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Contents

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Ajay Kumar Thakur is presently working as Senior Scientist (Biotechnlogy) at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan. He has obtained his graduation, PG and Doctoral degrees from Dr. Y.S. Parmar University of Horticulture & Forestry, Solan, H.P. He got induced into Agricultural Research Services (ARS) in 2008. He has published 40 research/review papers in various journals of International and National repute, authored one book 'Agricultural Biotechnology at a Glance', contributed 8 book chapters and 22 popular articles. He has developed high efficiency plant regeneration and genetic transformation protocols in a number of crops including *Populus ciliata*, *P. deltoides*, *Punica granatum*, *Capsicum annum* and *Cucumis sativus*. Dr. Thakur is associated with *Brassica juncea* improvement programme using biotechnological interventions from last 11 years. He has developed a core set of SSR markers for *B. juncea* genomics and is presently working on germplasm characterization and association mapping of various agronomically important traits in this oilseed crop. He has been granted with one Indian patent and associated in the development of a high yielding Indian mustard variety Giriraj, a white rust resistant Indian mustard genetic stock DRMR MJA 35, which is a *Moricandia* system- based cytoplasmic male sterile line of *B. juncea,* and a multiple disease (Alternaria blight, white rust and powdery mildew) resistant Indian mustard genetic stock, DRMRIJ 12-48. Dr. Thakur has received many awards from various societies and scientific organizations for his scientific contribution. He is also an elected Member of Plant Tissue Culture Association of India.

Pankaj Kumar is presently working as Assistant Professor (Biotechnology), Department of Biotechnology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. Formerly, he has worked as SERB- National Post Doctoral Fellow (DST Young Scientist Scheme) at Council of Scientific & Industrial Research - Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India. Dr. Kumar is working on plant secondary metabolite enhancement through cell and tissue engineering

approaches; Identification of molecular cues linked with enhanced metabolites using comparative transcriptomics approach and also have experience on hydro-aero cultivation of medicinal plants for industrial importance. During Doctoral Studies; he (research team) has generated technology at Dr. Yashwant Singh Parmar University of Horticulture & Forestry Nauni, Solan for developing insect pest-resistant transgenic plants in economically important vegetable crops of cauliflower cv. Pusa Snowball, cabbage cv. Pride of India and broccoli cv. Solan Green Head (with *cryIAa* gene) for insect pest resistance. He has been awarded DST INSPIRE JRF/SRF Fellowship, Department of Science and Technology, Ministry of Science and Technology, Government of India for Ph.D. full doctoral program. He has qualified ICAR AICE-SRF (PGS), ICAR ASRB National Eligibility Test (NET). He has published 35 research/review papers in various journals of International and National repute, contributed 13 book chapters and 5 popular articles. Dr. Kumar has received many awards from various societies and scientific organizations for his scientific contribution i.e. Young Scientist Award Biotechnology-2018 by Society for Plant Research, Young Scientist Award -2019 by the Society of Tropical Agriculture, New Delhi, India, Excellence in Research Award -2019 by Agro Environmental Development Society, India. etc.

3 In Vitro Production of Medicinal Compounds from Endangered and Commercially Important Medicinal Plants

Neha Sharma and Hemant Sood

Abstract

Medicinal plants are known for curing various disorders and ailments throughout the world. The therapeutic properties of these plants are attributed to the medicinal compounds produced by them. Growing demands of these medicinal compounds have urged a necessity to concentrate the research on enhancing their production by engaging different techniques. In this article, we have assessed different plant tissue culture techniques which could be applied for enhancing the production of commercially important secondary metabolites. Here, we have discussed the usage of cell/tissue culture techniques for mass propagation and bioactive metabolite production in endangered medicinal plants under in vitro conditions. Role of hairy root cultures, elicitation and precursor feeding, endophytes, and nanoparticles has also been studied for enhancement of commercially important bioactive compounds. Further, we have also highlighted the importance of metabolic engineering and CRISPR-based approaches for understanding the biology of biosynthesis of medicinal compounds.

