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The Role of Agricultural Biotechnology and Genetic Engineering in the Improvement of Medicinal Plants in Afghanistan

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ABSTRACT

Most pharmaceutical products are derived from plants, making plants an essential source for developing and discovering novel therapeutic compounds. The phytochemical components of medicinal plants (MPs), particularly the secondary metabolites (SMs), are linked to the pharmacological effects of MPs. The widespread interest in phytotherapy, consumer preference to use natural resources, the continuous exploitation of natural resources, the economic importance of MPs in the self-sufficiency of developing countries like Afghanistan, difficulties associated with the traditional breeding methods of MPs, and resulting insufficient plant yield have made wild MPs resources unable to meet the current requirements and led researchers to search for alternative solutions. The application of genetic engineering (GE) techniques and biotechnological tools, including combinatorial biosynthesis, CRISPR/Cas9-based systems, and genetically encoded biosensors to select, multiply, improve the bio-production, biodiversity preservation; conservation of the elite and rare genotypes of important MP species in extinction is considered a possible solution. Afghanistan is one of the main exporters of MPs due to its rich flora. Even though it's uncommon in the country to apply modern biotechnology and GE procedures to improve MPs, they may still be considered promising methods. This paper reviewed the recent successes and developments in the previously/at present use of various biotechnological and GE approaches for the improvement of MPs in Afghanistan and also to identify the main challenges the country's plant breeders and/or scientists may face during the use of these approaches to improve MPs shortly.

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Introduction

For thousands of years, plants have been a major source for discovering novel medical compounds (Salim *et al.*, 2008). According to estimates from the world health organization (WHO), almost eighty percent of the population still relies on traditional treatments,

particularly herbs, for their primary medical requirements (Farnsworth, 1990; Farnsworth *et al.*, 1985). Most modern medicines available in the pharmaceutical markets are primarily the result of straightforward chemical alteration or replication of naturally occurring compounds from plants.

Plant extracts or bioactive compounds derived from or modeled after plant components are thought to be included in approximately twenty-five percent of prescription medications (Farnsworth & Morris, 1976; Tripathi & Tripathi, 2003; Vanisree *et al.*, 2004; Nartop, 2018; Cragg & Newman, 2005). Furthermore, eleven percent of the 252 medications the WHO lists as basic and essential are extracted and used directly from plant sources . Furthermore, about 40% of the pharmaceutical lead chemicals utilized to make today's synthetic medications come from natural sources. These facts are the primary drivers behind the growing interest in optimizing plant secondary metabolism through modern technologies and the ever-increasing commercial value of plant chemicals. The other primary reasons for investigating alternative strategies for producing high-quality secondary metabolites (SMs) in lab conditions are the preservation of threatened medicinal plants (MPs) and their natural habitat due to the increasing demand for their usage and challenges with domestic cultivation (Canter *et al.*, 2005; Rikhari *et al.*, 2000).

Biotechnology has been found to be extensively used in agriculture, industry, environment, medicine, and marine biotechnology to solve concerns about food security, poverty reduction, climate change, and enhancing bioactive compounds in MPs. (ECLRC, 2010; Ahmad et al., 2019; Bourgaud et al., 2001; Giulietti & Ertola, 1999). MPs employ comparatively few genetic engineering (GE) techniques and several biotechnological tools compared to other crops. Yet, the selection, multiplication, genetic improvement, and analysis of MPs and, as a result, the continuous, dependable, and renewable production of plant SMs with the right and desired quality on a wide scale have become crucial strategies in recent years. These techniques, in contrast to classical methods, are not influenced by environmental or climatic circumstances, and they can yield greater quantities of plant metabolites while containing the fewest amount of undesirable substances (Ortiz, 1998; Khan *et al.*, 2009; Ahmad *et al.*, 2013).

High-quality phytomedicines can be produced by plant in vitro propagation (IVP). There are numerous IVP methods, such as micropropagation (MP). MP is the process of employing contemporary plant tissue culture (PTC) techniques to multiply stock plant material to produce many progeny plants quickly. Compared to traditional vegetative propagation, MP has several benefits, such as a higher multiplication rate, creating material free of pathogens, propagating yield plants that share the same genetic makeup as the donor plants, etc. The success of IVP of various MPs is influenced by several variables, including plant genotype, growth regulator type and concentration, explant type, condition, etc. (Tripathi & Tripathi, 2003). Some MPs have had protocols for their cloning created and examined (Table 1) (Kayser & Quax, 2007; Khan *et al.*, 2009).

Table 1: Different biotechnological tools for improvement of MPs (Tripathi & Tripathi, 2003; Canter et al., 2005; Kayser & Quax, 2007; Ahmad et al., 2013; Mittal & Sharma, 2017).

IVP[1] (MP)	CMO[2]	SE[3]	GT[4]
A. belladonna	*Adhatoda zeylanicum*	*Acacia catechu*	*Aconitum heterophyllum*
Bacopa monnieri	*Asparagus cooper*	*Acanthopanax koreanum*	*Artemisia annua*
Catharanthus roseus	*Centella asiatica*	*Adromischus cooperi*	*A. belladonna*
Chlorophytum borivilianum	*Cephaelis ipecacuanha*	*Aesculus hippocastanum*	*Azadirachta indica*
Cinchona ledgeriana	*Dioscorea alata*	*Asparagus officinalis*	*Camptotheca acuminata*
Datura metal	*Echinacea pallida*	*Bunium persicum*	*Datura innoxia*
Digitalis spp,	*Hyoscyamus muticus*	*C. roseus*	*Digitalis lanata*
Hoslundia opposite	*Lepidium sativum*	*Cayratia japonica*	*Echinacea purpurea*
Isoplexis canariensis	*Mentha arvensis*	*Medicago sativa*	*Eschscholzia californica*
Ranunculus asiaticus	*Plumbago spp.*	*P. corylifolia*	*Ginkgo biloba*
Rauvolfia serpentine	*Psoralea corylifolia*	*Podophyllum hexandrum*	*Hyoscyamus niger*
Rehmannia glutinosa	*Schizanthus hookeri*	*Typhonium trilobatum*	*Papaver somniferum*
S. nigrum	*Solanum laciniatum*	*Veratrum californicum*	*Pueraria phaseoloides*
Withania somnifera	*Tinospora cordifolia*		*Scrophularia sp*
	Zingiber officinale		*Solanum aviculare*
			Taraxacum platycarpum
			Taxus spp
			Thalictrum spp.

