Phytocannabinoids Biosynthesis in Plants

Subjects: Plant Sciences

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Phytocannabinoids are a structurally diverse class of bioactive naturally occurring compounds found in angiosperms, fungi, and liverworts and produced in several plant organs such as the flower and glandular trichrome of Cannabis sativa, the scales in Rhododendron, and oil bodies of liverworts such as Radula species; they show a diverse role in humans and plants. Moreover, phytocannabinoids are prenylated polyketides, i.e., terpenophenolics, which are derived from isoprenoid and fatty acid precursors. Additionally, targeted productions of active phytocannabinoids have beneficial properties via the genes involved and their expression in a heterologous host. Bioactive compounds show a remarkable non-hallucinogenic biological property that is determined by the variable nature of the side chain and prenyl group defined by the enzymes involved in their biosynthesis. Phytocannabinoids possess therapeutic, antibacterial, and antimicrobial properties; thus, they are used in treating several human diseases.

Keywords: abiotic stress ; cell homeostasis ; heterologous host synthetic approach ; terpenophenolics

1. Introduction

Phytocannabinoids are meroterpenoids bearing a resorcinol core with an isoprenyl, alkyl, or aralkyl para-positioned side chain, or alkyl group usually containing an odd number of carbon atoms—cannabinoids that have an even number of carbon atoms in the side chain are rare. Phytocannabinoids can be obtained from angiosperms (flowering plants), fungi, and liverworts (**Figure 1**). The first phytocannabinoid was isolated from the *Cannabis sativa* family Cannabaceae, but it has a long controversial history of its use and abuse [1][2]. From *C. sativa* more than 113 phytocannabinoids were isolated and classified into several groups such as cannabidiols (CBDs), cannabigerols (CBGs), cannabicyclols (CBLs), cannabidiols (CBNDs), cannabinols (CBNs), cannabitriols (CBTs), cannabichromenes (CBCs), (−)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) and miscellaneous cannabinoids [1][3][4][5]. Compounds obtained from *C. sativa* predominately generate alkyl-type phytocannabinoids with a monoterpene isoprenyl and the pentyl side chain [4][6]. In *C. sativa*, CBD, CBG, CBC, cannabichromevarine (CBCV), and Δ^9-THC are the most abundant cannabinoids in their respective acidic form. The acidic form of the cannabinoid (C22, “pre-cannabinoids”) is the final step of the cannabinoid biosynthetic pathway. Oxidation, decarboxylation, and cyclization lead to the development of modified phytocannabinoid via spontaneous breakdown or conversion product. The conversion mainly occurs due to the poor oxidative stability of phytocannabinoids, especially with the alkyl group. *C. sativa* produces the most common phytocannabinoids. In addition to this, the brains of mammals have receptors that respond to the *C. sativa* cannabinoid, so they were termed as cannabinoid receptor types 1 and 2 (CB_1R and CB_2R) and thus participated in the endocannabinoid system [1][3][4][7][8].

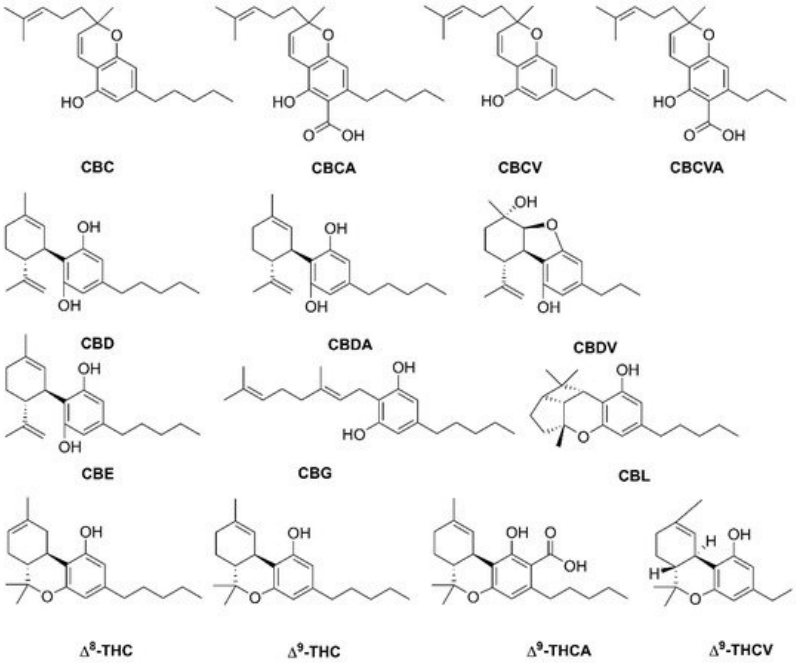

Figure 1. Structure of phytocannabinoids in *Cannabis sativa*. Abbreviations: CBC, cannabichromene; CBCA, cannabichromenic acid; CBCV, cannabichromevarine; CBCVA, cannabichromevarinic acid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarine, CBE, cannabielsoin; CBG, cannabigerol; CBL, cannabicyclol; Δ^8-THC, Δ^8-tetrahydrocannabinol; Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; Δ^9-THCV, Δ^9-tetrahydrocannabivarinic acid.

The Endocannabinoid system in humans and animals revealed that it participates in the regulation of biological functions such as memory, brain system, mood and addiction along with cellular and metabolic processes such as glycolysis, lipolysis, and the energy balance system [7][9]. Other angiosperms such as *Helichrysum umbraculigerum* (Asteraceae) native to South Africa, *Amorpha fruticosa* (Fabaceae), and *Glycyrrhiza foetida* (Fabaceae) contains a bioactive compound bearing a cannabinoid backbone (**Figure 2**); they are characterized as prenylated bibenzyl derivatives because the aralkyl side chain occurs [1][10].

Figure 2. Structure of phytocannabinoids in *Helichrysum* and *Glycyrrhiza* plants.

