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Genetic Enhancement of Plant Secondary Metabolites: Recent Developments and Future Perspectives

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ABSTRACT

Plant secondary metabolites (PSMs) are convenient intrinsic products synthesized through secondary metabolism in plants influenced by other environmental stressors and bio-geo-chemical factors. Various biotechnological techniques such as hairy root culture, ploidy engineering, genome editing, RNA interference, etc. are available for increased production of diverse secondary metabolites in plants having hitherto known medicinal properties. Plant tissue culture is useful for conservation, micro-propagation, and overproduction of medicinal plants. It also provides the primary platform for biotechnology-based breeding methods (BBBMs). Targeted genome editing has become a promising BBBM that can produce custom-tailored medicinal plants with desirable secondary metabolites. Here, we discuss the recent developments of BBBMs to increase the concentration of desired metabolites in important medicinal crops and the prospects of various genetic enhancement techniques. We also shed light on the recent progress in the genetic enhancement methods, their execution, industrial aspects, and international and national regulations for genetic manipulation to develop high-value crops for overall economic growth and sustainable utilization of hitherto explored bioresources. We pointed out the pitfalls and challenges in genetic modification of crops, success stories of genetic enhancement of PSMs, and future perspectives. Several techniques like endogenous target mimics (eTMs), CRISPR/Cas, PTC-based methods, NGS, and bioinformatics-based methods were tested for increased production rate and quality of various PSMs. Utilization of these techniques in combination may provide higher efficiency to develop genetically improved crops and enhance the production of PSMs for industrial scale and human health promotion.

Keywords: Genetic enhancement techniques; Plant secondary metabolites; Biotechnology-based breeding methods; Plant tissue culture; Genome editing; Ploidy engineering; Regulations of genetic manipulation

1. INTRODUCTION

Plants create green chemicals that are important for food, medicine, feed additives and other materials. Modern genetic modification can produce higher-quality crops. Medicinal plants produce plant secondary metabolites (PSMs) that have diverse pharmaceutical uses in defense, pollination, pigmentation, and drug development. They are categorized based on structure and synthesizing pathways[1-3]. Based on the biosynthetic pathway, PSMs are grouped into three major classes: terpenoids (or isoprenoids), phenolic compounds (phenylpropanoids and flavonoids), and nitrogenous compounds (alkaloids, glucosinolates, and cyanogenic glycosides). Biotechnology-based breeding methods (BBBMs) have a crucial effect on the enhancement of PSMs. Plant tissue culture (PTC) is an integral part of BBBMs, which also plays an important role in enhancing PSMs. Different PTC-based techniques like cryopreservation, micropropagation, synthetic seeds, hairy root culture, microRNA (amiRNA, eTMs), ploidy engineering, etc. have been employed for this purpose. Recently, with the advent of next-generation sequencing (NGS), a vast number of genetic modification techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) have been introduced to modify the medicinal plant as well as crop plant genome4. In addition, genome-wide association studies (GWAS), single nucleotide polymorphism (SNP) markers, DNA barcoding, restriction site-associated DNA sequencing (RAD-seq), RNA sequencing (RNA-seq), etc. have also been popularized (Fig. 1).

The PSMs have an immense role in plant resistance, defense mechanisms, product quality, etc. The PSM profile showed considerable variation, so genetic enhancement by manipulating a gene in a plant may be predictable only under laboratory conditions which might not be viable in the field. Production of PSMs by field cultivation of plants has various problems such as low yields, varied and unstable concentrations caused by seasonal, geographical, and environmental deviations, etc. Thus,

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how a range of BBBMs would have been established as a suitable choice for PSM production needs to be critically reviewed for future benefits.

Genetic enhancement and/or modifications result in increased production, reliability, and yields of crops; improved nutritional value; and reduced losses due to different abiotic and biotic stressors. Thus, new and/or novel genetic modification techniques have been designed by modern plant breeders and scientists for the identification, selection, experimentation, and development of plant varieties with genetically enhanced desirable traits within a short time.

However, there are several pitfalls and challenges in the genetic modification of crops. The release and cultivation of GM crops have become a debated and controversial topic due to several observations from all over the globe[5]. These challenges include the evolution of herbicide and pesticide-resistant strains, pest replacement, detrimental introgression of transgenes into the wild species, etc. To overcome these challenges modern breeding practices using molecular marker-assisted selection and breeding, advanced genetic engineering methods, development of Gene Use Restriction Technologies (GURTs), introducing site-specific genetic modifications with minimum exogenous DNA, development of new gene editing tools with high precision and accuracy, use of metabolic engineering, etc. have been routinely practiced to produce plant varieties with optimum desirable traits[5].

