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Review article

Terpenes in *Cannabis sativa* – From plant genome to humans

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ABSTRACT

Cannabis sativa (cannabis) produces a resin that is valued for its psychoactive and medicinal properties. Despite being the foundation of a multi-billion dollar global industry, scientific knowledge and research on cannabis is lagging behind compared to other high-value crops. This is largely due to legal restrictions that have prevented many researchers from studying cannabis, its products, and their effects in humans. Cannabis resin contains hundreds of different terpene and cannabinoid metabolites. Many of these metabolites have not been conclusively identified. Our understanding of the genomic and biosynthetic systems of these metabolites in cannabis, and the factors that affect their variability, is rudimentary. As a consequence, there is concern about lack of consistency with regard to the terpene and cannabinoid composition of different cannabis 'strains'. Likewise, claims of some of the medicinal properties attributed to cannabis metabolites would benefit from thorough scientific validation.

1. Introduction

Cannabis sativa (cannabis) is thought to have originated from central Asia, and has been domesticated for over 5000 years [1]. Cannabis varieties that are low in psychoactive cannabinoids are used for the production of fiber and oilseed. However, the most valuable cannabis product today is the terpene- and cannabinoid-rich resin with its various psychoactive and medicinal properties. The resin is produced and accumulates in glandular trichomes that densely cover the surfaces of female (pistillate) inflorescences and, to a lesser degree, the foliage of male and female plants (Fig. 1). In total, more than 150 different terpenes and approximately 100 different cannabinoids [2] (Fig. 2) have been identified in the resin of different cannabis types (Table 1). The predominant cannabinoids in cannabis grown for medicinal or recreational use are $^{\Delta}$9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). While cannabinoids are the primary psychoactive and medicinal components of cannabis resin, volatile terpenes (monoterpenes and sesquiterpenes) contribute many of the different fragrance attributes that influence consumer preferences.

Different cannabis types and their derived consumer products are commonly referred to with 'strain' names. These names often relate to fragrance attributes conferred, at least in part, by terpenes [3]. Different 'strains' may be distinguished by morphological features or differences in the chemical composition of the resin. However, due to a history of largely illicit cannabis production, cannabis 'strains' are often poorly defined genetically. 'Strains' may lack reproducibility with regard to profiles of terpenes and cannabinoids [4,5]. The species encompasses large genetic diversity, with most strains having high levels of heterozygosity and genetic admixture [5,6]. Cannabis is wind-pollinated, which also contributes to variability of cannabis metabolites. As a result, many cannabis 'strains' lack the level of standardization that producers and consumers are accustomed to with other crop plants, such as genetically and phenotypically well-defined grapevine varieties. In the absence of proper genetic or genomic characterization, some attempts have been made at chemotaxonomic classification of cannabis 'strains' based on terpenes, and cannabis plants have also been described as belonging to different chemotypes (Table 1). However, the complexity of terpene biosynthetic systems, and the many different sources of terpene variation, renders these efforts often futile; in general, concepts of chemotaxonomy have been outdated by genome sciences, and chemotypes cannot reliably substitute for properly genotyped plants.

With the lifting of some of the legal restrictions on cannabis research in Canada, and in some other jurisdictions, there is now an opportunity to build stronger scientific knowledge of the genomic, molecular and biochemical properties that define terpene and cannabinoid profiles in different cannabis 'strains'. This in turn can support the development of a larger number of well-defined cannabis varieties. Another aspect that requires new research are the various effects that are attributed to cannabis terpenes in humans. While some of the effects of the cannabinoids have been scientifically explained, there is a great deal of uncertainty about the effects of cannabis terpenes in humans

