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Cannabis cultivation: Methodological issues for obtaining medical-grade product

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ABSTRACT

As studies continue to reveal favorable findings for the use of cannabidiol in the management of childhood epilepsy syndromes and other disorders, best practices for the large-scale production of *Cannabis* are needed for timely product development and research purposes. The processes of two institutions with extensive experience in producing large-scale cannabidiol chemotype *Cannabis* crops—GW Pharmaceuticals and the University of Mississippi—are described, including breeding, indoor and outdoor growing, harvesting, and extraction methods. Such practices have yielded desirable outcomes in *Cannabis* breeding and production: GW Pharmaceuticals has a collection of chemotypes dominant in any one of eight cannabinoids, two of which—cannabidiol and cannabidivarin—are supporting epilepsy clinical trial research, whereas in addition to a germplasm bank of high-THC, high-CBD, and intermediate type cannabis varieties, the team at University of Mississippi has established an *in vitro* propagation protocol for cannabis with no detectable variations in morphologic, physiologic, biochemical, and genetic profiles as compared to the mother plants. Improvements in phytocannabinoid yields and growing efficiency are expected as research continues at these institutions.

This article is part of a Special Issue entitled "Cannabinoids and Epilepsy".

1. Introduction

Plant-based drugs present unusual challenges in the pharmaceutical world with respect to large-scale cultivation, processing, quality, and consistency. In the case of *Cannabis sativa* L., considerable additional complexity derives from regulatory concerns, depending on the countries of production and marketing. In recent years, the production of cannabidiol (CBD)-based medicinal materials for research as potential therapeutics in childhood epilepsy syndromes and other disorders has come into greater focus. This article will describe the botany and pharmacognosy of *Cannabis sativa* L. and approaches employed in the United States and the United Kingdom (UK) for biomass selection, cultivation, and harvest/processing biomass to ensure quality supplies for medical research and for pharmaceutical product development.

2. Historical background in the United States and United Kingdom

In the United States, cannabis was common in patent medicines in the late 1800s and was listed in the US Pharmacopeia from the 1850s up until 1942, prescribed for various pain conditions and nausea. In 1899, cannabis was listed in the first edition of *Merck's Manual* [1] and recommended for the management of several conditions including epilepsy. It also had some history of use as an intoxicant, but, in 1937, the Marihuana Tax Act made it illegal except for medical use, which was taxed. Those who produced, prescribed, or dispensed marijuana were required to buy a stamp and pay the tax. This requirement greatly restricted legal use of marijuana, and, gradually, in the mid-20th century, when the use of most crude botanical drugs in US medicine declined, cannabis medical use plummeted as well. Of course, illicit use continued, but no "dealers" for this purpose would buy the stamp and pay the tax, because doing so would incriminate themselves. This practice was a major basis on which the Marihuana Tax Act was ruled unconstitutional in 1969. In 1970, congress passed legislation that included marijuana on the "Schedule I" list with many other controlled substances, and it remains so to this date.

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During the 1960s, the US government had also initiated a research program on marijuana, but the sources for the plant material were variable and unreliable. The National Institute of Mental Health contracted with the University of Mississippi (UM) School of Pharmacy in 1968 to grow marijuana for research purposes. This work has continued for more than 47 yr as a competitively awarded contract, popularly known for many years as "the nation's only legal marijuana farm." Today, UM supplies Good Manufacturing Practice (GMP) grade, high-quality marijuana plant material, along with extracts and purified cannabinoids, for the National Institute on Drug Abuse (NIDA) Drug Supply Program; this program makes the materials available to researchers studying their harmful and beneficial effects. In recent years, these activities have been specifically expanded to include CBD-enriched extracts of cannabis intended for clinical research use by qualified investigators. Many states (including Mississippi [2]) have passed legislation designed to allow clinical research and treatment of patients with epilepsy and other disorders, but the sourcing of these materials, from a federal regulatory perspective, has been a daunting challenge. The work reported in the present article was supported in part by NIDA, the National Institutes of Health, and the US Department of Health and Human Services, Contract No. N01DA-10-7793.

