



Role of Secondary Metabolites and Prospects of Engineering Secondary Metabolite Production for Crop Improvement

Rakhi Chakraborty

Abstract

Plant secondary metabolites impart significant contributions in defense, stress tolerance, reproduction and are also involved in plant growth regulation and crop yield. They are of diverse chemical nature and biological functions, depending on their biosynthesis within a plant cell. Major classes of plant secondary metabolites that help regulate plant growth and development include—phytohormones, phenolics, terpenoids, nitrogen-containing compounds (alkaloids, non-protein amino acids and cyanogenic glycosides) and sulphur-containing compounds (glucosinolates, phytoalexin, defensin, etc.). They are synthesized in a relatively small amount within plant cells and exert their specific functions through several complex signaling pathways. Large-scale production of plant secondary metabolites is thus considered as one of the important strategies for crop improvement. Application of physical (UV-rays, gamma radiation, etc.), chemical (salicylic acid, jasmonic acid, melatonin, etc.) and biological elicitors (cyanobacteria, fungal and bacterial species), induction of polyploidy, nanotechnological approaches, epigenetic modification (gene overexpression, gene silencing, histone modification, etc.), transgenesis (homologous or heterologous transfer of one or more genes of specific secondary metabolite biosynthetic pathway), transcriptional and post-transcriptional regulation, etc., have been successfully applied in several crop plants for the increased production of desired secondary metabolites. However, most of the studies remain confined to the laboratory scale due to several constraints, viz. altered expression pattern, genetic instability, bioavailability, bioactivity, lack of desired quality and quantity of the products, etc. In this chapter, specific functions of plant secondary metabolites regulating the growth and development of crop plants have been

thoroughly discussed. Moreover, the engineering strategies for large-scale production of secondary metabolites along with the challenges have also been elaborated.

Keywords

Cellular signaling • Crop improvement • Genetic engineering • Metabolic regulation • Nanotechnology • Plant secondary metabolites

Abbreviations

ABA	Abcisic acid
AP2/ERF	APETALA 2/ethylene-responsive element binding factor
APX	Ascorbate peroxidase
BABA	β -Aminobutyric acid
BR	Brassinosteroid
CAT	Catalase
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9
DELLA	Aspartate-glutamate-leucine-leucine-alanine
DREB	Dehydration-responsive element-binding protein
2,4-D	2,4-Dichlorophenoxyacetic acid
ETR1	Ethylene receptor 1
ERS1	Ethylene response sensor 1
GABA	γ -Aminobutyric acid
GR	Glutathione reductase
GSA1	Grain size and abiotic stress tolerance1
HY5	Elongated hypocotyl 5
HLS1	Hookless 1
JA	Jasmonic acid
MYB	My elob lastosis
NO	Nitric oxide
ODC	Ornithine decarboxylase
ORCA3	Octadecanoid-derivative responsive Catharanthus APETALA2-domain

R. Chakraborty (✉)

Department of Botany, Acharya Prafulla Chandra Roy Government College, Himachal Vihar, Matigara, Siliguri, 734010, West Bengal, India

POX	Peroxidase
PVP	Polyvinyl pyrrolidone
ROS	Reactive oxygen species
SA	Salicylic acid
SBHA	Suberoyl- <i>bis</i> -hydroxamic acid
SOD	Superoxide dismutase
TIA	Terpenoid indole alkaloid
TILLING	Targeting induced local lesions in genomes
UDP	Uridine diphosphate

1 Introduction

Secondary metabolites in plants are defined as low molecular weight by-products of primary metabolism that are usually not directly involved in the primary growth of plants but play an immense role in crop protection or yield improvement by interacting with several biotic and abiotic factors. Secondary metabolites are produced from primary metabolites via various metabolic pathways under certain physiological conditions (Ahmed et al. 2017). According to Delgoda and Murray (2017), there are about 2,00,000 secondary metabolites of diverse chemical nature produced by different plants, though not all of them are biologically active. Their expression patterns are also differentially regulated by complex signaling pathways involving several enzymes and genes. Depending on their biological functions, secondary metabolites are grouped into several major classes. Plant growth regulators or phytohormones occupy an important group of secondary metabolites that can regulate growth and development and also aid in defense against a wide range of biotic and abiotic stresses (Erb and Kliebenstein 2020). Other major classes include nitrogen-containing compounds (alkaloids, cyanogenic glycosides and non-protein amino acids) terpenoids, phenolic compounds and sulphur-containing compounds (glucosinolates, defensin, phytoalexins, etc.) that directly or indirectly help in promoting crop improvement under several unfavourable environmental conditions. Diversity of plant secondary metabolites is also correlated with their multifaceted functional attributes, viz. protection against herbivores, pathogenic microorganisms and weeds, facilitating pollination and fertilization by attracting pollinators, establishing symbiotic association with mycorrhizal fungi and providing tolerance against environmental stress factors (Zhang et al. 2020a). Plant secondary metabolites also serve as excellent sources of pharmaceuticals, agrochemicals, food additives, flavouring agents, cosmetics and many other industrial products due to their effective antimicrobial, antioxidant, insecticidal and other beneficial properties (Tiwari and Rana 2015). The basic skeletons of all the secondary metabolites are synthesized via three major metabolic pathways, viz. shikimic

acid pathway, isoprenoid pathway or mevalonic acid pathway and the polyketide pathway, which are further modified by series of addition, deletion and substitution depending on the specific requirement (Teoh 2016). In general, plants produce secondary metabolites in very low concentrations, however, production increases under the adverse environmental condition that is attributed to increased stress tolerance. The complex interaction between several genes and transcription factors is known to be responsible for regulating the levels of secondary metabolite production in plants (Jan et al. 2021).

Crop production is influenced by a range of external and internal factors, viz. soil condition, temperature, light intensity, humidity, nutrient status, gene expression and regulation, signaling pathways, etc. Slight variation in any of these factors can cause significant alterations in the growth and yield of crops. Many advanced strategies have been adopted in the recent past to optimize crop yield throughout the year. Targeting secondary metabolite biosynthetic pathways is one such tool for yield improvement. Advancement in genetic engineering and biotechnological tools are being utilized to dissect the secondary metabolite biosynthetic pathways for decoding their specific functions in plants both in laboratory and field conditions. This review attempts to present an overview of major secondary metabolites in plants and their functions in plant growth and development. It also focuses on the advanced metabolic engineering techniques for their increased production and the problems for the implementation of these techniques in the agricultural sector.

2 Role of Secondary Metabolites in Plant Growth and Development

2.1 Phytohormones

Phytohormones play a significant role in plant growth and development (Table 1). They act as chemical messengers and target specific plant tissue to elicit certain physiological responses under several environmental conditions. For example, ethylene triggers the signaling pathway and interacts with other hormones for eliciting developmental responses. Ethylene is a gaseous hormone with a simple structure that regulates leaf development, flower development, fruit ripening, seed germination, etc. Dubois et al. (2018) showed that mutation in positive and negative regulators of the ethylene signaling pathway showed altered growth patterns. For example, the mutation in ethylene receptor proteins, viz. ETR1 and ERS1 showed decreased leaf growth, whereas overexpression of *Auxin-Regulated Gene involved in Organ Size (ARGOS)* and *ARGOS-LIKE (ARL)* caused a negative feedback regulation of ethylene

Table 1 An overview of the role of plant secondary metabolites in plant growth and development

