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Review

Releasing the Full Potential of Cannabis through Biotechnology

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Abstract: Cannabis (*Cannabis sativa* L.) is a dioecious plant cultivated worldwide for thousands of years. Besides the narcotic and therapeutic effects, Cannabis can be used as raw materials in multiple fields, including bioenergy, textiles, food, and ecological restoration. It is also an efficient bioremediation agent for contaminated soil, as well as greenhouse gas absorption. With the expansion of the market, there has been an increased demand to develop Cannabis cultivars with enhanced traits. As a major science breakthrough, the advent of the CRISPR/Cas system will revolutionize the basic and applied research in Cannabis. This article provides an overview of the recent advances in the optimization of a transformation system and in the gene editing of Cannabis. To achieve the full potential of this environmentally friendly and sustainable crop, we highlight future directions of genetic modification as well as several bottlenecks to overcome.

Keywords: *Cannabis sativa* L.; gene editing; CRISPR/Cas system

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1. Introduction

Cannabis sativa L. is diploid (2n = 20), with nine pairs of chromosomes and a pair of sex chromosomes (XY/XX chromosomal sex-determining system) [1,2]. This crop has been widely used in various fields including food, textiles, cosmetics as well as medicinal purposes from early Neolithic times in East Asia [3]. Recently, this plant has become known in the US for its popular use, where citizens prefer to eat the baby leaf hemp and the microgreens of hemp as nutritious salad greens [4,5]. Few species have ever been questioned thoroughly by journalists and the public as much as cannabis. The main reason is because its phytocannabinoid, D9-tetrahydrocannabinol (THC), is highly psychoactive with the risk of addiction and mental health disorders, while Cannabis used to be the most cultivated and trafficked drug crop globally [6].

As a potential drug, cannabidiol (CBD) was tested in various pharmacological assays targeted to treat different symptoms, including vomiting during chemotherapy, tremor in multiple sclerosis, and even posttraumatic stress [7,8]. Besides, more than 100 other cannabinoids were discovered, including cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), and so on, which are mainly biosynthesized in trichomes of the Cannabis floweret and leaves [9,10]. Cannabinoids were demonstrated to be terpenophenolics with mixed biosynthetic origins. These prenylated polyketides were derived from the methylerythritol-phosphate (MEP) and polyketide pathway producing the olivetolic acid, alkylresorcinolic acid, and monoterpene moiety. Olivetolic acid is synthesized by olivetol synthase and olivetolic acid cyclase, which are type III polyketide synthases [11]. Cannabigerolic acid (CBGA) is formed by a C-C Friedel-Craft alkylation of olivetolic acid at position C3. CBGA is also the first biosynthetic metabolite committed in the high diversity of cannabinoids. The

integral membrane protein cannabigerolic acid synthase (CBGAS) performed this reaction in the membranes of the plastids [12,13]. Finally, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) are produced from the oxidative cyclization of CBGA. These reactions are catalyzed by the tetrahydrocannabinolic acid synthase (THCAS) and cannabidiolic acid synthase (CBDAS), respectively. Besides, these enzymes are identified in the secretory cavities of glandular trichomes, suggesting the exportation of CBGA through the membranes by diffusion or active transport [14,15].

THCAS and CBDAS showed 83% similarity in the genome sequence, which indicates a common ancestor between them [16]. More recently, it was demonstrated that THCAS evolved from CBDAS through duplication and divergence mutations, which gave rise to the production of THCA instead of CBDA [17]. Another phenomenon that suggests they have a common ancestor is that both THCAS and CBDAS produced the same products (CBDA and THCA) at different ratios, depending on how to cyclize the C10 geranyl moiety in the same substrate (CBGA) [18]. One chemical bond was formed between C10 and C60 of the geranyl moiety in CBGA to produce CBDA. In the THCA production, Tyr484 in THCAS protein extracts the proton on O60′, which leads to the formation of two chemical bonds [16]. Finally, CBGA, the first branch-point in the cannabinoid biosynthetic pathway, serves as a common substrate to produce several cannabinoids such as CBDA, THCA or cannabichromenic acid (CBCA), which is achieved by cyclizing the C10 moiety from GPP via independent synthases. Out of these cannabinoid synthases identified, THCAS is the most well-studied cannabinoid synthase followed by CBDAS [19]. The crystallized THCAS and CBDAS have been isolated, purified and expressed in insect cells and cell-free systems [16,20,21]. In addition, Ren et al. (2021) conducted a study on the domestication history of cannabis using 110 different cannabis germplasm and found that farmers' intensive selection to increase fiber yield or psychoactive properties caused Cannabis to lose the complete sequence encoding THCAS or CBDAS, respectively [22].