In the UK in the late 19th and early 20th century, the use of cannabis as a medicine saw a similar rise and fall to that seen in the United States. Its use declined, however, and ceased when declared a Schedule I substance in 1971. In 1998–99, two major official investigations were carried out into cannabinoid science and, more broadly, the issues related to the potential medical benefits of cannabis. The investigations were performed by the House of Lords Science and Technology Select Committee in the UK and the Institute of Medicine's National Academy of Sciences in the United States [3–5]. Both of these investigations found strong evidence supporting the potential therapeutic effects of components of the *Cannabis* plant, particularly in the field of multiple sclerosis and pain management. GW Pharmaceuticals, formed in the UK in 1998, immediately set about addressing these issues. From its inception in 1998, however, GW also had a desire to develop plants for the possible future treatment of patients with epilepsy [6]. For the following 12 yr, however, all of GW's commercial crops were grown for production of Sativex®, a botanical drug used for managing symptoms of multiple sclerosis. More recently, the company has been developing the drug Epidiolex®, a liquid formulation of pure plant-derived CBD as a treatment for patients with various orphan pediatric epilepsy syndromes. The US Food and Drug Administration (FDA) has granted Epidiolex® orphan status for the treatment of patients with Dravet syndrome, Lennox-Gastaut syndrome, infantile spasms (West syndrome), and tuberous sclerosis complex, as well as a fast-track designation for Dravet syndrome.

The company is also performing clinical trials with a drug that features cannabidivarin (CBDV) as the primary cannabinoid. This drug has shown antiepileptic properties across a range of preclinical models of epilepsy. GW has advanced research efforts of the experimental CBDV-based drug GWP42006 into a Phase 2 study of epilepsy.

3. Cannabis physiology

Cannabis is predominantly an annual herb of central Asian origin, which, heavily influenced by man over several millennia, has adapted to grow in almost all parts of the world, from the tropics to the edge of the Arctic Circle [7]. It is one of the oldest plant sources for food, textile fiber, and medicine. It is only within the last century that the species has also become synonymous with use as a recreational drug.

In addition to its direct use as a foodstuff, cannabis seeds can be crushed to produce oil for a variety of purposes. This oil can contain large quantities of essential fatty acids, especially *gamma*-linolenic acid and stearidonic acid. Because of the presence of these highly valuable nutrients, hemp seed oil is widely marketed as a health food supplement. Hemp seed oil is not to be confused with the pharmaceutical material CBD oil, as dispensed at UM. CBD oil is made by solvent extraction of CBD-rich female cannabis flowers, which is then dissolved in an oil. It should also not be confused with hash oil, a liquid or semisolid concentrated extract of *Cannabis* plant material [8]. This is used recreationally, but those in possession of illicit samples often claim to have them for medicinal purposes.

Cannabis is predominantly dioecious, with male and female flowers by definition developing on separate plants if grown naturally from seed. It does, however, occasionally exhibit a monoecious (hermaphrodite) nature. Varieties bred specifically for fiber production are predominantly hermaphrodite, as this characteristic produces more uniform material.

Apart from plants derived from extreme equatorial or polar provenance, *Cannabis* is normally a so-called "short-day plant." Such plants naturally commence flowering at the end of summer in response to a detected increase in night length. In the past 15 yr, increasing numbers of so-called autoflowering varieties have become commercially available. These plants are not day-length sensitive and commence flowering when only approximately 2 wk old, irrespective of the day length. They can be grown in continuous lighting, producing very high yields. GW's research program includes trials with CBD chemotype plants of this type.

In general, it is impossible to discriminate male plants from female plants at the vegetative stage. However, flowering male and female plants can be easily differentiated based on their very different floral structures. Although of limited application, molecular techniques allow male and female plants to be differentiated at an early growth stage [9–11].

Cannabis sativa L. is a wind-pollinated species, which is highly allogamous in nature. A significant amount of plant-to-plant variation in its cannabinoids profile and content is observed, even when the crop is propagated through a single seed accession. For the production of cannabinoids, all-female crops are preferred. Male plants produce much lower quantities of cannabinoids, and pollinated females divert resources away from cannabinoid production for seed development. To avoid this process, one option is to remove male plants as they appear. Alternatively, the presence of male plants can be prevented by using vegetative propagation and/or micropropagation while ensuring in each case that the propagation material is female. It is also possible to propagate crops from specifically produced all-female seeds. The latter two practices are described in Section 6.

4. Cannabinoid biosynthesis

Cannabis sativa L. is considered a chemically complex species based on its numerous natural constituents. It contains a unique class of terpenophenolic compounds (cannabinoids or phytocannabinoids) that have been extensively studied since the discovery of the chemical structure of tetrahydrocannabinol (Δ^9-THC), commonly known as THC, the main constituent responsible for the psychoactive effects of cannabis. A total of 565 constituents, including 120 phytocannabinoids, have been reported in *Cannabis* so far [12]. Besides Δ^9-THC, CBD, and CBDV, other major cannabinoids of *Cannabis*, including Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) are showing potential pharmaceutical interest. In fresh plant material, these cannabinoids all exist in the cannabinoid acid forms, such as Δ^9–tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). As the plant material ages or is heated, the acid molecules lose a carboxyl moiety. Decarboxylation results in the conversion of the cannabinoid acids into their neutral forms (e.g., CBDA → CBD). As is common, this article hereafter refers to the cannabinoids in their neutral form only.