Secondary metabolites	Role in plant development
Phytohormones	<ul style="list-style-type: none"> • Growth and development of vegetative organs, viz. stem, root and leaves • Flower initiation and development, fruit ripening, seed germination and post-germination developmental changes • Alleviation of environmental stresses by ROS scavenging, ion homeostasis, accumulation of osmoprotectants, increased activity of antioxidative enzymes, etc • Regulation of major metabolic pathways—photosynthesis, glycolysis, pyruvate metabolism, TCA cycle, shikimic acid pathway, alkaloid biosynthesis, etc.
Terpenoids	<ul style="list-style-type: none"> • Regulation of photomorphogenetic responses- chloroplast organization and chlorophyll biosynthesis, apical hook opening, expansion of cotyledons, root development, etc. • Increased insect pollination, decreased spread of weeds, increased resistance against pests, fungal and microbial pathogens • Biosynthesis of phytoalexins, prevention of oxidative damage, increased biomass production under stress condition
Coumarins	<ul style="list-style-type: none"> • Growth regulation of vegetative organs, increased nutrient uptake from the soil • Stimulate symbiotic association with mycorrhizae and growth of beneficial soil microflora • ROS scavenging, reduced lipid peroxidation and membrane destabilization, reduced Na⁺ toxicity, increased activity of antioxidant enzymes under abiotic stress • Increased disease resistance
Flavonoids	<ul style="list-style-type: none"> • Growth promotion under stress condition • ROS scavenging, ion homeostasis, increased activity of enzymatic and non-enzymatic antioxidants, increased osmoregulation, reduced heavy metal toxicity • Enhanced resistance against insects, fungal and bacterial pathogens • Increased nutrient accumulation
Cyanogenic glycosides	<ul style="list-style-type: none"> • Accumulation and allocation of nitrogen in plants under stress condition • Stimulate growth and developmental responses under adverse environmental conditions • Increased defense against herbivores
Non-protein amino acid	<ul style="list-style-type: none"> • Reduce oxidative damage by preventing ROS formation, electrolyte leakage and lipid peroxidation • Confer stress tolerance by activation of antioxidative enzymes, maintaining osmotic balance and redox homeostasis, overexpression of heat-shock proteins • Increased chlorophyll content, photosynthetic efficiency, relative water content, regulation of carbohydrate and amino acid metabolism, regulation of expression of stress-responsive genes
Glucosinolates	<ul style="list-style-type: none"> • Promote root growth, chlorophyll content, biomass production • Increased osmoregulation, aquaporin synthesis, stomatal closure under drought condition • Increased resistance against fungal and bacterial pathogens

response and stimulated growth in *Arabidopsis* (Shi et al. 2015). Several reports are available depicting the complex interaction of ethylene with other phytohormones during growth and development. According to Iqbal et al. (2017), auxin stimulates endogenous ethylene biosynthesis and thereby regulates shoot apical meristem and leaf development in tomato and *Arabidopsis*. However, auxin-independent ethylene responses are also observed in common beans (*Phaseolus vulgaris*) (Keller et al. 2004). Ethylene also influences ABA sensitivity and acts synergistically with gibberellin during various stages of seed germination. Post-germination developmental changes, viz.

apical hook formation, hypocotyl growth, root initiation, etc. are also found to be regulated by the combined interaction of phytohormones, viz. auxin, cytokinin, ethylene, jasmonic acid, salicylic acid and brassinosteroids via regulation of several genes and transcription factors like *YUCCA1*, *YUCCA5*, *HLS1*, *HY5*, *MYC2*, *DELLA*, etc. (Ahammed et al. 2020). Sami et al. (2019) reported the crosstalk between the phytohormones with glucose in modulating plant developmental responses via hexose-dependent and hexose-independent signaling pathways. ABA, BR, SA, auxin, ethylene and GA help to minimize the adverse effects of salinity stress by interacting with signaling molecules like

NO, ROS and Ca^{+2} and stimulate plant growth by maintaining ion homeostasis, reducing oxidative damage, upregulation of genes for secondary metabolite biosynthesis, accumulation of osmoprotectants, increased biomass production under several abiotic stress condition (Amir et al. 2019). Interaction of NO with phytohormones like cytokinin, ethylene and auxin resulted in increased salinity tolerance in *Lactuca sativa* by regulating Na^{+} accumulation, antioxidant activities, mineral uptake and photosynthetic rate (Campos et al. 2019). Crosstalk between strigolactones and other phytohormone signaling pathways also led to plant growth promotion under normal and abiotic stress conditions by the formation of adventitious roots, regulation of shoot branching, inhibition of bud growth, promotion of secondary growth, leaf senescence, seed germination, internode elongation and nutrient uptake, etc. (Yang et al. 2019). Kumari and Parida (2018) showed that molecular crosstalk between ABA and JA brought about stomatal closure, reduced transpiration, regulation of major metabolic pathways, viz. glycolysis, pyruvate metabolism, TCA cycle, shikimic acid pathway, gluconeogenesis, alkaloid biosynthesis, etc. under saline condition. Exogenous application of gibberellic acid showed growth promotion in wheat cultivars by stimulating higher accumulation of proline along with the increased activity of Rubisco and antioxidant enzymes (peroxidase and superoxide dismutase) under saline conditions (Manjili et al. 2012).

2.2 Terpenoids

Terpenoids are the largest group of plant secondary metabolites and confer important roles in plant defense and crop vigour. Yu et al. (2018) reported the functional attribution of Terpenoid Indole Alkaloids (TIAs) during the process of photomorphogenesis in *Catharanthus roseus*. They observed the light stimulated activation of TIA biosynthetic enzymes and TIA accumulation (tabersonine, catharanthine, vindoline, vinblastine and vincristine) at the time of cotyledon opening. The combined interaction of auxin and cytokinin with terpenoid biosynthetic pathway in the morphogenesis and structural organization of chloroplasts in *Artemisia alba* has also been described by (Danova et al. 2018). Terpenoids were also known to improve the rate of plant reproduction and stimulate crop protection by increasing the rate of insect pollination, decreasing the spread of weeds and invader plants and reducing damages caused by pests and microbes in several agriculturally important crops, viz. apple, blueberry, tomato, etc. (Abbas et al. 2017). Terpenoid phytoalexins (zealexins and kauralexins) were known to induce root growth and biomass accumulation along with prevention of oxidative damage under various biotic and abiotic stress conditions (Akhi et al.

2021). Terpenes may also influence the expression of genes involved in plant defense mechanisms by acting as chemical messengers and regulating the adaptive features of plants under various biotic and abiotic stress (Zwenger and Basu 2008). For instance, 7-epizingiberene and R-curcumene were reported to increase insect resistance in tomatoes (Bleeker et al. 2011). β -caryophyllene could also induce resistance against microbial pathogens via jasmonic acid signaling, whereas isoprene and α - and β -pinene confer resistance via salicylic acid signaling in *Arabidopsis thaliana* (Frank et al. 2021). Wang et al. (2020) showed that sesquiterpenes secreted from glandular trichomes of wild tomato (*Solanum habrochaites*) could be responsible for conferring repellence against the potato aphid *Macrosiphum euphorbiae*. They highlighted the role of β -caryophyllene, α -humulene, α -santalene, α -bergamotene and β -bergamotene in affecting the survivorship and feeding behaviour of the aphid population. Capsidiol was known to be accumulated in *Nicotiana attenuata* in response to *Alternaria alternata* infection, and confer resistance against this fungal pathogen independent of jasmonic acid and ethylene signaling pathways (Song et al. 2019). Habash et al. (2020) evaluated the impact of a sesquiterpene, nootkatone against *Heterodera schachtii* parasitism on *A. thaliana*. They pointed out that nootkatone specifically decreased the number of nematodes and upregulated the defense-related genes involved in salicylic acid, jasmonic acid and ethylene biosynthetic pathways. The significance of terpenoids in plant growth and development is summarized in Table 1.

2.3 Phenolic Compounds

2.3.1 Coumarins

Coumarins are produced via phenylpropanoid pathway and involved in plant defense against pathogens, management of abiotic stresses and hormonal regulation. Coumarins along with reduced glutathione helped to ameliorate salinity stress and improve crop production in tomatoes by detoxifying ROS and methylglyoxal via enhancing the activities of glyoxalase enzymes (Table 1). Significant reduction in lipid peroxidation, membrane destabilization and Na^{+} toxicity was also observed (Parvin et al. 2020). Sultana et al. (2020) also observed that the exogenous application of coumarin effectively enhances vegetative growth and antioxidant enzyme activities (catalase, ascorbate peroxidase and guaiacol peroxidase) in sorghum seedlings on exposure to different concentrations of NaCl. Conversely, regulation of seed germination by coumarins was also observed in *Brassica parachinensis* via the reduction in ROS accumulation and decreased GA biosynthesis (Chen et al. 2021). Coumarin accumulation was also shown to confer increased resistance in *A. thaliana* against soft rot causing bacteria

