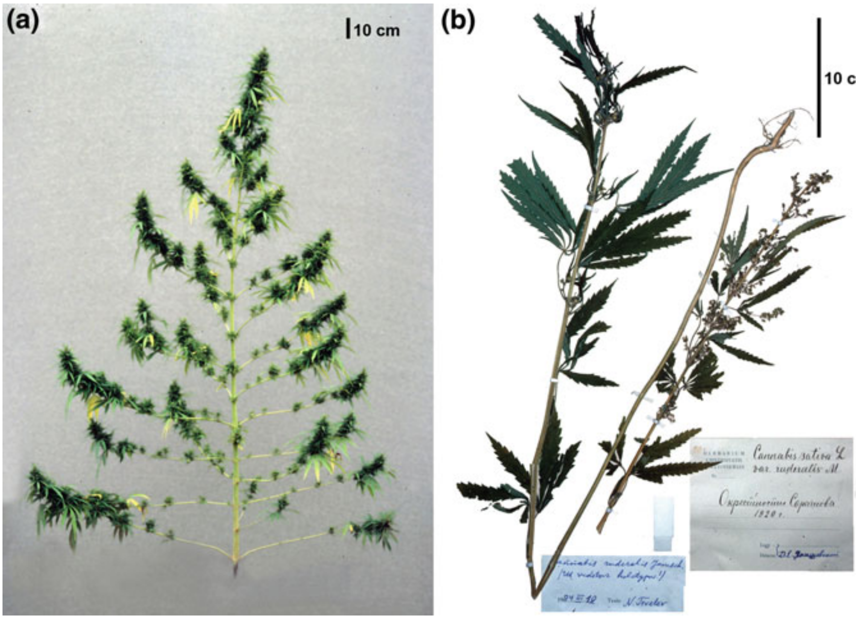

Fig. 1.2 Growth patterns of weedy forms of *Cannabis sativa*. **a** Strong branching pattern typical of a well-developed, open-grown, weedy female plant (cultivated near Toronto, Canada from seeds from Georgia, Eurasia). **b** A dwarfed, unbranched female plant (the type specimen of *C. ruderalis* Janischevsky; a male branch from another plant is at *right*). Note the narrow leaflets, typical of weedy plants

modified in some fiber, oilseed and marijuana selections, as noted later. In common with many other species with both domesticated and wild populations, the leaves of the domesticate tend to be larger and the leaflets broader, apparently to provide a greater photosynthetic area (Small 2015).

The "seeds" (achenes) of weedy plants differ dramatically from those of plants domesticated for fiber, oilseed or illicit drugs (Small 1975; Fig. 1.3). Usually the seeds of wild plants are smaller than 3.8 mm in length, in contrast to the larger seeds of domesticated selections. Large size of seeds in domesticated plants is usually the result of selection for a desired product in the seeds (frequently for food), but also larger seeds provide a greater store of food reserves for successful germination and establishment. Kluyver et al. (2013) proposed that ancient agricultural practices buried seeds quite deeply, leading to an increase in seed size under domestication so that seedlings would have the energy to grow out of the soil.

Most wild plants cast off their seeds or fruits at maturity, in order to disseminate them. Selecting mutations that inactivate the separation mechanisms (abscission, i.e. breaking away of fruits at their base so they fall away; or dehiscence, i.e. opening of fruits to release seeds) greatly facilitates harvest by humans because the