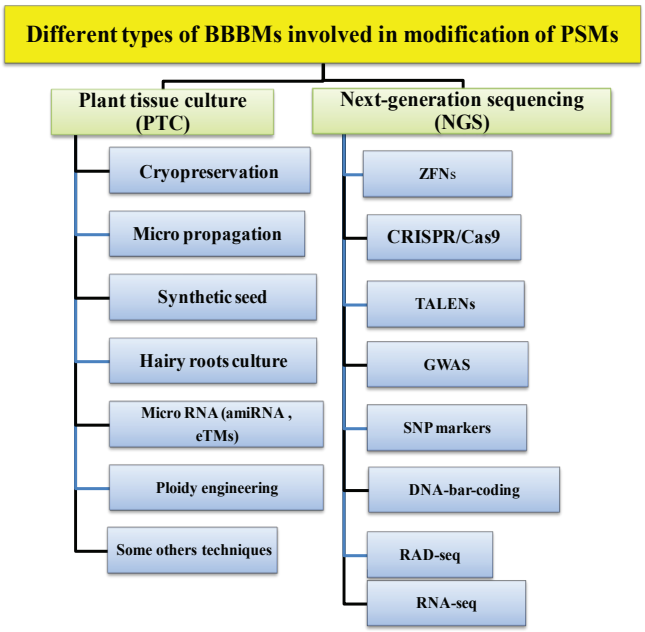

Figure 1. Different types of biotechnology-based breeding methods for the genetic enhancement of plants.

In this review, we talk about the recent developments in genetic enhancement techniques giving special emphasis on BBBMs to increase the concentration of desired metabolites in medicinally important crops. We also aim to explore the prospects of various techniques of genetic enhancement, its implementation for improved secondary metabolite production, and industrial aspects (Fig. 2).

2. NEXT-GENERATION SEQUENCING (NGS) TECHNIQUE AND ITS ROLE IN THE ENHANCEMENT OF PSMS

It is time for researchers to make use of advanced biotechnology-based methods to improve PSMs. The NGS is a strong area that has permitted the sequencing of a huge number of DNA molecules at the same time. The NGS technique and DNA barcoding are impressive molecular methods to measure the genetic diversity and ultimate classification and/or authentication of medicinal plants[6].

2.1 DNA Barcoding

DNA barcoding can effectively contribute to understanding a plant's genetic diversity in an efficient way[6]. In plants, it is used for phylogenetic studies, wild and cultivated genotypes classification, assessment of phytogeographical distribution patterns, and adulteration detection. Based on the taxon and species complexity, the barcode loci are useful to distinguish the closely related species and discover novel cryptic species[7].

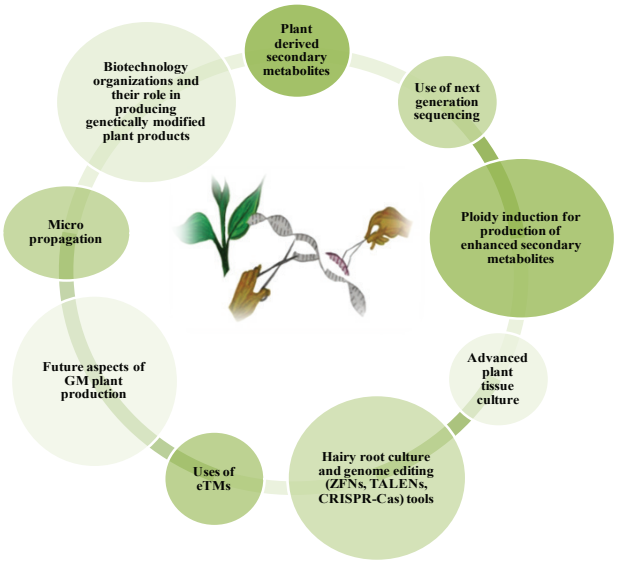

Figure 2. Genetic enhancement of plant secondary metabolites through various tools and techniques.

2.2 RNA-Sequencing

The cDNA sequencing or transcriptome profiling (RNA-seq) by using NGS platforms is a very popular and powerful technique to differentiate unknown genes and identify the differential expression of homoeologous and paralogous genes[8].