Dickeya spp. (Perkowska et al. 2021). Sarashgi et al. (2021) showed that coumarins present in root exudates of Brassicaceae plant species (*Brassica napus*, *Raphanus sativus* and *Sinapis alba*) play an important role in iron accumulation. Overexpression of genes involved in the coumarin biosynthetic pathway helped to mitigate the mycorrhizal incompatibility in *A. thaliana*. It was also experimentally proved that root secreted coumarins, especially scopoletin can improve pre-penetration signaling and established chemical communication between the host and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (Cosme et al. 2021). Coumarins were also known to promote lateral root growth in *A. thaliana* by inhibiting basipetal transport of auxin and altering microtubule cortical array organization (Bruno et al. 2021). Scopoletin exuded from roots could improve plant growth by establishing an association with rhizospheric microorganism *Pseudomonas simiae* and *Arabidopsis thaliana* (Stringlis et al. 2018). In vitro experiments with plant-derived coumarins showed improved soil characteristics and also stimulated the growth of beneficial soil microflora (Niro et al. 2016). Seed priming of *Vicia faba* with different concentrations of coumarin showed improved vegetative growth by the enhanced accumulation of primary and secondary metabolites, viz. carbohydrates, proteins, phytohormones, phenolics, etc. (Saleh et al. 2015). Abenavoli et al. (2004) showed that different concentrations of coumarin could affect root growth parameters in different types of maize seedlings in hydroponic cultures by regulating lateral root formation, root length and branching patterns. Coumarins also facilitate nitrate uptake from the soil, increased accumulation of nitrate in root cells and translocation of nitrates from root to shoot in durum wheat seedlings. The diameter of the xylem vessels of the root cells and the increased respiration rates were also found in response to coumarin treatment (Abenavoli et al. 2001).

2.3.2 Flavonoids

Flavonoids play a major role in plant growth in several adverse environmental conditions (Table 25.1). Liang and He (2018) experimentally demonstrated the protective role of nine anthocyanins, viz. pelargonidin 3-robinobioside, pelargonidin 3,5-di-(6-acetylglucoside), pelargonidin 3-(600-p-coumarylglucoside)-5-(6000-acetylglucoside), pelargonidin 3-(600-malonylglucoside)-7-(6000-caFFEylglucoside), cyanidin 3-rutinoside, cyanidin 3-O-[b-DXylopyranosyl-(1->2)-[(4-hydroxybenzoyl)-(->6)-b-D-glucopyranosyl-(1->6)]-b-D-galactopyranoside], cyanidin 3-lathyruside, cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside] and cyanidin 3,5-diglucoside (600,6000-malyl diester) during low nitrogen stress condition by enhancing seed germination rate in *A. thaliana*. Apigenin helped to mitigate the adverse effects of

salinity stress by improving the activities of enzymatic (catalase and ascorbate peroxidase) and non-enzymatic antioxidants (carotenoids and flavonoids) and maintaining K^+/Na^+ homeostasis in rice seedlings (Mekawy et al. 2018). Li et al. (2019) observed that the MYB-stimulated enhanced synthesis and accumulation of flavonoids help to increase plant growth in *A. thaliana* under saline conditions. They elucidated that the overexpression of MYB transcription factor (MYB111) during salinity stress condition, in turn, activate the major enzymes of flavonoid biosynthesis, viz. chalcone synthase, flavanone carboxylase and flavanol synthase 1. Caliskan et al. (2017) have revealed the significance of phenolic compounds in salinity tolerance in *Hypericum pruinatum*. Their study showed increased accumulation of phenolics, viz. chlorogenic acid, rutin, hyperoside, isoquercetin, quercitrine and quercetine in salt-stressed plants. Similar enhancement in flavonoid biosynthesis was also observed in *Camellia sinensis* subjected to drought stress. The activation of enzymes, viz. chalcone synthase1, cinnamate-4-hydroxylase, flavonoid 3'5' hydroxylase and flavanone-3-hydroxylase lead to increased accumulation of a good number of flavonoids (myricetin, quercetin and kaempferol) that successfully ameliorate the adverse effects of drought and promote plant growth by maintaining cellular antioxidative status (Sun et al. 2020). Increased expression of three key enzymes of phenolic metabolism, viz. Shikimate Dehydrogenase (SKDH), Cinnamyl Alcohol Dehydrogenase (CAD) and Polyphenol Oxidase (PPO) associated with enhanced accumulation of phenolics could alleviate the adverse effects of Zn and Cd toxicity in *Kandelia obovata* (Chen et al. 2019). Phenolic acids and flavonoids (quercetin, catechin, apigenin, o-coumaric acid, luteolin, etc.) showed enhanced scavenging of free radicals like superoxide and peroxide anions, that in turn provide better insect resistance and antibiosis in winter triticale (Czerniewicz et al. 2017). Anthocyanin accumulation in leaves of *Euphorbia pulcherrima* showed higher antioxidant activities associated with tolerance against photo-oxidative stress (Moustaka et al. 2020). Oleuropein was known to protect salt-stressed olive plants by accelerating biomass production via increased antioxidative defense and osmoregulation (Petridis et al. 2012). Munné-Bosch and Alegre (2003) showed that the synergistic action of carsonic acid and α -tocopherol prevent oxidative damages and promote the growth of rosemary and sage plants under drought stress. A similar accumulation of anthocyanins and flavonols was observed in *A. thaliana*, which might be responsible for increased antioxidant activities in response to drought (Nakabayashi et al. 2014). UV-B LED light pre-treatment in lettuce showed enhanced accumulation of flavonoids that in turn helped to confer resistance against downy mildew (McLay et al. 2020). Constitutive expression of a UDP-dependent glycosyltransferase (OsUGT706C2) stimulated flavonoid biosynthesis in rice, which in turn contributed to UV-B tolerance and crop improvement

(Zhang et al. 2020b). Li et al. (2021) showed that increased flavonoid content helped to enhance antioxidant activity and crop yield in drought-affected maize plants. Overexpression of *GSA1* that encodes a UDP-glucosyltransferase which helped to regulate flavonoid accumulation, grain size and abiotic stress tolerance in rice (Dong et al. 2020).

2.4 Nitrogen-Containing Compounds

2.4.1 Cyanogenic Glycosides

Cyanogenic glycosides are by-products of amino acid metabolism and can produce toxic hydrogen cyanides by enzymatic hydrolysis. Siegień et al. (2021) showed that a higher accumulation of two major cyanogenic glycosides, namely linamarin and lotaustralin could function as nitrogen reservoirs in flax leaves under low nitrogen conditions. According to the studies of Sohail et al. (2020), dhurrin stimulated the rapid growth of germinating seedlings of sorghum and helped to adapt to dry climatic conditions by serving as a nitrogen source. In another experiment, Myrans et al. (2021) also showed that cyanogenic glycosides play a divergent role in the allocation of nitrogen according to their availability in wild and cultivated sorghum species that eventually contribute to plant defense against environmental stresses and also improve crop growth. However, according to Cuny et al. (2019), two cyanogenic glycosides (limanarin and lotaustralin) played a significant role in defense against herbivores rather than in growth promotion in *Phaseolus lunatus*.

2.4.2 Non-protein Amino Acids and Derivatives

Non-protein amino acids are generally referred to the amino acids other than the proteinogenic amino acids. γ -aminobutyric acid (GABA) is a well-known amino acid that rapidly accumulates in plant tissues under stress and known to regulate plant growth and development. GABA has been known to alleviate oxidative damage and denaturation of chlorophyll molecules under high-temperature stress by stimulating activities of antioxidant enzymes (SOD, CAT, POX, APX and GR), overexpression of heat-shock proteins, accumulation of osmolytes and by lowering electrolyte leakage and lipid peroxidation in *Agrostis stolonifera* (Zeng et al. 2021). Similar heat tolerance was also conferred by exogenous application of GABA in wheat seedlings by regulation of amino acid metabolism and maintenance of redox homeostasis that in turn help to grow the plants under such adverse environmental conditions (Wang et al. 2021). GABA signaling could modulate stomatal movement in leaves of *A. thaliana* and suffice optimum water resilience under drought stress. Overexpression of Glutamate Decarboxylase 2 gene (*GAD2*) in turn was found to be responsible for increased biosynthesis of