Both phytocannabinoids and endocannabinoids are recognized as ligands for downstream signaling to regulate diverse physiological processes by G protein-coupled receptors (GPCRs). In recent years, Class A GPCRs CB1 and CB2 have caused extensive concern as cannabinoid receptors. CB1 shares 44% sequence similarity with CB2 [23–25]. Although phylogenetically related, the distribution of CB1 and CB2 are different [26]. CB1 is located all over the human organs as well as the central nervous system, while CB2 is usually found in the immune system and very scarcely in the nervous system [27]. This difference has been supposed to be the leading cause that most side effects of cannabinoids come from CB1 activation [26,28]. As the second most abundant cannabinoid after THC, CBD gained a growing interest due to its therapeutic potential, especially in treating epileptic seizures, even though its mechanism is not well understood [29]. Since CBD is demonstrated to be an antagonist to CB1 and a partial CB2 agonist, it is identified as a negative allosteric modulator of THC naturally [30,31].

Recently, the growing interest in the therapeutic properties of cannabinoids has shifted public attitudes [32]. Among all reported cannabis phytochemicals, CBD has therapeutic potential in treating mood disorders, pain, diabetes, neurodegenerative, and central nervous system diseases [33]. Unlike THC which can cause paranoia and memory loss, CBD has been proved highly safe in pharmacological properties [34]. Since there is an increased demand for medical products containing these chemicals, it is imperative to adopt biotechnologies, such as genetic engineering, to generate more varieties of Cannabis with desirable pharmacological properties to better meet the demand [35].

Considering the legal status of this plant, the synthesis of phytocannabinoids in other systems is less problematic, and relative research in heterologous biosynthesis has been going on for decades. Luo et al. engineered yeast strains to produce CBD from galactose, which laid foundation for the large-scale cannabinoid fermentation and enabled the pharmacological study of the cannabinoids in minor amounts [36]. This study promoted the possibility of cannabinoid synthesis using different systems. Recently, another two

intermediate compounds (olivetolic acid and cannabigerolic acid) have been synthesized using aromatic prenyltransferase in *Nicotiana benthamiana* and *Saccharomyces cerevisiae* [37].

Although the heterologous biosynthesis succeeded both in microorganism and in planta systems, challenges still need to be addressed to allow large-scale production, such as the high cost compared to agricultural production. Gene editing technology possesses a crucial potential to increase the production of valuable metabolites. This technology has been applied to engineer bacteria or fungi genomes by introducing desired sgRNA fragments with specific promoters or transcriptional regulators for novel functions [38]. As the common substrate to produce cannabinoids, CBGA is produced from the mevalonate-pathway. In comparison to non-edited strains, the mevalonate content in yeast cells can be increased by more than 41-fold using CRISPR [39]. Since Luo et al. established the biosynthetic approach to produce cannabinoid analogues, which demonstrated the potential of microorganism to produce cannabinoids [36], the upregulated mevalonate pathway would provide a great opportunity for large-scale production of major cannabinoids from the simple sugar galactose. Besides, CRISPR can be used to increase the production of desirable Cannabis metabolites while eliminating the synthesis of THCA and other psychedelic compounds [40]. This has been successfully used in fungi. In the modified *Monascus purpureus* strain KL-001, a dual-plasmid CRISPR/Cas system was designed to remove harmful contaminants formed in the production of Monascus red. The production of Mycotoxin, which caused disease and death in both humans and animals, was suppressed with the deletion of a 15-kb citrinin biosynthetic gene cluster [41].