2.3 Genome Editing Methods

The genome editing technique helps in the targeted alternation of secondary metabolite pathways in plants. These methods allow scientists to make interchanges to DNA. Scientists use various technologies to do genome editing for crop improvement all over the globe[9]. Acting like scissors, these technologies dissect the DNA at a specific spot. Scientists then can eliminate, add, or substitute

the DNA in the cutting portion. With this technology, disease models in many plants can be created and the function of genes can be determined. These genome editing techniques include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR/Cas9) (Fig. 3(a-c)). For instance, ZFNs repair the target gene ZmlPK1 in the Maize plant, and as a result the production of herbicide-tolerant crop[10]. In the rice plant, ZFNs edited the target gene OsQQRfot the production of enhanced variety[11]. In 2015, Čermák et al. introduced genetically modified (GM) purple tomatoes with the help of TALENs[12].

CRISPR/Cas9 is a more suitable genome editing technique than the ZFNs and TALENs[13,14]. We compared the main characteristics of ZFNs, TALLRNs, and CRISPR/Cas9 which confirmed that TALENs are advanced as compared to ZFNs and CRISPR/Cas9 is the most advanced technology among these (Fig. 4).

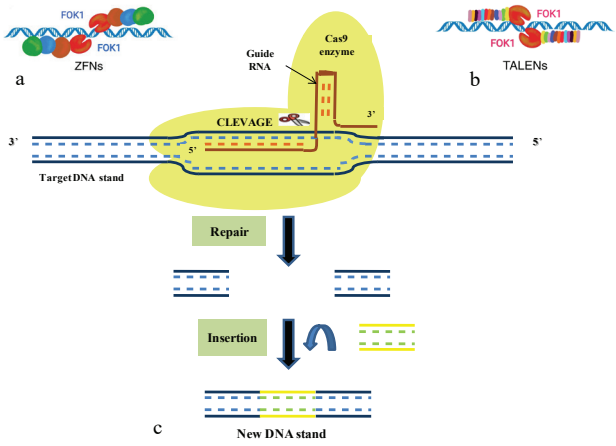

Figure 3. Schematic view of (a) Zinc-finger nucleases (ZFNs), (b) Transcription activator-like effecter nucleases (TALENs) and (c) CRISPR/Cas gene editing methods.

Genome editing

ZFN	TALEN	CRISPR/Cas9
Very high ratio of off-target mutations and cleavages.	TALEN is more straightforward than ZFN and binds to only single nucleotide rather than three bases.	Typically 20 bp guide sequence attached with PAM sequence.
Each zinc finger repeat recognizes only three bases making it error-prone.	Also, it is less convoluted than ZFN.	It is used for standard cloning procedures and oligonucleotide synthesis.
It requires substantial protein engineering.	TALEN is more time, effort and cost consuming technology than ZFN.	It is fast and cheap than the other gene-editing tools and can be designed easily.
It shows difficulties while targeting non-G-rich sites.	TALEN has some difficulties, such as it requires complex molecular cloning methods and difficult to deliver because of the large size of functional components.	It also offers high percentage of off-target mutations.

Figure 4. A comparative account of ZFN, TALEN, and CRISPR/Cas9 shows that CRISPR/Cas9 is the most advanced technology among the three genome editing methods.

3. PLANT TISSUE CULTURE (PTC) AND ITS APPLICATION FOR INCREASED PRODUCTION OF PSMS

Plant Tissue Culture (PTC) can be useful to understand the differential outcome experimental conditions for the enhanced production of PSMs[15], hormone metabolism, the signaling cascades, and transportation through the plant[16]. The PTC can help in the production of bioactive PSMs, which are of great value for food, pharmaceutical, and other industries[17,18]. The direct and indirect applications of PTC are displayed in Fig. 5.

3.1. Direct Application of Plant Tissue Culture (PTC)

3.1.1 Micropropagation

Micropropagation or *in vitro* propagation is the revival of the entire plant using tissue culture. Micropropagation can be done by the two procedures such as organogenesis, a two-step producer which requires several plant growth regulators for the shoot and root induction, and somatic embryogenesis which is more beneficial for the induced bipolar structure. Many factors can affect *in vitro* propagation such as explant type, explant age, the genotype of the plant, culture medium, and plant growth regulator, etc.

Through micropropagation, the swift multiplication of plantlets from explants on nutrient media under aseptic conditions was achieved successfully[19,20]. In recent times, with the help of plant tissue culture, the medicinal properties and nutritional value of *Phyllanthus emblica* L. were enhanced and utilized in the pharmaceutical industry[21].

