Plant Cell, Tissue and Organ Culture (PCTOC) (2022) 149:1–6
https://doi.org/10.1007/s11240-022-02289-2

PREFACE

Secondary metabolites: a boon from plants, the best chemist in nature: preface from the editors

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Published online: 28 March 2022

Plants synthesize a wide spectrum of specialized and often complex secondary metabolites (aka specialized metabolites), whose structures are sometimes difficult to reproduce synthetically. Most such metabolites do not necessarily participate in maintaining many of the fundamental plant life processes but could still play a central role in the interactions of plants with their changing environment. Certain metabolites possess antimicrobial or insecticidal activities that deter predators, and various allelopathic traits that may discourage competing weeds while, contrastingly, some secondary metabolites may attract pollinators or symbionts through their chromatic, olfactory, or chemotactic properties. Other than their direct impact for the plant, the secondary metabolites hold a paramount interest for human activities as flavors, fragrances, dyes, pesticides, antioxidants, or pharmaceuticals.

Recently, there is a global upsurge in the use of plants as a major source of complex bioactive molecules of pharmaceutical and cosmetic interest, given the difficulty in their chemical synthesis that renders this non-profitable. Consequently, most of these bioactive compounds are isolated from the wild, leading to an overexploitation of wild specimens, or sometimes from cultivated plants that may have low accumulation rates of the product of interest, often due to an incomplete knowledge of conditions most favorable for their biosynthesis. In this respect, in vitro biotechnology approaches may significantly improve the production and use of secondary metabolites. This special issue of Plant

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Cell Tissue and Organ Culture includes several reviews and original articles on various aspects of the development of biotechnology-based approaches for the increased biomass production and better understanding of the production and synthesis of secondary metabolites by in vitro cell and tissue cultures of medicinal plants.

Bhaskar et al. (https://doi.org/10.1007/s11240-021-02131-1) reviewed the state of the art on the use of biotic elicitors to foster the production of plant secondary metabolites in vitro, and in particular of those produced naturally as a defense mechanism to face environmental stress factors. Elicitors are chemicals that activate certain transcription factors (TFs) and upregulate the genes that trigger biosynthetic pathways thereby increasing secondary metabolite production. The authors extensively discussed the mechanism of biotic elicitation and surveyed the applications of bacterial, fungal, and algal elicitors, and of polysaccharides extracted from them. Examples included an increased genistein production due to the addition of *Rhizobium rhizogenes* elicitors and of diosgenin production by *Escherichia coli* elicitors, while fungal elicitors as *Aspergillus niger* increased thiophene production and *Botrytis* sp. sanguinarine production. Likewise, extracts of the algae *Haematococcus pluvialis* increased betalain production and those of *Botryococcus braunii* elicited vanillin, vanillylamine and capsaicin.

Luthra et al. (https://doi.org/10.1007/s11240-021-02187-z) reviewed the pharmaceutical applications and production enhancement strategies of centellosides from *Centella asiatica*, with a broad range of therapeutic properties. *C. asiatica* is under increasing danger of rapid depletion due to the increasing demand of its triterpenoid secondary metabolites and alternative sources of supply are required for an improved production of centellosides, including shoot culture, callus and cell suspension, and hairy root cultures. These were reviewed as also various culture methods like using elicitors and precursors to enhance centelloside production or commercial scale up in bioreactors.

Withania somnifera is a widely distributed solanaceous species of medicinal interest, with multiple pharmacological properties thanks to the secondary metabolites withanolide A, withanolide D, withaferin A, and withanone. This species is extensively used in the herbal industry which renders urgent to rapidly multiply elite cultivars, to develop and exploit alternative tissue sources, and also to fine-tune the requirements for an optimized production of withanolides. These subjects were comprehensively reviewed by K. Kaur et al. (https://doi.org/10.1007/s11240-021-02225-w).