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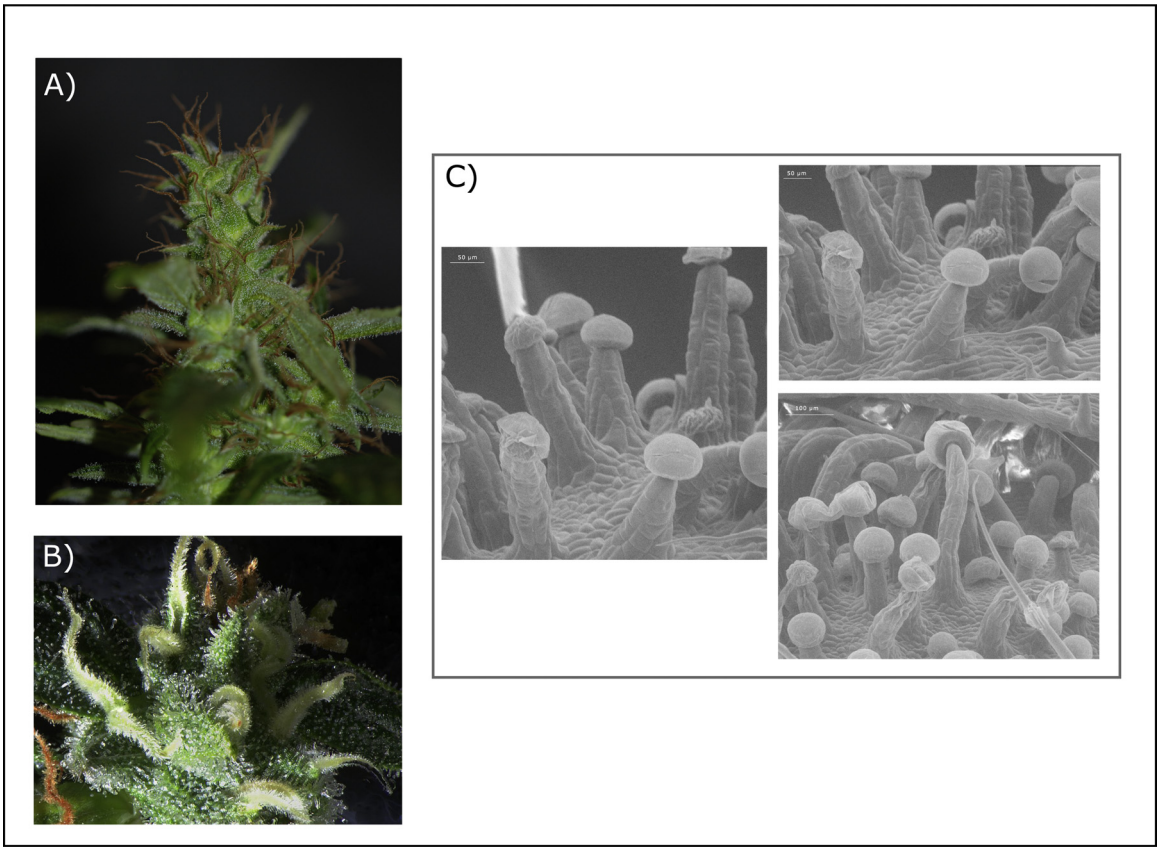

Fig. 1. Cannabis inflorescence and stalked glandular trichomes. A) Apical inflorescence from the strain Purple Kush, eight weeks post onset of flowering. B) Floret cluster from the strain Lemon Skunk, five weeks post onset of flowering. C) Stalked glandular trichomes on the surface of strain Finola pistillate flowers. Scanning electron microscopy and image credit for C) thanks to Samuel Livingston, UBC, Department of Botany.

beyond fragrance perception.

2. Chemistry, biosynthesis and genomics of terpene diversity and variation in cannabis

Terpene composition is a phenotypic trait that shows much variation across different cannabis 'strains' (Table 1). The majority of terpenes found in cannabis are hydrocarbons, which are the direct products of terpene synthase (TPS) enzymes [7,8], as opposed to more complex terpenes that require modification by other enzymes such as cytochrome P450s. Therefore, the chemical diversity of cannabis terpenes reflects the diversity of TPS enzymes encoded in the cannabis (Cs)TPS gene family.

The monoterpene myrcene as well as the sesquiterpenes β-caryophyllene and α-humulene appear to be present in most cannabis 'strains'. Other common compounds include the monoterpenes α-pinene, limonene, and linalool as well as the sesquiterpenes bisabolol and (*E*)-β-farnesene. It is important to note that some terpenes, in particular sesquiterpenes, remain difficult to identify due to the lack of authentic standards for many of these compounds. As a result, reports of terpene profiles in cannabis may include unknown compounds, rely on tentative identification, or present incomplete profiles of selected compounds. Stereochemistry is also not consistently described, or is often ignored, in reports on cannabis terpenes. These issues make it difficult to fully assess the diversity of terpenes in cannabis using the available data and make it problematic to compare the results of different studies.