GABA via Ca^{+2} /calmodulin signaling (Xu et al. 2021). Wu et al. (2020) also found that exogenous application of GABA into tomato plants helped to reduce Na^{+} uptake and accumulation in roots and leaves, prevent ROS formation and lipid peroxidation and also improve plant growth under NaCl stress. Sita and Kumar (2020) pointed out the role of GABA in the alleviation of multiple abiotic stress factors in leguminous plants by modulating carbohydrate and amino acid metabolism and maintaining antioxidative mechanisms and membrane stability. In this context, Priya et al. (2019) also elaborated the thermo-protective effect of GABA on *Vigna radiata* by enhancement of reproductive function under high-temperature stress. Post-harvest exogenous application of GABA could mitigate the toxic impacts of low-temperature storage by reducing the activities of lipooxygenases and phospholipases and accelerating antioxidative activities of SOD and CAT in cucumber fruits (Malekzadeh et al. 2017). Dopamine, an amino acid derivative, could act as a signal molecule in plant growth and development and protect against various abiotic stresses by regulating the expression of major stress-responsive genes involved in senescence, chlorophyll degradation, nitrate transport, etc. (Liu et al. 2020). Dopamine application could increase biomass production of apple seedlings by enhancing the rate of photosynthesis and chlorophyll content and decreasing the accumulation of ROS under alkali stress (Jiao et al. 2019). Exogenous application of β -Aminobutyric Acid (BABA) has been shown to improve Relative Water Content (RWC), photosynthetic efficiency and antioxidant activities in *Vicia faba* through the over-expression of stress-responsive genes, viz. *VfGST*, *VfMYB*, *VfDHN*, *VfLEA*, *VfERF*, *VfNCED*, *VfWRKY*, *VfHSP* and *VfNAC* under drought stress (Abid et al. 2020). Kim et al. (2013) also studied the effect of BABA on seedling growth of Kimchi cabbage by inducing resistance against *Alternaria brassicicola* and *Colletotrichum higginsianum* (Table 1).

2.5 Sulphur-Containing Compounds

2.5.1 Glucosinolates

Glucosinolates are a group of plant secondary metabolites with S- β -d-glucopyrano unit anomericly connected to an O-sulphated (Z)-thiohydroximate (Blažević et al. 2020). Glucosinolates are found to be indirectly involved in crop improvement by increasing resistance against ZnO nanoparticle toxicity in *A. thaliana* (Tao et al. 2021). Tao and co-workers also showed that treatment with ZnO nanoparticles promoted increased accumulation of glucosinolates that in turn helped to mitigate the adverse effect of the nanoparticles on root growth, chlorophyll content and plant biomass. Glucosinolates like isothiocyanates, glucobrassicin, sinigrin,

glucoiberin, etc. were known to confer greater resistance against a wide range of plant pathogens (*Rhizoctonia solani*, *Fusarium* sp., *Alternaria solani*, *Aspergillus flavus*, etc.) and indirectly involved in crop protection and improvement (Poveda et al. 2020). Eom et al. (2018) conducted a transcriptome analysis study to elucidate the role of glucosinolate metabolism in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) in response to drought stress. Differential expression of several drought-responsive genes, viz. *AP2/ERFs*, *bHLHs*, *NACs*, *bZIPs* and particularly, *BrbZIPs* were found to be involved in improving plant tolerance by increased accumulation of glucosinolates in leaves and preventing water loss by inducing stomatal closure. In this connection, a marked increase in glucosinolate accumulation was also observed under the influence of drought and salinity stress in different Brassicaceae crops, viz. *Brassica rapa*, *B. juncea*, *B. oleracea*, *B. napus*, etc. (Essoh et al. 2020). It was also shown that the glucosinolates confer abiotic stress tolerance by the overexpression of *MYB* genes (especially

MYB28 and *MYB29*), involved in increased aquaporin synthesis and osmoregulation (Essoh et al. 2020) (Table 1).

3 Strategies to Regulate Secondary Metabolite Production

Several strategies have been adopted to increase the production of secondary metabolites beneficial for crop growth and improvement (Fig. 1). A detailed account of these strategies is discussed in the following sub-sections and the major studies depicting the use of these elicitors in the improvement of crop plants are also enlisted in Table 2.

3.1 Chemical Elicitors

Exogenous application of plant growth regulators (salicylic acid and 24-epi-brassinolide) showed improved secondary metabolite production in *Brassica nigra* under salinity stress

Fig. 1 Commonly known elicitors that are being used for the engineering of secondary metabolites in plants for the improvement of crop production

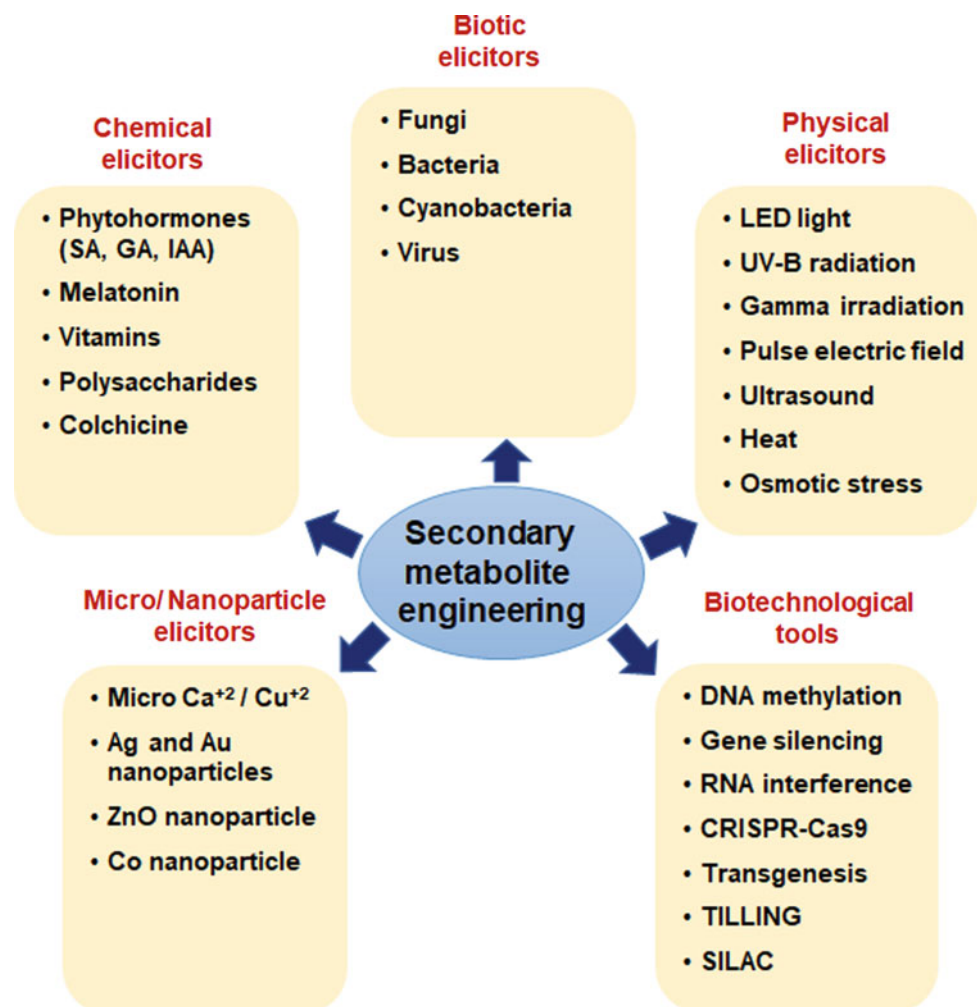


Table 2 Studies depicting the strategies to engineer secondary metabolite production in plants for crop improvement