The cytotoxic effect of cannabinoids is another challenge that needs to be addressed. In suspension-cultured Cannabis cells, treatment with 50 μM CBGA and THCA caused 100% cell death and similar results were observed in tobacco and insect cells treated with CBGA and THCA [42]. Like many other secondary metabolites synthesized in glandular trichomes, cannabinoids act as defense compounds and protect young tissues from predator damages by inducing cell apoptosis processes. Therefore, organisms or plants with storage cavities are appropriate candidates for the mass production of cannabinoids in heterologous systems, such as the glandular trichomes of mint, basil, lavender, oregano, and thyme. However, increasing trichome formation is still the most feasible approach to enhance cannabinoid synthesis, which can be completed by site-specific editing of targeted genes [12]. A stable transformation is necessary to facilitate cultivar improvements and obtain desirable traits, but a Cannabis transformation protocol was not well established due to the low shoot regeneration rate and notorious recalcitrance to genetic engineering technologies.

2. The Advances of Cannabis Tissue Culture

The increase in shoot regeneration rate is critical to establish an efficient transgenic system. Since existing studies have implied its recalcitrant nature to regeneration, combinations of different plant hormones were tested with various explants in Cannabis, such as shoot tips, axillary bud, leaf, and cotyledon [40,42]. Leaf is the most widely used one in Cannabis tissue culture among all the explants reported [43]. Compared with other cash crops, Cannabis has low regeneration efficiency of tissue culture and wide variation among cultivars. Zhang et al. (2021) performed a regeneration assay for one hundred Cannabis varieties and observed significant differences among all the varieties estimated [44]. Lata et al. (2016) demonstrated the highest shoot regeneration rate induced from callus axillary buds using thidiazuron [43]. Chaohua et al. (2016) obtained the highest shoot induction rate in the cotyledon [45]. Kodym and Leeb (2019) developed a photoautotrophic system, and a 97.5% rooting rate was obtained from regenerated shoots [46]. Nevertheless, Cannabis regenerations are still limited to specific varieties. Results of previous investigations showed that the process of plantlet regeneration requires a detailed evaluation of genotype response to medium, and even the same combination of plant hormones can result in different recovery rates among varieties [45,46]. To overcome the bottleneck imposed by tissue culture-based regeneration, Zhang et al. (2021) developed a

transgenic method through coexpressing developmental regulators (DRs) and genome editing (GE) components, which simplified tissue culture by reprogramming somatic cells into meristems, thus enabling direct regeneration of shoots from callus [44]. The overexpression of DRs, such as *ZmWUS2* and *OsGRF4*, showed positive influences on shoot organogenesis in several plant species recalcitrant to regeneration [47–50]. To increase the regeneration efficiency, Zhang et al. (2021) cloned the homologous genes of five DRs in Cannabis, which were reported to be effective in several monocot or dicot species and delivered these DRs into hypocotyls [44]. The average regeneration efficiency for the *CsGRF3*–*CsGIF1* chimera demonstrated a twofold improvement compared to the control. This alteration of DR gene expression facilitated the acquisition of regenerated clones and opened the door to transformed Cannabis.

3. The Genetic Improvement of Cannabis

The current breakthroughs in GE tools, especially the CRISPR/Cas system, are characterized with the high efficiency, simple target design, multiplex roles in gene knock-in/-out, and low cost, which has significantly boosted research across plant science and crop improvement by optimizing functional genes and creating genetic variations.

THCAS and CBDAS genes were mapped to the loci with tight linkage and multiple copies of CBDAS- and THCAS- related sequences were cloned within the same region. Some of them contributed to the final chemotype, while the others were pseudogenes or partially functional alleles [50]. The CRISPR/Cas9 system enables the knockout of several homologous genes in one editing step. Shen et al. (2017) edited eight targeted rice genes simultaneously and isolated mutants carrying these homozygous mutated alleles [51]. The high efficiencies in specific editing would be beneficial for Cannabis metabolic engineering.