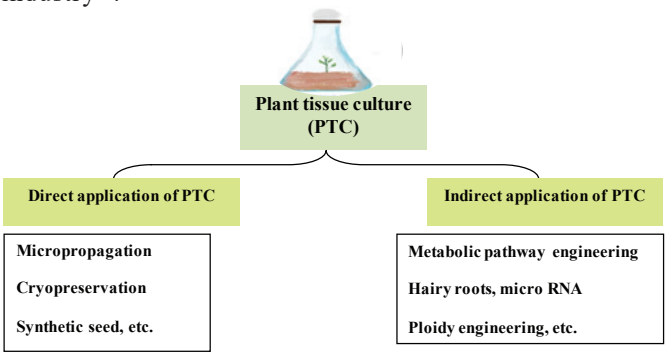

Figure 5. Major types of plant tissue culture (PTC) techniques and their applications to enhance plant secondary metabolites (PSMs).

3.1.2 Cryopreservation

Cryopreservation means the preservation in a frozen state and storage of germplasm. For long-term conservation of cell cultures that produce plant secondary metabolites, it is the most ideal technique. Identification of cold-resistant mutant cell lines can be achieved through cryopreservation. Various plant materials can be maintained for several years through cryopreservation and could be used when required. The three main purposes of cryopreservation on the medicinal plant are genetic stability of soma clones, retention of biosynthetic potential, and germplasm conservation[22].

3.1.3 Synthetic Seed

An artificial seed or a synthetic seed is a plant that originated from a somatic embryo. It is encapsulated in hydrogel and then used as a seed. Apical and basal meristematic regions of the bipolar somatic embryos produce shoot and root, respectively. In the encapsulation medium, plant growth regulators (PGRs) are applied as vital supplements to augment the efficacy of synthetic seeds[23]. Gantait *et al.* (2017) reported that synthetic seed was produced from the shoot tip of *Rauvolfia serpentina* in the 0.5 MSL, basal medium[24].

3.2. Indirect Application of Plant Tissue Culture (PTC)

3.2.1 Metabolic Pathway Engineering

Metabolic engineering is a biotechnological tool used for optimizing genetic and regulatory courses by the application of genetic analysis, biochemical engineering, and metabolic regulation to enhance the assembly of target molecules. This technique was applied to *Catharanthus roseus*, an important medicinal herb that synthesizes two anticancer Monoterpenoid Indole Alkaloids (MIAs) namely vinblastine and vincristine. Through metabolic pathway engineering, the MIA pathway in *C. roseus* could be modulated for increased production of these bioactive compounds[25].

3.2.2 Hairy Roots Culture

It is also called transformed root culture and is produced after infection of explant by gram-negative soil bacterium *Agrobacterium rhizogenus*. Due to their genetic stability and high growth rate, hairy root cultures can be useful in the production of root-associated metabolites. In medicinal plants, the production of hairy roots represents a good replacement for genetic methods to produce target molecules. Hairy root growth is more advanced as compared to adventitious roots and conventional plant cultures[13,26,27]. Hairy root cultures are generally utilized to identify the function of novel genes[28]. A recent review by Biswas, et al. in 2023 showed the potential applications of hairy root culture for augmented production of secondary metabolites from Solanaceous plants[29]. Also, by the genetic manipulation of the gene TRI-PMT in the plant *Anisodus acutangulus*, a 2-3-fold increased level of hyoscyamine was obtained[30].

3.2.3 Micro RNA

RNA interference (RNAi) is one of the most potent methods for genetically enhancing plants with medicinal properties. The RNAi can be induced by microRNAs (miRNAs) and short-interfering RNAs (siRNAs). The miRNA can regulate secondary metabolite biosynthesis in plants[31]. The design of the silencing construct, the construct's delivery into the plant, and the silencing of the artificial micro RNAs or eTMs and/or silencing of the target are vital for the gene silencing process through miRNA[31,32]. Singh and Sharma (2017) reported that in *Curcuma longa* (turmeric) 16 miRNA families

regulate 238 targets which modulated the synthesis of plant secondary metabolites[33].

3.2.3.1 Artificial microRNA (amiRNA)

The silencing construct mainly contains artificial microRNA (amiRNA) or endogenous target mimics (eTMs), developed by genetic engineering. Previously amiRNA methods utilized miRNA precursor to produce single-stranded guide RNA for the gene silencing process but recently there are various software like WMD3 (Web-based MicroRNA Designer 3) and DART (Designer Artificial miRNA Tool) to design amiRNA[34,35].