Jakovljević et al. (https://doi.org/10.1007/s11240-022-02286-5) reviewed the literature on secondary metabolite production from cultures of different species of *Ocimum* L., basil, which they called "the king of herbs". Basils are widely cultivated due to their large content of essential oils, phenolic acids, isoprenoids, and flavonoids. This manuscript critically surveyed the available data about the mass propagation, somatic embryogenesis, cell, and organ cultures of basil as well as the conditions for an enhanced synthesis of its secondary metabolites under tissue culture conditions. They evoked the limited information regarding the genotypes used or the origin of the plant material studied and underlined the importance of completing this gap to design better protocols to further enhance the production of secondary metabolites in basil cultures.

Alzheimer's disease is a reason for increasing concern worldwide and recent medical studies have shown that acetylcholinesterase inhibitors can be used for its treatment. Galanthamine, a selective and reversible inhibitor, is a substance of choice and H. Kaur et al. (https://doi.org/10.1007/s11240-022-02229-0) reviewed the various biotechnological strategies that may be used for an optimized production of this alkaloid. Galanthamine is produced in low quantities by several species in the Amaryllidaceae, and in vitro culture offers an alternative tool for its sustainable production besides being environmentally sustainable and protecting the native biodiversity in a circular bioeconomy context. The authors discussed the manipulation of plant growth regulators, photoperiod, elicitors, and bioreactors systems, in view of the efficient scaling-up of galanthamine production.

Timely, Dou and Weathers (https://doi.org/10.1007/s11240-022-02287-4) examined the regulatory considerations for specialty plant molecules and in vitro cultures which have made their way into the pharmaceutical market as new drugs. Examples include paclitaxel and ginseng-derived compounds, but the list is not long, often due to regulatory barriers, and the authors discussed the potential of specialty molecules to be provided as dietary supplements, nutraceuticals, herbal medicines, botanical drugs, and pure molecules, with a focus on the United States Food and Drug Administration (US FDA) regulatory categories, that could prove useful for taking such specialty molecules to the global market.

These first broad-spectrum articles are followed by several more specific manuscripts addressing various aspects of the tissue culture methodology applied and its impact on the production of different secondary metabolites of interest.

Thus, Klimek-Szczykutowicz et al. (https://doi.org/10.1007/s11240-021-02148-6) examined the effect of varying wavelengths of LED light on biomass growth and on the production of glucosinolates and phenolic compounds, as well as on the production of photosynthetic pigments and soluble sugars, and on the antioxidant potential of in vitro cultures of watercress (*Nasturtium officinale*). They showed that 50% yellow, 35% red and 15% blue light LEDs were significantly beneficial for biomass growth and glucosinolates production, while phenolics, photosynthetic pigments, sugars, and the antioxidant potential were comparable to the control cultures.

Biswal et al. (https://doi.org/10.1007/s11240-022-02274-9) also assessed LED lights to elicit biomass accumulation, antioxidant activity, pigment concentration and secondary metabolite production in callus culture of *Operculina turpethum* (L.). However, in this case monochromatic blue light was best, compared with callus grown in the dark or under control light conditions, and enhanced the synthesis of total phenolics and flavonoids, showing also the highest in vitro antioxidant activities. The highest production of gallic acid, quercetin, coumarin, and salicylic acid in callus occurred under blue light.

Ahmed et al. (https://doi.org/10.1007/s11240-022-02239-y) developed a novel surface sterilization strategy for in vitro culture of the endangered seaweed *Sargassum fusiforme*, using crude extracts of *Artemisia dracunculus* which has natural and medicinal properties against contaminating microbes. The *A. dracunculus* extract was less toxic to plant tissues and overperformed ethanol as well as various chemical sterilants and antibiotics. Its antimicrobial power may be due to several newly identified (by UPLC-QTOF MS analysis) compounds, among which monoethyl acetylmalate was the most abundant followed by sodium dodecenate, cinnzeylanone and trichilin A.

Sethy and Kullu (https://doi.org/10.1007/s11240-022-02231-6) reported on the micropropagation of two common ethnomedicinal species of *Calotropis*, *C. procera* and *C. gigantea*, coupled with a better production of stigmasterol R, that will help the large-scale production of this pharmaceutically significant bioactive metabolite without depleting natural populations of *Calotropis* sp.