Gene editing reagents are delivered to plant cells by agroinfiltration, which can express a gene of interest rapidly. To achieve transient gene expression, agroinfiltration protocols for Cannabis have been optimized recently. Using optimized *Agrobacterium*-mediated transformation system, T-DNA of the corresponding plasmid was delivered into the hairy roots and tumors of Cannabis successfully [52]. VIGS-vectors were reported to be delivered to mature leaf with a 70% reduction of gene expression in magnesium chelatase subunit I [53]. Besides, the agroinfiltration system was developed and utilized to overexpress and silence genes with high efficacy in the aerial parts of Cannabis [31].

The RNA interference (RNAi) tool is an alternative way to knockdown targeted gene expression, which enables the generation of novel cannabinoid profiles. The bottlenecks for the success are efficient delivery of dsRNA into the cell and instability of naked dsRNA in plants. Attempts regarding the RNAi spray or soaking protocols were reported from some companies, but details were not further disclosed. Most recently, Matchett-Oates et al. (2021) modulated cannabinoid biosynthesis genes using RNAi via agroinfiltration [54]. The vacuum infiltrated and transfected leaf segments with different RNAi constructs targeting *CBDAS* and *THCAS* genes, which showed significant downregulation quantified by real-time qPCR. Although RNAi technology provides an exceptional tool to study the gene function, the stable transformation of exogenous genes is still the barrier to enhance Cannabis phenotypes through genetic engineering.

Despite several critical genes involved in cannabinoid biosynthesis being identified [47], the functions of these genes were not fully validated yet, mainly due to few reports of stable transformation in this species. Recently, several approaches were used in the transient transformation to introduce foreign DNA fragments, such as the vacuum infiltration or nanoparticle-based method [55]. In addition, the regeneration of transgenic plantlets from transformed cells is a time-consuming process, as well as varied between different Cannabis varieties [40]. Although there are some problems to solve, editing of THCAS is still an attractive target for researchers. Transgenic plants with zero THC production and increased production of other cannabinoids could be guaranteed simultaneously if the function of THCAS can be completely silenced.

Recently, Zhang et al. (2021) reported the first generation of edited Cannabis plants by optimizing the *Agrobacterium*-mediated transformation method [44]. The shoot regeneration efficiency was increased through the integration of co-expression of DR chimera and CRISPR/Cas9 tools in the embryo hypocotyls of immature grains. The phytoene desaturase gene was edited to generate four seedlings with albino phenotype, which validated the stable integration of T-DNA in the Cannabis genome. Further optimization of this approach will help to release the potential of Cannabis.

4. Overcoming Limitations in Cannabis Gene Editing

4.1. Transformation Strategies

Particle bombardment is a conventional tool to deliver reagents to somatic tissue, which has transformed a wide range of species without biological limitations. Besides, it enabled the production of DNA-free gene editing through the direct delivery of RNAs and proteins into plants [56]. However, the biolistic delivery is often inefficient, and the gold particles are expensive with specific protocols, making it less widely used as a valuable tool. Poly-ethylenimine cationic polymer-modified silicon dioxide-coated gold nanoparticles have been demonstrated as an efficient alternative to gold particles for passively infiltrating DNA and RNA into the Cannabis cells [55]. Since grafting DNA onto the surface of the nanoparticles is difficult, it is still a skill demanded process and far from routine in most laboratories. Thus, most of the transient and stable transformations reported in Cannabis were developed using *Agrobacterium*-mediated methods. Among them, sonication or vacuum-assisted *Agrobacterium*-mediated transformations were widely attempted in mature leaves, male and female flowers, proving to be an easy and low-cost method to for non-susceptible plant species, such as Cannabis [57].