3.2.3.2 Endogenous Target Mimics (eTMs)

It is a recently discovered gene regulatory mechanism that can control miRNA activity[36]. The TraceRNA software is available to explore eTMs[34]. In addition, the PeTMbase (a database of plant eTMs, http://tools.ibg.deu.edu.tr/petmbase/) has also been developed with an effective search engine[37]. On plant eTMs, very limited numbers of studies have been conducted till now. In 2015, Reichel and Millar silenced miR319 and miR159 with MIM319 and MIM159 in *Arabidopsis* to understand the mechanism of target mimicry[38].

3.2.3 Ploidy Engineering

In medicinal plants, the production of PSMs per unit of biomass has enormous economic value. Hence, genome doubling of cells advocates genome multiplication, enhancement of protein synthesis, as well as an increase in the production of PSMs. The source of plant breeding is the variation in plants. Heritably and desirably variations occur in nature by mutation, polyploidy, recombination, and chromosomal aberrations. When the number of chromosomes is increased twofold by itself in the same plant, it is called autopolyploidy. The schematic representation of different gene expressions in autopolyploids and diploids, generating different PSMs has been depicted in Fig. 6.

Colchicine is suitable for producing steady and effective autopolyploids in higher frequencies among the various antimitotic agents[39,40]. For instance, two hours of treatment with 0.1% of colchicines resulted in a four-fold increased level of bacoside, a secondary metabolite product in *Bacopa monnieri*[41]. The shooty teratoma of *C. roseus*, treated with 0.01% colchicine for 24-48 hours, resulted in a double-fold increment in the production of secondary metabolite vincristine[42].

4. SUCCESS STORIES OF GENETIC ENHANCEMENT OF PSMS

Several researchers successfully applied the methods of genetic manipulation to enhance the PSM profile of various plants. The enhanced synthesis of a variety of PSMs such as flavonoid, anthocyanin, vitamins, pigments, phenylalanine, cyanogenic glucosides, etc. was reported in *Arabidopsis thaliana* through metabolic and genetic engineering of secondary metabolic pathways. Improved production of alkaloids, carotenoids, terpenoids, carboxybenzene formatives, and phytoanticipins were also reported in *Catharanthus roseus, Coptis japonica, Thalictrum tuberosum, Hyoscyamus muticus, Atropa belladonna, Nicotiana sylvestris* etc. through genetic enhancement[43].

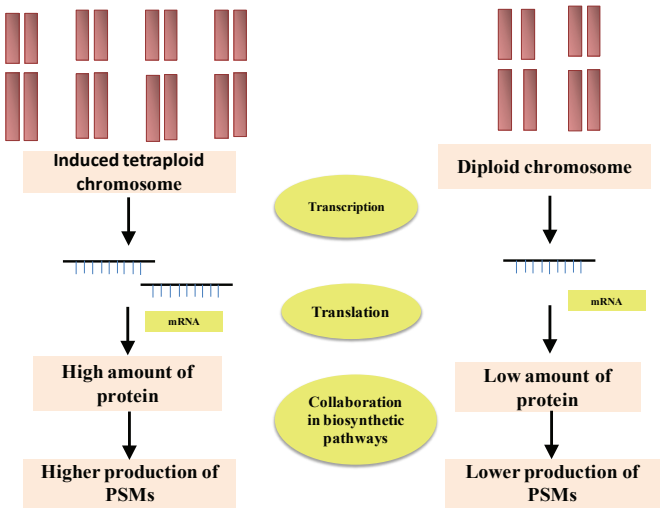

Figure 6. Schematic representation of different gene expressions in autopolyploids and diploids, generating different PSMs

The CRISPR/Cas genome editing method was successfully employed on various plants like *Arachis hypogaea, Atropa belladonna, Brassica napus, Camellia sinensis, Camelina sativa, Cichorium intybus, Dendrobium officinale, Dioscorea alata, Dioscorea zingiberensis, Euphorbia pulcherrima, Fagopyrum tataricum, Hordeum vulgare, Humulus lupulus, Ipomoea nil, Papaver somniferum, Salvia miltiorrhiza, Symphytum officinale, Vitis vinifera,* etc. for improved production of several PSMs that include oleic acid, linolenic acid, linolenic acid, hyoscyamine, caffeine, phytocannabinoids, sesquiterpene lactones, alkaloids, phenanthrenes, polysaccharides, bibenzyls essential oils, glycoside, diosgenin, squalene, pelargonidin, flavonoids, isoflavone, tocopherol, carotenoid, alkaloids, rosmarinic acid, phenolic acids, diterpenoids, tanshinones, etc.[44]. The details of genetic enhancement techniques to improve PSMs and crop plant production have been described in Table 1.