[1]In vitro propagation; [2]Callus-mediated organogenesis; [3]Somatic embryogenesis; [4]Genetic transformation

Plant tissue culture, or PTC, is the aseptic, controlled environment in which plant cells, tissues, and organs are grown and multiplied on a specified solid or liquid medium. PTC has been employed for research on MP phytoconstituents' bioconservation, biotransformation, bioproduction, and biosynthetic studies (Hasnain *et al.*, 2022; Chandran *et al.*, 2020; Mulabagal *et al.*, 2004; Smetanska, 2008). PTC is the ideal source for the bioproduction of pure and safe phytoconstituents. Because of this method, it is possible to isolate numerous components from a single culture and produce less contaminated biomass by pathogens and the environment. Some examples of plants from which common active ingredients for cosmetics are derived using PTC technology are *Symphytum officinale*, *Saponaria pumila*, *Morinda citrifolia*, *Gossypium herbaceum*, *Dolichos biflorus*, *Daphne odora*, *Rubus ideaus*, *Malus domesticus*, *Hisbiscus syriacus*, *Buddleja davidii*, *Coffea bengalensis* and *Lotus japonicas* (Hasnain et al., 2022).

The production of SMs on a commercial scale using PTC is possible in bioreactors (Yancheva *et al.*, 2018; Kreis & Reinhard, 1989; Ruffoni *et al.*, 2010). The mass cultivation of plant cells can benefit from the use of bioreactors in a number of ways, such as automaticity, cost-effectiveness, improved control over cell suspension cultures (CSCs) for the production of stem cells (SMs), continuous condition regulation at different stages of bioreactor operation, simple and quick product harvesting and inoculation, increased multiplication rate, and higher yields of SMs and plantlets. This method produces some expensive compounds budoes not apply to synthesizing allioactive compounds Table 2 below.

Table 2 Examples of MPs growth and production of SMs in cell cultures (Kayser & Quax, 2007; Valluri et al., 1991; Valluri, 2009).

No.	Type of culture and SMs Produced			
1	Bioreactors	SM	Callus culture	SM
2	*A. annua*	Artemisinin	*Allium sativum*	Proteolytic enzymes
3	*Aspidosperma*	Indole alkaloids	*A. indica*	Azadirachtin and Nimbin
4	*A. belladonna*	Atropine	*C. roseus*	Indole alkaloids
5	*Capsicum frutescens*	Capsaicinoid	*C. ipecacuanha*	Cephaelin and emetine
6	*C. roseus*	Vinblastine and vincristine	*C. ledgeriana*	Quinoline alkaloids
7	*Coleuis blumei*	Rosmarinic acid	*D. lanata*	Cardenolides
8	*Coptis japonica*	Berberine	*Dioscorea deltoidea*	Diosgenin
9	*Datura stramonium*	Hyoscyamine	*Glycyrrhiza glabra*	Triterpenes
10	*E. californica.*	Berberine, (S) reticuline	*L. sativum*	Lepidine
11	*P. ginseng*	Ginsenoside	*Piper methysticum*	Kavapyrones
12	*P. somniferum*	Sanguinarine	*Panax ginseng*	Ginsenoside
13	*Santalum album*	Phenolic compounds	*Senecio sp.*	Pyrrolizidine alkaloids
14	*Scopolia parviflora*	scopolamine and hyoscyamine	*Solanum eleagnifolium*	Solasodine
15	*S. aviculare*	Solasodine		
16	*Tabernaemontana sp.*	Indole alkaloids		
17	*T. baccata*	Taxol		
18	*Vernonia cinerea*	Alkaloids		

Collections of somatic cells or tissues give rise to somatic embryos through somatic embryogenesis (SE). In the correct circumstances, these embryos resemble entire seed zygotic embryos and can grow into seedlings. Plant regeneration through stem elongation (SE) from single cells, which may be encouraged to generate an embryo and, ultimately, a fully developed plant, has been demonstrated by several MPs species. Effective somatic embryo development and germination are necessary for commercial plantlet production, which can be done even without growth regulators Table 1 above .

The long-term liquid nitrogen conservation technique known as cryopreservation (CP) offers a chance to preserve MPs that are in danger of extinction. According to certain studies, the CP approach is used to conserve a number of MPs, such as *Rauwolfia serpentine, Atropa belladonna, Hyoscyamus spp*, *D. lanata*, etc. (Baust *et al.*, 2009; Edesi *et al.*, 2020).

Plant breeders now have direct access to a large pool of valuable genes previously unavailable to them offered by genetic transformation (GT). Using multiple desirable genes at once in a single event, modern GE technologies allow the coordinated introduction of novel genes or characteristics into the elite background. To modify, add, or remove a particular characteristic to increase production, applied transgenic research aims to introduce a desired trait from closely related plants, related species, or wholly unrelated species, even in different taxonomic phyla (Sharma *et al.*, 2002).

Table 1 above lists the MPs for whom GT has been recorded (Kayser & Quax, 2007; Khan *et al.*, 2009). This method, especially the hairy roots that *Agrobacterium rhizogenes* induces, could be a powerful tool for boosting the productivity of novel SMs with low yields (Khan *et al.*, 2009). In addition to improving SMs production, GT may improve post-harvest quality, nutrient uptake, nutritional quality, photosynthetic rate, and plant tolerance to insect pests and illnesses. (Sharma *et al.*, 2002; Saito, 1994; Saito, 1992; Veronese & et al., 2001). It may also modify plant tolerance to herbicides and abiotic stressors.