In the sonication method, when tensile stress is superimposed on plant tissues with the ambient pressure, cavitations created thousands of micro-wounds, allowing *Agrobacterium* to penetrate into or even throughout the tissue completely, increasing the probability to infecting Cannabis cells compared to conventional wounds by cutting. Tests were performed to determine the best sonication time, and it was reported that thirty seconds of sonication led to 20% higher beta-glucuronidase (GUS) expression than that without sonication [57]. For vacuum treatment, explant tissues were submerged into the suspension of *Agrobacterium* harboring binary vectors. Vacuum resulted in air evacuation from interstitial space of the submerged tissues, and then *Agrobacterium* suspension entered plant cells to replace the evacuated air when breaking the vacuum [42]. Excessive vacuum time damages plant tissues, while short vacuum time results in insufficient evacuation with low efficiency. In Deguchi's study, both 10 min and 15 min of the vacuum treatment resulted in seven times higher GUS expression compared to non-vacuum control, also higher than those after shorter vacuum application [57].

4.2. Target Tissue Selection

The development of a stable and robust regeneration system is critical to successful transformation. Since Cannabis plants are highly recalcitrant to *Agrobacterium*-mediated transformation, a variety of explants have been tested with the alteration of the tissue culture conditions for the purpose of regeneration. The totipotency of plant tissues is the foundation of most plant regeneration. DNA, dsRNA, or plasmid are delivered to isolated somatic tissue of Cannabis (be it hairy roots, leaves, flowers, stem, immature embryos) and selected for positive transgenic plants regenerated from the modified tissues [54]. Despite being developed over decades, the process is still genotype-dependent and far from routine in laboratories. Since Cannabis explants have been demonstrated to be susceptible to transient expression instead of stable transformation, the focus of researchers turned to the calli tissue developed from embryos [54,57]. Calli derived from immature embryos or mature plants were two main sources for *Agrobacterium*-mediated transformation in several plant species [58]. It has been demonstrated that both cotyledons and young true leaves are amenable to transient transformation, while notoriously recalcitrant to

traditional genetic transformation methods [57]. To increase transformation rates, our focus turned to the calli developed from immature embryos. The embryogenic calli can provide competent cells, extremely susceptible to transformation by *Agrobacterium* [59]. To increase the regeneration rate and optimize the protocol, Zhang et al. (2021) performed a regeneration assay for one hundred Cannabis varieties [44]. These Cannabis varieties were obtained from the national germplasm bank of the Institute of Bast Fiber Crops. The immature grains were collected about 15 days after flowering because the hypocotyls collected at this time can produce more shoots. After sterilization, these hypocotyls were isolated and incubated in the mediums to estimate the regeneration ability. Among all the varieties evaluated, DMG278 has the highest shoot induction rate of 7.09%, suitable for the *Agrobacterium*-mediated transformation [46]. As one of the major limitations of regeneration and transformation, this barrier needs to be overcome before releasing the full potential of gene-editing technology in Cannabis.

In addition, the genetic background of the *Agrobacterium* strains influenced the efficiency of T-DNA transfer into cellular nuclei. Some *Agrobacterium* strains were demonstrated to be more virulent than others for specific species [46]. Because of the limited transformation studies in this species, few reports mentioned about the appropriate *Agrobacterium* strains for Cannabis. Deguchi et al. tested the transformation efficiency of EHA105, LBA4404, and GV3101, which belonged to three types of *Agrobacterium* strains, succinamopine, octopine, and nopaline respectively [1]. The GV3101 strain is the optimal choice for Cannabis due to a significantly higher GUS expression than the other strains, which means the octopine-type strains might have better compatibility with Cannabis. Other *Agrobacterium* strains in this type, such as GV3100, GV3850, C58C1, A136, and EHA 101, should be further tested for Cannabis transformation efficiency.