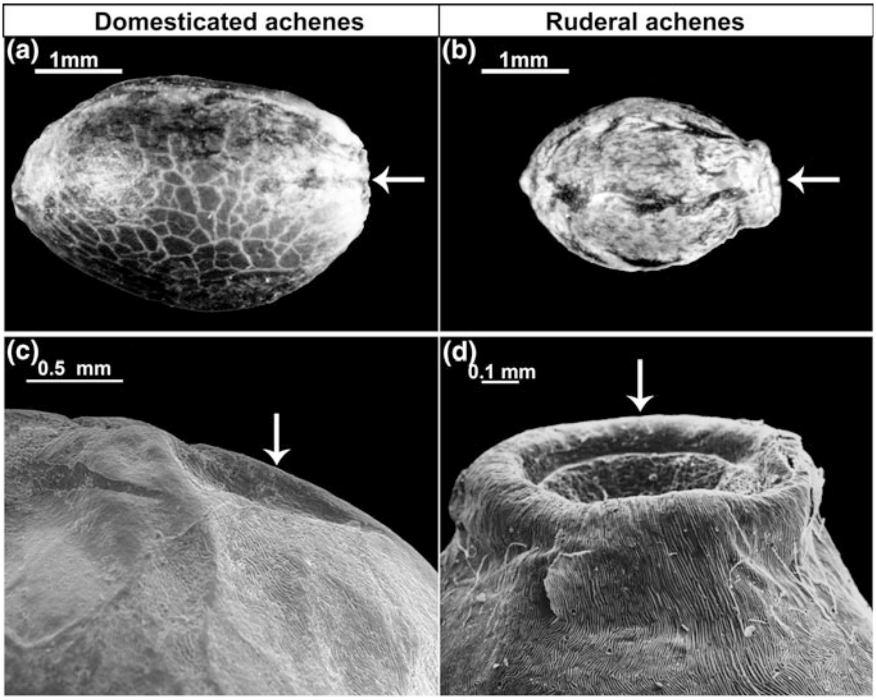

Fig. 1.3 Achenes ("seeds") of *Cannabis sativa* (areas of attachment to the plant are indicated by *arrows*). *Left column* shows achenes of domesticated plants, *right column* shows achenes of ruderal plants. *Top row* (**a**, **b**) shows light photomicrographs, *bottom row* (**c**, **d**) shows scanning electron photomicrographs. The domesticated fruits are larger, lack a camouflagic persistent covering layer derived from the perianth, and lack an elongated attachment base that facilitates disarticulation in the wild form

mature seeds or fruits remain on the plant. This reduction of "shattering" (natural shedding of seeds at maturity) is the most important way that humans have domesticated the majority of seed crops (Harlan 1995; Fuller and Allaby 2009). In cereals, a "domesticated syndrome" of characteristics is recognizable whereby the "grains" (fruits technically termed caryopses in the grass family) have lost the features in their wild ancestors that cause them to detach and scatter away (see, for example, Sakuma et al. 2011). A parallel syndrome of characteristics promotes seed retention in domesticated *C. sativa*. The fruits of wild plants possess a well-developed abscission zone and a basal "neck" (attenuated area), both facilitating disarticulation as soon as the fruits are ripe, and this is essential given the considerable predation by birds on seeds that remain attached to the plant.

A camouflagic mottled layer covers the achenes of wild *C. sativa*, providing some protection for the fallen seeds against mammalian, insect and avian herbivores. The layer is developmentally homologous with the perianth—the petals and sepals of conventional flowers (female flowers of *C. sativa* lack normal petals or sepals, although the male flowers have sepals). The dark appearance of wild seeds also contributes to their being inconspicuous. By contrast, the achenes of domesticated *C. sativa* tend to slough off the adherent perianth layer, and have often been selected for a lighter shade of exposed hull (Small 2015).

Wild plants are virtually always either staminate (male) or pistillate (female), and hermaphrodites are rare, outbreeding clearly representing the natural condition in nature. By contrast, there are numerous fiber and oilseed cultivars that have been selected for monoecy (male plants usually considered undesirable) and (in monoecious plants) for minimal development of male flowers. Indeed, most modern hemp cultivars are monoecious, and so are easily distinguishable from wild plants (as well as marijuana strains).

1.6.1.4 Adaptive Physiological Differences Between Wild and Domesticated Plants

Unlike the seeds of cultivated varieties of *C. sativa*, wild seeds of the species are generally at least somewhat dormant and germinate irregularly (Small et al. 2003; Small and Brookes 2012), features that obviously adapt the plants to the environmental fluctuations typical of wild habitats. In most respects, domesticated forms of *C. sativa* have narrower physiological tolerances to stresses than their wild-growing counterparts. Wild plants tend to be comparatively resistant to drought, cold, shade and wind, and probably also to damaging biotic agents ranging from microorganisms to large grazing mammals (Small 2015).

1.6.2 Fiber Plants

Two basic classes of fiber occur in the stems of *C. sativa*: phloem ("bast" or "bark") in the outer stem, and xylem (wood) in the core, as illustrated in Fig. 1.4b. These are associated with the two vascular (fluid transportation) systems of plants: xylem tissue, which functions to transport water and solutes from the roots to other parts of the plant, and phloem tissue, which transport photosynthetic metabolites from the foliage to nourish other parts of the plant. Historically, phloem fiber was very widely employed for cordage and textiles, and the woody core was of limited value, although today both kinds of fiber are considered valuable.