Keywords

Secondary metabolites · Medicinal compounds · Micropropagation · Elicitors · Endophytes · Metabolic engineering

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3.1 Introduction

The importance of medicinal plants has been known to humans since ancient times and is also recommended for their medicinal properties by traditional medicinal systems like Unani, Ayurveda, and other ancient texts including Atharva Veda, Charaka Samhita, Rig Veda, Sushruta Samhita, etc. Conferring by World Health Organization (WHO), 65–80% people of developing nations cannot access the expensive modern pharmaceutical products and depend on medicinal plants to endure their prime healthcare needs (Palhares et al. 2015). The herbal medicines are less expensive and safer than synthetic or modern drugs (Karimi et al. 2015). Approximately 25% of modern medicines and 60% of anticancer medicines are obtained from natural resources (Palhares et al. 2015). Medicinal compounds are mainly secondary metabolites, which are the resultant of primary metabolites and involved in directly in the life of plant cells by playing role in their defense, signaling, and chemical adaptations to environmental stresses. These medicinal compounds are obtained from various plant organs like bark, flower, leaves, stem, root, seed, or whole medicinal plant and can vary in a tissue and organ specific way during different developmental stages (Wink 2003). Secondary metabolites are mainly categorized as terpenoids (derived from acetyl coenzyme A or glycolysis cycle intermediates), phenolics (aromatic rings 11 bearing a hydroxyl functional group), and alkaloids (nitrogen containing compounds).

Due to emergence of new products like health care, personal care, and immunity-boosting formulations, industrial demand for herbal products has increased and will further tend to rise in future. Estimates show that the international demand for plant derived drugs is likely to approach ~US$5000 billion by end of the year 2050 (Pan et al. 2014). Currently, majority of herbal collections are obtained from their natural habitat and only 10% are contributed by the cultivated medicinal plants. The escalating demand of herbal products is affecting the dwindling population of medicinal plants in wild. Slow growth rate, complex accumulation patterns, narrow geographic ranges along with unorganized and extensive harvesting of medicinal plants are making them more prone to extinction and loss of genetic diversity. Also, uneconomical chemical synthesis and inaccessibility of quality plant material in adequate amounts hinder in satisfying the increasing industrial demands. Therefore, biotechnological tools offer valuable alternatives for production of desirable medicinal compounds by enhancing their biosynthesis and accumulation. In this chapter, we have reviewed different techniques and approaches used for production as well as enhancement of commercially important compounds in medicinal plants.

3.2 Medicinal Compounds Production Using Cell/Tissue Culture Techniques

In medicinal plants, cell/tissue culture techniques are promising methods for controlled biosynthesis of numerous bioactive compounds. These are environment friendly and act as cost-effective alternative to chemical synthesis of medicinal

compounds. These techniques enable the in vitro grown plants to biosynthesize particular medicinal compounds analogous to the intact plants and also aid in producing secondary metabolites at large scale without any seasonal constraints. Utilizing cell/tissue culture techniques, various medicinal plants can be established under aseptic conditions using different explants including meristems, leaves, stems, and roots for mass propagation and production of bioactive metabolites. Secondary metabolite accumulation is genotype specific; therefore, high metabolite content producing genotypes or cell/organ lines needs to be selected for induction of in vitro cultures of medicinal plants. Various valuable medicinal compounds are biosynthesized in callus or suspension cultures, while some metabolites are produced in more organized structures like shoots, roots, glands, or somatic embryos. Callus or suspension cultures have been widely utilized for production of secondary metabolites and can be efficiently upscaled for production of important medicinal compounds under in vitro conditions (Kapoor et al. 2018; Kumar et al. 2020). Organ culture under aseptic conditions is required for biosynthesis of some medicinal compounds which need differentiated structure or organ for production of medicinal compound. For example, *Panax ginseng* roots for saponins, foliar glands of *Hypericum perforatum* for hypericins and hyperforins, *Nicotiana tabacum roots for* biosynthesis of lysine to anabasine and its leaves for conversion of anabasine to nicotine (Hussain et al. 2012). Numerous studies have been attempted on medicinal compounds production in medicinal plants using cell suspension or organ/tissue culture techniques and some of these studies have been summarized in Table 3.1. Biosynthesis of bioactive compounds under in vitro conditions is dependent on various factors including culture media composition (carbon source, macronutrients, micronutrients, other organic compounds and plant growth hormones), pH of media, explant or inoculum concentration, and other suitable environmental conditions like light, temperature, aeration, and agitation. Optimization of these factors aids in improvement of metabolite productivity. There are studies where higher metabolite content in cultured cells has been reported compared to native medicinal plants like camptothecin in *Ophiorrhiza mungos* (Deepthi and Satheeshkumar 2017), shikonin in *Onosma bulbotrichom* (Bagheri et al. 2018), and vinblastine and vincristine produced in *Catharanthus roseus* (Mekky et al. 2018). Cell/tissue culture techniques offer various advantages for medicinal compound production such as biosynthesis of secondary metabolites under in vitro conditions is more reliable, simpler, and predictable. Also, the process of extraction of valuable phytochemicals from in vitro grown cell suspensions or tissue culture plants is fast and effective in comparison to isolation from whole plants. Interfering secondary metabolites can also be avoided in cell cultures. However, cost-effective parameters need to be considered for their economic production at large scale.