4.3. Gene Editing Strategies

Recent advances in plant gene-editing tools extended the target range and increased the tissue specificity of delivering CRISPR reagents. The CAS12 and CASΦ nucleases were reported to install all possible mutations in target sites precisely [60]. However, these tool kits were applied to plant species with well-developed transformation systems, such as Arabidopsis, tobacco, wheat, and rice. By far, most reports of genetic engineering in Cannabis concerned transient expression, which means foreign DNA was delivered into the plant cells successfully. However, these cells failed to develop into embryogenic callus, which could be one of the main barriers to increase the regeneration rate in Cannabis. An alternative approach is to deliver the developmental regulators into plant cells and induce meristem formation. These regulating genes showed increased transformation frequencies in monocots and dicots, most likely by promoting the transition between stem cells and embryogenic calli [58].

An approach by coexpressing developmental regulators and genome editing components might be useful for genome manipulation and regeneration, which has been reported to be effective in several monocot and dicot species such as wheat, rice, and maize [53]. The transformation of BABY BOOM (BBM) and WUSCHEL (WUS) into immature maize embryos promoted the germination of seedlings without a callus phase. However, expressions of BBM and WUS demonstrated negative pleiotropic effects on plant growth while improving plant regeneration efficiencies. Therefore, these regulators had to be excised from generation lines [47]. To overcome these shortcomings, additional developmental genes were proposed to improve the regeneration efficiency. In *N. benthamiana*, overexpression of isopentenyl transferase (IPT) and shoot meristemless (STM) induced the regeneration of shoots [57]. The transcription factors grf-interacting factor (GIF) and growth-regulating factor (GRF) were demonstrated to form a functional transcriptional complex, which performed well under various transformation protocols by regulating the transition between stem cells, and to boost genetic transformation in various crop species [58,61].

To increase regeneration efficiency, the Cannabis genes homologous to *WUS2*, *STM*, *IPT*, *GRF4*, and *GIF1*, were co-delivered with gene editing reagents into hypocotyls [44]. The

regeneration efficiency of the *CsGRF3–CsGIF1* chimera performed the best among all the regulators, which constitutes the first report of successful and stable genetic transformation with *CsPDS1* editing. Although impressive work was presented, the gains were still modest. The expression of gene regulators only increased regeneration 1.7-fold, which was still below 0.2%. Further research is necessary to increase the regeneration efficiency by estimating new developmental regulators robust to induce Cannabis roots and shoots from callus.

4.4. Rapid Detection System for Editing Specificity

Transient transformation of protoplasts is an important method to test the effectiveness of the designed guide RNA (gRNA), which is a quick method to assess the nuclease specificity in crop trait improvement. Protoplast isolation of Cannabis is a complicated procedure. There are several factors that can influence the viability and quantity of the isolated cells, such as the growth circumstances, genotype, pretreatment conditions, source tissue, composition of cell-wall digestion enzymes, and enzymolysis solution [8]. Beard et al. (2021) completed the in vitro micropropagation of Cannabis nodal and shoot-tip explants and quantifies the transient gene expression in a low-THC cultivar using the flow-cytometric [62]. Through transient expression in the Cannabis protoplasts, Zhang et al. (2021) established the first detection system for editing specificity in Cannabis successfully and assessed the mutagenesis efficiency of targeting regions designed for *CsPDS1* [44]. The candidate target sites within *CsPDS1* were selected through the online software CHOP-CHOP Version3 (https://chopchop.cbu.uib.no/ (accessed on 25 March 2021). Each of the selected portions was synthesized as a pair of reverse complementary oligonucleotides, and then assembled into the genome-editing vector. After the co-transformation of constructs expressing gRNA, protoplasts were incubated in the dark for 48 h. The *CsPDS1* gene was amplified by PCR and detected for the mutagenesis efficiency by deep sequencing. Based on the mutation result in protoplast, the efficiency of the designed gRNAs in Cannabis can be determined, which can be applied to test the efficiency of gene-editing tools and even regenerate transgenic plants through protoplast culture directly.