Many *Rhododendron* species (family Ericaceae) such as *Rh. dauricum* native to Northeastern Asia, *Rh. adamsii* found in Eastern Siberia and Mongolia, *Rh. rubiginosum* var. *rubiginosum* native to Southwest China, and *Rh. anthopogonoides* grown in Southern China, all generate active monoterpenoids that have a cannabinoid backbone. Phytocannabinoids are CBC types with an orcinol or methyl group side chain (**Figure 3**). *Rh. dauricum* particularly produces cannabinoids bearing sesquiterpene moiety such as daurichromenic acid (DCA), grifolic acid (GFA), confluentin, and rhododaurichromenic acid [11][12][13]. *Rh. adamsii* produces cannabigerorcinic acid, DCA, cannabigerorcinic acid methylase, chromane, and chromene monoterpenoids; *Rh. rubiginosum* produces cannabinoid rubiginosins A–G [14][15]. *Rh. anthopogonoides* contains chromane/chromene derivatives such as cannabiorcicyclolic acid, cannabiorcichromenic acid, anthopogochromenic acid, and anthopogocyclolic acid (**Figure 3**) [16].

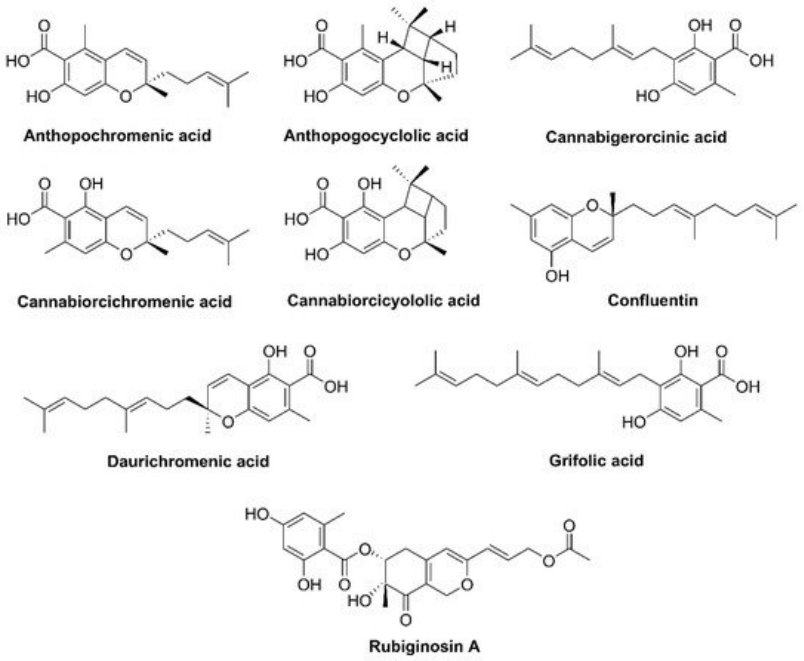

Figure 3. Structure of phytocannabinoids in *Rhododendron* plants.

Liverworts, such as *Radula marginata*, *R. perrottetii,* and *R. laxirameae* native to New Zealand, produce active cannabinoids with bibenzyl backbones such as lunularic acid and its dimeric form—vittatin (**Figure 4**) [17][18][19][20]. Some fungi, e.g., *Albatrellus* (Albatrellaceae, mycorrhizal fungi) species, also produce GFA along with its derivative confluentin, grifolin, and neogrifolin (**Figure 4**). Additionally, *Cylindrocarpon olidum* generates cannabiorcichromenic acid and halogenated cannabinoid, i.e., 8-chlorocannabiorcichromenic acid (**Figure 4**) [1][21].

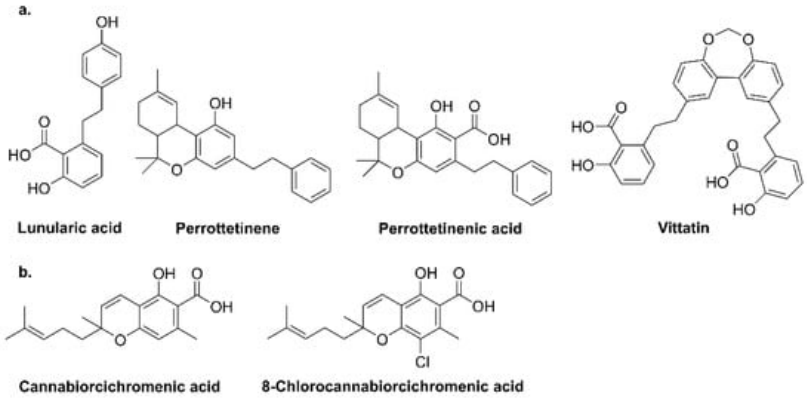

Figure 4. Structure of phytocannabinoids in (**a**) liverworts and (**b**) fungi.

This review focuses on the biosynthesis of different active phytocannabinoids in several cellular compartments of *C. sativa*, *Rhododendron*, and *Radula* species. In this topic framework, the most crucial criterion is the synthetic and biotechnological techniques for the production of phytocannabinoids. The current review highlights the multi-faceted role of different active phytocannabinoids in humans and plants. Interestingly, this review briefly highlights the antimicrobial, antibacterial, and antibiotic properties of phytocannabinoid based on recent papers. Additionally, the role of phytocannabinoids in ameliorating pathogenic attack, and environmental stresses, e.g., cold, heat, and UV radiation, is also briefly assessed.