Traditional biotechnology methods have been utilized to breed MPs. Targeted genome editing techniques and other new biotechnology-based breeding strategies can produce customized MPs with distinct SM profiles. The evaluation of genetic diversity, conservation, proliferation, and overproduction are the main ways that genetics and biotechnology might contribute to the faster improvement of MPs (Carvalho *et al.*, 2019). Through PTC, MPs might readily implement emerging biotechnological breeding techniques such as artificial polyploidy and agrobacterium-mediated gene modification. It is also possible to manipulate SM pathways in MPs by regulating endogens and altered genes using transcription activator-like or modified zinc-finger proteins (Niazian, 2019).

It is commonly known that next-generation sequencing techniques have been used to look into genetic diversity and find genes and enzymes connected to the MPs' SM biosynthesis pathway. (Niazian, 2019). Like a dazzling dawn, microRNAs and the clustered regularly interspaced short palindromic repeat-associated endonuclease 9 (CRISPR/Cas9) systems have appeared, greatly assisting not only in the regulation of protein-coding genes but also in the targeted modification of certain traits. Reverse breeding techniques enable the creation of new cultivars faster to address the issues resulting from a changing environment (Ouyang *et al.*, 2017).

Few studies have been conducted on biotechnology to address abiotic stresses and other aspects of MPs agronomic performance. By introducing the genes encoding the enzyme phosphinothricin acetyl transferase, transgenic *A. belladonna* resistant to the herbicides bi-alap-hos and glufosinate and *Panax ginseng* resistant to the herbicide Basta were created. Some recognized examples of the aforementioned treatments include the regeneration of herbicide-tolerant *Solanum nigrum*, fungal-resistant *P. quinquefolium* transformed with either a chitinase or thaumatin-like antifungal genes, and *M. piperita* (Canter et al., 2005).

A new technique for creating and producing novel, rare, and expensive natural products is combinatorial biosynthesis (CB) (Khan *et al.*, 2009). Combining metabolic pathways from several organisms at the genetic level is the fundamental idea behind CB. Important families of natural compounds such as carotenoids, flavonoids, terpenoids (artemisinin, paclitaxel), alkaloids (vinblastine, vincristine, ajmaline, morphine), and flavonoids have all been treated with this approach (Khan *et al.*, 2009).

The development of amorphadiene synthase in *Escherichia coli*, which produces artemisinin (Picaud *et al.* , 2005); production of taxadiene by co-expressing the taxadiene

synthase from *Taxus brevifolia* with an endogenous deoxyxylulose 5-phosphate synthase from *E. coli*, isopentenyl diphosphate synthase from *Schizosaccharomyces pombe*, and geranylgeranyl diphosphate synthase isolated from *Erwinia herbicola* (Wildung & Croteau , 1996; Math et al., 1992; Hahn & Poulter , 1995; Huang , st al., 2001); expression of the CrtE gene, which codes for geranygeranyl diphosphate synthase from *Erwinia sp.*, and the gps gene, which codes for GGDP synthase from *Archaeoglobus fulgidi*, for the synthesis of carotemoids; synthesis of lycopene, ß-carotene, and astaxantin in engineered yeast *Candida utilis* (Miura et al., 1998; Misawa et al., 1990; Wang et al., 1999); cloning of the *Thalictrum flavum* enzyme (S)-norcoclaurine synthase in *E. coli*; expression of the codeinone reductase gene in *E. coli* and insect cells for morphine production (Samanani et al., 2004; Samanani & Facchini , 2002; Zhang et al., 2005); and the cloning of genes that code for vinca alkaloids' production-related enzymes (Verpoorte et al., 1993; Kutchan et al., 1994) are the examples of CB of SMs in microorganisms.

Alkaloids, terpenoids, and phenolics are the three main classes into which SMs are often divided based on their natural distributions and biosynthesis routes. (Justin et al., 2014; Rodney et al., 2000; Hussain et al., 2012).

Afghanistan is a landlocked country with a variety of MPs. The importance of these plants, especially endemic ones, is very clear in different aspects of the country's self-sufficiency. Low-level economy, rich plant flora of the country, increasing desire and demand for products of natural origin, value of MPs as main exported items, and extinction threat of some MPs in use are the key points that highlight the need for improving MPs yield and bioproduction. Despite being an agricultural nation, there are not many arable fields in Afghanistan to cultivate vast and enough MPs to fulfill the growing need for these items. Improvement of MPs using classical breeding techniques is labor-extensive and low yielded. On the other hand, modern biotechnological techniques compensate for these drawbacks and offer more technical feasibility. However, as a developing country, Afghanistan struggles to apply these approaches due to various limitations.

Considering the high demand for MPs improvement at the national level, the technical feasibility of novel biotechnological methods in this context, and the challenges faced by the country to practice these techniques, we reviewed the recent successes and developments of applying various biotechnological and GE approaches for the improvement of SM production by MPs and, to some extent, highlighted the current situation in Afghanistan in this regard. The paper was motivated by MPs' medicinal, industrial, and economic value and the current lack of facilities regarding modern biotechnological techniques to enhance the bioproduction of SMs in some developing countries, including Afghanistan. The main purpose of the current research was to report the application of various biotechnological and GE-based techniques previously/at present in use to improve MPs in Afghanistan and also to identify the main challenges the country's plant breeders and/or scientists may face during the use of these approaches to improve MPs, based on the documented findings of the other researchers, shortly. The aims of this research are as follows:

1. To report the application of various biotechnological and GE-based techniques previously/at present in improving the MPs in Afghanistan.
2. To identify the main challenges the country's plant breeders and/or scientists may face while using different biotechnological and GE-based approaches to improve MPs in the near future.

Application of biotechnological methods in the production of alkaloids

Alkaloids are an important group of secondary metabolites (SMs) found in plants with nitrogen in their chemical structure and a broad range of physiological activities. They are also extensively distributed in nature . According to the definition given by German pharmacist FW Carl in 1819, alkaloids are substances originating from plants that have alkali characteristics . According to SW Pelletier, alkaloids are cyclic chemical compounds that contain nitrogen in negative oxidation and are occasionally found in living organisms .