The terpenes found in the cannabis resin, as well as the isoprenoid moiety of the cannabinoid structure, are produced through the isoprenoid biosynthetic system, which originates in the mevalonic acid (MEV) pathway in the cytosol and the methylerythritol phosphate (MEP) pathway in plastids. Monoterpenes and cannabinoids have a common ten-carbon isoprenoid precursor, geranyl diphosphate (GPP, C_{10}), while sesquiterpenes are produced from the fifteen-carbon isoprenoid farnesyl diphosphate (FPP, C_{15}). Using GPP or FPP as substrates, monoterpene synthases (mono-TPS) and sesquiterpene synthases (sesqui-TPS) produce the different structures of mono- and sesquiterpenes found in the cannabis resin (Fig. 2). A recent analysis of the Purple Kush cannabis genome and transcriptome sequences identified more than 30 different CsTPS genes [8]. Only nine CsTPS have been functionally characterized and published to date [8,9]. As with many other plant TPS [7], eight of the nine characterized CsTPS are multi-product enzymes that generate several different terpene structures from either GPP or FPP [8]. The multi-product nature of CsTPS can explain why some terpenes, such as α-humulene and β-caryophyllene, typically co-occur in different cannabis samples. The CsTPS responsible for many of the different terpenes found in cannabis are still unknown.

Variation of the composition of the CsTPS gene family and variation in CsTPS gene expression is likely to explain observed variations of terpene profiles across the species. However, the level of variation of the size, composition and expression of the CsTPS gene family, and factors that influence CsTPS gene expression, are for the most part unknown. For example, variation of terpene biosynthesis at the genome, transcriptome, proteome and biochemical levels have been shown in other plants to account for phenotypic intra-specific variation of terpene profiles [e.g. 10,11]. Terpene profiles may also substantially change as a result of differential CsTPS gene expression over the course of plant development or in response to environmental factors. In addition, developmental or tissue specific expression of CsTPS may affect variation of terpene profiles in cannabis products. None of these factors of terpene variation, which may contribute to poor reproducibility of