Plant species	Approach used for elicitation	Effect on crop improvement	References
<i>Arabidopsis thaliana</i>	Overexpression of <i>TOGT1</i> gene	Enhanced scopoletin production	Wang and Hou (2009)
<i>Arabidopsis thaliana</i>	Complete pathway transfer of dhurrin biosynthesis from sorghum	Enhanced dhurrin production	Tattersall et al. (2001)
<i>Arabidopsis thaliana</i>	Heterologous overexpression of <i>PnJAZ1</i> gene from <i>Pohlia nutans</i>	Helped in seed germination and seedling growth under salt stress by regulating JA and ABA biosynthesis	Liu et al. (2019)
<i>Arabidopsis thaliana</i>	Heterologous overexpression of <i>CrUGT87A1</i> gene from <i>Carex rigescens</i>	Increased flavonoid accumulation, antioxidative activities and improved salinity tolerance	Zhang et al. (2021)
<i>Atropa belladonna</i>	Transgenesis and overexpression of <i>ODC</i> gene	Increased production of putrescine, N-methyl putrescine, hyoscyamine and anisodamine	Zhao et al. (2020)
<i>Bambusa multiplex</i>	Histone deacetylase inhibition by suberoyl bis-hydroxamic acid and trichostatin A	Increased production of 3-O-p-coumaroyl quinic acid and 3-O-feruloyl quinic acid	Nomura et al. (2021)
<i>Brassica nigra</i>	Exogenous application of gibberellic acid salicylic acid (1 mM) and 24-epi-brassinolide (0.1 µM)	Improved secondary metabolite production under salt stress	Ghassemi-Golezani et al. (2020)
<i>Brassica rapa</i>	Foliar application of thiamine (100 mM)	Enhanced secondary metabolite production, increased photosynthetic rate and antioxidant activity under drought stress	Jabeen et al. (2021)
<i>Brassica rapa</i>	Single amino acid modification of CAX1a transporter by TILLING technique	Increased IAA and GA content with improved salinity tolerance	Navarro-León et al. (2020)
<i>Capsicum frutescens</i>	Seed treatment with colchicine (300 mg/L) and oryzalin (30 mg/L)	Increased capsaicin production by tetraploid plants	Pilankong et al. (2017)
<i>Catharanthus roseus</i>	Application of PVP-coated cobalt nanoparticles (10, 15 and 20 mg/L)	Concentration-dependent increase in accumulation of alkaloids in cell suspension culture	Fouad and Hafez (2018)
<i>Catharanthus roseus</i>	Transgenesis and overexpression of geranyl diphosphate synthase and geraniol synthase	Increased accumulation of vindoline and catharanthine	Kumar et al. (2018)
<i>Catharanthus roseus</i>	Seed treatment with colchicine (0.2% aqueous solution)	Tetraploid explants showed increased production of terpenoid indole alkaloids along with an increased number of stomata and larger leaves	Xing et al. (2011)
<i>Centella asiatica</i>	Elicitation with <i>Colletotricum gloeosporioides</i>	Promotes biosynthesis of asiaticoside	Gupta and Chaturvedi (2019)
<i>Convolvulus sepium</i> , <i>Withania somnifera</i> , <i>A. thaliana</i> , <i>Tylophora tanakae</i>	Transgenesis and overexpression of fungal <i>crypt</i> gene	Enhanced biosynthesis of calystegine, withaferin, tylophorin, etc. in hairy root culture	Chaudhuri et al. (2009)
<i>Coriandrum sativum</i>	Regulation of photosynthetic photon flux density (300 µL) and root-zone temperature (30 °C)	Increased accumulation of phenolics and flavonoids and biomass production along with enhanced antioxidant activity	Nguyen et al. (2019)
<i>Cuminum cyminum</i>	Treatment with 2,4-D (2.5 mg/L) and kinetin (0.5 mg/L)	Increased essential oil synthesis and callus induction	Farvardin et al. (2017)
<i>Datura metel</i> , <i>Hyoscyamus muticus</i>	Transgenesis with tobacco <i>pmt</i> gene	Increased production of scopolamine (in <i>Datura</i>) and hyoscyamine (in <i>Hyoscyamus</i>) in hairy root culture	Moyano et al. (2003)
<i>Dracocephalum forrestii</i>	LED light (blue, red, blue + red and white) exposure	Increased phenolics and flavonoid content in shoot culture	Weremczuk-Jeżyna et al. (2021)
<i>Echinacea purpurea</i>	Exogenous application of gibberellic acid (0.025 µM)	Accumulation of caffeic acid and lignin in hairy root culture	Abbasi et al. (2012)

(continued)

Table 2 (continued)

Plant species	Approach used for elicitation	Effect on crop improvement	References
<i>Echinacea purpurea</i>	Treatment with ZnO microparticles (150 mg/L) and ZnO nanoparticles (75 mg/L)	Increased biomass and flavonoid accumulation in in vitro callus culture	Karimi et al. (2018)
<i>Glycyrrhiza uralensis</i> , <i>Tropaeolum majus</i> , <i>Ocimum basilicum</i>	UV-B treatment (280–320 nm)	Stimulated secondary metabolite biosynthesis	Yavas et al. (2020)
<i>Helianthus annuus</i>	Application of biofabricated silver nanoparticles with <i>Euphorbia helioscopia</i> leaf extract (60 mg/L)	Enhanced growth and secondary metabolite production	Batool et al. (2021)
<i>Hyoscyamus reticulatus</i>	Treatment with ZnO nanoparticles (100 mg/L)	Growth promotion and increased synthesis of hyoscyamine and scopolamine	Asl et al. (2019)
<i>Hypericum perforatum</i>	Treatment with dextran, pectin and chitin (100 mg/L)	Increased biosynthesis of hypericin and pseudohypericin in shoot culture	Simic et al. (2014)
<i>Hypericum perforatum</i>	Elicitation with <i>Colletotrichum gloeosporioides</i> , <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> , <i>Saccharomyces cerevisiae</i> , and <i>Botrytis cinerea</i>	Enhanced accumulation of xanthones, flavonoids and phenolics	Shakya et al. (2019)
<i>Hypericum perforatum</i>	Elicitation with <i>Agrobacterium tumefaciens</i> , <i>A. rhizogenes</i> and <i>Stenotrophomonas maltophilia</i>	Increased accumulation of flavonols, flavanols, lignin, etc	Shakya et al. (2019)
<i>Lactuca sativa</i>	Chitosan-coated microcapsules combined with calcium and copper ions and <i>Trichoderma viridae</i>	Enhanced secondary metabolite production and increased antioxidant activities	Jurić et al. (2020)
<i>Leucosium aestivum</i>	Exogenous melatonin treatment (10 µM)	Increased alkaloid content in in vitro cell culture	Ptak et al. (2019)
<i>Lithospermum erythrorhizon</i>	<i>Agrobacterium</i> -mediated transformation of <i>ubiA</i> gene from <i>E. coli</i>	Promotes shikonin production	Boehm et al. (2000)
<i>Mentha spicata</i>	Silencing of <i>MSYABBY5</i> gene	Enhances terpene biosynthesis in peltate glandular trichomes	Wang et al. (2016)
<i>Nicotiana benthamiana</i>	Heterologous expression of <i>IbC4H</i> gene from <i>Ipomoea batatas</i>	Enhanced polyphenol biosynthesis and increased antioxidant activity	Wang et al. (2017)
<i>Nicotiana tabacum</i> , <i>A. thaliana</i>	Overexpression of <i>PAP1</i> gene	Increased accumulation of flavonoids	Gantent and Memelink (2002)
<i>Ocimum basilicum</i> , <i>Origanum vulgare</i>	Exogenous application of IBA (0.1 mg/L) and BA (2 and 4 mg/L)	Increased phenolic content in vitro shoot culture	Karalija et al. (2016)
<i>Panax ginseng</i>	Exogenous application of linoleic acid and α-linolenic acid (5 µM/L)	Increased biosynthesis of ginsenoside along with biomass production	Wu et al. (2009)
<i>Papaver somniferum</i>	Silencing of 4'OMT2 gene	Increased biosynthesis of benzyloquinoline alkaloids	Alagoz et al. (2016)
<i>Papaver somniferum</i>	Elicitation with poppy mosaic virus	Stimulates production of alkaloids—codeine, papaverine, narcotine, etc	Zaim et al. (2014)
<i>Passiflora edulis</i>	Elicitation with TMV, telosma mosaic virus and cucumber mosaic virus	Increased biosynthesis of polyphenols and flavonoids	Mishra et al. (2020)
<i>Pelargonium</i> spp., <i>Withania somnifera</i>	Homologous and heterologous overexpression of <i>DXS</i> gene from <i>Pelargonium</i>	Increased biosynthesis of essential oil (in <i>Pelargonium</i>) and withanolide (<i>Withania somnifera</i>)	Jadaun et al. (2017)
<i>Prunella vulgaris</i>	Application of silver and gold nanoparticles	Enhanced phenolic and flavonoids accumulation and increased antioxidant activity	Fazal et al. (2016)
<i>Psoralea corylifolia</i> , <i>Capsicum annum</i> , <i>Stevia rebaudiana</i> , <i>Panax ginseng</i>	Gamma irradiation (20 kGy)	Enhanced production of psoralen, capsaicinoids, stevioside and ginsenoside, respectively	Vardhan and Shukla (2017)