5. INDUSTRIAL ASPECT

Genetically enhanced plants have huge industrial aspects. Several industrial organizations used different genetic enhancement techniques to improve plants. Some of them are as follows.

i. Monsanto, an agrochemical and agricultural biotechnology corporation, introduced GM wheat (Bioceres HB4, MON71800) in the year 2020, which Argentina approved. Monsanto also introduced polyploidy-engineered Duram wheat. Monsanto introduced and commercialized GM seeds which produced the crystalline insecticidal Bt protein from *Bacillus thuringiensis*. In 1995, the Environmental Protection

Table 1 Genetic enhancement techniques for improved production of plant secondary metabolites

Sl. no.	Technique	Plant name	Efficiency induction	Target site	Country	Reference
1	CRISPR-Cas9	*Papaver somniferum* (Opium poppy)	Silencing enzymes involved in biosynthetic pathway	4OMT gene	Iran	45
2	cDNA sequencing	*Trachyspermum ammi* L. (Ajowan)	To identify the unigenes for the biosynthesis of monoterpenoids	na	Iran	8
3	Plant tissue culture	*Catharanthus roseus* (Periwinkle)	Enhancement in the production of monoterpene indole alkaloids vinblastine	Overexpression of G(G)PPS and GES genes	India	46
4	ZFNs	*Zea mays* (Sweet corn)	Production of herbicide-tolerant and phytate-reduced maize	ZmIPK1gene	USA	10
5	CRISPR-Cas9	Artemisia annua (Sweet wormwood)	To improve the artemisinin content in *Artemisia annua*	SQS, BFS, and CPS genes	Iran	47-49
6	CRISPR-Cas9	*Salvia miltiorrhiza* (Sage weed)	To identify important genes and metabolic networks in the water-soluble phenolic acid biosynthetic pathway	SmRAS gene	China	50
7	Hairy root culture	*Isatis indigotica* (Dyer's woad)	8-9-fold enhancement of lariciresinol	Ii049 genes	China	51
8	Ploidy engineering	*Tetradenia riparia* (Ginger bush)	3.5-fold increased essential oil production	na	South Africa	52
9	Ploidy engineering	*Eclipta alba* (L.) Hassk. (Bhringoraj)	An approximately 3-fold increase in wedelolactone	na	USA	53
10	miRNAs	*Nicotiana tabacum* (Tobacco)	Increased production of nicotine	CYP82E4, QPT1, QPT2, NAC-148 genes	China	36
11	miRNAs	*Taxus baccata* (Himalayan yew)	Enhancement of taxol	Taxane 2α-O-benzoyl-transferase, Taxane 13α hydroxylase genes	China	54
12	Genetic engineering (pre-plasmolytic treatment)	*Catharanthus roseus* (Periwinkle)	Modulation of monoterpenoid indole alkaloids (MIA) pathway	na	India	25
13	TALENs	*Solanum lycopersicum* (Purple tomato)	Production of purple tomatoes with high anthocyanin	ANT1gene	USA	12
14	Micropropagation	*Phyllanthus emblica L.* (Amla)	na	na	India	21
15	Micropropagation	*Costus speciosus* (Crepe ginger)	Drives to the enhancement of the bioactive constituent of this plant	na	India	55
16	Hairy root culture	*Salvia miltiorrhiza* (Sage weed)	Overexpression of the gene MYB98	MYB98 gene	China	13
17	Hairy root culture	*Ferula pseudalliacea* (Ferula)	The ATCC strain 15834 produced an elevated amount of hairy roots and fresh and dried biomass as compared to strain 1724	rolB gene	Iran	56

18	Hairy root culture	*Echinacea purpurea* (Eastern purple coneflower)	Heterologous expression of an acid phosphatase gene and limitation of phosphate drive considerable production of chicoric acid	R15834 gene	Iran	57
19	Hairy root culture	*Tanacetum parthenium* (Feverfew)	Overexpression of parthenolide synthase (TpPTS)	TpPTS gene	USA	58

na: not available

Agency (EPA) approved Monsanto's potato plants producing Bt toxin. After getting approval from The United States Food and Drug Administration (FDA), this crop became the first pesticide-producing crop in the United States. Monsanto also developed Bt maize (MON 802, MON 809, MON 810, MON 863), Bt cotton, and Bt soybean. In the year 2016, German chemical company Bayer acquired Monsanto (https://www.cropscience.bayer.com/).