More than 20,000 substances are classified as alkaloids according to the general criteria for alkaloids. While approximately twenty-five percent of plant species contain alkaloids, higher plants are the primary producers and extractors of these chemicals; nonetheless, it has also been documented that mammals, insects, microbes, and low plants can also produce alkaloids .

The necessity for alkaloids production has grown dramatically due to their significance in medicine and high economic worth. However, the lack of sufficient information regarding the synthesis and evaluation of alkaloids in plant cells, the time-consuming and unprofitable chemical process of alkaloid extraction, the complex process of extracting alkaloids from resource biomass, the quantitative and qualitative affectability of alkaloids from environmental and geographic, and genetic diversity of source plants, etc., are among the main factors that have led scientists to search for alternative strategies to produce alkaloids (Srivastava & Srivastava, 2013; Dehghan et al, 2010). While many alkaloids can be chemically synthesized, it is not practicable to produce most alkaloids on an industrial scale .

The advancement of molecular biology and biotechnology methods has led scientists to investigate workable ways to boost alkaloids' commercial production. An environmentally friendly alternative to traditional methods of producing alkaloids is the application of biotechnological methods, including plant cell/tissue culture, hairy root culture, bioreactors, etc. **Error! Reference source not found.** above (Hulvová *et al.*, 2013; Ozyigit *et al.*, 2023). But thus far, not much has been successful.

An in vitro-cultured alkaloid-producing plant can synthesize alkaloids in the same way as an intact plant. Alkaloid production can be optimized with the use of callus culture. The media composition helps induce callus to enhance the production of alkaloids and the conservation of threatened genotypes (Thengane *et al.*, 2003). In CSC, cells are suspended alone or in groups in a liquid culture medium. T cells can be genetically modified to include one or more foreign genes (Table 3 and Table **4**).

Table 3. Production of Alkaloids in plant culture under in- vitro conditions.

Name of Alkaloid	Pharmacological Activity	Source Plant	Type of Culture	Ref.
Atropine	Sympathomimetic	Solanaceae species	[1]CC of *H. niger*	(Kirsi-Marja & Oksman-Caldentey, 2007; Hussain et al., 2012)
Berberine	Anti- microbial	– *C. japonica* – *Phellondendron amurense*	CC of – *C. japonica* – *Thalictrum spp.* – *Berberis spp*	(Hussain, etal., 2012)
Camptothecin	Anti-cancer	*C. acuminata*	[2]CaC of *C. acuminata*	(Gonçalves & Romano, 2018; Kowalczyk et al., 2020)
Capsaicine	Analgesic, GI stimulant	*Capsicum spp*	[3]CSC of *C. frutescens*	(Hussain, etal., 2012)
Codeine	Antitussive	*P. somniferum*	CaC and CSC of *P. somniferum*	(Hussain et al., 2012; Gonçalves & Romano, 2018)
Ergot alkaloids	Anti-migraine, Anti-parkinson, Oxytocic	*Claviceps purpurea*	[4]SC of *C. purpurea*	(Hulvová et al., 2013; Řeháček, 1984)
Galantamine	Anti-cancer, Anti-viral, Anti-microbial	*Leucojum aestivum*	CC of *L. aestivum*	
Hyoscyamine	Sympathomimetic	Solanaceae species	CC and [5]HRC of *H. niger*	
Lycorine	Anti-cancer, anti-viral, anti-microbial	*L. aestivum*	CC of *L. aestivum*	
Morphine	Analgesic	*P. somniferum*	CaC & CSC of *P.somniferum*	(Hussain et al., 2012)
Nicotine	Parasymp*athomimetic	*N. tabacum*	CC & CaC of *N. tabacum*	(Hussain et al., 2012)
Podophyllotoxin	Anti-cancer	*Linum spp.*	CSC of – *L. album* HRC of – *Linum* spp.	
Quinoline alkaloids	Antimalarial	*Cinchona sp.*	CSC of *Cinchona sp.*	
Reserpine	Antihypertensive	*Rauwolfia serpentina*	CSC of *R. serpentina*	
Scopolamine	Sympathomimetic	Solanaceae species	CC of *H. niger*	
Taxines (Taxol)	Anti-cancer	*Taxus* spp.	CSC of – *Taxus spp.* – *Corylus avellana*	(Gonçalves & Romano, 2018; Kowalczyk et al., 2020;

Vinblastine	Anti-cancer	*C. roseus*	HRC of *C. roseus*	(Gonçalves & Romano, 2018; Heijden et al., 2004)
Vincristine	Anti-cancer	*C. roseus*	HRC of *C. roseus*	(Gonçalves & Romano, 2018; Heijden et al., 2004)

[1]cell culture; [2] callus culture; [3]cell suspension culture; [4]suspension culture;; [5]hairy root culture.

Table 4 Examples of Alkaloids produced in hairy root culture of transgenic plants.

Alkaloid	Source Plant	Transgenic Plant	Target Genes	Reference
Camptothecin	*C. acuminate*	*C. acuminate*	ORCA and AOC[1]	(Sakato et al., 1974; Ni et al., 2011; Yan, 2012)
		Ophiorrhiza pumila	STR[2] and G10H[3]	
Hyoscyamine	Solanaceae species	*H. niger*	Vitreoscilla hemoglobin	
Paclitaxel	*T. brevifolia*	*T. marei*	DBAT[4] and TXS[5]	(Wani et al., 1971; Ho, Chang, & Lung, 2005)
		Taxus x *media* var. *hicksii*	TXS	(Sykłowska-*et al.*, 2015)
		T. chinensis	ECOx[6]	
		N. benthamiana	TXS	(Kowalczyk et al., 2020)
Scopolamine	Solanaceae species	*H. niger*	Vitreoscilla hemoglobin	
Taxadiene	*Taxus spp.*	*T. chinensis*	NINV and taxadiene synthase	(Hidalgo et al., 2017; Dong et al., 2015)
Vinblastine	*C. roseus*	*C. roseus*	CrMYC1 and CrERF5 transcription factors	(Sazegari et al., 2018; Pan et al., 2019)
Vincristine	*C. roseus*	*C. roseus*	TD[7] and STR	(Tomioka et al., 2013)