Sharma et al. (https://doi.org/10.1007/s11240-022-02288-3) optimized a single step protocol for high frequency propagation through rhizome buds of *Podophyllum hexandrum* Royle, performed molecular analysis using SCoT markers to assess the genetic stability of produced plants, and analysed podophyllotoxin production by HPLC in roots, leaves and callus produced in vitro. The mother plants were similar to their tissue culture-derived progeny, and podophyllotoxin production, that was maximum from in vitro roots and root calli, could be increased further by elicitation with methyl jasmonate (MeJA).

Liu et al. (https://doi.org/10.1007/s11240-021-02215-y) developed suspension cultures for red *Cyclocarya paliurus* cells and studied through metabolomics the effects of hormones on anthocyanin biosynthesis. They identified and quantified 23 compounds in cells, among which the content of cyanidin-3-O-galactoside and cyanidin-3-O-glucoside was significantly increased compared to the control. Also, the optimized hormone combination upregulated the expression levels of key genes in the anthocyanin biosynthesis pathway including flavonoid 3′-hydroxylase (*F3'H*), dihydroflavonol 4-reductase (*DFR*), leucocyanidin reductase (*LAR*), leucoanthocyanidin dioxygenase (*LDOX*), and anthocyanin-3-O-glucosyltransferase (*3GT*), and TFs (*MYB5*, *MYB86*, *ERF003*, *ERF024*, and *bZIP44*), and downregulated the expression levels of the pathway genes flavonoid 3′,5′-hydroxylase (*F3'5'H*) and anthocyanin reductase (ANR) and TFs (*MYB4*, *MYC2*, and *bHLH137*).

Strigolactones have been recently described as a new group of plant hormones. In an innovative work, Duran and Issah (https://doi.org/10.1007/s11240-021-02212-1) analysed the effect of using synthetic strigolactone GR24 alone and combined with NAA and BAP on the callus growth and phenolic compound contents in caper (*Capparis spinosa* L.). Media with strigolactone GR24 at 0.1 µM favored the highest callus production, coupled with the highest contents of rutin, quercetin and chlorogenic acid. In addition, aromatic compounds in caper calluses were grouped as sulfur compounds, aldehydes, ketones, hydrocarbons and derivatives, alcohols, and others, their amounts varying dependent on hormone treatment, with an optimum at 0.2 µM GR24.

Chatterjee et al. (https://doi.org/10.1007/s11240-022-02237-0) reported on the regeneration in vitro of *Piper longum* L., a well-known spice of the family Piperaceae with high pharmacognosy potential. They performed a comparative RP-HPLC analysis of piperine production of in vitro and in vivo grown plants. Plants were regenerated from internode callus by somatic embryogenesis as shown by a detailed field emission scanning electron microscopic study. The quantification of piperine in root and fruit of both in vitro and in vivo grown plants revealed the maximum amount in the fruit of in vitro produced plants.

Tomilova et al. (https://doi.org/10.1007/s11240-022-02271-y) developed callus and cell suspension cultures of the two rare foxglove species, *Digitalis grandiflora* Mill. and *D. ciliata* Trautv. S.V., using leaves, cotyledons and hypocotyls from in vitro seedlings as initial explants. Leaf explants were the most responsive although with differences between the two species. The authors identified ten bioactive compounds using UPLC-ESI-Q-TOF–MS, and the major constituents were phenylethanoid glycosides and steroid furostanol glycosides, whose production remained stable even after two years of cultivation in vitro.

In an innovative work, Abdelsalam et al. (https://doi.org/10.1007/s11240-021-02202-3) characterized the metabolic differences between embryogenic and organogenic calli and their regenerated shoots in *Cymbopogon schoenanthus* subsp. *proximus* using nuclear magnetic resonance. This technique allowed them to identify 52 bioactive metabolites, some of which were unique to somatic embryo-derived (serine and lactate) and organogenesis-derived shoots (2-hydroxyisobutyrate, tyrosine, histamine and homoserine). Then, they compared callus from the two regeneration pathways and showed that proline, asparagine and arginine were increased in embryogenic callus while sucrose was augmented in organogenic callus. On the other hand, within

embryogenic tissues, monosaccharides were increased in callus, and proline, pyroglutamate and 4-aminobutyrate in shoots. Likewise, in organogenic tissues, trigonelline was significantly increased in shoots, and monosaccharides in callus. In comparisons among shoots 4-aminobutyrate, betaine and proline were higher in embryogenic shoots, while mono- and disaccharides were increased in organogenic shoots. Finally, the embryogenic system was characterized by an accumulation of stress related metabolites (i.e., proline), while glycolate was identified only in the organogenic material.