3.3 Hairy Root Culture

Hairy roots are comprised of differentiated transformed roots which are produced by infection caused by *Agrobacterium rhizogenes*. T-DNA located in root-inducing (Ri) plasmid of this pathogen is transferred and integrated into the genome of host plant, thereby leading to formation of hairy roots at the wounded site. These proliferating roots show lateral branching, self-regulating rapid growth, absence of geotropism and are genetically stable (Hussain et al. 2012). Unlike natural roots, which are accessible at particular period of the year, these proliferating roots could biosynthesize bioactive compounds throughout the year without the effect of seasonal variations. Attributed to high productivity of secondary metabolites, this technique has become popular tool to produce same medicinal compounds corresponding to the wild type roots. Therefore, hairy roots provide an imperative alternative to natural plant material for biosynthesizing many valuable medicinal compounds. Production of some important medicinal compounds using this technique has been listed in Table 3.2.

3.4 Role of Elicitors and Precursor Feeding for Enhancement of Medicinal Compounds Production

Elicitors act as the signal molecules which can be biotic or abiotic in nature and are capable of inducing or enhancing the production of specific secondary metabolites by initiating defense or stress related responses. Biotic elicitors are partially purified extracts of biological origin like fungus, bacteria, yeast, or the plant itself. Some examples of biotic elicitors include polysaccharides, glycoproteins, inactivated enzymes, alginate, xanthan, yeast extract, fungal homogenate, etc. Abiotic elicitors are substances which are of non-biological origin and are categorized in physical, chemical, and hormonal factors such as UV rays, light, temperature, heavy metal salts, osmotic stress, antibiotics, jasmonates, methyl jasmonic acid, salicylic acid, acetyl salicylic acid, abscisic acid, etc. (Halder et al. 2019). They have been extensively used in cell/organ and hairy root cultures of different plant species to enhance the production of medicinal compounds. Further, they can also trigger the outflow of intracellular products and ease the extraction and purification of the targeted compounds. Application of different biotic and abiotic elicitors for production of various medicinal compounds has been summarized in Table 3.3.

The approach of supplementing the extrinsic precursors to growth medium is also used to enhance the production of bioactive compounds. For production of desired metabolites, if the endogenous levels of certain precursors are low, then supplying the precursors of key biosynthetic steps enhances the production of those metabolites (Bourgaud et al. 2001). Several reports have shown the effect of feeding suitable precursor for increasing the productivity and accumulation of target metabolites in different medicinal plants under in vitro conditions. Exogenous feeding of tryptamine and loganin has enhanced the secologanin production in mutant cell lines of *Catharanthus roseus* (Whitmer et al. 1998). In another study, exogenous

Table 3.2 Medicinal compounds produced in some plants using hairy root culture techniques

Plant name	Medicinal Compound	Properties	References
Aconitum heterophyllum	Aconites	Anticancer, anti-inflammatory, antimicrobial, pesticidal	Giri et al. (1997)
Agastache rugosa	Rosmarinate	Antioxidant, anti-inflammatory, antimutagenic, antimicrobial, antiviral, astringent	Lee et al. (2007a, b)
Angelica gigas	Deoursin	Anticancer, antibacterial, antinematodal	Xu et al. (2008)
Artemisia annua	Artemisinins	Antimalarial	Liu et al. (2002)
Astragalus mongholicus	Cycloartane saponin	Antimicrobial, antifungal	Ionkova et al. (1997)
Beta vulgaris	Betalains	Antioxidant, anti-inflammatory	Pavlov et al. (2005)
Brugmansia candida	Tropane alkaloids, hyoscyamine, scopolamine Scopolamine and hyoscyamine Scopolamine and hyoscyamine	Anti-asthmatic, anticholinergic, narcotic, anesthetic	Marconi et al. (2008)
Catharanthus roseus	Alkaloids	Anticancer	Hanafy et al. (2016)
Centella asiatica	Asiaticoside	Wound healing, memory improvement, cognition, and mood modulation	Nguyen et al. (2019)
Coleus forskohlii	Forskolin and rosmarinic acid	Anti-inflammatory and antipyretic	Li et al. (2005)
Echinacea purpurea	Alkamides	Immunostimulatory, anti-inflammatory	Romero et al. (2009)
Fagopyrum esculentum	Rutin	Antioxidant, anti-inflammatory, anticancer, antimicrobial, cardioprotective, hypolipidemic, antidiabetic, reno-protective, wound healing, anti-stress	Lee et al. (2007a, b)
Gentiana macrophylla	Gentiopicroside	Antimicrobial	Zhang et al. (2010)
Gingko biloba	Terpenoids	Antioxidant, improve blood flow to brain	Ayadi et al. (2003)
Linum flavum	Lignans	Anticancer	Renouard et al. (2018)
Lithospermum erythrorhizon	Shikonin	Antimicrobial	Tatsumi et al. (2016)
Panax ginseng	Ginsenosides	Neuroprotection, anticancer, antidiabetic, hepatoprotective, immunomodulatory	Murthy et al. (2017)