[1]allene oxide cyclase; [2]strictosidine synthase; [3]geraniol- 10- hydroxylase; [4]10-deacetylbaccatin III-10-O-acetyltransferase; [5]taxadiene synthase; [6]9- cis- epoxycarotenoid dioxygenase; [7]tryptophan decarboxylase

It has been proposed that using plant cell/tissue culture methodology is an environmentally beneficial substitute for other methods when producing various alkaloids. Numerous factors influence the biosynthesis of alkaloids employing PTC approaches, including culture conditions, strain selection for high metabolite production, precursor feeding, growth regulator type and concentration, elicitation, permeabilization, etc. Optimizing these factors may enhance the yield for commercial utilization (Ahmad *et al.*, 2013; Ciddi *et al.*, 1995; Holden *et al.*, 1988; Narayani & Srivastava, 2017).

Since the synthesis of the necessary compounds cannot be met by the alkaloids produced in cell/plant tissue culture alone, strategies such as using transcriptome and metabolome data for metabolic engineering of alkaloid-producing PTC, transferring and expressing foreign genes that produce alkaloids to desired plant cells, cloning genes that produce alkaloids or their precursors in recombinant microorganisms like bacteria and yeasts,

modifying the environment and culture conditions, etc., are among those suggested to enhance the synthesis of alkaloids in plant cell/ tissue culture (Dörnenburg & Knorr, 1995; Schäfer & Wink, 2009; Ptak *et al.*, 2020; Heijden *et al.*, 2004; Khalil, 2017; Kirsi-Marja & Oksman-Caldentey, 2007).

Patients and customers reject using genetically modified plants as a source for medications and extract preparations produced and sold in pharmacies. Conversely, MPs' biotechnological methods have focused more on certain natural products and biosynthetic pathways because of the increasing adoption of phytotherapy and the belief that natural items are safe. Although the biogenetic routes of alkaloids in plants are generally complex and involve multiple biosynthetic steps, alkaloids have been a key target of metabolic engineering (ME) research. Many vital genes that produce certain alkaloids, such as morphine, nicotine, scopolamine, and berberine, have been identified (Croteau *et al.*, 2000; Verpoorte *et al.*, 2002).

Genetic engineering of aromatic amino decarboxylase levels (Facchini et al., 2000), strictosidine synthase , hyoscyamine 6-β-hydroxylase (Yun et al., 1992) which caused levels of tyramine, serotonin, and tryptamine to rise; production of strictosidine, a glucoalkaloid, and several of its derivatives in a *C. roseus* transgenic cell culture; exclusive accumulation of scopolamine in transgenic *A. belladonna* instead of hyoscyamine; GE of *P. somniferum* to overexpress (S)- N -methylcoclaurine 3' hydroxylase and codeinone reductase; silencing of berberine bridge enzyme, which respectively led to increased alkaloid yield in latex of transformed plants; increase in the concentration of several pathway intermediates from all biosynthetic branches of benzylisoquinoline alkaloids in the latex of transgenic plants, as well as an increase in the dry weight basis of morphinan alkaloid content in transgenic plants (Dehghan et al., 2010) are some examples of metabolically engineered MPs producing alkaloids.

It has been demonstrated that several genes required for synthesizing scopolamine, nicotine, and berberine have been cloned, allowing the ME of these alkaloids. Putrescine N-methyl transferase (PMT) in transgenic *A. belladonna* and *Nicotiana sylvestris* and (S)-scoulerine 9-Omethyltransferase (SMT) in cultured cells of *C. japonica* and *E. californica* were both effectively engineered to express branching-point enzymes. The findings demonstrated that whereas endogenous PMT activity was significantly suppressed, the amount of nicotine in *N. sylvestris* was increased considerably due to overexpression of PMT. Moreover, the ectopic expression of SMT increased the accumulation of benzylisoquinoline alkaloids in *E. californica* (Sato et al., 2001).

Application of biotechnological methods in the production of terpenoids

Terpenes and terpenoids made by plants have hundreds of different types and are the largest and most structurally diverse category of SMs. These substances are the primary components of essential oils (EOs). EOs are volatile chemicals isolated from plants using steam distillation or organic solvent extraction (Paul et al., 2020). Even though over 3000

different plant species have yielded distinct essential oils (EOs), only around 300 of those species have been the subject of in-depth study, and only about 20 of those species have been recognized as valuable sources for commercial application.

EOs are vital in shielding plants from herbivores since they are insecticides and antibacterial, anti-viral, antifungal, and attractant agents (Gutensohn et al., 2013; Verdeguer et al., 2020). In addition, they find extensive applications as anti-microbial, pheromones, perfumes, food preservatives, insecticides, antioxidants, analgesics, sedatives, anti-inflammatory, antispasmodic, and local anesthetic agents. Their antibacterial, fungicidal, and therapeutic properties are also acknowledged (Pandey et al., 2020).

Since EO compounds are typically exclusively generated in certain plant tissues (Raut & Karuppayil, 2014), it can be expensive to extract valuable compounds from the essential oils (EOs) of aromatic plants (APs). The need for natural EO production became critical due to consumer preference for natural EOs and the wide range of uses of essential oils (EOs) in a variety of industries, such as perfumery, aromatherapy, pharmaceuticals, healthcare, cosmetics, food flavoring, and food preservation (Aziz et al., 2018; Campêlo et al., 2019).

The conventional, labor-intensive, and constrained plant breeding approach increases APs' capacity to generate SMs. The development of GE, advancements in APs tissue culture, and modern biotechnological techniques can enable the cost-effective, large-scale, and high-efficiency biosynthesis of desirable, rare, and high-value natural EOs through a variety of environmentally friendly methods (Di Gioia et al., 2011; Lubbers et al., 2019). Other benefits of using biotechnological-based methods for AP enhancement of SMs include the independence of these techniques from seasonal and geographical conditions, the practical application of genetic modifications (Fierascu et al., 2020), an increase in the quantity or size of EO-producing cells and tissues, polyploidy (Kuluev et al., 2013), as well as the creation of breeding lines and resistant varieties (Shelepova et al., 2022).