2. Phytocannabinoid Biosynthesis Sites

In *C. sativa*, phytocannabinoids are stored in glandular trichomes, located all over the aerial part of the plant, so root surface and root tissues do not keep phytocannabinoid. Female flowers possess a high density of phytocannabinoid [22][23]. Glandular trichomes have balloon shaped secretory vessicle which store cannabinoid. High temperature or herbivory leads to trichome rupture, which releases the sticky contents on the plant parts with viscous and non-crystallizing properties [24][25]. Higher temperatures increase cannabinoid production. Furthermore, cannabinoid production is raised in the cannabis flower after UV-B exposure. Nevertheless, phytocannabinoids act as a sun shield that absorbs lethal UV radiation [26]. *Rhododendron* genus lepidote consists of small leaves surrounded by glandular scales on both the abaxial and adaxial surfaces. These scales have lipophilic globules that contain major bioactive compounds such as

cannabinoids, terpenes; the apoplastic space of the glandular scale also contains cannabinoids such as DCA in the *Rhododendron* [27]. Liverworts have oil bodies that are membrane-bound cellular structures that contain cannabinoids, aromatic oil, and terpenoid (*cis* configuration), mostly sesquiterpenoids and diterpenoids. Oil bodies are odiferous bitter, pungent compounds, which make them biologically active. Furthermore, these possess several ecological advantages such as tolerance from temperature, light, or radiation [28][29].

3. Biosynthesis of Phytocannabinoids

This section focuses on detailed events undergone in the production of several phytocannabinoids inside *C. sativa*, *Rhododendron*, and liverworts. Moreover, biosynthesis of phytocannabinoids via biotechnological approaches in a heterologous host and synthetic methods are discussed.

3.1. Cannabis sativa

Phytocannabinoids are prenylated polyketides, i.e., terpenophenolic compounds, which are derived from isoprenoid and fatty acid precursors. Phytocannabinoid biosynthesis occurs in different cellular compartments: gland cells cytosol, the plastids, and the extracellular storage cavity. In the cytosol, oxidative cleavage of fatty acid such as palmitic acid yields hexanoic acid; it further synthesizes olivetolic acid (OA). The next step is prenylation of phenolic moiety (the polyketide derivatives, 5-pentenyl resorcinolic acid, and OA) with the terpenoid geranyl pyrophosphate (GPP). This step originates from the methylerythritol-4-phosphate (MEP) pathway in plastids. Cyclization (oxidative) and storage of the final products take place outside the gland cells. Transport proteins and vesicle trafficking participate in mobilizing intermediates across the morphologically highly specialized interface between the gland cells and storage cavity [30][31][32].

In *C. sativa*, phytocannabinoids biosynthesis is divided into three important places: cytosol (for polyketide pathway), plastids (MEP pathway for prenylation), and apoplastic spaces (oxidocyclization and storage) (**Figure 5**). Inside cytosol, biosynthesis of phytocannabinoids participates in the integration of major steps in polyketide and isoprenoid metabolism. Fatty acids (C18) are sequentially desaturated, peroxygenated, and cleaved into the hexanoic acid (C6) and C12 product via enzyme desaturase, lipoxygenase (LOX), and hydroperoxide lyases, respectively. Hexanoic acid is converted into thioester hexanoyl-CoA; this reaction is catalyzed by acyl-activated enzyme 1 (AAE1). Later, hexanoyl-CoA and malonyl-CoA (C2 donor) together via the action of olivetol synthase (OLS) and olivetolic acid cyclase (OAC) synthesizes OA. Moreover, it was reported that OAC is the dimeric (α+β) barrel protein, it is the first plant enzyme that catalyzes (C2-C7) intramolecular aldol condensation along with carboxylate retention; OAC contains distinctive active-site bearing the pentyl-binding hydrophobic pocket and polyketide binding site, whereas it is devoid of aromatase and thioesterase activities [31][33][34].

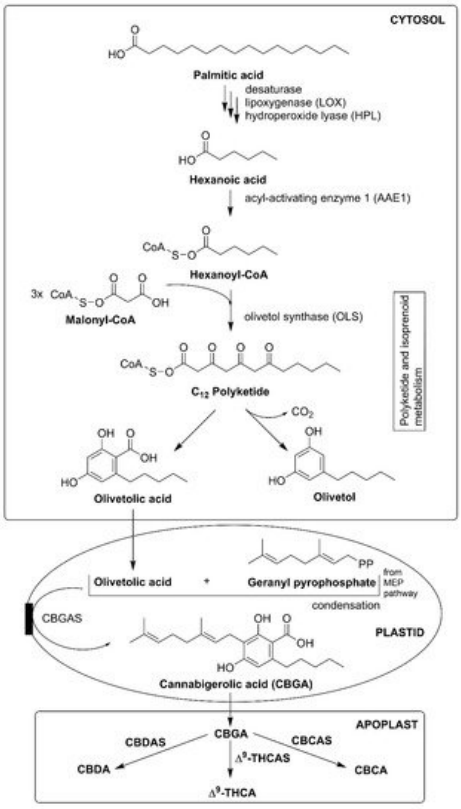

Figure 5. Phytocannabinoids biosynthesis in *Cannabis sativa*. Abbreviations: CBCA, cannabichromenic acid; CBCAS, cannabichromenic acid synthase; CBDA, cannabidiolic acid; CBDAS, cannabidiolic acid synthase; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; Δ^9-THCAS, Δ^9-tetrahydrocannabinolic acid synthase.

Inside plastids, the MEP pathway synthesizes GPP. It prenylates OA, which forms the intermediate branch-point and first cannabinoid which is cannabigerolic acid (CBGA), and this reaction is catalyzed by cannabigerolic acid synthase (CBGAS). CBGA is an essential cannabinoid because it acts as the precursor of several cannabinoids with an alkylic pentyl side chain. In contrast, CBGAS is a transmembrane aromatic prenyltransferase (PT) that transfers plastid signals. Then CBGA is converted into Δ^9-THCA and cannabidiolic acid (CBDA) with the help of two enzymes which are CBDAS and Δ^9-tetrahydrocannabinolic acid synthase (Δ^9-THCAS). This conversion continues by reducing oxygen (O_2) into hydrogen peroxide (H_2O_2) via oxidative cyclization reactions. Additionally, CBDAS and Δ^9-THCAS are necessary flavoprotein enzymes that are dependent on O_2 (electron acceptor) [1][30][31].