ii. Corteva Agriscience and Purdue University collaborative project introduced a fungal disease resistance soybean gene called Rps11 with the help of molecular marker-based breeding which is resistant to multiple types of *Phytophthora sojae* in the year 2017. In 2018, the joint partnership of Corteva Agriscience and the International Rice Research Institute (IRRI) developed advanced rice technology and programmed collaboration to accelerate the discovery of new advanced breeding technology, which aims to improve global rice production and quality (https://www.corteva.com).

iii. Corteva Agriscience and PepsiCo unlock the potential of the oat by genome sequencing.

iv. DuPont Pioneer announced (in the year 2016) its first commercial agricultural product, waxy corn hybrids developed by the application of CRISPR/Cas licensed advanced breeding technology[59] (https://www.pioneer.com).

v. Mitsui Petrochemical Ind. Ltd., a Japan-originated company, enhanced Shikonin production of the plant Lithospermum erythrorhizon through hairy root culture (https://jp.mitsuichemicals.com/en/).

Several companies worked on plant improvement techniques. However, fewer industrial agencies in India for crop improvement. Globally, these companies are associated with the genetic enhancement and development of biotechnological processes to improve some common crop plants and the production of some medicinal plants (Table 2).

6. REGULATORY GUIDELINES FOR GENETIC MANIPULATION

Globally, the recent regulations for GE crops in different countries have some discrepancies. Three countries namely the United States of America (USA), China, and Japan are the global leaders in GE crop production. The SDN1 (small indels), SDN2 (change in few nucleotides), and SDN3 (template-guided repair of targeted DSB using a donor template) are the basic three categories of GE (Importation, Interstate Movement, and Environmental Release of Certain Genetically Engineered Organisms[60]. Here, we discuss the status of gene-edited crops in a few countries. The United States Department of Agriculture (USDA) exempted SDN1 and SDN2 categories of gene-edited crops from agreed regulations in 2017. After that, the Animal and Plant Health Inspection Service of USDA (USDA-APHIS) also agreed to exempt many gene-edited crops such as antibrowning mushrooms, waxy corn, etc. from the regulations. Likewise, Canada has also exempted the SDN1 and SDN2 groups of genetically manipulated crops from any regulation[61]. However, despite being the leading country for research on gene-edited crops, China has not formulated any regulatory guidelines or policy for GE crops. According to the regulation of a few South American countries such as Brazil, Argentina, Colombia, and Chile, the SDN1 and SDN3 group of crops are known as GMOs. However, SDN2 group of crops are not classified[62]. In addition, countries like Canada, England, Nigeria, South Africa, and Kenya showed distinguished views in formulating their regulations regarding genetic manipulations[63].

India is a signatory to the "Cartagena Protocol on Biosafety" and several regulations, acts, and policies are formulated for the genetic manipulation technologies in India[64]. The biosafety assessment of GE crops will be completed through several essential processes such as data submission on molecular characterization, delivery techniques, integrated donor DNA type, off-target investigation, etc. Recently, the Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India notified the "Guidelines for the Safety Assessment of Genome Edited Plants, 2022" for R&D of genome-edited plants in India. The "Standard Operating Procedures (SOPs) for regulatory review of genome-edited plants under SDN-1 and SDN-2 categories" was also prepared by the DBT constituted an expert committee for finalizing the SOPs and status on Genome Edited (GE) Plants, to provide support to applicants and Institutional Biosafety Committees (IBSCs) for R&D activities. The main requirements in the SOPs were that the GE plant(s) ought to be within SDN-1 or SDN-2 categories and should not hold any exogenously introduced DNA[65,66].

The Indian guidelines for genetic manipulation of plants have diminished the risks and harvest the utmost benefits

of producing transgenic, GM, and GE crops. Experiments on mustard, groundnut, cotton, rice, banana, chickpea, and many more were successful for developing GE varieties with improved desirable traits. The international trade of these GE crops has tremendous benefit with the global legal classification. Recently, most biotechnology-based companies have been willing to establish and reallocate to the USA due to their obvious regulations and policies for GE crops. Therefore, to establish a promising global market for GE crops, a uniform and integrated global regulation on GE crops is essential[63,64].