The biotransformation of secondary metabolic routes in general and particular terpene pathways have been studied using GE of APs. Metabolic and combinatorial engineering techniques have been used to *E. coli* as the principal microbe. Improvements in omic methods produced better foundations for the growth of SMs production. It is widely recognized that genetic modification of bacteria and yeast as substitute hosts is crucial for synthesizing large-scale plant EO components (Shelepova et al., 2022). Advances in transgenic research have made it possible for ME to investigate biosynthetic pathways and generate important synthetic molecular markers. ME is a technique that shows promise for boosting yields and endogenous SM production in the plant system. Using this technique, the desired SMs can be synthesized by isolating and expressing particular genes in intact and cultured APs. Table 5 provides some instances of EOs ME in APs.

Table 5 Examples of APs and EO content modification after genetic transformation.

Medicinal Plant	Gene	Transgenic Plant	Result of Transgenesis

A. annua	Trichome-specific LTP genes (AaLTP3 and AaLTP4)	*A. annua*	Enhanced production of: – Arteannuin B, – Artemisinin, – Dihydroartemisinic acid – Artemisinic acid
	TLR1 and TLR2		Decreased artemisinin production
	TfGA2ox2		Increased yields of EO and artemisinin production
	AaWRKY1		Increased production of artemisinin
	cyp71av1 and cpr		Increased artemisinin content
Lavandula spp.	Linalool synthase (LIS)		Higher EO yield and linalool synthesis
Lallemantia iberica	NtLTP1	*M. aquatic* var. *citrate*	Increased monoterpene accumulation in the glandular trichomes
M. citrate (*M. piperita* f. *citrata*)	DXPS	*M. piperita* (elite line MFS7A)	Increased EO yield
	MFS		Reduction in the proportions of (+)-pulegone and (+)-menthofuran
M. piperita	MsYABBY5		Decreased terpene levels
	MsMYB		Decreased monoterpene biosynthesis.
O. basilicum	β-glucuronidase (GUS)		Reduction in eugenol content and accumulation of coniferous alcohol
S. fruticosa	SaDXR		Enhanced production of sesquiterpenoids unique to sandalwood
S. album	Terpene synthase (TPS)		A rise in the amount of thymol
	IPT		Modification of EO quality

Terpenoids are biosynthesized by the mevalonate (MVA) or deoxyxylulose phosphate (DOXP) pathway. Recent expression of the MVA pathway in *Escherichia coli* led to the successful synthesis of the terpenoids amorpha-4,11diene and taxadiene (Khan et al., 2009). The primary instances of the ME of the monoterpene pathway could be the silencing of the enzyme menthofuran synthase in transgenic *Mentha×piperita* and the ectopic expression of the deoxyxylulose phosphate reductoisomerase gene, which led to the synthesis of EO with a reduced quantity of menthofuran and a fifty percent rise in the accumulation of total EO levels in peppermint that is transgenic and *Clarkia breweri*'s utilization of the floral gene linalool synthase (LIS)., which displayed a distinct chemical profile upon overexpression in multiple plants (Dudareva et al., 1996).

Sesquiterpenes are one of the other main types of SMs produced by APs. An example of the ME of the sesquiterpene route is the two- to three-fold increase in artemisinin synthesis by the parent plant in transgenic *A. annua* caused by overexpression of a cotton gene encoding farnesyl diphosphate synthase (Chen et al., 2000; Canter et al., 2005).

Plants resistant to many diseases and pests can develop when APs with ME produce enough SMs in their organs and tissues. For example, the overexpression of the linalool synthase gene (CuSTS3-1) in transgenic *Citrus sinensis* resulted in high linalool content and resistance to citrus *Xanthomonas citri* subsp. *citri* cancer ; increased (E)-beta-farnesene release in *Matricaria recutita*, which enhanced insect repellent activity (Su et al., 2015);

induction of water deficiency in *Ocimum basilicum* due to β-glucuronidase gene expression (Khakdan et al., 2022) could be noted.

The production of cis-abienol in microorganisms ; the extensive geraniol cultivation process in bioreactors; the production of the EO component of valuable and endangered plants in cell culture of *Ajuga bracteosa*, *Nepenthes khasiana*, and *Zataria multiflora* (Ghasemi et al., 2014; Máthé et al., 2015); the generation of SMs in *M. spicata* hairy root culture (Yousefian et al., 2020), *Artemisia spp*., and *Salvia spp*. are some examples of the production of SMs by APs employing various biotechnological strategies.

Application of biotechnological methods in the production of phenolics

Plant SMs called flavonoids, which are generated from phenylpropanoid and have a low molecular weight, are commonly present in fruits, vegetables, and meat products . Flavonoids' medicinal significance makes them desirable targets for large-scale production through a variety of biotechnological and bioengineering approaches; their use as antioxidants, anti-inflammatory, anti-cancer, and anti-diabetic agent, leads to high commercial values, despite their low bioavailability and the impracticality of chemical synthesis or plant extraction.

The production of phenolic extracts in vitro plant cultures has been the subject of numerous investigations (Dias et al., 2016; Valanciene et al., 2020), but due to the complexity of the biosynthetic pathways and extraction techniques, there is a deficiency in the synthesis of distinct phenolic chemicals. Elicitation methods are widely used to increase the synthesis of phenols, usually producing higher yields than in non-elicitated cultures. An overview of multiple cases of economically relevant phenolic compound production using different biotechnological approaches is given in Table 6.

Engineering model plants to identify critical elements in the general flavonoid biosynthetic pathways; engineering ornamental plants for decorative purposes; engineering flavonoids with strong pigment; engineering plants for improved tolerance toward biotic and abiotic stress; and engineering crop plants to increase flavonoid accumulation are the five main goals of engineering the biosynthesis of flavonoids in plants, according to Nabavi *et al.* (2020).