Another important enzyme, cannabichromenic acid synthase (CBCAS), dependent from FAD and O_2, takes part in the synthesis of cannabichromenic (CBCA). Additionally, enzymes Δ^9-THCAS and CBCAS have high sequence similarity at about a 96% nucleotide level. Both remain active inside resin space, which shows that CBCAS participates as an O_2 dependent flavoprotein that converts CBGA to cannabichromenic acid (CBCA) with H_2O_2 as the side product via an oxidocyclization reaction. Active cannabinoid Δ^9-THCA, CBDA, and CBCA with a pentyl side chain are synthesized in the apoplastic cannabis space. Furthermore, these active phytocannabinoids undergo decarboxylation and spontaneous rearrangement reactions on exposure to heat, radiation, or during storage. Some phytocannabinoid having unknown C1-C4 alkyl side chain are synthesized from acetyl-CoA, propanoyl-CoA, or pentanoyl-CoA [1][5][31][35].

3.2. Rhododendron

DCA and its derivative are produced and stored inside specialized glandular scales in *Rh. dauricum*. DCA utilizes carbon atoms from acetyl-CoA and farnesyl-CoA, with two significant intermediates, i.e., orsellinic acid (OSA) and GFA. DCA biosynthesis in *Rhododendron* is split between the cytosol, plastid, and apoplastic spaces (**Figure 6**) [11][36].

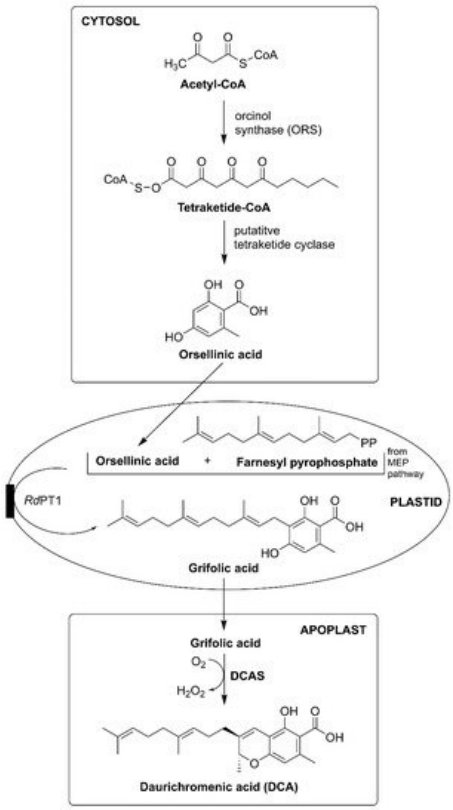

Figure 6. Phytocannabinoids biosynthesis in Rhododendron. Abbreviations: DCAS, daurichromenic acid synthase; MEP, methylerythritol-4-phosphate; PT, prenyltransferase.

The biosynthesis of DCA starts in the cytosol with polyketide formation, type III polyketide synthase (PKS) helps in acetyl-CoA chain extension. Then another enzyme, orcinol synthase (ORS), catalyzes orcinol, OSA, triacetic acid, tetracetic acid, lactone and phloroacetophenone, where malonyl-CoA (three units) act as a carbon donor. Furthermore, tetraketide cyclase catalyzes OSA from ORS [36][37]. OA is transported to the plastid via a transporter, which is still unknown. Inside the plastids, the MEP pathway derives farnesyl-CoA. The inhibition of the MEP pathway via clomazone decreases OSA

and DCA synthesis. In contrast, inhibition of the mevalonate-dependent pathway via mevastatin led to an increment in OSA and DCA biosynthesis. Moreover, aromatic farnesyltransferase *Rh. dauricum* prenyltransferase (PT) helps in regiospecific farnesylation; this enzyme moderates sequence identity with UbiA aromatic PTs that lie within chloroplasts. Geranyl-CoA and geranylgeranyl-CoA serve as the alternative prenyl donors used by PT, but their activity rate is 13%, and 2.5% of the activity acquired by farnesyl-CoA. GFA is synthesized as the intermediate within the plastids. Then, within apoplastic spaces, an oxidocyclization reaction takes place via DCA synthase (DCAS) forming CBC scaffold; reaction moves forward by H_2O_2 release. Like Δ^9-THCAS and CBDAS, DCAS is active enzymatically outside apoplastic spaces and dependent on O_2. In *Rh. dauricum*, DCA decarboxylated forms produce confluentin; spontaneous decarboxylation occurs via heat, irradiation, and during storage, similar to the decarboxylation acidic to neutral phytocannabinoids in *C. sativa* trichomes [5][11][37].

Apoplastic spaces serve as storage for many metabolites, essential oils, DCA, and confluentin. Moreover, GFA and DCA act as phytotoxic compounds in *Rh. dauricum* cell culture, as they induce cell death. Similarly, H_2O_2 formed as a side product in DCA biosynthesis also increases cell death by enhancing apoptosis-related reactions. However, to overcome cell death, autotoxicity, and cell damage, DCA storage occurs in the apoplast, and H_2O_2 is released to participate in the plant-defense system and provide plant immunity. In *Rh. dauricum*, transport proteins and vesicle trafficking mechanisms are still not well understood and remain a valuable and exciting approach for further future investigations [1][2][37].

3.3. Liverworts

It was reported that *Radula marignata* possesses enzymes for GPP biosynthesis and helps in the biosynthesis of bibenzyl cannabinoid. Moreover, the production of bibenzyl CBGA analog (i.e., lunularic acid, perrottetinenic acid, and perrottetinene) needs precursor stilbene acid or dihydrostilbene acid, which is very rare, and compounds of this type were found in *Hydrangea macrophylla* var. *thunbergii* and liverworts such as *Marchantia polymorpha* and *Convolvulus hystrix* (**Figure 7**) [1][17][38].