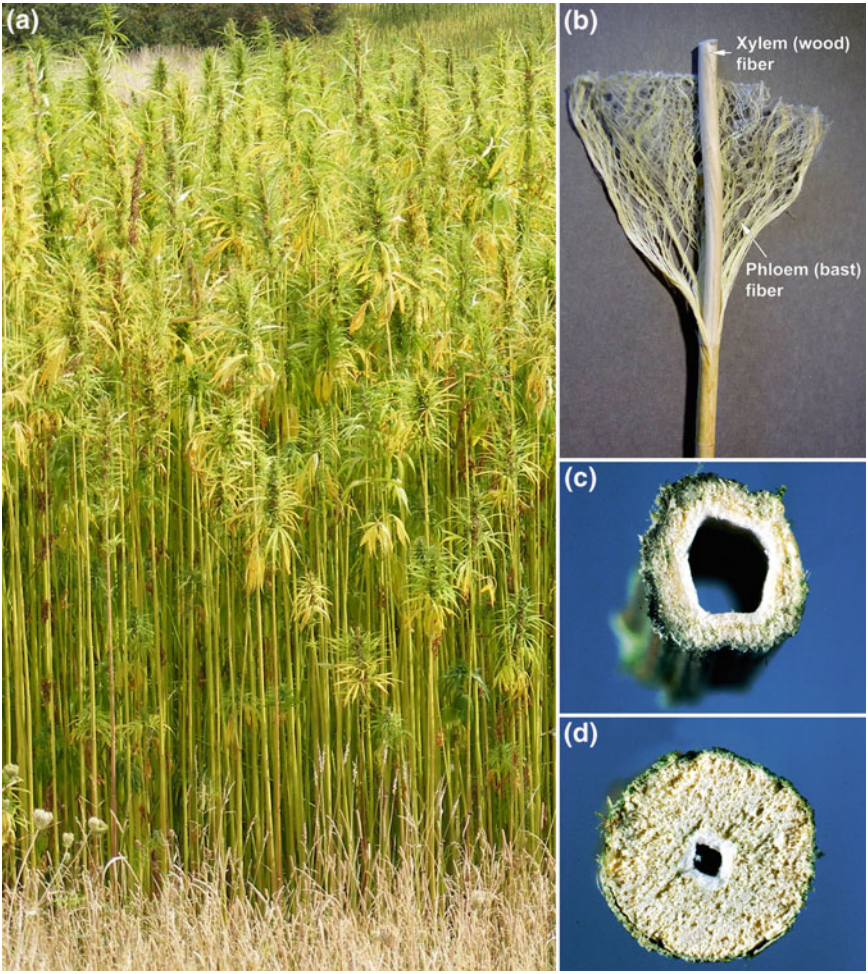

Fig. 1.4 Notable features of fiber hemp. **a** Densely grown hemp, illustrating development of tall, slim stalks and suppression of branching. Photo by Adrian Cable (CC BY 2.0 license). **b** Hemp stalk, showing the valuable phloem (bast) fiber separated from the woody core. Photo by Natrij, released into the public domain. **c**, **d** Cross sections of stems at internodes of, respectively, a fiber plant and of a marijuana plant. Fiber cultivars have stems that are hollower at the internodes, i.e. with less woody tissues, since this allows more energy to be directed into the production of phloem fiber

1.6.2.1 Historical Review

For most of recorded history, *C. sativa* was primarily valued as a fiber source, considerably less so as an intoxicant, and only to a limited extent as an oilseed crop. Hemp is one of the oldest sources of textile fiber, with extant remains of hempen cloth trailing back at least 6 millennia. For thousands of years, hemp has been most valued for rope, because of its strength, durability and water-resistance (Bócsa and Karus 1998).

Estimates of the time that hemp was first harvested by the Chinese range from 6000 years (Li 1974) to 8500 years (Schultes 1970; Schultes and Hofmann 1980), or even 10,000 years (Allegret 2013). For millennia, hemp has been a respected crop in China (Touw 1981; Clarke and Merlin 2013), where it became a very important fiber for clothing. To this day, China remains the world's chief producer of hemp fiber.

Hemp grown for fiber was introduced to western Asia and Egypt, and subsequently to Europe between 1000 and 2000 BC. Cultivation in Europe became widespread after 500 AD. The crop was first brought to South America in 1545, in Chile, and to North America in 1606, in Port Royal, Acadia (Small 1979b).

Hemp was one of the leading fiber crops of temperate regions from the sixteenth through the eighteenth centuries. It was an important European crop until the middle of the nineteenth century. Hemp was widely used for rot-resistant, coarse fabrics as well as for paper, and was the world's leading cordage fiber (used for rope, twine and similar purposes) until the beginning of the nineteenth century. Until the middle of the nineteenth century, hemp rivalled flax as the chief textile fiber of vegetable origin.