5. Releasing the Full Potential of Cannabis

Recently, renewed interest emerged in the medical effects of cannabinoids CBD [63]. Although best known for the psychoactive effects of THC, other phytocannabinoids such as CBD showed therapeutic potential for disease treatments from neurological diseases to cancer [63]. By 2021, medical Cannabis and cannabinoids were fully or partially decriminalized in most developed countries and major developed economies [64]. At present, the medical Cannabis market is growing fast worldwide, with $3.5 billion at retail prices last year, which is expected to be $20.2 billion after five years [65].

Currently, the investigation of gene and trait associations in Cannabis is lagging due to its highly heterogeneous genomes [66]. Despite the large variability observed in important traits, genetic mechanisms controlling these traits remain mostly unknown in Cannabis, making it difficult to improve desirable traits through conventional breeding. Due to the multiple usages of this plant, studies are directed to decipher proper ways to fix its desirable characteristics, such as the CRISPR technology.

As shown in Figure 1, CRISPR technology can help to release the full potential of Cannabis with traits improved. In response to the burgeoning market of medicine cannabinoids, research on Cannabis and cannabinoid biosynthesis have expanded significantly in recent ten years. The multiplex mutation system in CRISPR can be useful for cannabinoid metabolic engineering. For instance, the CRISPR/Cas9 technology can be applied to knock out the duplicates of THCAS gene, silence the THCAS gene via a single editing step, and even create transgenic Cannabis plants with zero THC. Increasing the accumulation of cannabinoids is another hot spot in the current study. It has been reported that upregulations of cannabinoid synthases (CBDAS and THCAS) would not promote cannabinoid biosynthesis, and the enzymatic steps for CBG production are critical for the biosynthesis

of CBD and THC [67]. Gene editing allows breeders to modify the rate-limiting enzymes which participate in CBGA biosynthesis, such as GPP synthase, prenyltransferase, tetraketide synthase, or olivetolic acid cyclase. Besides, cannabinoids are synthesized and stored in the storage cavity of glandular trichomes, which can avoid the cytotoxicity of these metabolites [67]. Increasing trichome density is another feasible approach to enhance endogenous phytocannabinoid synthesis and storage capacity. By overexpressing the MYB1 gene from *Artemisia annua*, transgenic Cannabis displayed a higher average number of trichomes than the empty vector control [68]. As the homologous genes of AaMYB1, several MYB genes in hemp have been predicted to enhance trichome formation as well as CBDA synthesis [68,69]. Engineering these genes would result in overproduction or accumulation of cannabinoids above wild-type levels. Another widely used product of Cannabis is fiber, made from the plant stem. Suppression or knock out of genes responsible for lignin formation, such as the patatin-related phospholipase AIIIs, would affect Cannabis growth with less lignified stems and finally improve the fiber quality [70].