The cannabinoids are predominantly, if not entirely, synthesized and sequestered in small structures called glandular trichomes [13]. Evidence suggests that these secondary metabolites play a major role in the defense of the cannabis plant. Plant species generally tend to optimize defense by allocating secondary metabolites

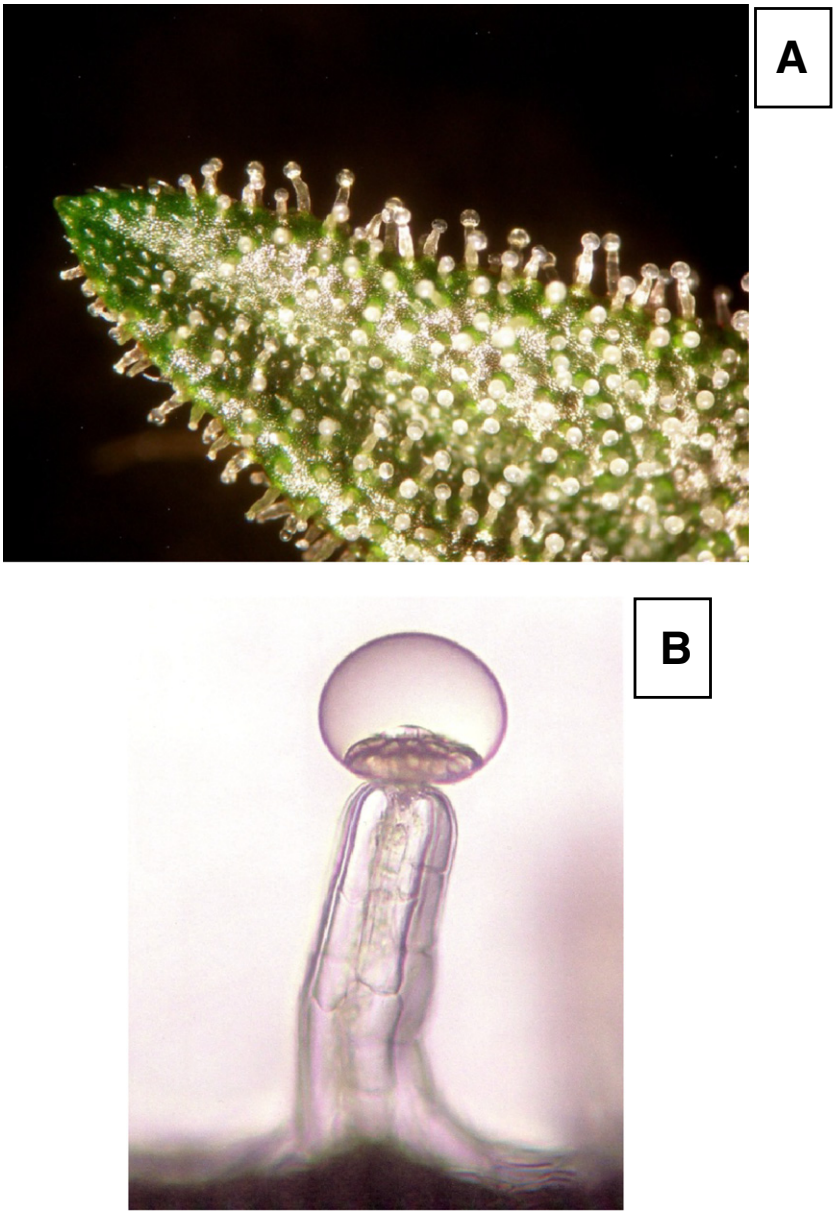

Fig. 1. A: A pubescence of *Cannabis* trichomes on the cannabis surface, B: a single trichome. CBD and other cannabinoids are secreted and sequestered in the resin head at the top of the trichome stalk.

to tissues in direct proportion to their value [14]. In *Cannabis*, this tissue is the female flower. In the highest performing genotypes, the inflorescences develop a dense pubescence of these cannabinoid-containing structures (Fig. 1). A full review of cannabis trichome form and function is available elsewhere [15,16].

5. Cannabis chemotype and plant breeding

Thanks to human influence, as well as natural diversity, plants within the species differ widely in structure and appearance. Varieties grown for fiber are typically tall, unbranched, and grown to produce a high ratio of fibrous stem-to-floral material. Plants grown for fiber and seed are commonly referred to as *hemp*. Recreational users, however, desire female floral material, so "drug plants" have been selected and bred to produce a higher proportion of flowers. The term hemp does not normally apply to this form of the species. However, throughout the world, the words used to describe the various forms of cannabis plant materials and products vary widely.