Scaling-up of the production of secondary metabolites is a prerequisite for their commercial exploitation by pharmaceutical, medicinal or cosmetic industries, and a few articles in this special issue were devoted to the study and optimization of culturing of tissues of various plants of interest for these industries in bioreactors.

Amini et al. (https://doi.org/10.1007/s11240-022-02233-4) carried out one such study with saffron, to increase cell biomass and crocin production, where they compared cultures kept in Erlenmeyer flasks and in a stirred bioreactor. First, they examined the buffering effects of various concentrations of 2-(N-morpholino) ethanesulfonic acid (MES) and of sucrose in Erlenmeyer flasks and, once, these were optimized they continued their research in a stirred bioreactor to evaluate the effect of aeration and pH medium adjustment. In Erlenmeyer flasks, the highest cell biomass and crocin production occurred when the medium was buffered with 2.5 mM of MES and sucrose was increased gradually from 3 to 6%. The stirred bioreactor experiments proved that constant pH (5.8) during the growth period was a limiting factor. The same was true for aeration that inhibited the production of crocin, due to an excessive medium evaporation, but could apparently be countered if the evaporated water was replaced.

Steviol glycoside (SG), the natural sweetener present in *Stevia rebaudiana* Bertoni, is a diterpenoid secondary metabolite synthesized from ent-kaurenoic acid, which is also the precursor of gibberellin (GA). Saptari et al. (https://doi.org/10.1007/s11240-022-02276-7) used the GA inhibitor, Daminozide to block conversion of ent-kaurenoic acid towards GA synthesis to increase SG content of stevia propagated in temporary immersion bioreactor. At 10 mg/L, Daminozide increased biomass weight and SG content (stevioside and rebaudioside A) up to 40%, and also the transcripts accumulation of genes implicated in SG biosynthesis (*SrKA13H*, *SrUGT85C2*, and *SrUGT76G1*). This was more active due to the inhibition of GA pathway, evidenced by the upregulated expression of GA biosynthesis gene (*GA3ox*) due to feedback regulation, and the downregulated expression of GA catabolism gene (*GA2ox2*) as a result of feedforward regulation provoked by the Daminozide treatment.

Using the Plantform bioreactor, Grzegorczyk-Karolak et al. (https://doi.org/10.1007/s11240-021-02168-2) optimized the cultivation conditions of shoots to increase polyphenol production of the sage species, *Salvia viridis* L. They evaluated the effect of culture duration, use of a semi-continuous system and immersion frequency in liquid medium on the growth and accumulation of secondary metabolites (determined by HPLC). Optimum growth was observed for shoots grown in a fed-batch system and immersed every 80 min, while production of bioactive compounds was best for shoots grown for three weeks in a batch system, immersed every 80 min. Within three weeks in these conditions total phenolic acid level was almost 10-times greater than that found in the aerial parts of four-month-old soil-grown plants with a similar phenylethanoid level.

Irrespective of the species and secondary metabolite being studied, it is important to ensure the conservation of medicinal plants, particularly when overharvesting of wild material puts its future in jeopardy. Mishra et al. (https://doi.org/10.1007/s11240-022-02244-1) developed a method for conservation of germplasm of *Nyctanthes arbor-tristis* L., an antiviral medicinal plant in the Oleaceae, by encapsulation and slow growth conditions. A gel matrix with 3% sodium alginate and 100 mM calcium chloride was best for encapsulation of nodal segments, and their storage at 4 C on one-eighth strength Murashige and Skoog (MS) medium with 0.5% sucrose permitted their conservation for up to 180 days, with little impact on the viability and recovery of rooted plants capable of field survival. Importantly, the clonal fidelity of such in-vitro derived plantlets was ascertained with start codon targeted primer profile.