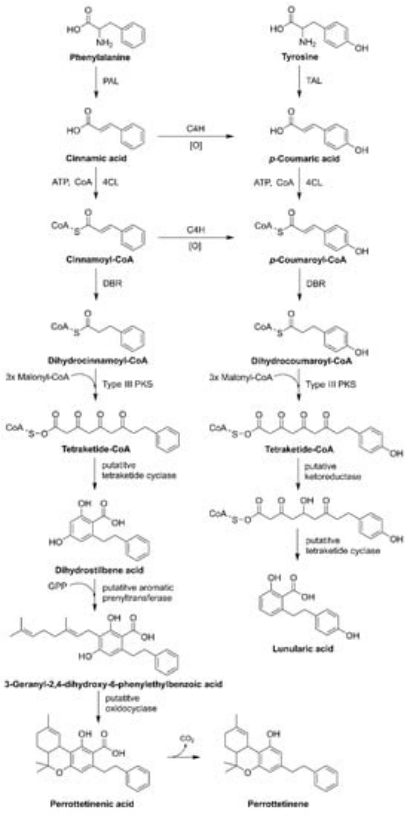

Figure 7. Phytocannabinoids biosynthesis in liverworts. Abbreviations: C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase.

Stilbene acid is synthesized from type III PKS via coumaroyl-CoA or dihydrocoumaroyl-CoA, CoA-activated precursors. Starter molecules are extended by malonyl-CoA with decarboxylation, followed by a condensation reaction, which produces polyketide intermediate, which synthesizes different core structures. Hydrangic acid is the starter molecule that acts as coumaroyl-CoA. It is extended utilizing malonyl-CoA (three units) as a C2 donor to synthesize tetraketide intermediate. These reactions are catalyzed via stilbene synthase (STS)-type PKS enzymes. Ketoreductase (KR) leads to polyketide reduction followed by STS-like C2 to C7 intramolecular aldol condensation, here retention of the carboxylic group produces hydrangic acid. KR is involved in the loss of the C5-hydroxyl group on the aromatic ring structure of

hydrangic acid, contrary to the stilbene acid structure. In *R. marginata*, the precursor of stilbene acid or dihydrostilbene is derived from the coumaroyl CoA or cinnamoyl-CoA; type III PKS enzyme helps in chain elongation, later putative tetraketide cyclase or (dihydrostilbene acid cyclase, DHAC) helps in cyclization [1][18][38][39].

The lunularic acid precursor, prelunularic acid is produced by the type III PKS, named bibenzyl synthase (BBS). Moreover, BBS catalyzes the reaction where dihydrocoumaroyl-CoA serves as the starter molecule for the extension utilizing malonyl-CoA (3-units), which serves as the carbon donor. Later, cyclization occurs on reduced polyketide, with a lack of C5-hydroxyl group on the aromatic ring structure. Furthermore, type III PKS plays a crucial role in the bibenzyl cannabinoid and its analog synthesis by also catalyzing the carboxylate retaining reaction mechanism. KR is involved in the cyclization and assists proper ring formation. Thus, after cyclization, lunularic acid is synthesized [40][41][42].

Perrottetinenic acid (PA) is synthesized in *R. marginate* [43]. The transcriptomic approach of liverworts bears the mRNA encoding for type III PKS (responsible for chain elongation), which were later recognized as STS. Furthermore, it also exhibits a 60% homology of the amino acid sequence to stilbene-carboxylate synthase in *Marchantia polymorpha*. Other enzymes, such as double-bond reductase (DBR)—aromatic PT and oxidocyclase (perrottetinenic acid synthase, PAS), are also responsible for the production of PA. DBR catalyzes compounds that are precursors of phenylpropanoid, and generates dihydrocinnamoyl-CoA. The production of bibenzyl phytocannabinoid is dispersed across the liverworts cells in the same way as in cannabis and *Rhododendron*. Therefore, DHAC and DBR reside in the cytosol, PT is localized in the plastids and PAS inside the oil body. Signal peptides act as the crucial indicators for the encoding genes selection [18][39][41][44]. Another bibenzyl *cis*-THC, (−)-*cis*-perrottetinene (*cis*-PET) was isolated from the liverwort *R. perrottetii* [45]. The *cis* configuration in the cyclohexene ring in *cis*-PET is comparable with Δ^9-*trans*-THC. PET resembles Δ^9-THC in its 3D shape, and can bind to many of the same cannabinoid receptors (CBRs) as Δ^9-THC. Interestingly, PET also reduces the level of prostaglandins in the brain—a compound with inflammatory properties that increase in response to Δ^9-THC and may be responsible for adverse effects [17].

3.4. Application of Biotechnological Approaches to Phytocannabinoids Production in Heterologous Hosts

Phytocannabinoids can be generated in different heterologous hosts such as fungi, bacteria, and plants with the help of biotechnological techniques. Several investigations reported that in vitro culture of phytocannabinoids biosynthesis in *C. sativa*, with the help of explants and micropropagation, is a widely used biotechnological approach for phytocannabinoid production. Moreover, apart from *C. sativa*, *Nicotiana benthamiana* also emerged as the favorable heterologous host for the production of phytocannabinoids. It exhibits the production of several proteins and bioactive compounds and has glandular trichomes that help overcome cell death, autotoxicity, and cell damage caused due to intermediates produced during phytocannabinoids biosynthesis. In recent research, it was found that a major biotechnological tool for phytocannabinoids production and inducing genetic modification is established via the micropropagation technique. Additionally, cell suspension culture, hairy root, and adventitious root culture also produce a small quantity of cannabinoids. In *Saccharomyces cerevisiae*, galactose produces phytocannabinoids such as CBG, CBDA, Δ^9-THCA, and minor phytocannabinoids such as cannabidivarinic acid (CBDVA) and Δ^9-tetrahydrocannabidivarinic acid (Δ^9-THCVA) [46][47][48][49][50][51].