Table 6 *Examples of commercially important phenolic compounds production using different biotechnological methods (Dias et al., 2016; Al-Jibouri et al., 2016; Ghimire, Yu & Chung, 2017; Amer, 2018; Sitarek et al., 2018; Suzuki et al., 2019).*

Source Plant	Culture Type	Compound Name	Plant Culture
Cyclocarya paliurus	[1]CaC	Acylated anthocyanin pigments	CaC of *Ipomoea batatas*
Genista spp.	CaC	Anthocyanin	CC of *Daucus Carota*
H. perforatum var. *angustifolium*	CaC	Betalain	HRC of *Beta vulgaris*
L. tenuis	CaC	Betulinic acid	Transgenic *Lotus japonicu*
M. sativa	CaC	Caffeic acid	CaC of *I. batatas*

Maackia amurensis	CaC	Chlorogenic acid	– CaC of *I. batatas* – CC & [8]OC of *Eryngium planum*
Nepeta spp.	CaC	Coumarin	CaC of *Verbascum thapsus*
Plantago spp.	CaC	Coumestrol	HRC of *Psoralea spp.*
Ononis arvensis	CaC	Daidzein	HRC of *Psoralea spp.*
Pluchea lanceolata	CaC	Ellagic acid	[9]SC of *R. chamaemorus*
Scutellaria baicalensis	CaC	Eugenol	CaC of *V, Thapsus*
Sophora flavescens	CaC	Hypericin	CSCs of *H. perforatum*
Stevia rebaudiana	CaC	p-coumaric acid	CaC of *Ruta graveolens* ssp. *Divaricate*
Maclura pomifera	CaC & [2]CC	Phenolic acids	Transgenic *Leonurus sibiricus*
G. biloba	CaC & CSCs	Phenolic compounds	– HRC of *Fagopyrum tataricum* – Transgenic *Perilla frutescens*
Momordica charantia	CaC & [3]CSC	Phytoalexins (medicarpin)	CSC of *M. sativa*
D. lanata	CC	Protocatechuic acid	SC of *R. graveolens*
V. myrtillus	CC	Quercetin	– HRC of *F. tataricum* – CaC of *P. lanceolata*
Fragaria ananassa	CSC	Rosmarinic acid	– CC and OC of *E. planum* – IVC of Lamiaceae species – IVC of Boraginaceae species – CC of *Coleus Blumei*
Iphiona mucronata	[4]EC and CSC	Rutin	HRC of: – F. esculentum – *F. tataricum*
G. uralensis	[5]HRC	Shikonin	CC of *Lithospermum erythrorhizon*
Eucommia ulmoides	[6]HCC	Sophora Flavanone G (SFG)	CC of *S. flavescens*
Astragalus missouriensis	[7]IVC	Thiophene	HRC of *Tagetes patula*
Cyclopia genistoides	IVC	Thymol	CaC of *V. Thapsus*
Dionaea muscipula	IVC		
Drosera capensis	IVC		
H. muticus	IVC		
Sanicula graveolens	IVC		
Schisandra chinensis	IVC		

[1]Calluc Culture; [2]Cell Culture, [3]Cell Suspension Culture; [4]Embryogenic Callus; [5]Hairy Roots Culture; [6]Hypocotyl Callus Culture; [7]In vitro Culture; [8]Organ Culture; [9]Shoot Culture

Overexpressing transcriptional factors, reverse genetic engineering strategies (gene silencing, insertional mutagenesis, knock-out, point mutation or target-induced local lesions in genome (TILLING), gene targeting and using transgenics for ectopic expression), plant suspension-, callus-, cell suspension-, and hairy root-cultures, transformation, nano-treatment, and engineering a flavonoid biosynthetic pathway in microbial hosts are the main applied biotechnological techniques to improve the production of flavonoids in plants. Expression systems utilized to create recombinant products include *Streptomyces spp.*, *E. coli*,

and *Saccharomyces cerevisiae*. Furthermore, a range of bioengineering methods are used to create flavonoid molecules, including genome engineering based on CRISPR and genetically encoded biosensors to identify flavonoid production (Amer, 2018; Shah et al., 2019; Marsafari et al., 2020).

The R2R3-MYB transcription factor anthocyanin1 (CsAN1) is activated in *Camellia sinensis*, causing an ectopic accumulation of pigments. This activation, which is followed by an up-regulation of the bHLH transcription factor CsGL3 and anthocyanin LBGs ; enhanced flavonoid production in CSC of *Hypericum perforatum* with methyl jasmonate (MeJA) treatment through the inhibition of catalase (CAT) and induction of phenylalanine ammonia-lyase (PAL) (Wang et al., 2015); after elicitation with MeJA or methylated β-cyclodextrins, the production of t-resveratrol from CSC of transgenic plant *Silybum marianum* harboring the gene stilbene synthase from *Vitis vinifera* (Hidalgo et al., 2017); boosting the expression of stilbene synthase genes, which increases the amount of resveratrol extracted from the callus culture of *V. amurensis* (Suprun et al., 2019) are some examples of biotechnological tools and GE strategies to improve flavonoid biosynthesis in MPs.

Increasing the levels of medicarpin and sativan in *M. sativa*, cajanol and stilbene in *Cajanus cajan*, deoxyanthocyanidin flavonoids (luteolinidin, apigenindin, etc.) in *Sorghum bicolor*, and stilbene in *Cicer arietinum* are a few examples of metabolic pathway engineering to alter host-plant resistance to insect pests and diseases (Sharma et al., 2002). According to reports, the richest sources of antioxidant metabolites are the MP of Vaccinium berry crops. The experiment's outcome has shown that the fruits of in vitro-grown plants had a higher overall antioxidant activity .

Challenges faced by biotechnological tools for bio-production of SMs

There are several issues with using biotechnological techniques to increase the yield output of SMs by different MPs. These obstacles could be categorized as socioeconomic and technological considerations, how the general public views biotechnology, regulatory concerns, research agendas, and issues connected to the distribution of research findings. For example, the main problem with CB stems from the incomplete understanding of most biosynthetic pathways at the genetic level and the lack of complete sequences and functional explanations of genes involved in plant regulation and biosynthesis. It is, therefore, difficult to transfer the entire biosynthetic pathway to a heterologous host. .