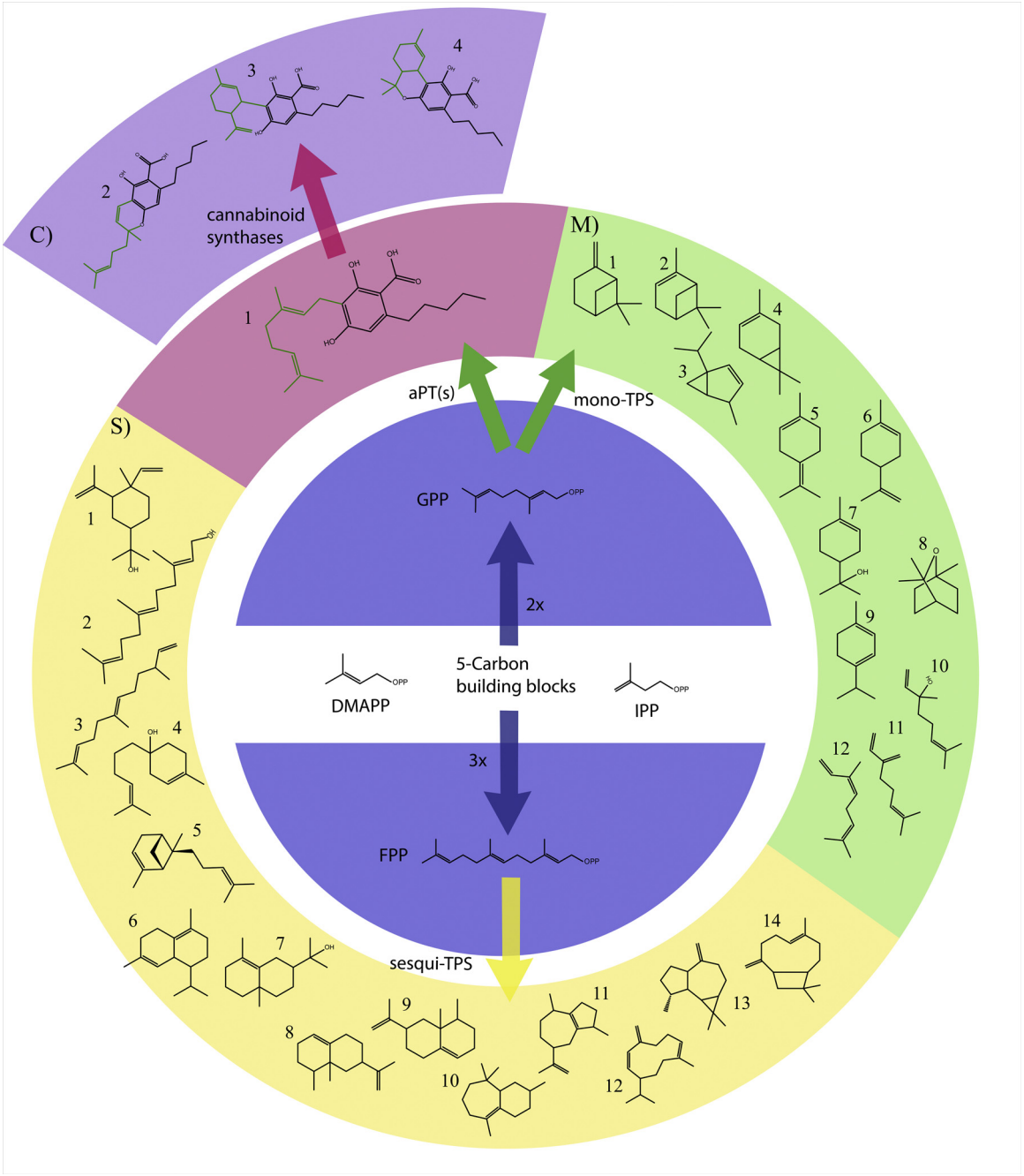

Fig. 2. Schematic of terpene and cannabinoid biosynthesis in cannabis. 5-Carbon isoprenoid building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are condensed to form geranyl diphosphate (GPP) (C10) or farnesyl diphosphate (FPP) (C15). Terpene synthases (TPS) convert GPP or FPP into terpenes. Aromatic prenyltransferases (aPTs) condense GPP with olivetolic acid to form cannabigerolic acid (CBGA), which is cyclized by cannabinoid synthases to produce cannabinoids. Cannabinoids: C1: cannabigerolic acid, C2: cannabichromenic acid, C3: cannabidiolic acid, C4: tetrahydrocannabinolic acid. Monoterpenes: M1: β-pinene, M2: α-pinene, M3: β-thujone, M4: 3-carene, M5: terpinolene, M6: limonene, M7: terpineol, M8: 1,8-cineole, M9: α-terpinene, M10: linalool, M11: myrcene, M12: (*Z*)-β-ocimene. Sesquiterpenes S1: α-elemol, S2: (*E*)-β-farnesol, S3: (*E*)-β-farnesene, S4: bisabolol, S5: (+)-α-bergamotene, S6: δ-cadinene, S7: γ-eudesmol, S8: valencene, S9: eremophilene, S10: β-himachalene, S11: α-guaiene, S12: germacrene D, S13: alloaromadendrene, S14: β-caryophyllene.

terpene composition, have been systematically studied in cannabis.

The oxygen functionality of simple terpene alcohols found in cannabis such as linalool or bisabolol may result from the enzymatic activity of CsTPS as has also been shown for TPS in other plants species [8]. Other terpene derivatives detected in cannabis may arise non-enzymatically due to oxidation or due to thermal- or UV-induced rearrangements during processing or storage, such as caryophyllene oxide, β-elemene, or derivatives of myrcene [8,12]. These non-enzymatic modifications may add a level of variation that is independent of the plant genome and biochemistry. When terpene analysis is performed with dried plant material, variable quantitative losses of terpenes, especially the more volatile monoterpenes [13], may be another cause of terpene variation.

To resolve issues of poor reproducibility of terpene profiles in cannabis, it will be essential to perform rigorous studies with a diversity of cannabis genotypes grown under controlled environmental conditions and analyze terpene profiles quantitatively and qualitatively over the course of plant development. This would need to include organ-, tissue- and cell-type specific terpene analysis, and would have to include controlled experiments to assess effects of environmental conditions

Table 1
Publications listing cannabis terpene profiles. Purpose refers to the stated objective of the study. Origin of plant material indicates what the authors stated as the source of their cannabis or extracts. Number of terpenes identified includes all named or numbered compounds listed by the authors, including those not identified using authentic standards. Publications are listed in order of date published, from earliest to most recent.