3.5. Production of Phytocannabinoids through Synthetic Approaches

Phytocannabinoids are terpenophenolic compounds that are produced by polyketide and the MEP pathway. In the heterologous biosynthesis approach, two important pathways that are optimizing and engineering provide abundant precursors for cannabinoid production. Additionally, these pathways are linked via aromatic PT, ubiquitous in plants, animals, bacteria, and fungi [52][53]. Thus, this helps in the production of aromatic metabolites such as coumarin, flavonoid, and phenylpropanoid at different reaction spectra [54]. As discussed, biosynthesis of phytocannabinoid is different in plants, fungi, and liverworts; thus, the recent techniques which emerge out to be beneficial is by using the aromatic PT-based approach to generate novel phytocannabinoids in the heterologous hosts formed on combinational utilization of module over several species [52][53][54].

Similar to *Humulus lupulus*, inside trichomes of *C. sativa*, chalcone isomerases such as proteins (CHILs) are expressed. Additionally, CHILs are polyketide binding proteins, and their co-expression in heterologous cannabinoid production mechanism plays a pivotal role in augmenting biosynthesis. Apart from combinatorial biochemistry techniques, enzyme-based approaches also play a remarkable role in phytocannabinoid production. Furthermore, some non-natural precursors such as pentanoic acid, hexanoic acid, heptanoic acid are incorporated inside the generated cannabinoid. Derivatization of phytocannabinoid diversifies their functionality. Glycosylation of phytocannabinoid reduces cell damage and autotoxicity and increases cell stability and life; in a therapeutic context, glycosylated phytocannabinoid enhances absorption, distribution, metabolism and, excretion (ADME) features. Halogenation of cannabinoids (like DCA) by co-

expression of halogenase AscD derived from *Fusarium* exhibits several antifungal, antibacterial, antitumor, antiparasitic properties. Thus, the synthetic biology technique serves as a new insight into the production and designing of phytocannabinoids [55][56][57][58].

References

1. Gülck, T.; Møller, B.L. Phytocannabinoids: Origins and biosynthesis. Trends Plant Sci. 2020, 25, 985–1004.
2. Hanuš, L.O.; Meyer, S.M.; Muñoz, E.; Taglialatela-Scafati, O.; Appendino, G. Phytocannabinoids: A unified critical inventory. Nat. Prod. Rep. 2016, 33, 1357–1392.
3. ElSohly, M.A.; Slade, D. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. Life Sci. 2005, 78, 539–548.
4. Berman, P.; Futoran, K.; Lewitus, G.M.; Mukha, D.; Benami, M.; Shlomi, T.; Meiri, D. A new ESI-LC/MS approach for comprehensive metabolic profiling of phytocannabinoids in Cannabis. Sci. Rep. 2018, 8, 14280.
5. Pollastro, F.; Caprioglio, D.; Del Prete, D.; Rogati, F.; Minassi, A.; Taglialatela-Scafati, O.; Munoz, E.; Appendino, G. Cannabichromene. Nat. Prod. Commun. 2018, 13, 1189–1194.
6. Happyana, N.; Kayser, O. Monitoring metabolite profiles of Cannabis sativa L. trichomes during flowering period using 1H NMR-based metabolomics and real-time PCR. Planta Med. 2016, 82, 1217–1223.
7. Chanda, D.; Neumann, D.; Glatz, J.F.C. The endocannabinoid system: Overview of an emerging multi-faceted therapeutic target. Prostaglandins Leukot. Essent. Fatty Acids 2019, 140, 51–56.
8. Flores-Sanchez, I.J.; Verpoorte, R. Secondary metabolism in Cannabis. Phytochem. Rev. 2008, 7, 615–639.
9. Dei Cas, M.; Casagni, E.; Casiraghi, A.; Minghetti, P.; Fornasari, D.M.M.; Ferri, F.; Arnoldi, S.; Gambaro, V.; Roda, G. Phytocannabinoids profile in medicinal cannabis oils: The impact of plant varieties and preparation methods. Front. Pharmacol. 2020, 11, 570616.
10. Pollastro, F.; De Petrocellis, L.; Schiano-Moriello, A.; Chianese, G.; Heyman, H.; Appendino, G.; Taglialatela-Scafati, O. Amorfrutin-type phytocannabinoids from Helichrysum umbraculigerum. Fitoterapia 2017, 123, 13–17.
11. Taura, F.; Iijima, M.; Kurosaki, F. Daurichromenic acid and grifolic acid: Phytotoxic meroterpenoids that induce cell death in cell culture of their producer Rhododendron dauricum. Plant Signal. Behav. 2018, 13, e1422463.
12. Taura, F.; Iijima, M.; Lee, J.-B.; Hashimoto, T.; Asakawa, Y.; Kurosaki, F. Daurichromenic acid-producing oxidocyclase in the young leaves of Rhododendron dauricum. Nat. Prod. Commun. 2014, 9, 1329–1332.
13. Kashiwada, Y.; Yamazaki, K.; Ikeshiro, Y.; Yamagishi, T.; Fujioka, T.; Mihashi, K.; Mizuki, K.; Cosentino, L.M.; Fowke, K.; Morris-Natschke, S.L.; et al. Isolation of rhododaurichromanic acid B and the anti-HIV principles rhododaurichromanic acid A and rhododaurichromenic acid from Rhododendron dauricum. Tetrahedron 2001, 57, 1559–1563.
14. Rogachev, A.; Komarova, N.; Korchagina, D.; Dolgikh, M.; Sorokina, I.; Baev, D.; Tolstikova, T.; Fomenko, V.; Salakhutdinov, N. Some prenylated phenols of Rhododendron adamsii: Isolation, modification and pharmacological tests. Chem. Sustain. Dev. 2009, 17, 185–193.
15. Yang, Y.-X.; Wang, J.-X.; Wang, Q.; Li, H.-L.; Tao, M.; Luo, Q.; Liu, H. New chromane and chromene meroterpenoids from flowers of Rhododendron rubiginosum Franch. var. rubiginosum. Fitoterapia 2018, 127, 396–401.
16. Iwata, N.; Kitanaka, S. New cannabinoid-like chromane and chromene derivatives from Rhododendron anthopogonoides. Chem. Pharm. Bull. 2011, 59, 1409–1412.
17. Chicca, A.; Schafroth, M.A.; Reynoso-Moreno, I.; Erni, R.; Petrucci, V.; Carreira, E.M.; Gertsch, J. Uncovering the psychoactivity of a cannabinoid from liverworts associated with a legal high. Sci. Adv. 2018, 4, eaat2166.
18. Hussain, T.; Espley, R.V.; Gertsch, J.; Whare, T.; Stehle, F.; Kayser, O. Demystifying the liverwort Radula marginata, a critical review on its taxonomy, genetics, cannabinoid phytochemistry and pharmacology. Phytochem. Rev. 2019, 18, 953–965.
19. Métoyer, B.; Lebouvier, N.; Hnawia, E.; Herbette, G.; Thouvenot, L.; Asakawa, Y.; Nour, M.; Raharivelomanana, P. Chemotypes and biomarkers of seven species of new Caledonian liverworts from the Bazzanioideae subfamily. Molecules 2018, 23, 1353.
20. Pryce, R.J. Lunularic acid, a common endogenous growth inhibitor of liverworts. Planta 1971, 97, 354–357.
21. Quaghebeur, K.; Coosemans, J.; Toppet, S.; Compernolle, F. Cannabiorci- and 8-chlorocannabiorcichromenic acid as fungal antagonists from Cylindrocarpon olidum. Phytochemistry 1994, 37, 159–161.