(continued)

Table 2 (continued)

Plant species	Approach used for elicitation	Effect on crop improvement	References
<i>Rubia cordifolia</i>	Transgenesis with <i>rol</i> genes	Stimulates increased biosynthesis of anthraquinones	Bulgakov et al. (2010)
<i>Salvia dolomitica</i>	Controlled exposure to drought	Increased production of terpenoids, phenolics and flavonoids	Caser et al. (2019)
<i>Salvia miltiorrhiza</i>	Inhibition of DNA methylation by 5-azacytosine (10 μ M)	Increased phenolic acid biosynthesis in hairy root culture	Yang et al. (2018)
<i>Salvia miltiorrhiza</i>	CRISPR/Cas9 mediated targeted mutagenesis of <i>SmRAS</i> gene	Increased production of rosmarinic acid	Zhou et al. (2018)
<i>Saussurea involucreta</i>	Transgenesis and overexpression of <i>chi</i> gene from <i>Saussurea medusa</i>	Increased production of naringenin, apigenin and total flavonoid in hairy root culture	Li et al. (2006)
<i>Solanum chrysotrichum</i>	Cell immobilization within calcium-alginate gel beads (0.1 to 0.8 mol/L Ca and 1–1.5% w/v alginate)	Enhanced spirostanol production	Charlet et al. (2000)
<i>Solanum tuberosum</i>	Transgenesis and overexpression of <i>RIP</i> gene	Increased production of sesquiterpenes and glycoalkaloids	Matthews et al. (2005)
<i>Solanum tuberosum</i>	Induction of polyploidy by colchicine and oryzalin	Increased sesquiterpene production by tetraploid plants in vitro	Cara et al. (2020)
<i>Stevia rebaudiana</i>	Seed treatment with colchicine (0.6% aqueous solution)	Tetraploid plants showed increased stevioside production along with increased leaf size and chlorophyll content	Yadav et al. (2013)
<i>Tanacetum parthenium</i>	Treatment with ZnO nanoparticles (2000 ppm)	Increased production of terpenolide and essential oil, mineral absorption and crop yield	Shahhoseini et al. (2020)
<i>Taxus chinensis</i>	Application of pulse electric field (50 Hz, 10 V/m)	Enhanced taxuyunnanine C production	Ye et al. (2004)
<i>Tripterygium wilfordii</i> , <i>Catharanthus roseus</i>	Homologous and heterologous overexpression of class I TGA transcription factor from <i>Tripterygium wilfordii</i>	Stimulates biosynthesis of sesquiterpene and pyridine alkaloids	Han et al. (2020)
<i>Vitis vinifera</i>	Exogenous melatonin treatment	Promotes endogenous melatonin and flavonoid level coupled with fruit ripening	Xu et al. (2017)
<i>Vitis vinifera</i>	Elicitation with Grapevine red blotch-associated virus	Increased biosynthesis of flavonoids and anthocyanin	Blanco-Ulate et al. (2017)

(Ghassemi-Golezani et al. 2020). Application of Gibberellic Acid (GA_3) in hairy root cultures of *Echinacea purpurea* showed enhanced accumulation of caffeic acid derivatives and lignin (Abbasi et al. 2012). Indole Butyric Acid (IBA) and Benzyladenine (BA) also showed elevation in the accumulation of phenolics and flavonoids along with increased antimicrobial activity in *Ocimum basilicum* and *Origanum vulgare* (Karalija et al. 2016). 2,4-D and kinetin also showed increased essential oil synthesis in *Cuminum cyminum* (Farvardin et al. 2017). Exogenous application of melatonin has been shown to stimulate biomass production and accumulation of many Amaryllidaceae alkaloids (especially lycorine and galanthamine) in in vitro cell cultures of *Leucojum aestivum* (Ptak et al. 2019). Melatonin could also enhance phenolics and flavonoid content and antioxidant activity by upregulation of the *STS* gene and ethylene signaling in grape berries (Xu et al. 2017). Foliar application of

vitamin B₁ (thiamin) also stimulated secondary metabolite production, antioxidant activity and growth promotion in *Brassica rapa*, subjected to drought stress (Jabeen et al. 2021). Polysaccharides such as dextran, pectin and chitin also acted as elicitors for the biosynthesis of phenolics, flavonoids and naphodianthrones (hypericin and pseudohypericin) in *Hypericum perforatum* shoot cultures (Simic et al. 2014). Exogenous application of essential fatty acids (linoleic acid and α -linolenic acid) also helped to elicit the production of phenolics, flavonoids and ginsenoside along with increased biomass production and antioxidant activity in *Panax ginseng*, cultured in bioreactors (Wu et al. 2009). Immobilization of cells of *Solanum chrysotrichum* within the calcium-alginate gel matrix showed significant enhancement in the production of antimycotic saponin—spirostanol (Charlet et al. 2000). Salicylic acid, jasmonic acid and methyl jasmonate also act as regulators of secondary

metabolite biosynthesis in *Withania somnifera*, *Gymnema sylvestre*, *Panax ginseng*, etc. (Chodisetti et al. 2015; Sivanandhan et al. 2013; Thanh et al. 2005). According to Gantait and Mukherjee (2021), inhibition of spindle fibre formation or induction of polyploidy can also be considered effective in manipulating secondary metabolite production in several plants. They showed that treatment with colchicine and oryzanol in different culture conditions can significantly increase biosynthesis of alkaloids (capsaicin, vincristine, etc.), terpenoids (andrographolide, α -gualene, limonene, etc.), glycosides (stevioside, rebaudioside, bacoside etc.), lactones (artemisinin, wedelolactone, etc.) total phenolics, flavonoids (quercetin, kaempferol, chlorogenic acid, etc.) in several polyploid plant species—*Catharanthus roseus*, *Capsicum frutescens*, *Aquilaria malaccensis*, *Sphagneticola calendulacea*, *Andrographis paniculata*, *Stevia rebaudiana*, *Bacopa monnieri*, *Eucommia sp.*, *Citrus limon*, etc.

3.2 Physical Elicitors

Induction of light stress by exposure with LED lights of different wavelengths (blue, red, blue-red 30%:70% and white; $\lambda = 430$ nm, 670 nm, and $\lambda C = 430$ –670 nm) showed significant enhancement in the biosynthesis of phenolic and flavonoid derivatives (chlorogenic acid, caffeic acid, salvianolic acid, apigenin *p*-coumarylrhannoside, methyl rosmarinate, etc.) in in vitro shoot culture of *Dracocephalum forrestii* (Weremczuk-Jeżyna et al. 2021). UV-B radiation was also found to induce biosynthesis and accumulation of flavonoids, tannins, isoprenoids, glucosinolates in *Glycyrrhiza uralensis*, *Tropaeolum majus*, *Ocimum basilicum* and many others (Yavas et al. 2020). Gamma irradiation also showed similar enhancement in biosynthesis and accumulation of psoralen in *Psoralea corylifolia*, capsaicinoids in *Capsicum annum*, stevioside in *Stevia rebaudiana*, ginsenoside in *Panax ginseng* by activating the key enzymes of secondary metabolism, viz. phenylalanine ammonia-lyase, chalcone synthase, squalene synthase, etc. (Vardhan and Shukla 2017). Ye et al. (2004) elucidated the efficiency of pulse electric field for stimulation of secondary metabolite biosynthesis in *Taxus chinensis*. Regulation of photosynthetic photon flux density and root temperature was shown to stimulate the biosynthesis and accumulation of total flavonoids, chlorogenic acid, rutin, trans-2-decenal in addition to increased antioxidant activity and crop yield in coriander (Nguyen et al. 2019). Caser et al. (2019) reported the implication of controlled drought conditions could modulate terpenoid, phenolics and flavonoid biosynthesis in *Salvia dolomitica*. Narayani and Srivastava (2017) reported similar elicitation of secondary metabolite production by several abiotic (heat shock, osmotic stress, ultrasound, ozone exposure, etc.) and biotic factors (extract of algae, fungi, bacteria, microbe-derived chitosan, pectin, cyclodextrin, etc.) in in vitro cell culture.