Within the Western world, CBD is the main cannabinoid found in hemp floral tissue. The concentration of CBD in the dry floral tissue would typically be 2% weight for weight (w/w) or less. Cannabis grown for recreational use, however, is dominated by the presence of THC. The THC content of floral tissue in modern varieties typically exceeds 20% w/w. This difference in cannabinoid profile has been used by some taxonomists to suggest the existence of two different cannabis species or subspecies. However, the differences in cannabinoid profile are purely the result of human intervention. No strict natural relationships between fiber characteristics and cannabinoid content exist, only artificial associations for which exceptions occur [17,18]. Historically, very little effort has been placed on finding and breeding plants with high CBD concentrations. The recreational market simply had no demand for this nonpsychoactive chemical.

An in-depth understanding of the genetic control of cannabinoid biosynthesis has resulted from a long-term medicinal cannabis breeding and research program, commencing in the Netherlands in the 1990s and thereafter at GW Pharmaceuticals [18]. The findings of this research program are reported in de Meijer et al. [19–21] and de Meijer and Hammond [22].

As stated previously, fiber hemp as a source of CBD typically contains approximately 2% CBD *w*/w within dried inflorescences. The first GW genotypes were bred from atypically resinous Turkish genotypes containing up to 4.5% CBD. The best selections bred in 1998 contained approximately 7% CBD, and that material has been used since for the drug Sativex® [23]. As a result of breeding at GW, the materials used to produce CBD for Epidiolex® contain a similar cannabinoid concentration to the most potent THC varieties, with floral material typically containing over 20% CBD.

The alleles purported to control THC or CBD synthesis are codominant. As a result, landrace populations consist of homozygous plants dominant in THC or CBD (and referred to as being of the THC or CBD chemotype) alongside heterozygous "mixed chemotype" individuals synthesizing a more even balance of both THC and CBD. The fact that CBD is the dominant cannabinoid in Western hemp is purely the result of plant breeding and local politics. Licenses to grow hemp in Europe are only issued if growers use varieties that are capable of producing no more than 0.2% w/w THC. In Canada, the limit has been 0.3% w/w. In contrast, recreational users have increasingly selected seeds and clones capable of producing plants with the greatest psychoactive effects. The allele for CBD biosynthesis has been selectively rejected. As a result, surveys of seized illicit cannabis in the United States and the UK found minimal CBD in sinsemilla [8,24]. As both publications show, traditional resin still contains a more even balance of THC and CBD. Much is made by traditional methods from landrace materials (natural plant populations), and growers of plants used for hashish respond to some pressure to select plants based upon quantity of resin produced, not just the THC content.

Until recently, the commercial acquisition of seeds capable of producing significant quantities of CBD was all but impossible. When packets of seed of 52 recreational varieties were acquired and sown, 97% of seedlings produced were of the THC chemotype, the other 3% having a mixed THC-CBD profile. Not a single CBD chemotype seedling was produced [25]. More recently, seeds advertised as being of the CBD chemotype have become available. Some of those acquired by GW Pharmaceuticals and grown in the UK for research purposes proved to be of the THC chemotype. As the European cannabis seed market is unregulated [26], an illicit grower would be in no position to challenge the supplier's seed description.

A recently developed accession, bred at GW, can produce dry floral tissue containing over 25% w/w of total cannabinoids. This composition is similar to that achieved in today's best THC chemotype plants. However, whereas the purity level of THC within the cannabinoid profile of the THC chemotype can reach 98%, the purity of CBD in the profile of the CBD chemotype is lower (i.e., 85%–90%) [18, p. 99]. All CBD chemotype plants produce some THC, in a ratio of approximately 25:1 CBD:THC. These plants are often described in the literature as being "high CBD low THC" plants. It appears that little scope exists for this THC content to be removed by conventional plant breeding. Many of the less–well-regulated CBD oil products circulating therefore contain potentially undesirable levels of THC.

As a result of the breeding program, GW Pharmaceuticals now has a collection of chemotypes dominant in any one of eight cannabinoids [18, p. 92]. Purity levels of 71% CBDV were reported in the floral material of one genotype [27], but further breeding has raised purity levels to 84%. The new material will support the ongoing GW research involving CBDV as a drug for the treatment of epilepsy. GW will further improve its ability to increase the CBDV in plant extracts by selective purification of cannabinoids.

6. Cannabis cultivation

The growing methods used by the two contributors are described in Sections 6.1 and 6.2. Since 1999, GW has optimized methods for growing cannabis indoors in a tightly controlled growing environment. That method has been adopted for growing the CBD chemotype plants used to make Epidiolex®. Research has been performed to evaluate growing methods in a simpler greenhouse with minimal supplementary lighting. Crops of high-CBD, all female *Cannabis* have also been grown outdoors. Each of the three GW growing methods is described before describing indoor and outdoor cultivation at UM.