22. Elhendawy, M.A.; Wanas, A.S.; Radwan, M.M.; Azzaz, N.A.; Toson, E.S.; ElSohly, M.A. Chemical and biological studies of Cannabis sativa roots. Med. Cannabis Cannabinoids 2019, 1, 104–111.

23. Happyana, N.; Agnolet, S.; Muntendam, R.; Van Dam, A.; Schneider, B.; Kayser, O. Analysis of cannabinoids in laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. Phytochemistry 2013, 87, 51–59.

24. Livingston, S.J.; Quilichini, T.D.; Booth, J.K.; Wong, D.C.J.; Rensing, K.H.; Laflamme-Yonkman, J.; Castellarin, S.D.; Bohlmann, J.; Page, J.E.; Samuels, A.L. Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. Plant J. 2019, 101, 37–56.

25. Mahlberg, P.G.; Kim, E.-S. Cuticle development on glandular trichomes of Cannabis sativa (Cannabaceae). Am. J. Bot. 1991, 78, 1113–1122.

26. Eichhorn Bilodeau, S.; Wu, B.-S.; Rufyikiri, A.-S.; MacPherson, S.; Lefsrud, M. An update on plant photobiology and implications for cannabis production. Front. Plant Sci. 2019, 10, 296.

27. Iijima, M.; Munakata, R.; Takahashi, H.; Kenmoku, H.; Nakagawa, R.; Kodama, T.; Asakawa, Y.; Abe, I.; Yazaki, K.; Kurosaki, F.; et al. Identification and characterization of daurichromenic acid synthase active in anti-HIV biosynthesis. Plant Physiol. 2017, 174, 2213–2230.

28. Asakawa, Y.; Ludwiczuk, A. Chemical constituents of bryophytes: Structures and biological activity. J. Nat. Prod. 2017, 81, 641–660.

29. He, X.; Sun, Y.; Zhu, R.-L. The oil bodies of liverworts: Unique and important organelles in land plants. Crit. Rev. Plant Sci. 2013, 32, 293–302.

30. Geissler, M.; Volk, J.; Stehle, F.; Kayser, O.; Warzecha, H. Subcellular localization defines modification and production of Δ9-tetrahydrocannabinolic acid synthase in transiently transformed Nicotiana benthamiana. Biotechnol. Lett. 2018, 40, 981–987.

31. Luo, X.; Reiter, M.A.; d'Espaux, L.; Wong, J.; Denby, C.M.; Lechner, A.; Zhang, Y.; Grzybowski, A.T.; Harth, S.; Lin, W.; et al. Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. Nature 2019, 567, 123–126.

32. Rodziewicz, P.; Loroch, S.; Marczak, Ł.; Sickmann, A.; Kayser, O. Cannabinoid synthases and osmoprotective metabolites accumulate in the exudates of Cannabis sativa L. glandular trichomes. Plant Sci. 2019, 284, 108–116.

33. Gagne, S.J.; Stout, J.M.; Liu, E.; Boubakir, Z.; Clark, S.M.; Page, J.E. Identification of olivetolic acid cyclase from Cannabis sativa reveals a unique catalytic route to plant polyketides. Proc. Natl. Acad. Sci. USA 2012, 109, 12811–12816.

34. Raharjo, T.J.; Chang, W.-T.; Choi, Y.H.; Peltenburg-Looman, A.M.G.; Verpoorte, R. Olivetol as product of a polyketide synthase in Cannabis sativa L. Plant Sci. 2004, 166, 381–385.

35. Laverty, K.U.; Stout, J.M.; Sullivan, M.J.; Shah, H.; Gill, N.; Holbrook, L.; Deikus, G.; Sebra, R.; Hughes, T.R.; Page, J.E.; et al. A physical and genetic map of Cannabis sativa identifies extensive rearrangements at the THC/CBD acid synthase loci. Genome Res. 2019, 29, 146–156.

36. Saeki, H.; Hara, R.; Takahashi, H.; Iijima, M.; Munakata, R.; Kenmoku, H.; Fuku, K.; Sekihara, A.; Yasuno, Y.; Shinada, T.; et al. An aromatic farnesyltransferase functions in biosynthesis of the anti-HIV meroterpenoid daurichromenic acid. Plant Physiol. 2018, 178, 535–551.