(continued)

Table 3.2 (continued)

Plant name	Medicinal Compound	Properties	References
Papaver somniferum	Morphine, sanguinarine	Analgesic, narcotic, sedative, stimulant	Le Flem-Bonhomme et al. (2004)
Picrorhiza kurroa	Picroliv	Hepatoprotective, anti-inflammatory, anticholestatic, antiulcerogenic, anti-asthmatic, antidiabetic, immunomodulatory	Verma et al. (2015)
Rauvolfia micrantha	Ajmalicine and ajmaline	Neuroprotective	Sudha et al. (2003)
Rubia akane	Alizarin and purpurin	Anticancer, antimalarial, antimicrobial, antifungal, antioxidant	Lee et al. 2010
Silybum marianum	Flavonolignan	Hepatoprotective, anticancer, antihepatitis	Rahnama et al. (2008)
Swertia japonica	Amarogenetin	Antibacterial, antihepatitis, anticholinergic, chemopreventive, anti-lieshmanial	Ishimaru et al. (1990)
Withania somnifera	Withanoloid A	Aphrodisiac, liver tonic, anti-inflammatory	Murthy et al. (2008)

supplementation of geraniol, 10-hydroxygeraniol, or loganin significantly increased tabersonine levels in hairy roots of *Catharanthus roseus* (Morgan et al. 2000). Bemani et al. (2013) have observed that application of phenylalanine to suspension cell cultures of *Corylus avellana* L. has increased the production of taxol along with the antioxidant activity and cytotoxic effects. Lately, Kumar et al. (2016) have found that exogenous supply of precursors like cinnamic acid and catalpol influences the synthesis of picroside-I in shoot cultures of *Picrorhiza kurroa*. Recently, Thakur et al. (2019) have reported enhanced picrosides content via nutrition feeding method in aeroponic and hydroponic system of *Picrorhiza kurroa*. Hence, elicitation and precursor feeding under in vitro conditions can be used as a viable tool for enhancing the production of pharmaceutically important secondary metabolites which are otherwise produced in low quantities.

3.5 Role of Endophytes in In Vitro Production of Medicinal Compounds

Endophytes are microbes that exist inside the plant living tissues without causing any negative effect to them. These microbes have the capability to biosynthesize secondary metabolites similar to that produced by their host plants (Köberl et al. 2013). They produce secondary metabolites due to the horizontal gene transfer between host and endophyte. These secondary metabolites empower them to create supremacy over other invading pathogens or enhance plant defense and consequently securing themselves against various pathogens (Mousa et al. 2016; Naik

Table 3.3 Different elicitors utilized in production of secondary metabolites in plants