7. FUTURE PROSPECT

To maintain the health of a growing population, we need to find alternatives to synthetic medicines. Using secondary metabolites from medicinal plants can help without sacrificing commercial value. BBBM-based breeding methods and bioinformatics can improve plant growth and population. PTC helps conserve rare and threatened medicinal plants. Techniques for genetic improvement and increased production of PSMs can be used for future crop improvement. Advanced techniques like *in-vitro* polyploidy induction, bioreactor, gene transformation, and targeted gene editing have greatly improved the production of secondary metabolites in medicinal plants. PLAZA is an online platform for plant genomic study, providing functional and structural annotation of published plant genomes[67]. Potential miRNAs can be screened *in silico* to avoid high costs and time-consuming processes. The regulation of secondary metabolites by miRNAs and eTMs is a new approach and useful for modifying nicotine production in *Nicotiana tabacum*. Further research is needed to develop databases for important medicinal plants (Fig. 2).

8. FUTURE PERSPECTIVES OF GM TECHNOLOGY IN INDIAN SCENARIOS

India has initiated several R&D initiatives and innovations in various areas of GM technology. Several food crops, fruits and vegetables, livestock feed, fuel and fiber, medicinal and dietary purpose plants used for this purpose and more than 20 crops with some desirable traits such as abiotic stress tolerance, production of hybrid seeds, resistance to insect pests, tolerance to herbicide, nutritional enrichment, etc. were improved successfully by different genetic enhancement technologies[64,68,69]. Field trials of several GE plants such as Brinjal, Cabbage,

Table 2 Industrial organizations developing plant-based products using genetic enhancement techniques

Sl. No.	Company	Technique	Plant used	Quality developed	Country	Website
1	Mitsui Petrochemical Ind. Ltd.	Hairy root culture	*Lithospermum erythrorhizon* (Purple gromwell)	Shikonin production and Erythrorhizon cell growth on Linsmaier and Skoog agar medium is improved with a rich supply of oxygen	Japan	https://jp.mitsuichemicals.com/en/
2	DuPont Pioneer	CRISPR/Cas	*Zea mays* var. *ceratina* (Waxy corn)	It is a hybrid corn variety that contains larger amounts of amylopectin	USA	https://www.pioneer.com
3	Syngenta	CRISPR/Cas9	*Zea mays* (Waxy corn), *Triticum sp.* (Wheat), *Solanum lycopersicum* (Tomato)	With the help of genome editing technique, it promotes the incensement of the production of some crop plants	Switzerland	https://www.syngenta.com
4	Phyton Biotech GmbH	Plant Cell Fermentation Technology (PCF®)	*Taxus sp.* (Himalayan yew)	Synthesis of 100% natural plant-based paclitaxel which is GMO-free	Germany	https://phytonbiotech.com

5	Corteva AgriscienceTM	Molecular marker-based breeding	*Glycine max* (Soybean)	Improvement of global rice production and quality, Production of a fungal disease resistance soybean gene called Rps11	USA	https://www.corteva.com
6	Nitto Denko Corporation	Plant cell and organ cultures	*Rubia akane* (Asian Madder)	Alteration of ginsenosides purpurin	Japan	https://www.nitto.com
7	Bayer (Monsanto)	Polyploidy engineering	*Zea mays* (Waxy corn), *Triticum sp.* (Wheat)	It introduced genetically modified wheat (Bioceres HB4, MON71800), and also Bt maize, Bt soybean, and Bt cotton	Germany	https://www.cropscience.bayer.com/

Castor, Cauliflower, Chickpea, Corn, Cotton, Groundnut, Mustard, Okra, Papaya, Pigeon pea, Potato, Rice, Rubber, Sorghum, Tomato, Watermelon, Wheat, etc. with different desirable traits got approval by the regulatory agencies in the last few years. Both public and private-funded organizations are actively involved in the products of GM technology to indicate its promising prospect in India and all over the globe.

9. CONCLUSION

It is crucial to recognize human metabolic processes and discover targets of PSMs that may be useful in pharmacology, nutrition, and food science. Also, promotion of human health can be accomplished by improved food crop production systems via genetic modification. Different genetic manipulation technologies like eTMs, advanced CRISPR/Cas, PTC-based methods, bioinformatics-related methods, etc., are employed to increase production rate and quality of various PSMs. Utilizing these techniques in combination may provide higher efficiency to develop genetically improved crops, enhance the production of PSMs for industrial scale, and human health promotion.

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