37. Taura, F.; Iijima, M.; Yamanaka, E.; Takahashi, H.; Kenmoku, H.; Saeki, H.; Morimoto, S.; Asakawa, Y.; Kurosaki, F.; Morita, H. A novel class of plant type III polyketide synthase involved in orsellinic acid biosynthesis from Rhododendron dauricum. Front. Plant Sci. 2016, 7, 1452.

38. Katsuyama, Y.; Kita, T.; Funa, N.; Horinouchi, S. Curcuminoid biosynthesis by two type III polyketide synthases in the herb Curcuma longa. J. Biol. Chem. 2009, 284, 11160–11170.

39. Hussain, T.; Plunkett, B.; Ejaz, M.; Espley, R.V.; Kayser, O. Identification of putative precursor genes for the biosynthesis of cannabinoid-like compound in Radula marginata. Front. Plant Sci. 2018, 9, 537.

40. Akiyama, T.; Shibuya, M.; Liu, H.-M.; Ebizuka, Y. p-Coumaroyltriacetic acid synthase, a new homologue of chalcone synthase, from Hydrangea macrophylla var. thunbergii. Eur. J. Biochem. 1999, 263, 834–839.

41. Austin, M.B.; Noel, J.P. The chalcone synthase superfamily of type III polyketide synthases. Nat. Prod. Rep. 2002, 20, 79–110.

42. Imoto, S.A.; Ohta, Y. Intracellular localization of lunularic acid and prelunularic acid in suspension cultured cells of Marchantia polymorpha. Plant Physiol. 1985, 79, 751–755.

43. Toyota, M.; Shimamura, T.; Ishii, H.; Renner, M.; Braggins, J.; Asakawa, Y. New bibenzyl cannabinoid from the New Zealand liverwort Radula marginata. Chem. Pharm. Bull. 2002, 50, 1390–1392.

44. Yang, X.; Matsui, T.; Kodama, T.; Mori, T.; Zhou, X.; Taura, F.; Noguchi, H.; Abe, I.; Morita, H. Structural basis for oliveto lic acid formation by a polyketide cyclase from Cannabis sativa. FEBS J. 2016, 283, 1088–1106.
45. Toyota, M.; Kinugawa, T.; Asakawa, Y. Bibenzyl cannabinoid and bisbibenzyl derivative from the liverwort Radula perrot tetii. Phytochemistry 1994, 37, 859–862.
46. Fuentes, P.; Armarego-Marriott, T.; Bock, R. Plastid transformation and its application in metabolic engineering. Curr. O pin. Biotechnol. 2018, 49, 10–15.
47. Kodym, A.; Leeb, C.J. Back to the roots: Protocol for the photoautotrophic micropropagation of medicinal Cannabis. Pla nt Cell Tissue Organ Cult. 2019, 138, 399–402.
48. Lange, K.; Schmid, A.; Julsing, M.K. Δ9-Tetrahydrocannabinolic acid synthase: The application of a plant secondary me tabolite enzyme in biocatalytic chemical synthesis. J. Biotechnol. 2016, 233, 42–48.
49. Lata, H.; Chandra, S.; Khan, I.A.; ElSohly, M.A. Micropropagation of Cannabis sativa L. -An update. In Cannabis sativa L. Botany and Biotechnology; Chandra, S., Lata, H., ElSohly, M.A., Eds.; Springer International Publishing: Cham, Swit zerland, 2017; pp. 285–297.
50. Wróbel, T.; Dreger, M.; Wielgus, K.; Słomski, R. The application of plant in vitro cultures in cannabinoid production. Biot echnol. Lett. 2017, 40, 445–454.
51. Andersen-Ranberg, J.; Kongstad, K.T.; Nielsen, M.T.; Jensen, N.B.; Pateraki, I.; Bach, S.S.; Hamberger, B.; Zerbe, P.; Staerk, D.; Bohlmann, J.; et al. Expanding the landscape of diterpene structural diversity through stereochemically cont rolled combinatorial biosynthesis. Angew. Chem. Int. Ed. 2016, 55, 2142–2146.
52. Chen, R.; Gao, B.; Liu, X.; Ruan, F.; Zhang, Y.; Lou, J.; Feng, K.; Wunsch, C.; Li, S.-M.; Dai, J.; et al. Molecular insights into the enzyme promiscuity of an aromatic prenyltransferase. Nat. Chem. Biol. 2017, 13, 226–234.
53. Kannangara, R.; Siukstaite, L.; Borch-Jensen, J.; Madsen, B.; Kongstad, K.T.; Staerk, D.; Bennedsen, M.; Okkels, F.T.; Rasmussen, S.A.; Larsen, T.O.; et al. Characterization of a membrane-bound C-glucosyltransferase responsible for car minic acid biosynthesis in Dactylopius coccus Costa. Nat. Commun. 2017, 8, 1987.
54. Yazaki, K.; Sasaki, K.; Tsurumaru, Y. Prenylation of aromatic compounds, a key diversification of plant secondary meta bolites. Phytochemistry 2009, 70, 1739–1745.
55. Ban, Z.; Qin, H.; Mitchell, A.J.; Liu, B.; Zhang, F.; Weng, J.-K.; Dixon, R.A.; Wang, G. Noncatalytic chalcone isomerase-fold proteins in Humulus lupulus are auxiliary components in prenylated flavonoid biosynthesis. Proc. Natl. Acad. Sci. U SA 2018, 115, E5223–E5232.
56. Gribble, G.W. Biological activity of recently discovered halogenated marine natural products. Mar. Drugs 2015, 13, 404 4–4136.
57. Hansen, E.H.; Osmani, S.A.; Kristensen, C.; Møller, B.L.; Hansen, J. Substrate specificities of family 1 UGTs gained by domain swapping. Phytochemistry 2009, 70, 473–482.
58. Khan, B.A.; Warner, P.; Wang, H. Antibacterial properties of hemp and other natural fibre plants: A review. BioResource s 2014, 9, 3642–3659.