# of terpenes identified	Origin of plant material	Purpose of analysis	Reference
25	Wild-grown in Kashmir	Plant Biology	[37]
50	Forensic samples	Classification	[38]
66	Grown by researchers	Plant Biology	[13]
48	Breeders, researchers, law enforcement	Classification	[39]
16	Grown by researchers	Plant Biology	[40]
27	Bedrocan BV	Classification	[41]
49	Grown by researchers outdoors	Metabolite survey	[42]
28	Grown by researchers	Metabolite survey	[43]
20	Coffee shops in the Netherlands and Bedrocan BV	Classification	[44]
12	Bedrocan BV	Industrial	[45]
53	Forensic samples	Metabolite survey	[12]
13	Grown outdoors	Industrial	[46]
27	Indoor cultivator in California	Industrial	[47]
28	Submissions from medical patients	Classification	[4]
28	Grown by researchers	Plant Biology	[48]
17	Bedrocan BV	Industrial	[49]
50	Bedrocan BV	Classification	[50]
16	Submitted by dispensary	Classification	[3]
14	Licensed producers in Canada	Classification	[51]
20	Indoor cultivator in New Mexico, assorted growers	Classification	[52]
21	Dispensary in California	Medical	[53]
45	Grown outdoors	Medical	[54]

such as light, irrigation, and nutrients. Such experiments should include not only terpene metabolite analysis, but also a comprehensive transcriptome profiling of CsTPS gene expression. The results of such a study would enable much needed proper assignment of reproducible terpene profiles to different 'strains' and support the standardization of cannabis varieties and derived consumer products.

3. Biosynthesis of cannabinoids

Compared to terpene biosynthesis, cannabinoid biosynthesis has been a priority of the limited research on metabolite biosynthesis in cannabis to date. Much of the core cannabinoid biosynthetic pathway has been characterized [14–17]. The primary branch-point intermediate for cannabinoid biosynthesis is cannabigerolic acid (CBGA). CBGA is produced by the prenylation of the aromatic olivetolic acid with a geranyl moiety. An aromatic prenyltransferase (aPT) was recently cloned and shown to be active in a metabolically engineered yeast to produce CBGA [16], and a related cannabis membrane protein with prenyltransferase activity was previously reported in the patent literature [18]. Similar enzymes were shown to prenylate acylphloroglucinols to produce bitter acids in hop, a close relative of cannabis [19,20]. The precise origin of the fatty acid precursors of olivetolic acid is unknown. Genes of three different cannabinoid synthases, specifically THCA synthase (THCAS), CBDA synthase (CBDAS) and cannabichromenic acid synthase (CBCAS), have been published [21–23]. However, the genes and enzymes responsible for the many minor cannabinoids, including propyl sidechain variants, remain unknown.

4. Effects attributed to terpenes in cannabis

Arguably, the only effect of cannabis terpenes on humans that is unquestionable are the fragrance attributes of different mono- and sesquiterpene volatiles and their mixtures. Depending on the variable composition of cannabis terpene profiles, different 'strains' elicit different fragrance impressions, which may affect consumer preference [24]. However, other attributes assigned to terpenes in cannabis products, including medicinal properties, remain for now outside of the space of scientific evidence.

The so-called 'entourage effect' is a popular idea. It suggests a pharmacological synergy between cannabinoids and other components of cannabis resin, in particular terpenes [25,26]. Putative aspects of the entourage effect include the treatment of depression, anxiety, addiction, epilepsy, cancer, and infections. The anecdotal notion of a synergistic effect appears to stem from the perception among cannabis users that different 'strains' have different physiological effects. There is no doubt that the large chemical space of thousands of plant terpenes and terpenoids includes many biologically active molecules. Some terpenoids, such as the anticancer drug Taxol, are potent and highly valuable pharmaceuticals, the effects of which are supported by the full range of pharmacological and clinical studies. In one of the few examples of the entourage effect being tested, terpenes were found not to contribute to cannabinoid-mediated analgesia in rats [27]. With the possible exception of the sesquiterpene β-caryophyllene, no molecular mechanism has been demonstrated to explain a potential synergy of terpenes with cannabinoids. One potential explanation for the effects attributed to terpenes is revealed in a recent review [28], pointing out that the placebo effect is partially mediated through the endocannabinoid system, which may explain some of the perceived effects of cannabis products.