Plant name	Medicinal compound	Elicitor	Culture type	References
Andrographis paniculata	Andrographolide	Methyl jasmonate	Cell suspension	Sharma et al. (2015a, b)
Amni majus	Xanthotoxin	Copper	Shoot	Purohit et al. (1995)
Ambrosia artemisiifolia	Thiarubrine A	*Protomyces gravidus*	Hairy root	Bhagwath et al. (2000)
Azadirachta indica	Azadirachtin	*Claviceps purpurea*	Hairy root	Satdive et al. (2007)
Bacopa monnieri	Bacosides	Jasmonic acid, methyl jasmonate, pH, sucrose, copper	Shoot	Naik et al. (2010), Sharma et al. (2013), Munish et al. (2015)
Catharanthus roseus	Vinblastine and vincristine	Sodium chloride	Embryogenic tissues	Fatima et al. (2015)
Corylus avellana	Paclitaxel	Sucrose	Cell suspension	Sara-Alsadat et al. (2015)
Digitalis purpurea	Digitoxin	Salicylic acid	Shoot	Patil et al. (2013)
Datura metel	Hyoscyamines, scopolamines	Salicylic acid	Root	Ajungla et al. (2009)
	Atropine	Silver, *Escherichia* coli, *Bacillus cereus, Staphylococcus aureus*	Hairy root	Zahra et al. (2015)
Echinacea pupurea	Derivatives of caffeic acid	Gibberellic acid	Hairy root	Abbasi et al. (2012)
Gymnema sylvestre	Gymnemic acid	Methyl jasmonate, salicylic acid, *Aspergillus niger, Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae*	Cell suspension	Chodisetti et al. (2013), Chodisetti et al. (2015)
Glycyrrhiza uralensis	Glycyrrhizic acid	Salicylic acid	Adventitious root	Li et al. (2015a)
Hypericum adenotrichum	Hypericin and pseudohypericin	Sucrose, polyethylene glycol	Seedling	Omer et al. (2013)
Hypericum hirsutum	Hypericin and pseudohypericin	Salicylic acid	Shoot	Coste et al. (2011)
Hypericum perforatum	Hypericin and pseudohypericin	Chitin, pectin, dextran	Shoot	Sonja et al. (2015)
	Phenylpropanoid and naphtodianthrone	Chitin, *Fusarium oxysporum, Phoma*	Cell suspension	Sonja et al. (2015)

(continued)

Table 3.3 (continued)

Plant name	Medicinal compound	Elicitor	Culture type	References
		exigua, Botrytis cinerea		
Melissa officinalis	Rosmarinate	Ozone	Shoot	Tonelli et al. (2015)
Perovskia abrotanoides	Cryptotanshinone and tanshinone IIA	Methyl jasmonate, sorbitol, silver, yeast extract	Adventitious roots	Arehzoo et al. (2015)
Picrorhiza kurroa	Picroside-I	Methyl jasmonate, sodium nitroprusside, abscisic acid, seaweed extract	Shoot	Sharma et al. (2016)
Plumbago indica	Plumbagin	Jasmonic acid	Hairy root	Gangopadhyay et al. (2011)
Plumbago rosea	Plumbagin	Jasmonic acid, yeast extract	Cell suspension	Silja et al. (2014)
Pueraria thomsnii	Puerarin	Ozone	Cell suspension	Sun et al. (2012)
Salvia castanea	Tanshinone	Methyl jasmonic acid, silver	Hairy root	Li et al. (2015b)
Salvia miltiorrhiza	Tanshinone	Methyl jasmonate, salicylic acid, gibberellic acid, *Trichoderma atroviride*	Hairy root	Yuan et al. (2008), Qianliang et al. (2013), Xiaolong et al. (2015)
Salvia officinalis	Diterpenoid	Methyl jasmonate, sodium salicylate	Shoot	Izabela et al. (2009), Kracun-Kolarevic et al. (2015)
Silybum marianum	Silymarin	Methyl jasmonate, yeast extract	Cell suspension	Firouzi et al. (2013)
Stevia rebaudiana	Steviol glycoside	Proline, polyethylene glycol	Callus and cell suspension	Pratibha et al. (2015)
Taverniera cuneifolia	Glycyrrhizic acid	Methyl jasmonate, *Mucor hiemalis, Rhizobium leguminosarum*	Root	Awad et al. (2014)
Vitis vinifera	Resveratrol, trans-resveratrol, stilbene, viniferins	UV radiations, methyl jasmonate, salicylic acid, chitin, silver, cadmium, cobalt	Cell suspension	Taurino et al. (2015), Xu et al. (2015)

(continued)

playing role in manipulating metabolic pathways to biosynthesize desired medicinal compounds. CRISPR technology can be employed in different approaches for manipulating the biosynthetic pathways which include controlling transcription factors to trigger or block specific effectors of metabolic pathways, enzyme manipulation, enzyme inhibition, blocking branch pathways, switching path to alien metabolite, removing limited availability to precursor, translational regulation, protein modification, etc. (Sabzehzari et al. 2020a, b). Alagoz et al. (2016) have knocked out benzyl isoquinoline alkaloids pathway gene, viz. *4OMT2* in *Papaver somniferum* and observed reduced morphine, thebaine levels in edited plants. Similarly, in *Salvia miltiorrhizarosmarinic acid synthase* (*SmRAS*) gene of phenolic acid metabolic pathway has been edited using CRISPR/Cas9 technology (Zhou et al. 2018). They have found declined rosmarinate and lithospermic acid B contentsin edited hairy root lines, specifically in the homozygous mutants. Additionally, enhanced level of rosmarinic acid precursor 3,4-dihydroxy phenyl lactic acid was observed. Therefore, the study suggested that the rosmarinic acid synthase enzyme is important for biosynthesis of rosmarinic acid and can be targeted to increase the level of desired metabolites.