Adventitious roots as well as hairy root cultures have frequently been studied as an interesting source tissue to produce secondary metabolites, and several articles in this special issue dealt with this kind of culture.

Demirci et al. (https://doi.org/10.1007/s11240-021-02173-5) studied the effect of L-phenylalanine and culture duration on the root growth and production of tropane alkaloids and phenolics in adventitious root cultures of *Hyoscyamus niger* L., derived from petiole explants of in vitro seedlings. Scopolamine and hyoscyamine amounts were highest in the cultures treated with 0.50 mM L-phenylalanine for 3 days, while 0.50 mM and 1.00 mM L-phenylalanine for one and 3 days were best for phenolics contents.

Valdevite et al. (https://doi.org/10.1007/s11240-021-02214-z) examined the effect of exogenous inhibitors on the biosynthesis of anticancer quinonemethide triterpenes by root cultures of *Monteverdia foribunda*. The inhibitors tested were terbinafne hydrochloride, ancymidol, paclobutrazol, gibberellin (GA), and the association of GA + paclobutrazol. Ancymidol, GA, and GA + paclobutrazol favored the best elicitation results, while GA had a positive effect on the

production of terpenoids from the mevalonate pathway, via which maytenin biosynthesis occurs.

Panax ginseng contains two major bioactive compounds, ginsenosides Rg1 and Re, and the key enzymes in their biosynthesis are Dammarendiol synthase (DDS) and protopanaxatriol synthase (PPTS). Li et al. (https://doi.org/10.1007/s11240-021-02222-z) investigated the role of ABA on ginsenoside biosynthesis in adventitious roots of gingseng at 5 °C. Cold stress induced an accumulation of ginsenosides Rg1 and Re, increased expression levels of *DDS* and *PPTS* genes, and triggered the accumulation of ABA prior to ginsenosides Rg1 and Re accumulation. Inhibiting ABA biosynthesis with nordihydroguaiaretic acid (NDGA) in cold-exposed ginseng roots down-regulated *DDS* and *PPTS* expression levels and suppressed the accumulation of ginsenosides Rg1 and Re but was restored by exogenous addition of ABA such roots. These results indicated that ABA can be used as an elicitor for production of ginsenosides Rg1 and Re in ginseng adventitious roots.

Using adventitious roots of *Andrographis paniculata* (Burm. F.) Nees, Srinath et al. (https://doi.org/10.1007/s11240-022-02241-4) examined the contribution of two biosynthetic pathways (MVA and MEP) towards the accumulation of andrographolide as influence by light, and developed an alternative strategy for mass cultivation of plants to enhance production of secondary metabolites in general and diterpene lactones, andrographolide in particular. Root culturing on a platform without direct immersion in liquid medium was best for improved biomass generation. Elicitation of adventitious roots with ethrel enhanced both biomass and andrographolide content. Under light, the MEP pathway was dominant for andrographolide biosynthesis via the up regulation of the *DXR*, *DXS*, *HDR*, *HDS* genes as well as down-stream *GGPS* gene expression. In dark, MVA pathway was the main contributor for andrographolide.

Reyes-Pérez et al. (https://doi.org/10.1007/s11240-021-02162-8) studied the production of anti-inflammatory compounds in hairy roots of the traditional Mexican medicinal plant *Sphaeralcea angustifolia*, whose properties are attributed mainly to scopoletin. *Agrobacterium rhizogenes* ATCC15834/pTDT enabled a high transformation frequency from nodal segments and leaves of 2-month-old plantlets. One of the seven hairy root lines selected exhibited a sphaeralcic acid content 440-fold higher than that found in *S. angustifolia* wild plants and also much higher than sphaeralcic acid production by *S. angustifolia* cell suspensions in flasks or in a stirred tank bioreactor. Even after 2 years in culture, such hairy roots were still productive, and they could be stressed by nitrate reduction and/or copper to further stimulate the scopoletin and sphaeralcic acid production.