6.1. Cannabis production at GW pharmaceuticals

6.1.1. Intensive indoor cultivation

In this operation, great emphasis is placed in producing a uniform product. Growing temperature, irradiance levels, day lengths, planting densities, and harvest timings are kept tightly specified, as are the growth medium ingredients. Cannabidiol chemotype plants are grown from cloned female material. To ensure traceability, every aspect of the growing process is rigorously documented. A small number of genotypes are used, which are currently derived from a single accession. To start the process, so-called mother plants of each genotype are grown vegetatively in batches. When sufficiently large, these plants are cut into sections to produce cuttings. A small amount of rooting hormone is applied, and the cuttings are placed in moist rooting coir plugs. After 2 wk of retention in high humidity and continuous light, a high proportion of these cuttings have produced a vigorous root system [15,28]. They are then ready to be potted into a peat-based growth medium.

The genotypes used can be described as true short-day plants, so mother plants and newly potted plants, for the first 3 wk of growth, are kept in continuous light to keep them vegetative. Some cannabis growers recommend growing their plants in an artificial day length of 18 h as opposed to 24 h. In some cases, this practice is believed to reduce electrical consumption [29]. However, it slows down plant growth proportionally [30].

Throughout the night, and also when daytime light conditions are poor, a supplementary lighting system ensures minimum irradiance levels of 70 W m^{-2} PAR (i.e., photosynthetically active radiation). This supplementary lighting is at least twice as bright as that used in culinary or ornamental crops in the UK. As stated earlier [31], cannabis requires high light levels to produce optimum yield. Average daily temperatures of 25 °C are maintained. The optimum temperature for cannabis growth is up to 35 °C, depending on geographic origin [31]. However, such high temperatures are unfavorable for staff welfare and encourage rapid populations of insect pests [32].

Plants are induced to flower by moving whole benches of plants into areas of the greenhouse where blinds and lights in the greenhouse roof deliver an alternating 12-h light, 12-h dark regimen. Induction of flowering is rapid. Foliar and stem growth slows dramatically and all but ceases within 3 wk. Floral development is rapid and within 8–10 wk the bulk of the plant material present is resinous floral tissue [33]. The plants are then cut down and dried, and the combined flower and leaf material (i.e., botanical raw material) stripped from the stem. The latter is discarded. Harvests take place weekly, with benches of plants being moved forward through the greenhouse continuously on a production-line basis. A view of the flowering crops in one such greenhouse is shown in Fig. 2.

Throughout this process, plants are irrigated with potable water. The growth medium requires a certificate of conformity before use, to ensure it is of the correct structure, pH, and nutrient content and free of pesticide or heavy metal residues. Growing conditions that would

Fig. 2. A flowering CBD crop. The youngest plants in the production line are the closest to the camera.

favor disease problems are avoided, and insect pests are controlled by the introduction of beneficial insects that predate on them.

To regulate the rate of gas and water vapor exchange between leaf and microenvironment, it is important to have air flow around the leaf surface. The air movement affects the leaf boundary layer thermal conductance, energy budget, and, ultimately, the physiology and growth of the whole plant. This movement is achieved by the installation of numerous fans within the growing environment. Good air circulation also helps prevent the establishment of plant disease. The growing conditions are tightly monitored at all times and recorded for each batch. The high level of monitoring ensures that good growing conditions are maintained at all times, and alarms are raised if any unplanned deviation is detected.

At the end of the growing process, plants are cut at the base and hung to dry in a warm dehumidified environment. Fig. 3 shows one bench of CBD-chemotype plants being inspected prior to harvest. This 10-m^2 bench carries enough feedstock for production of just 1 to 2 yr of Epidiolex® treatment for a pediatric patient. The growing process is summarized schematically in Fig. 4.

6.1.2. Less-intensive greenhouse growing options

Preparing for a possible response to the clinical trial success of Epidiolex® for managing childhood epilepsy syndromes, GW Pharmaceuticals investigated the feasibility of commissioning a large greenhouse previously used to grow culinary crops. After small-scale greenhouse trials, it was shown that crops of its favored genotype could be grown without supplementary lighting during the flowering phase. This approach had provisos, however:

1. Supplementary lighting was required for the mother plant and vegetating crops, with the irradiance level being 70 W m^{-2} PAR, as in other sophisticated production greenhouses.
2. Roof blinds were required to enable an artificial 12-h day length to be maintained where necessary.

Fig. 3. A bench of mature CBD plants being collected for harvest.