Several developments, listed in decreasing order of importance in the following, drastically curtailed the importance of hemp fiber outside of Asia. (1) The use of steam- and petroleum-powered motorized ships greatly reduced the need for hemp fiber for naval purposes. (2) Hemp rope tends to hold water in the interior and to prevent internal rotting the ropes were tarred, a laborious process that was made unnecessary when abaca was substituted. Abaca rope proved preferable for marine use because it was lighter, could float and had greater resistance to salt water corrosion. (3) The Industrial Revolution (approximately 1760–1840 in Britain) initiated sustained economic growth and living standards in the Western world, but also accentuated differences for the cost of fiber production between rich temperate regions and poor tropical and semi-tropical regions. As a fiber crop, hemp (like flax) is best adapted to temperate areas, in contrast to other leading fiber crops such as cotton, jute and sisal. Outside of Asia, production costs (largely determined by labor) in recent centuries have been much cheaper for tropical and semi-tropical fiber crops, and this contributed to making hemp much less competitive. (4) Hemp fiber was once important for production of coarse but durable clothing fabric. In the nineteenth century softer fabrics took over the clothing market. As the world has judged, cotton is a remarkably more attractive choice for apparel. The invention of the modern cotton gin by Eli Whitney in 1793 enormously increased the efficiency of cotton production, and has been claimed to have contributed to the demise of hemp fiber, which is relatively difficult to separate cleanly from other parts of the plant. Increasing limitation of cheap labor for traditional production in Europe and the New World led to the creation of some mechanical inventions for preparing hemp fiber, but too late to counter growing interest in competitive crops. (5) Human-made fibers began influencing the marketplace with the development of rayon from wood cellulose in the 1890s. Largely during the twentieth century, commercial synthetic fiber technology increasingly became dominant (acetate in 1924, nylon in 1936, acrylic in 1944, polyester in the 1950s), providing competition for all natural fibers, not just hemp. (6) Hemp rag had been much used for paper, but the nineteenth century introduction of the chemical woodpulping process considerably lowered demand for hemp. (7) A variety of other, minor usages of hemp became obsolete. For example, the use of hemp as a waterproof packing (oakum), once desirable because of resistance to water and decay, became antiquated. (8) The growing use of the cannabis plant as a source of marijuana drugs in the Western world in the early twentieth century gave hemp a very bad image, and led to legislation prohibiting cultivation of hemp.

During the two World Wars there were brief revivals of hemp cultivation by both the allies and Germany, because of difficulties importing tropical fibers. In particular, abaca and sisal fiber from the Philippines and Netherlands Indies were cut off in late 1941, and there was a concerted effort to re-establish the industry in the U.S. (Hackleman and Domingo 1943; Wilsie et al. 1942, 1944). In 1952, the U.S. Department of Agriculture issued a revision of Robinson's (1935) guide to cultivating hemp in the U.S., but lost interest in the crop subsequently. After the war, however, hemp cultivation essentially ceased in most of Western Europe, all of North America, and indeed in most non-Asian countries, although production continued at a diminished level in Asia, eastern Europe, and the Soviet Union.

In Asia (particularly in China), in most of the Soviet Union, and in most of Eastern Europe, hemp cultivation was not prohibited as it was in most of the remaining world during the twentieth century. In these areas hemp production continued to a lesser or greater degree depending on local markets (Ceapoiu 1958; de Meijer et al. 1995). A surge of interest in re-establishing the hemp industry in Western countries began in the 1990s, particularly in Europe and the British Commonwealth. At the time, governments generally were hostile to growing any form of *C. sativa* for fear that this was a subterfuge for making marijuana more acceptable. Throughout Western nations in the 1990s, interest in reviving traditional non-drug uses of *C. sativa*, as well as developing new uses, has had to contend with the dominating image of the plant as a source of marijuana. Nevertheless, cultivation resumed in the temperate-climate regions of many Western countries. Some Western European countries, such as France and Spain, never prohibited hemp cultivation, and also participated in the 1990s in the revival of hemp cultivation. About 3 dozen countries currently grow significant commercial hemp crops. As of 2016, the United States has been the only notable Western nation to persist in prohibiting hemp cultivation, although, the majority of U.S. states have enacted resolutions or legislation favoring the resumption of hemp cultivation, and cultivation has been initiated in some states. However, federal U.S. laws have precedence. The reluctance to authorize hemp cultivation has been particularly related to continuing suspicion that cultivating hemp would facilitate and promote "narcotic" usage of the species.