3.3 Microparticles and Nanoparticles as Elicitors

Sustainable utilization of chitosan-coated microcapsules combined with calcium and copper ions and *Trichoderma viridae* on the enhanced production of secondary metabolites and significant increments in antioxidant activities was observed in *Lactuca sativa* (Jurić et al. 2020). Application of silver and gold nanoparticles in callus culture of *Prunella vulgaris* showed enhanced production of phenolics and flavonoid content along with increased antioxidant activity (Fazal et al. 2016). Seedling treatment of *Tanacetum parthenium* with zinc oxide nanoparticles showed improved crop yield, mineral absorption along with the biosynthesis of essential oil and sesquiterpene lactones (parthenolide) (Shahhoseini et al. 2020). Nano ZnO also helped to increase root growth coupled with increased phenolic content, antioxidant activity and increased accumulation of tropane alkaloids (hyoscyamine and scopolamine) by upregulation of hyoscyamine-6-beta-hydroxylase (*h6h*) gene in *Hyoscyamus reticulatus* (Asl et al. 2019). Application of different concentrations of ZnO nanoparticles and microparticles on the *Echinacea purpurea* callus extract showed enhanced accumulation of flavonoids, which could be correlated with its anticancer activity (Karimi et al. 2018). Biofabrication of silver nanoparticles with the leaf extract of *Euphorbia helioscopia* showed enhanced growth parameters and secondary metabolite production in *Helianthus annuus*, in different developmental stages (Batool et al. 2021). Polyvinyl Pyrrolidone (PVP) coated cobalt nanoparticles also stimulated alkaloid biosynthesis in cell suspension culture of *Catharanthus roseus* under oxidative stress conditions (Fouad and Hafez 2018).

3.4 Biotic Elicitors

Elicitation of secondary metabolite production with the help of microbial extract was found to be useful in *Hypericum perforatum* (Shakya et al. 2019). The addition of cell culture filtrates of *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, and *Botrytis cinerea* have shown increased accumulation of xanthenes, flavonoids and phenolic substances in shoot and suspension culture of *H. perforatum*. Apart from the fungal elicitors, several bacterial species, viz. *Agrobacterium tumefaciens*, *A. rhizogenes* and *Stenotrophomonas maltophilia* also showed the enhanced synthesis of flavonol, flavanol, lignin and other phenolics in *H. perforatum* (Shakya et al. 2019). A Co-cultivation system using an endophytic fungus *Colletotrichum gloeosporioides* stimulated the biosynthesis of asiaticoside content in *Centella asiatica* (Gupta and Chaturvedi 2019). Many cyanobacterial genera, viz. *Synechocystis*, *Synechococcus* and *Anabaena* are

successfully transformed with plant-derived genes to produce important secondary metabolites like- limonene, p-coumaric acid, caffeine, carotenoid, etc. (Xue and He 2015). Viral infection often acted as elicitors for secondary metabolite production in the host plants. For example, cucumber mosaic virus elicits the production of polyphenols and flavonoids in *Passiflora edulis*, Grapevine red blotch-associated virus elicits flavonoid and anthocyanin synthesis in *Vitis vinifera*, poppy mosaic virus elicits alkaloid production in *Papaver somniferum*, etc. (Mishra et al. 2020).

3.5 Biotechnological Approaches

3.5.1 Regulation at DNA Level

Epigenetic regulation (DNA methylation, histone modification, RNAi transcription) of selected metabolic pathways was also found to be significant for modulation of secondary metabolites production in the plant cell (Brzycki et al. 2021). Yang et al. (2018) have confirmed the role of a DNA methylation inhibitor (5-azacytosine) in increasing the expression of major genes involved in phenolic acid biosynthesis in *Salvia miltiorrhiza*. However, the opposite result was observed when a donor for DNA methylation, SAM (S-adenosyl methionine), was added. Similarly, treatment of suspension culture of *Bambusa multiplex* cells with two histone deacetylase inhibitors (SBHA and trichostatin A) showed enhanced biosynthesis of cryptic secondary metabolites (Nomura et al. 2021). Gene silencing approaches through RNA interference are also extensively used to enhance the production of plant secondary metabolites for crop improvement (Rajam 2020). The silencing of a novel gene (*MSYABBY5*) showed increased terpene biosynthesis in the peltate glandular trichome of *Mentha spicata*. On the contrary, heterologous expression of *MSYABBY5* repressed secondary metabolite production in *Ocimum basilicum* and *Nicotiana glauca* (Wang et al. 2016). Attempts have also been made through gene silencing via RNA interference by topical application of dsRNA (Deguchi et al. 2020).

3.5.2 Transcriptional Regulation and Transgenesis

Transcriptional regulation of *MYB* and *bHLH* protein-encoding genes (*C1* and *R*), AP2/ERF-domain transcription factor ORCA3, DREB2A and DREB2B proteins are also being successfully implemented in *Catharanthus roseus*, *Arabidopsis thaliana* and several other plants for engineering biosynthesis of anthocyanins, flavonoids, terpenoid indole alkaloids, etc. (Memelink et al. 2001). Overexpression of genes of secoiridoid pathway (geraniol synthase and geranyl diphosphate synthase) stimulated the accumulation of commercially important monoterpene

indole alkaloids (vindoline and catharanthin) in transgenic *C. roseus* (Kumar et al. 2018). Similar overexpression of ornithine decarboxylase (*ODC*) gene showed increased biosynthesis of tropane alkaloids, viz. putrescine, N-methyl putrescine, hyoscyamine and anisodamine in *Atropa belladonna* hairy root cultures. Transcriptional and post-transcriptional regulation of several transcription factors, viz. WRKY, MYB, bHLH, APETALA2/Ethylene Responsive-Factor (AP2/ERF), Jasmonate-responsive ERF (JRE), Basic Leucine Zipper (bZIP), SQUAMOSA Promoter-binding protein-Like (SPL), etc. could help modulate the biosynthesis of several classes of terpenoids and flavonoids—monoterpenes, sesquiterpenes, diterpenes, triterpenes, steroidal lactones, etc. in several crop species (Nagegowda and Gupta 2020). Similarly, homologous and heterologous overexpression of class I TGA transcription factor from *Tripterygium wilfordii* stimulated the biosynthesis of sesquiterpene and pyridine alkaloids (Han et al. 2020). *Agrobacterium*-mediated transgenesis of cannabinoid-synthesizing genes was also successfully applied for increased metabolite production in hemp (*Cannabis sativa*) in tissue culture. Manipulation of the shikonin biosynthetic pathway with the introduction of the 4-hydroxybenzoate-3-polyprenyltransferase (*ubiA*) gene from *E. coli* had successfully enhanced shikonin production in *Lithospermum erythrorhizon* (Boehm et al. 2000). Activation of cryptic gene clusters by co-cultivation or epigenetic modification of several endophytes (*Fusarium mairei*, *Trichoderma atroviridae*, *Enterophosphospora* sp., etc.) also helped in large-scale production of high-value plant secondary metabolites like taxol, berberine, camptothecin, vincamine (Venugopalan and Srivastava 2015).

According to Chandra and Chandra (2011), the formation of hairy root lines by the transformation of T-DNA from *Agrobacterium rhizogenes* could offer a useful strategy for the increased production of secondary metabolites. For instance, incorporation of the chalcone isomerase (*chi*) gene from *Saussurea medusa* into the genome of *Saussurea involucreata* showed increased production of naringenin, apigenin and total flavonoid content. Moreover, binary vectors formed by combining the T-DNA of the Ri-plasmid and putrescine N-methyltransferase (*pmt*) gene showed enhanced production of scopolamine and hyoscyamine by upregulation of tropane alkaloid biosynthetic pathway in *Datura metel* and *Hyoscyamus muticus* (Moyano et al. 2003). Transformation of *Rubia cordifolia* hairy root cultures with *rol* genes boosted the plant cells for increased production of secondary metabolites (Bulgakov et al. 2010). Transgenic *A. belladonna* also showed enhanced biosynthesis of hyoscyamine and anisodamine due to overexpression of the *ODC* gene (Zhao et al. 2020). Transgenic mimicry of pathogen attack could also lead to increased secondary metabolite production in several crop species,