There is less research on APs than on traditional crops, where such processes are rare. Because of the rapid buildup of specialized metabolites and the peculiar ways that viruses and agrobacteria interact with plant cells and tissues, it is difficult to develop transgenic and modified APs in an in vitro environment. These interactions lead the metabolites of EOs to suppress the activities of agrobacteria while promoting the effective operation of the antioxidant system. (Ahmad et al., 2013). Other constraints of in vitro plant growth techniques include challenges with continuous operation, product removal, maintaining an

aseptic state, and somaclonal variance of populations created from tissue culture (Hasnain et al., 2022).

Although bioreactor-based techniques have been proposed to enable high-yield flavonoid synthesis in certain species with PTCs, large-scale culture presents technical hurdles that have prevented the establishment of commercial feasibility to date. The reasons behind the failure of commercial and large-scale bio-production of flavonoids through these systems are the poor cultural productivity rate, competing pathways, metabolic flux diversion, and hereditary stability in plant CSC (Marsafari et al., 2020). The problems with flavonoid bio-production using plant cell systems include time consumption, high costs associated with the use of natural resources, an expensive and environmentally unfriendly process of isolation and purification that may lead to product loss and degradation, and limited yield accumulation in the absence of developmental or environmental stimuli (Wang et al., 2011; Lim et al., 2001; Mavel et al., 2006).

The role of agricultural biotechnology and genetic engineering in the improvement of medicinal plants in Afghanistan

Afghanistan is landlocked with mountains and a diverse flora, with over 29% of its plants being endemic (Freitag et al., 2010). The country's economy depends mainly and fundamentally on these resources to grow and become self-sufficient. The reports that are now available indicate that the most significant components of the country's gross domestic product (GDP) are agricultural and livestock. Approximately 85 percent of the population in this nation works as farmers. Just 1/8 of the nation's land is arable, even though agricultural items account for most of its domestic production .

Around 5,000 species of vascular and flowering plants are found in Afghanistan's plant flora, according to the currently available data. These species include 1086 plant genera, 23 genera and 50 species of Pteridophytes, 8 genera and 24 species of gymnosperms, 195 genera and 817 species of monocotyledons, and 860 genera and 3935 species of dicotyledons, of which 700 species are aromatic and MPs, and 120 species are currently used in traditional medicine in the country. Certain plants, like licorice, caraway, asafoetida, colchicum, parsnip, cumin, and others that grow wild, can be domesticated. Other species, like garlic, saffron, coriander, anise, etc., are cultivated. Caraway is now an annual plant that was domesticated in the province of Herat .

Twelve hundred and fifteen (15 percent) of the five thousand species of plants that have been identified are endemic (Breckle et al., 2013). These endemic species are primarily found in eight plant families: Asteraceae, Fabaceae, Brassicaceae, Apiaceae, Lamiaceae, Caryophyllacea, Boraginaceae, and Poaceae . Among the most prevalent MPs in Afghanistan's flora are plant genera like *Artemisia*, *Cousinia* (Asteraceae family) (Amiri et al., 2020), *Astragalus* (Fabaceae family), *Brassica* (Brassicaceae family), *Cymbopogon*, *Andropogon* (family Poaceae), *Phlomis*, *Thymus*, *Mentha*, *Nepeta*, *Origanum* and

Eremostachys (Lamiaceae family), *Ferula*, *Dorema* (Apiaceae family), etc. which may be considered as promising tools for research and biotechnological studies at country level.

To the best of our knowledge, no research hasn't been published on modern biotechnological and genetic engineering approaches to improve MPs in Afghanistan. The country is lagging in using these national treasures and improving the yield of products using modern methods. These include other technical obstacles and challenges, the lack of sufficient information regarding important MPs nationally, the extinction of some important plant species due to their irrational and excessive use, the lack of technical and trained personnel, the lack of sufficient budget to launch field-related scientific studies, etc.

Conclusion

A lot of attention is currently being paid to the selection, multiplication, bio-production of SMs, biodiversity preservation, and the conservation of elite and rare genotypes of important medicinal plant species that are facing extinction using various biotechnological tools and GE-based techniques, such as CB, CRISPR/Cas9-based systems, and genetically encoded biosensors. The primary motivations behind investigating contemporary alternative methods to replace conventional techniques to improve SMs biosynthesis by MPs are the following: the challenges associated with the classic plant breeding process; the significant consumer demand for natural origin pharmaceutical and nutraceutical items; threatened biodiversity; the extinction of MPs; and the environmentally friendly and, in some cases, economically advantageous process of SMs bio-production. Nevertheless, compared to other crops, fewer examples demonstrate the use of biotechnological approaches by MPs to enhance SM production. Afghanistan is landlocked with a diverse MP flora that includes over 29% endemic species. The country's economy depends heavily and fundamentally on these resources to grow and become self-sufficient. Despite the lack of published research on the use of contemporary biotechnological and genetically engineered (GE) approaches to increase MPs yield production in Afghanistan, it is crucial to begin applying biotechnology-based methods for the improvement of MPs, especially endemic ones, given the abundance of natural resources in the nation and the viability of the aforementioned techniques.

Recommendations

- At the national level, medicinal plants (MPs), particularly endemic plant species, should be thoroughly investigated.
- Appropriate usage of these plants is necessary to preserve biodiversity and keep certain MPs species from going extinct.
- Several MPs from the country's flora must be domesticated and cultivated in order to improve yield production.

- Extensive research initiatives employing cutting-edge scientific techniques should be carried out in pertinent scientific facilities in order to domesticate the significant MP species.
- Another fundamental issue is the absence of appropriate training resources and equipment, which necessitates the organization of short-term training programs overseen by knowledgeable and experienced staff.
- To achieve a high success rate when applying biotechnological techniques to boost MPs productivity, it is crucial to have access to well-trained personnel, well-equipped labs, MPs germplasms, etc.

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