Seed accessions selected
↓
Seeds germinated
↓
Best candidates selected
↓
Mother plants raised
↓
Branches removed and cuttings produced
↓
Cuttings rooted 14 days in rooting plug—*25°C, 24-h day length, high humidity*
↓
Rooted cuttings—*potted up in growth medium*
↓
Vegetative growth period—*3 weeks, 24-h day length, 25°C*
↓
Flower formation and maturation—*10 weeks, 12-h day length, 25°C*
↓
Harvest—*whole plant cut at base*
↓
Drying—*plants hung in dry ventilated area in darkness 25°–30°C, 1 week*
↓
Stripping— *flowers and leaves stripped from stem*
↓
Botanical raw material inspection and garbling—*stem fragments and contaminants removed*

Fig. 4. Selection and propagation of high-quality plant material: the essential steps.

3. Plants could only be supported through the flowering stage between the vernal and fall equinoxes, when natural irradiance levels are sufficient for good plant growth.
4. A gas-fuelled heater was required to enable rapid drying of large weekly harvests during spring and summer.

Without supplementary lighting during the flowering phase, yields were approximately one-third lower than those of illuminated crops. However, large tonnages of good quality material were produced. Fig. 5 shows part of one crop growing in this environment.

Fig. 5. An area of high-yielding CBD chemotype crop, growing without supplementary lighting.

6.1.3. Outdoor propagation

The United Nations Office on Drugs and Crime has described one-third of the earth's dry surface as being suitable for cannabis cultivation. In Europe, a latitude of 55 °N was suggested to be the northerly limit [34].

Since 2000, GW has regularly grown small areas of research crops outdoors at a latitude closer to 50°N. Initially, these crops were from cloned material. However, when performing research to examine the future possibility of growing CBD-chemotype cannabis outdoors for drug production, it was recognized that many kilograms of CBD are required for each child being treated for Lennox-Gastaut syndrome or Dravet syndrome. A predicted demand for 10,000 patients would require a vast acreage of crop if it was grown outdoors. Producing and planting sufficient clones to populate this area would present massive logistical problems. Despite a lower CBD yield per unit area, growing a crop from seed presents fewer agronomic difficulties. Successful plant breeding, and the development of improved seed production methods, have enabled the efficient production of many millions of all-female cannabis seeds—each capable of producing a CBD concentration in the harvested flower and leaf material of 10% w/w. The production of all-female seeds relies on the industry-wide practice of treating one female parent with silver thiosulfate, as described by Mohan Ram and Sett [35]. This practice causes the female to produce pollen, each grain carrying a female sex chromosome only. All seeds formed as a result of crosses with this pollen will be female.

Experimental crops, covering many tens of acres, have been planted with all-female seeds produced within the company. The grower is not able to majorly influence harvest date, so large volumes mature around the same time. This crop is harvested by machine and force dried promptly within a rotary drier. Wet material entering the drier is totally dry within 10 min and is then baled before transport. A view of the harvester moving through the crop is seen in Fig. 6. Fig. 7 shows a seed plant festooned with the seed needed to establish such a crop.

6.1.4. Onward processing of harvested material

After drying, the plant material is milled and heated to convert (i.e., decarboxylate) CBDA within the plant material to CBD. The material is then mixed with liquid carbon dioxide under extreme pressures for several hours. Cannabinoids, essential oils, and waxes in the plant dissolve in the solvent. When brought back to atmospheric pressure, the cannabinoids and other ingredients precipitate from the rapidly diminishing solvent. The purification of CBD from the precipitate is a proprietary method.

6.2. Cannabis production at UM

6.2.1. Vegetative propagation: indoor and outdoor

At UM, plants of different *Cannabis* varieties (high-THC varieties, intermediate varieties [i.e., THC–CBD] and high-CBD varieties) are being grown from seeds and/or cuttings for the purpose of screening and selection of elite mother clones. They are also grown for the production of GMP-grade biomass, extracts, and pure cannabinoids. Screening of elite clones is achieved by making a few cuttings of each seedling after enough vegetative growth has developed, and these are kept under a vegetative light cycle (18 h or more, photoperiod). Meanwhile, the original mother plants are subjected to flowering (in a 12-h photoperiod so that their gender can be identified). Male plants are removed as they appear, and females are kept until maturity. Biomass samples of fully mature plants are analyzed using gas chromatography with a flame ionization detector (GC-FID). On the basis of the cannabinoid profile, high-yielding elite plants of different varieties are identified. Their related cuttings, taken at vegetative stage, are selected as mother plants for future use.