3.7 Nanoparticles Mediated Secondary Metabolite Production

Nanoparticles are 1–100 nm sized particles, which can interfere with various signaling pathways and can modulate secondary metabolite production in plants; however, the exact mode of action behind this modulation is yet to be uncovered (Marslin et al. 2017). Zhang et al. (2013) have reported 3.9-fold enhanced artemisinin content by silver nanoparticle treatment in *Artemisia annua* L. hairy roots. In *A. thaliana*, silver nanoparticles have upregulated anthocyanin and flavonoid biosynthetic genes (Garcia-Sanchez et al. 2015). Recently, Jasim et al. (2017) have studied the consequence of silver nanoparticles treatment in *Trigonella foenum graecum* L. and observed a substantial increase in diosgenin concentration. Nanoparticles have potential to increase secondary metabolite production in plants; however, deeper understanding is still required to fully exploit this technique to produce commercially important medicinal compounds.

3.8 National and International Status of Medicinal Compounds in Commercially Important Medicinal Plants

India harbors diverse potential for medicinal plants due to its different climatic zones, geographical variance, and rich biodiversity. Medicinal compounds of these plants are used in modern therapeutic drugs throughout the world, for example, use of berberine from *Coptis japonica* as cardioprotective, hepatoprotective, and renoprotective (Morimoto et al. 1988), reserpine, serpentine from *Rauwolfia serpentina* root in hypertension (Salma et al. 2008), vinblastine and vincristine, from *Catharanthus roseus* for cancer treatment (Mekky et al. 2018). Several types of

research have been conducted by national and international research community to enhance medicinal compounds production in commercially important medicinal plants. The demand of medicinal plants is constantly increasing around the globe. China, India, Nigeria, the United States of America (USA), and WHO are significantly investing on herbal medicinal research, so that it can contribute to global health (WHO 2002).

3.9 Conclusion

Medicinal plants have been used to cure various diseases since early times. Slow growth rate and threat of extinction of some medicinal plant species along with the inaccessibility of superior plant material in suitable amounts hamper in meeting the increasing industrial demands. Biotechnological approaches provide an additional production system to prevail over inadequate availability of bioactive and commercially important medicinal compounds. Cell/tissue culture techniques aid in multiplication of medicinal plants under in vitro conditions which provide an incessant, reliable source of natural products and thus relieve pressure from their natural habitat. Advances in hairy root cultures and endophytes can lay out new modes for cost efficient, viable production of low volume pharmaceutically important secondary metabolites. Elicitation with various biotic and abiotic elicitors has been broadly practiced for production of medicinal compounds in different species and will further aid in meeting growing demand of these natural metabolites. Metabolic engineering has opened new gateways to produce important metabolites by manipulating endogenous biochemical pathways. The emergence of CRISPR/Cas system has also opened new doors towards next-generation metabolic engineering which can assist in better elucidation of secondary metabolite production in plants.

References

Abbasi BH, Stiles AR, Saxena PK, Liu CZ (2012) Gibberellic acid increases secondary metabolite production in *Echinacea purpurea* hairy roots. Appl Biochem Biotechnol 168(7):2057–2066

Ajungla L, Patil PP, Barmukh RB, Nikam TD (2009) Influence of biotic and abiotic on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metel* L. Indian J Biotechnol 8 (3):317–322

Alagoz Y, Gurkok T, Zhang B, Unver T (2016) Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in *Opium poppy* using CRISPR-Cas9 genome editing technology. Sci Rep 6:30910

Amna T, Puri SC, Verma V, Sharma JP, Khajuria RK, Musarrat J, Spiteller M, Qazi GN (2006) Bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of an anticancer alkaloid camptothecin. Can J Microbiol 52:189–196