1.6.2.2 Architecture and Anatomy

Fiber hemp plants, by contrast with *C. sativa* plants grown for marijuana or oilseed, and also in contrast with wild plants, have been selected for features maximising stem fiber production. Selection for fiber has resulted in biotypes that have much more primary phloem fiber (Fig. 1.4b) and much less woody core than encountered in marijuana strains, oilseed cultivars and wild plants. Fiber varieties may have less than half of the stem made up of woody core, while in non-fiber strains more than three quarters of the stem can be woody core (de Meijer 1994; Fig. 1.4d). Moreover, in fiber plants more than half of the stem exclusive of the woody core can be fiber, while non-fiber plants rarely have as much as 15% fiber in the corresponding tissues. Also important is the fact that in fiber selections, most of the fiber can be the particularly desirable long primary fibers (de Meijer 1995). Since the stem nodes tend to disrupt the length of the fiber bundles, thereby limiting quality, tall, relatively unbranched plants with long internodes have been selected. Another strategy has been to select stems that are especially hollow at the internodes (Fig. 1.4c), with limited hurds (wood and associated pith), since this maximises production of long phloem fiber (although the decrease in woody tissues makes the stems less resistant to lodging by wind). Similarly, limited seed productivity concentrates the plant's energy into production of fiber, and fiber cultivars often have low genetic propensity for seed output. Selecting monoecious strains overcomes the problem of differential maturation times and quality of male and female plants (males mature 1–3 weeks earlier). Male plants in general are taller, albeit slimmer, less robust, and less productive (although they tend to have superior fiber). Except for the troublesome characteristic of dying after anthesis, male traits are favored for fiber production. In former, labor-intensive times, the male plants were harvested earlier than the females, to produce the best fiber. Fiber strains have been selected to grow well at extremely high densities (Fig. 1.4a), which increases the length of the internodes (contributing to fiber length) and increases the length of the main stem (fiber cells are amalgamated into bundles, so this contributes to fiber bundle length) while limiting branching (making harvesting easier). The high density of stems also increases resistance to lodging, desirable because woody supporting hurd tissue has been decreased by selection. The limited branching of fiber cultivars is often compensated for by possession of large leaves with wide leaflets, which increase the photosynthetic ability of the plants.

1.6.2.3 Physiology

Both wild and cultivated plants that grow for many generations in a particular location have evolved adaptations to their local climates, and these adaptations may make a given biotype quite unsuitable for a foreign location. Compared to marijuana strains, which typically originate from semi-tropical and/or very dry regions, most hemp biotypes are comparatively better adapted to temperate, mild, relatively cool, moist conditions. Nevertheless, optimal temperature for hemp germination is frequently about 24°C, a rather elevated temperature reflecting adaptation to a relatively warm subtropical climate. However, comparative cold-resistance of most hemp cultivars indicates adaptation to a temperate climate: light frosts of short exposure can be tolerated by seedlings (as low as −10°C) and mature plants (as low as −6°C, or even −10°C in Siberian cultivars) (Van der Werf 1993; Bócsa and Karus 1998).

1.6.2.4 Cannabinoid Profile

Since fiber plants have not generally been selected for drug purposes, the level of THC is often limited, usually much less than 1%. The majority of cultivars licensed in Western nations by law must have a content of less than 0.3% THC (dry weight) in the upper, flowering portion, and in some jurisdictions regulations require less than 0.2%. However, some hemp strains grown in subtropical Asia (where fiber hemp is a very minor crop and the strains are mostly unimproved land races with fiber content below 20%) are of variable THC content, and may have a content of THC as high as 3%.

1.6.2.5 Economic Status and Potential

China has dominated fiber hemp production for millennia, largely for textile applications, mostly for clothing and other woven applications. China probably will remain dominant in this niche for the foreseeable future, although hemp textiles are obsolescent. Since the early 1980's, the EU provided considerable subsidization for the creation of new hemp harvesting and fiber processing technologies, and Europe (particularly France) has developed non-woven applications of hemp fiber. Nevertheless, fiber applications of hemp are very limited because of competition with synthetic fibers and with other natural fibers. Although fiber hemp is a niche crop, of relatively minor importance today, it has experienced a limited economic resurgence based on non-traditional usages, particularly in the production of a very wide range of pressed fiber and insulation products, and plastics, employed especially in the automobile, construction, and agriculture industries (Small and Marcus 2002; Small 2014).

1.6.3 Oilseed Plants

"Oil" has three meanings with respect to *C. sativa*. (1) "Essential oil" (also known as volatile oil and ethereal oil) from the glandular secretory trichomes. Essential oil is an indistinct category of compounds synthesized primarily as secondary metabolites in plants, and includes complex mixtures of organic (hydrocarbon) chemicals. Essential oil is said to be "non-fixed" (meaning that it can evaporate