Once a particular clone is selected, the bulking up of plant material using vegetative propagation is similar to that used by GW Pharmaceuticals. Cuttings taken from the mother plant are dipped in rooting hormone and placed in soil using small (4-in) biodegradable peat-based pots for rooting in a climate-controlled indoor growing facility. A well-rooted batch of a vegetatively propagated crop is shown in Fig. 8. Well-rooted cuttings are propagated in larger pots for further indoor cultivation or taken out in the field and planted in soil using automated planters. Alternatively, cuttings taken from mother plants can be directly planted in a hydroponics system using rockwool as a supporting medium. During the year 2014, a range of high-CBD and intermediate (CBD–THC) variety clones were screened, and their vegetative crop was grown in a 1.5-acre plot to produce plant material for research under a contract with NIDA (Fig. 9). The germplasm of these varieties is being maintained in a strict climate-controlled indoor growing room at a vegetative stage for future use. *In vitro* propagation is also being used as an alternative means for the conservation and mass propagation of *Cannabis* [36–38].

6.2.2. In vitro propagation

Most of the *in vitro* regeneration protocols developed so far for *Cannabis* have been via the callus phase [9,39–41]. Indirect organogenesis developed for *Cannabis* at the UM laboratory uses young leaves as the source explant [42]. Because callus-mediated regeneration may sometimes lead to somaclonal variations, the propagation of *Cannabis*

Fig. 6. Harvesting of a CBD field crop.

Fig. 7. A side branch of a CBD seed plant, bearing vast quantities of all-female CBD chemotype cannabis seeds.

through nodal explants, compared with calli, allows recovery of genetically stable and true-to-type progeny plants.

Although different routes are available for plant tissue culture regeneration, direct organogenesis is a common method of micropropagation that involves tissue regeneration of adventitious organs or axillary buds directly or indirectly from the explants. Direct organogenesis holds advantages, including less culture stages (no callus stage) and less or no chances of somaclonal variations and thereby higher genetic stability [43]. Direct organogenesis is a two-step protocol for *Cannabis* that has been established at UM using nodal segments [44]. A one-step regeneration protocol, however, has been further developed, based on adventitious shoot induction as well as an effective rooting using a novel aromatic cytokinin, meta-Topolin [45]. Synthetic seed technology has also been used for *in vitro* conservation and propagation of elite cannabis germplasm [36,37].

The team at UM has successfully hardened and acclimatized the direct organogenesis regenerated plants in soil with 95% survival frequency (Fig. 10). The regenerated plants did not show any detectable variation in morphologic or growth characteristics and were highly comparable with the mother plants in terms of physiological, biochemical, and genetic profiles [45–47]. The protocols developed would be helpful for large-scale mass propagation of elite *Cannabis* varieties for further use in phytopharmaceuticals (Fig. 10).

6.2.3. Outdoor cultivation

The UM produces GMP-grade marijuana outdoors in compliance with regulations of the FDA and the Drug Enforcement Administration. Production facilities include a 14-acre fenced garden, which is secured by armed guards and electronic surveillance systems. Within the garden is a polyhouse, a climate-controlled vegetative propagation building, industrial grade biomass driers, and a 1900-square-foot production building for processing plant material. Outdoor cultivation of *Cannabis* normally starts in late March or early April with arrival of warmer weather and lasts until November or early December, depending on the variety. Starting from seeds, plants are raised in small biodegradable 2-in peat-based pots. After enough growth, these pots can be directly planted outdoors. Male flowers start appearing within 2 to 3 months (around the middle of July), followed by female flowers. Male plants are removed manually before pollination can occur. Alternatively and preferably, vegetative cuttings of selected mother plants having specific chemical profiles (high-CBD type and/or CBD–THC type) are raised in the same size peat-based pots and planted using automatic

Fig. 8. Vegetative propagation of *Cannabis sativa* L.

Fig. 9. An aerial view of outdoor cultivation of CBD rich *Cannabis sativa* L. at UM.

planters. Cannabinoid content and profile is periodically monitored (using GC-FID) by taking random samples of plants from different areas of the growing field. Samples are tested more frequently as plants approach the budding/maturity stage.

The amount of cannabinoid has been found to generally increase with the age of plant, reaching the highest level at the budding stage and achieving a plateau before the onset of senescence. The maturity of the crop is determined visually and confirmed based on the major cannabinoid content of samples collected at different growth stages. Because the whole plant does not mature at the same time, mature upper buds are harvested first and other branches are given more time to achieve their maturity (Fig. 11).