The sesquiterpene β-caryophyllene is prominent in many cannabis 'strains' and products. The molecule binds to the mammalian CB_2 cannabinoid receptor, which may provide a plausible mechanism for interaction with cannabinoids and a starting point for future research [29]. β-caryophyllene is one of the least variable terpene components of cannabis (Table 1), which would suggest that it cannot explain 'strain'-specific effects in humans. The proposed synergistic effects of terpenes in the effects of cannabis in humans is an area that will require careful research, which will now be possible in those jurisdictions in which some of the legal restrictions have been lifted.

5. Claims of anticancer effects of cannabis and cannabis terpenes may do more harm than good

Certain monoterpenes have been shown to block tumor formation or inhibit cell cycle progression in vivo and in rats [30–32]. However, the amounts of terpenes required to produce anti-proliferative effects in rats are excessively high with up to 10% of the animals' diet [30]. Similarly, cannabinoids may inhibit tumor formation in animal models of cancer [33]. Laboratory studies such as these may have led to the suggestion that cannabis extracts, with their combination of

cannabinoids and terpenes, have anti-cancer properties [25,26]. However, to our knowledge, there is no conclusive evidence to support claims of anticancer activity of terpenes consumed with cannabis products. While the ethanolic extract of cannabis flowers has higher antitumor activity than pure THC, this effect was not attributed to any of the five most abundant terpenes [34].

In general, it is important to remember that cannabis is often consumed by smoking or as a vapor. This includes cannabis consumption by young adults. Consumer habits such as inhaling combusted or vaporized cannabis products must be considered a health risk, including the potential risk of causing cancer or other health issues [35,36], before promoting unsupported claims of anti-cancer effects of cannabis.

6. Perspective and future directions

Genomics has been slow to reach cannabis, largely due to legal restrictions on funding agencies and researchers. A first reference quality cannabis genome was published in 2018 [23], enabling the genome-wide analysis of genes for metabolic pathways systems in cannabis. More genotyping and sequencing studies are required to encompass the full diversity of the species. A special emphasis is needed on Eurasian and African landraces, which have been under-sampled. Critical tools for functional genomics of metabolic systems, and ultimately crop improvement, such as genetic transformation or genome editing, are not yet established for cannabis research in the public domain. Beyond the genes that encode enzymes for the biosynthesis of terpenes and cannabinoids in cannabis, research is needed to elucidate the factors that control expression of these biosynthetic systems. This would include, for example, the regulation of cell-type specific gene expression in the context of the development of glandular trichomes, plant architecture, and onset of female flowering.

As restrictions on research with cannabis relax, cannabis is likely to become a more popular research organism both for the gain of basic knowledge and parctical applications. Cannabis is a useful system for terpene research as it produces a large volume of a diverse terpene-rich resin on its trichome-covered surfaces. The abundance and size of its glandular trichomes make it a useful system for research in cell specialization and regulation of terpene and cannabinoid metabolism.

At present, of the hundreds of terpene and cannabinoid metabolites that have been identified in cannabis, the biosynthesis of less than 30 has been characterized. Future biochemical and functional work on biosynthetic systems in cannabis would benefit from a focused community effort to produce and archive a complete and reproducible set of metabolite and genomic data for one or a few genotypes that will serve as a reference framework. In parallel, a larger number of cannabis types need to be properly genotyped and phenotypically characterized (e.g. with regard to their metabolites) to overcome current issues with inconsistencies in what is referred to as ‘strains’. The goal would be to establish reproducible cannabis varieties for use in research and in the industry, comparable to the well-defined grapevine varieties that are used in viticulture. Moving from ‘strains’ to varieties will require the cooperation of cannabis researchers, breeders and growers. To our knowledge, so far, no industry association has taken a lead to set community standards and practices or define community-accessible varieties. Researchers and industry in Canada, as the first developed nation to have fully legalized cannabis, are uniquely positioned to lead this effort.

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