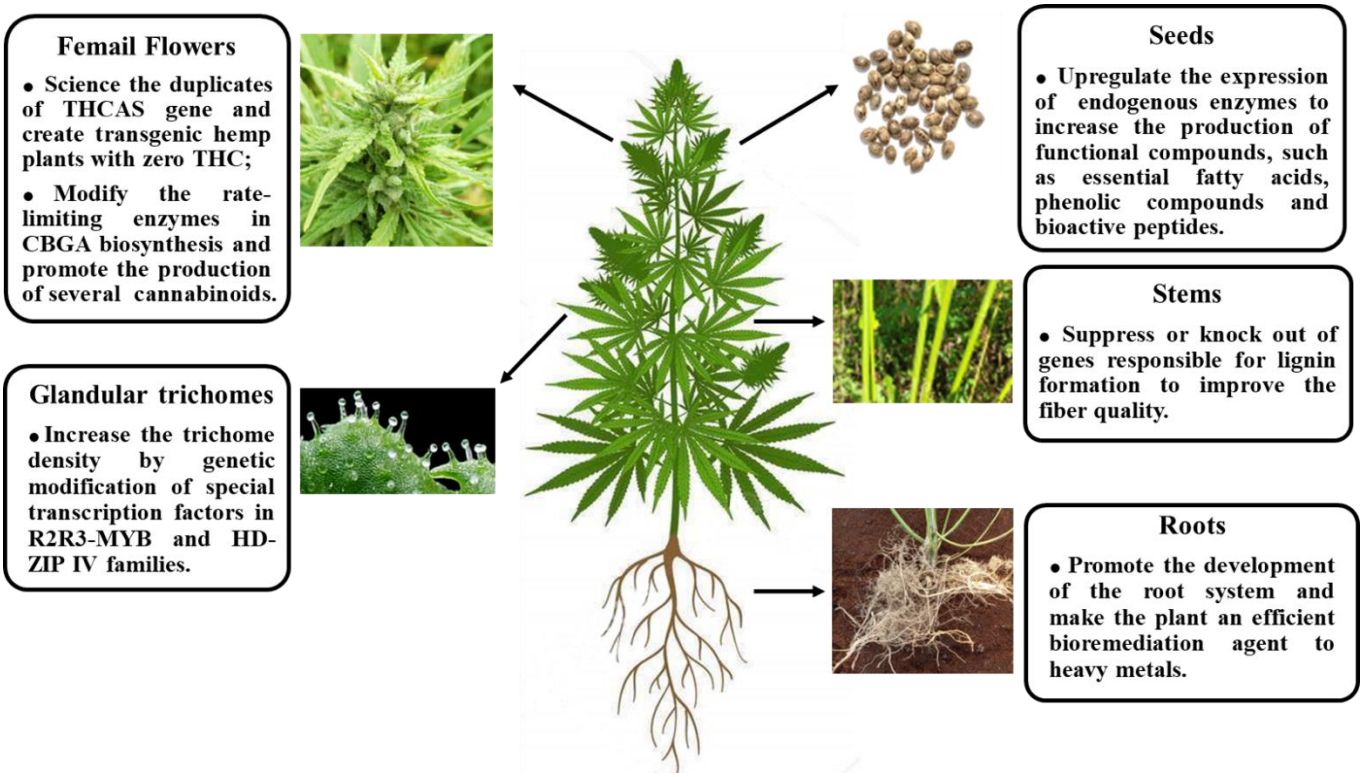

Figure 1. Gene editing can help to fulfill the potential usefulness of Cannabis in multiple fields, including bioenergy, textiles, food, and ecological restoration.

In releasing the full potential of Cannabis, another issue that needs to be addressed is the breeding time. For this question, Simiyu et al. (2022) proposed that Doubled haploid (DH) technology could be used as it has been successfully applied in a variety of other major traditional crops [71]. Moreover, Simiyu et al. (2022) envisioned that once DH technology is successfully established in Cannabis, it could also be applied in gene editing [71]. The combination of the two technologies would allow for more rapid genetic improvement of this plant to better meet demand. Speeding up the breeding of Cannabis requires a more thorough understanding of its biology, which is poor now. Shiels et al. (2022) suggested that interdisciplinarity approaches have great potential for Cannabis biological research [72].

6. Conclusions

Compared to other cash crops, Cannabis is more suitable for bioenergy production. Concurrently, there is increased interest in its medicinal and commercial use. With the devel-

opment of biotechnology, especially the continuous optimization of gene editing technology, the full potential of cannabis is expected to be released through its genetic improvement.

This review focuses on the advances in the regeneration, transformation and genetic engineering of Cannabis recently, including (1) the optimization of explant, variety, and developmental regulator, (2) the transient and stable transformation, and (3) the advent of Cannabis genetic modification. Although much effort has been expended to mutate hemp or marijuana plants using CRISPR technology, there is only one successful report generating seedlings with albino phenotype, which shed light on future gene editing applications in Cannabis molecular breeding. To release the full potential of Cannabis by gene editing, we highlight the bottlenecks to overcome, including the delivery system, target tissue, and editing tools. With the rapid development in genetic transformation methods, gene editing will fulfill the gaps in usefulness of this valuable plant.

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