6.2.4. Harvesting, processing, and storage

Optimum harvest time is very important to maximize the yield of secondary metabolites in any crop of pharmaceutical importance. This is also true for cannabis; harvesting is done based on the desired cannabinoids content as described earlier. If the biomass is being used as a

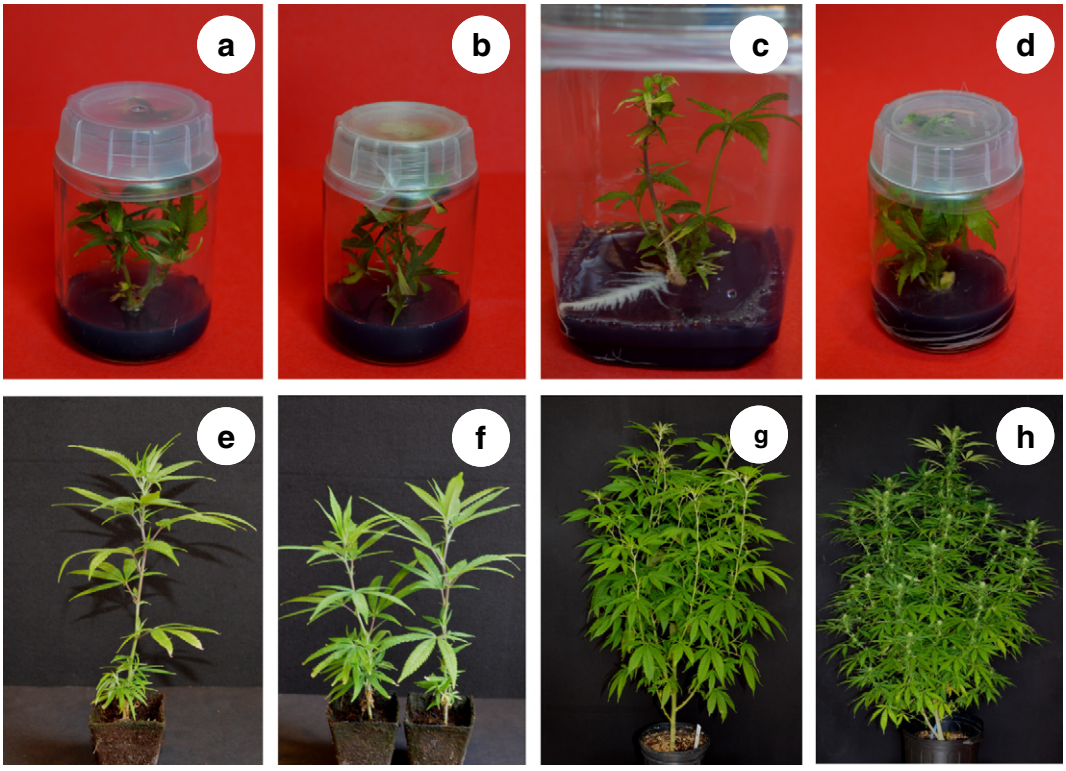

Fig. 10. In vitro propagation of *Cannabis sativa*. a and b: Shoot formation, c and d: rooting, e and f: acclimatized plants stabilized in peat-based pots, g: fully grown cannabis plant at the vegetative stage, and h: flowering cannabis plant.

Fig. 11. Outdoor cultivation of *Cannabis sativa* L. at UM. A: Crop at the vegetative stage, B: crop at the flowering stage.

starting material for pharmaceutical interest, its contact with the ground should be avoided. Avoidance of soil contamination is a stated requirement of the Good Agricultural and Collection Practice guidelines [48]. Dry and large leaves may be removed from mature buds before drying. The type of drying facility selected depends on the quantity of crop being harvested. For large scale growing, the plants are dried in industrial grade "forced-air" dryers, but for small samples, a simple laboratory oven is used for overnight drying at 40 °C. Dried plant material is packaged in FDA-approved barrels and labeled with a unique lot number. Plant material is stored in a refrigerated vault until needed for the extraction process. All processes involved in a batch of *Cannabis* biomass production, from selection of a seed or clone to labeling and packaging, is recorded in a batch production record.

6.2.5. Extraction of phytocannabinoids

Extraction of plant material at UM is accomplished by one of two ways, either by solvent extraction or by supercritical fluid extraction, the details of which are proprietary. Decarboxylation of the acid cannabinoids to the neutral cannabinoids is accomplished using the extract, unlike the process at GW Pharmaceuticals, where the plant material itself is subjected to the decarboxylation step before extraction. All the steps involving the manufacture of a single batch of cannabis extract are recorded in a batch production record. Pure CBD or THC is isolated following proprietary methods.

7. Conclusion and looking ahead

The protocols developed by GW Pharmaceuticals and UM will be useful for future research and mass propagation of *Cannabis* varieties for phytopharmaceuticals. Research actively continues at UM and GW Pharmaceuticals, and further optimization of cannabis growing methods will probably see increases in CBD and CBDV yields and improvements in growing efficiency in a range of horticultural and agricultural settings.

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