Anderson LA, Roberts MF, Phillipson JD (1987) Studies on *Ailanthus altissima* cell suspension cultures. The effect of basal media on growth and alkaloid production. Plant Cell Rep 6:239–241

Arehzoo Z, Christina S, Florian G, Parvaneh A, Javad A, Seyed HM, Christoph W (2015) Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. Ind Crop Prod 67:97–102

Awad V, Kuvalekar A, Harsulkar A (2014) Microbial elicitation in root cultures of *Taverniera cuneifolia* (Roth) Arn. for elevated glycyrrhizic acid production. Ind Crop Prod 54:13–16

Ayadi R, Guiller JT (2003) Root formation from transgenic calli of *Ginkgo biloba*. Tree Physiol 23:713–718

Bagheri F, Tahvilian R, Karimi N, Chalabi M, Azami M (2018) Shikonin production by callus culture of *Onosma bulbotrichom* as active pharmaceutical ingredient. Iran J Pharm Res 17 (2):495–504

Baldi A, Dixit VK (2008) Enhanced artemisinin production by cell cultures of *Artemisia annua*. Curr Trends Biotechnol Pharmacol 2:341–348

Bemani E, Ghanati F, Rezaei A et al (2013) Effect of phenylalanine on taxol production and antioxidant activity of extracts of suspension-cultured hazel (*Corylus avellana* L.) cells. J Nat Med 67:446–451

Bhagwath SG, Hjortsø MA (2000) Statistical analysis of elicitation strategies for thiarubrine a production in hairy root cultures of *Ambrosia artemisiifolia*. J Biotechnol 80:159–167

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 161:839–851

Chakravarthi BVSK, Das P, Surendranath K, Karande AA, Jayabaskaran C (2008) Production of paclitaxel by *Fusarium solani* isolated from *Taxus celebica*. J Biosci 33:259–267

Chandran H, Meena M, Barupal T, Sharma K (2020) Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnol Rep 26:e00450

Chang HC, Xie HM, Lee MR, Lin CY, Yip MK, Agrawal DC, Tsay HS (2020) In vitro propagation of bulblets and LC–MS/MS analysis of isosteroidal alkaloids in tissue culture derived materials of Chinese medicinal herb Fritillaria cirrhosa D. Don. Bot Stud 61:9

Chattopadhyay S, Srivastava A, Bhojwani SVS, Bisaria (2001) Development of suspension culture of *Podophyllum hexandrum* for production of podophyllotoxin. Biotechnol Lett 23:2063–2066

Chodisetti B, Rao K, Gandi S, Giri A (2013) Improved gymnemic acid production in the suspension cultures of *Gymnema sylvestre* through biotic elicitation. Plant Biotechnol Rep 7:519–525

Chodisetti B, Rao K, Gandi S, Giri A (2015) Gymnemic acid enhancement in the suspension cultures of *Gymnema sylvestre* by using the signaling molecules—methyl jasmonate and salicylic acid. In Vitro Cell Dev Biol Plant 51:88–92

Coste A, Vlase L, Halmagyi A, Deliu C, Coldea G (2011) Effects of plant growth regulators and elicitors on production of secondary metabolites in shoot cultures of *Hypericum hirsutum* and *Hypericum maculatum*. Plant Cell Tiss Org Cult 106:279–282

Cui Y, Yi D, Bai X, Sun B, Zhao Y, Zhang Y (2012) Ginkgolide B produced endophytic fungus (*Fusarium oxysporum*) isolated from *Ginkgo biloba*. Fitoterapia 83:913–920

Deepthi S, Satheeshkumar K (2017) Cell line selection combined with jasmonic acid elicitation enhance camptothecin production in cell suspension cultures of *Ophiorrhiza mungos* L. Appl Microbiol Biotechnol 101:545–558

Deng BW, Liu KH, Chen WQ, Ding XW, Xie XC (2009) *Fusarium solani*, Tax-3, a new endophytic taxol-producing fungus from *Taxus chinensis*. World J Microbiol Biotechnol 25:139–143

Elavarasi A, Rathna GS, Kalaiselvam M et al (2012) Taxol producing mangrove endophytic fungi *Fusarium oxysporum* from *Rhizophora annamalayana*. Asian Pac J Trop Biomed 2:1081–1085

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J Nat Prod 69(8):1121–1124

Fatima S, Mujib A, Dipti T (2015) NaCl amendment improves vinblastine and vincristine synthesis in *Catharanthus roseus*: a case of stress signalling as evidenced by antioxidant enzymes activities. Plant Cell Tiss Org Cult 121:445–458