Also working with hairy root cultures, Maciel et al. (https://doi.org/10.1007/s11240-021-02201-4) examined the enhancement of bioactive cafeoylquinic acid derivative production by jasmonates in *Eclipta prostrata* L. The antioxidant, anti-inflammatory and anticancer activities of extracts of this species have been ascribed to various phenylpropanoids, including flavonoids, coumestans and the cafeoylquinic acid derivatives wedelolactone, demethylwedelolactone and 3,5-di-O-cafeoylquinic acid (recently reported to have anti Covid-19 activity too). All of these were increased after addition of jasmonic acid or MeJA as eliciting agents.

MeJA, at 100 µM, was also shown to increase the accumulation of rosmarinic acid, total phenolics and total flavonoids in hairy root cultures of *Prunella vulgaris* by Ru et al. (https://doi.org/10.1007/s11240-022-02273-w) When checking the expression levels of gene transcripts associated with the rosmarinic acid synthetic pathway, correlation analysis showed that its accumulation is significantly correlated with the transcripts expression of *PvHPPR*, *PvPAL*, *PvC4H*, *Pv4CL1*, *Pv4CL2*, and *PvCYP98A101* genes, but not with the transcripts of *PvTAT* and *PvRAS* genes. These results pave the way for the use of MeJA as an efficient elicitor for a scale-up of hairy root cultures of *P. vulgaris* for an increased yield of rosmarinic acid in a bioreactor system.

As evoked at the onset of this Preface, the use of elicitors/inhibitors was shown to significantly impact the efficiency of the production of secondary metabolites and, in this context, several articles in this Special Issue were devoted to this area of research.

Shahkarami et al. (https://doi.org/10.1007/s11240-021-02213-0) targeted biotic elicitation of *Scrophularia striata* with *Piriformospora indica* to induce the production of phenylethanoid glycosides as well as defense responses in cell cultures. Such biotic elicitation activated antioxidant enzymes (e.g., superoxide dismutase and guaiacol peroxidase) thereby regulating the oxidative status of cells in response to the fungal culture filtrate via a shift in primary and secondary metabolites profile towards the accumulation of defense compounds. Such reprogramming resulted in an increased level of different precursor molecules coupled with an increased level of sucrose and defensive compounds flavonoids, and of the phenylethanoid glycosides echinacoside and acteoside.

In a simple, yet original study, Kakade et al. (https://doi.org/10.1007/s11240-022-02242-3) described how the use of extract from the seaweed *Sargassum ilicifolium* enhanced seed germination and mass propagation in vitro, while it also improved the accumulation of plumbagin in *Plumbago zeylanica* L. The protocol developed is reproducible and cost-effective; more importantly, it shows for the first time the use of *S. ilicifolium* extract as an alternative to conventional plant growth regulators with a potential to improve both growth and bioactive compound accumulation.

On the other hand, Khattab et al. (https://doi.org/10.1007/s11240-021-02224-x) used silicon dioxide and silver

nanoparticles to enhance the growth and multiplication of lavender (*Lavandula officinalis*) in vitro plants and to elicit the production of antimicrobial secondary metabolites. The soothing and antimicrobial properties of lavender essential oil are due to beta-linalool, cineol, camphor, and many other bioactive compounds. Addition of 50 mg/L Si nanoparticles significantly improved explant multiplication, while 20 mg/L Ag nanoparticles were less efficient. In addition, extract from plantlets grown in presence of 100 mg/L Si nanoparticles exhibited the highest activity against *Escherichia coli* and *Staphylococcus aureus*, while the highest antimicrobial activity against *S. pneumoniae* was seen with extract from plantlets grown without nanoparticles.

Shoja et al. (https://doi.org/10.1007/s11240-022-02251-2), in turn, first optimized callus proliferation from the apical meristems and leaves of *Salvia tebesana* and thereafter assessed the use of nano-TiO_2 and MeJA as elicitors to enhance the production of phenolic compounds, rosmarinic acid (RA), and various flavonoids as well as the antioxidant capacity of callus. They found that elicitation with 10 and 60 mg L^{-1} nano-TiO_2 (for apical meristem and leaf, respectively), and 50 µM MeJa significantly promoted the content of total phenolics, *O*-diphenols, phenolic acid, flavonoid, flavone and the flavonol, proanthocyanidin in *S. tebesana* calli. Interestingly, nano-TiO_2 treatment was best for the production of RA, quercetin and rutin, while MeJa elicited the highest content of apigenin, all exhibiting a strongly positive correlation with antioxidant activity (DPPH and FRAP).

Also studying a sage species, *Salvia abrotanoides* (Kar.) Sytsma, rich in phenolic acids, and RA in particular, Rostami et al. (https://doi.org/10.1007/s11240-022-02252-1) showed the stimulatory effects of sodium nitroprusside (SNP), a donor of nitric oxide (NO), on their accumulation in shoot cultures. The highest RA content occurred 144 h after elicitation of shoots with 100 µM SNP, while for salvianolic acid A and B maximum values were obtained with shoot exposure to 50 and 25 µM SNP, respectively. SNP elicitation was also shown to significantly upregulate various crucial genes (*PAL*, *TAT*, *RAS* and *CYP98A14*) involved in phenolic acids biosynthesis, although without a correlation with phenolic acids accumulation.

Tragacanth's (*Astragalus gossypinus*) richness in phenolic compounds, saponins, and polysaccharide gum is endangering the plant due to uncontrolled collection from the wild. Therefore, Maassoumi et al. (https://doi.org/10.1007/s11240-022-02253-0) developed cell suspensions as an alternative source of bioactive compounds of interest and examined the impact of selenium on the production of gum tragacanth and secondary metabolites (phenolic and saponin compounds). A supply of more than 2.5 µM Se significantly increased soluble sugar and amino acid content, as well as the total amount of exopolysaccharides, and the enzymatic and non-enzymatic radical scavenging system components. Moreover, glutathione was the main non-enzymatic compartment that functions in an intricate network, where the increased oxidant scavenging capacity was initiated by caffeic acid, tannins, and phlobaphene while salicylic acid increased saponin production.

A mutant in vitro seedling of *Atractylodes lancea* (Thunb.) DC. with early stem growth and higher sesquiterpenoid content than the wild type was used to study the mechanisms underlying such efficient sesquiterpenoids synthesis in this mutant by Wang et al. (https://doi.org/10.1007/s11240-022-02240-5). The photosynthetic efficiency, central carbon metabolism efficiency, and energy metabolism efficiency were significantly improved in the mutant compared with wild type *A. lancea* plants, concomitant with a significant change in the content of endogenous hormones, such as gibberellin and jasmonic acid. All of those responses were coupled with significantly higher levels of key metabolites and expression level of key genes in the mevalonate and 2-C-methyl-D-erythritol-4-phosphate pathways.

Finally, Kashani et al. (https://doi.org/10.1007/s11240-022-02279-4) improved the production of paclitaxel, a diterpenoid broadly used for anticancer treatment, by transforming *Taxus baccata* in order to remove the bottleneck identified for paclitaxel production. For this purpose, they first integrated the full length coding sequence of gene *DBTNBT* downstream of the CaMV 35S promoter (pCAMBIA1304-DBTNBT) and transiently expressed it in the *Taxus* leaves via *Agrobacterium tumefaciens* and vacuum infiltration. Thus, transient overexpression of the DBTNBT gene, associated with dual elicitation with methyl-β-cyclodextrin and coronatine resulting in 7.4-fold more paclitaxel production compared with the no-inoculation/no-elicitation control. They also observed an increased expression level for several genes including *DBTNBT*, followed by *ABC* and *BAPT* genes.

Depletion of natural stands of medicinal plants in the wild is critical and, in this sense, looking for innovative answers is a valid resource. Secondary metabolites production in vitro is part of this search as proven by the number of recent publications on this topic in recent times. In this respect, this SI set out to provide the latest advances in Secondary Metabolism and Medicinal Plant Biotechnology in species known for their valuable phytochemicals like sage, ginseng or saffron, to others that are part of traditional vernacular medicines such as *Ajowa* sp., *Hancornia* sp. or *Sphaeralcea* sp.