Firouzi A, Mohammadi SA, Khosrowchahli M, Movafeghi A, Hasanloo T (2013) Enhancement of silymarin production in cell culture of *Silybum marianum* (L) Gaertn by elicitation and precursor feeding. Int J Geogr Inf Syst 19(3):262–274

Gangopadhyay M, Dewanjee S, Bhattacharya S (2011) Enhanced plumbagin production in elicited *Plumbago indica* hairy root cultures. J Biosci Bioeng 111:706–710

Gao SL, Zhu DN, Cai ZH, Jiang Y, Xu DR (2004) Organ culture of a precious Chinese medicinal plant – *Fritillaria unibracteata*. Plant Cell Tiss Org Cult 59:197–201

García-Granados R, Lerma-Escalera JA, Morones-Ramírez JR (2019) Metabolic engineering and synthetic biology: synergies, future, and challenges. Front Bioeng Biotechnol 7:36

Garcia-Sanchez S, Bernales I, Cristobal S (2015) Early response to nanoparticles in the *Arabidopsis* transcriptome compromises plant defence and root-hair development through salicylic acid signalling. BMC Genomics 16:341

Garyali S, Kumar A, Reddy MS (2013) Taxol production by an endophytic fungus, *Fusarium redolens*, isolated from Himalayan yew. J Microbiol Biotechnol 23(10):1372–1380

Giri A, Banerjee S, Ahuja PS, Giri CC (1997) Production of hairy roots in *Aconitum heterophyllum* wall using *Agrobacterium rhizogenes*. In Vitro Cell Dev Biol Plant 33:280–284

Grotewold E (2008) Transcription factors for predictive plant metabolic engineering: are we there yet? Curr Opin Biotechnol 19:138–144

Grzegorczyk I, Wysokinska H (2008) Liquid shoot culture of *Salvia officinalis* L. for micropropagation and production of antioxidant compounds: effect of triacontanol. Acta Soc Botanicorum Poloniae 73:99–104

Halder M, Sarkar S, Jha S (2019) Elicitation: a biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. Eng Life Sci 19:880–895

Hanafy MS, Matter MA, Asker MS, Rady MR (2016) Production of indole alkaloids in hairy root cultures of *Catharanthus roseus* L. and their antimicrobial activity. South Afr J Bot 105:9–18

Hirasuna TJ, Pestchanker LJ, Srinivasan V et al (1996) Taxol production in suspension cultures of *Taxus baccata*. Plant Cell Tiss Org Cult 44:95–102

Hohtola A, Jalonen J, Tolnen A, Jaakola L, Kamarainen T, Pakonen M, Karppinen K, Laine K, Neubauer P, Myllykoshi L, Gyorgy Z, Rautio A, Peltonen O (2005) Natural product formation by plants, enhancement, analysis, processing and testing. In: Jalkanen A, Nygren P (eds) Sustainable use renewable natural resources – from principles to practices. University of Helsinki Publication, Helsinki, pp 34–69

Hu K, Tan F, Tang K et al (2006) Isolation and screening of endophytic fungi synthesizing taxol from *Taxus chinensis* var. mairei. J Southwest China Normal Univ Nat Sci Edit 31:134–137

Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M (2012) Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci 4(1):10–20

Ionkova I, Karting T, Alfermann W (1997) Cycloartane saponin production in hairy root cultures of *Astragalus mongholicus*. Phytochemistry 45:1597–15600

Ishimaru K, Sudo H, Salake M, Malsugama Y, Hasagewa Y, Takamoto S, Shimomura K (1990) Amarogenetin and amaroswertin and four xanthones from hairy root cultures of *Swertia japonica*. Phytochemistry 29:1563–1565

Izabela G, Halina W (2009) The effect of methyl jasmonate on production of antioxidant compounds in shoot cultures of *Salvia officinalis*L. Herba Polonica 55:238–243

Jasim B, Thomas R, Mathew J, Radhakrishnan EK (2017) Plant growth and diosgenin enhancement effect of silver nanoparticles in fenugreek (*Trigonella foenum-graecum* L.). Saudi Pharm J 25:443–447

Kapoor S, Raghuvanshi R, Bhardwaj P, Sood H, Saxena S, Chaurasia OP (2018) Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of *Rhodiola imbricata*. J Photochem Photobiol B Biol 183:258–265

Karimi A, Majlesi M, Rafieian-Kopaei M (2015) Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol 4(1):27–30