suggesting a suitable method for eliciting defense responses in plants. *A. rhizogenes* mediated transfer of fungal β -cryptogein gene (*crypt*) resulted in improved growth and enhanced accumulation of calystegine, polyphenols, withaferin, tylophorin, etc. in *Convolvulus sepium*, *Withania somnifera*, *A. thaliana* and *Tylophora tanakae* (Chaudhuri et al. 2009). Genetically modified potato cultivars constructed using Ribosome-Inactivating Protein-coding gene (*RIP*) from maize, showed enhanced synthesis and accumulation of sesquiterpenes and glycoalkaloids under a range of biotic and abiotic stress conditions (Matthews et al. 2005). Adventitious root culture using bioreactors is considered useful for large-scale production of plant secondary metabolites, viz. ginsenoside, resveratrol, camptothecin, vindoline, etc. (Kumar 2015). Catalytic synthesis of glycoconjugate involving the multigene superfamily of glycosyltransferases can also be employed in the production and modification of plant secondary metabolites. For instance, reduced expression of Tobacco Glycosyltransferase (*TOGT*) in transgenic tobacco plants showed decreased glycosylation of scopoletin that impaired resistance against TMV. Whereas overexpression of the *TOGT1* gene led to increased resistance against Potato Virus Y by enhancing scopoletin synthesis (Wang and Hou 2009). Transgenic overexpression of *1-deoxy-D-Xylulose-5-phosphate Synthase (DXS)* gene from *Pelargonium* spp. showed enhanced production of secondary metabolites in both homologous (essential oil in *Pelargonium* spp.) and heterologous conditions (withanolide in *W. somnifera*) (Jadaun et al. 2017). Heterologous expression of cinnamate 4-hydroxylase gene from *Ipomoea batatas (IbC4H)* showed enhanced drought tolerance associated with increased polyphenol biosynthesis and antioxidative activities in transgenic tobacco (Wang et al. 2017). Liu et al. (2019) showed that overexpression of a jasmonate ZIM-domain gene from a moss *Pohlia nutans (PnJAZ1)* in *A. thaliana* regulated ABA signaling pathways and induced seed germination and seedling growth under salinity stress. Overexpression of an anthocyanin-producing gene (*production of anthocyanin pigment 1, PAPI*) showed enhanced accumulation of anthocyanin by regulating the enzymes of flavonoid biosynthetic pathways in *Nicotiana tabacum* and *A. thaliana* (Gantet and Memelink 2002). Ma et al. (2019) reported the efficient application of a fungal host *Yarrowia lipolytica* for the heterologous synthesis of plant terpenoids by the introduction of terpene synthase and modifying the mevalonate pathway. Nascimento and Fett-Neto (2010) pointed out the effective transformation of entire plant-derived metabolic pathway genes from one species to another. For instance, complete pathway transfer of cyanogenic glycoside dhurrin from *Sorghum bicolor* to *Arabidopsis thaliana* was carried out successfully for increased herbicide resistance (Tattersall et al. 2001). Zhang et al. (2021) reported that overexpression of a UV-B responsive

UDP-sugar glycosyltransferase gene from *Carex rigescens (CrUGT87A1)* showed increased salinity tolerance in *A. thaliana* by stimulating flavonoid biosynthesis and antioxidative activity. Bleeker et al. (2012) successfully transfer the biosynthetic pathway to produce a sesquiterpene (7-epizingiberene) from the wild tomato into a greenhouse cultivated variety for enhanced herbivore resistance.

3.5.3 Molecular Engineering Techniques

Several reports are depicting the significant contributions of molecular engineering approaches in the modulation of secondary metabolite biosynthesis for crop improvement. Sabzehzari et al. (2020) have demonstrated the significance of Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-mediated transcriptional regulation of secondary metabolite production in several crop plants by silencing the enzymes of biosynthetic pathways. In this context, genome editing of *4'OMT2* (3'-hydroxyl-N-methylcoclaurine 4'-O-methyltransferase) gene by CRISPR/Cas9 system helped in mass production of bioactive benzylisoquinoline alkaloids (papaverine, codeine, thebaine, laudanosine, noscapine, s-reticuline and morphine) in *Papaver somniferum* by converting them into biofactories (Alagoz et al. 2016). Genome editing of *SmRAS* (rosmarinic acid synthase) gene through CRISPR/Cas9 helped in regulating the biosynthetic pathway in *Salvia miltiorhiza* (Zhou et al. 2018). Genetic manipulation of Morphogenic Regulator (*MR*) gene during somatic embryogenesis was found to be helpful in increased production of cannabinoids in vitro. Targeting Induced Local Lesions In Genomes (TILLING) mutation technique of cation/H⁺ exchangers transporters showed improved salinity tolerance and crop growth in *Brassica rapa* by influencing phytohormone signaling and ion homeostasis (Navarro-León et al. 2020). Stable Isotope Labelling by Amino acids in Cell culture (SILAC) approach was successfully employed for enhancing the secondary metabolism pathways in plants (Martínez-Esteso et al. 2015). Multiple Reaction Monitoring (MRM) would also be an innovative approach in targeting the key enzymes and transporter proteins of plant secondary metabolic pathways for enhanced production and crop improvement (Martínez-Esteso et al. 2015). Isolation of plant extracellular vesicles and nanovesicles from different plant sources have also been proved to be useful for plant bioprocess engineering of secondary metabolite production (Woith et al. 2021). Wany et al. (2014) mentioned the significance of functional genomic approaches for enhancing secondary metabolite production via antisense- or sense gene suppression of metabolic pathways, production of novel compounds, regulation by compartmentalization in many plants (*A. thaliana*, *Ocimum americanum*, etc.). Jain et al. (2013) mentioned that cell suspension culture in bioreactors could be helpful for large-scale bioproduction of saponins from *Bacopa*

monnieri. Biotechnological approaches were applied to identify and characterize the genes responsible for climate resilience (*SiATG8a*, *SiASR4*, *SiMYB56*, *EcbZIP17*, *EcGBF3*, *PgNAC21*, *PgeIF4A*, etc.) in millets and were successfully introduced into several crop plants (tobacco, rice, *Arabidopsis*, etc.) for improved stress tolerance and crop performance (Singh et al. 2021). Ferrari (2010) summarized the importance of the data pool retrieved from transcriptomics, proteomics and metabolomics for developing novel techniques of secondary metabolite production. Careful dissection of the signaling network could offer a detailed understanding of elicitor induction for the modulation of metabolic pathways.

4 Constraints of Plant Secondary Metabolite Production and Future Prospects

A large number of external and internal factors regulate the biosynthesis of secondary metabolites in plants. Verma and Shukla (2015) have classified the factors into four broad categories—genetic, ontogenic, morphogenetic and environmental factors. The complex interplay between these factors, in turn, affects plant secondary metabolism in several different manners. Metabolic engineering of secondary metabolite biosynthetic pathways has faced some challenges regarding the desired concentration, bioavailability, absorption criteria and bioactivity of the compounds specifically used as food products (Davies and Espley 2013). In this connection, Nascimento and Fett-Neto (2010) pointed out some major drawbacks of developing novel engineering strategies for plant secondary metabolite production. These include—lack of availability of sustainable plant sources, constraints regarding transformation and regeneration, proper evaluation of synthesis and accumulation in different developmental and environmental conditions, problems in proper identification of metabolic intermediates, difficulties in separating the actual plant metabolic reactions from that of the endophytes or plant–endophyte interaction and the lack of knowledge regarding the intracellular and intercellular transport mechanism of secondary metabolites in the plant. Plant tissue and organ culture have been successfully employed for large-scale production of medicinally important plant secondary metabolites for many years. However, the culture conditions, media requirements, cultivation techniques showed great variation in yield and quality of the final products (Isah et al. 2018). Large-scale production of secondary metabolites is still not obtained by using cyanobacterial biofactories due to the absence of specific proteins and transcription factors required for

post-translational modifications of some enzymes involved in plant secondary metabolism (Xue and He 2015). Tiago et al. (2017) pointed out several limitations regarding the production of secondary metabolites in plants, viz. complex interaction of soil and environmental factors on the biosynthesis of secondary metabolites, pleiotropy of genes encoding the biosynthesis of secondary metabolites, multiple regulations of biosynthetic genes, obtaining superior genotypes, toxicity symptoms, etc. According to Brzycki et al. (2021), a combination of traditional approaches such as metabolic engineering and cellular engineering techniques with modern targeted epigenetic engineering could overcome the production deficit of secondary metabolites in plants. Recently, the extensive use of mathematical modelling approaches like Response Surface Method (RSM), Artificial Neural Network (ANN), Kriging and the ANN-RSM combined approach in plant biotechnology helped to maximize the yield of secondary metabolites by selecting high-performance cell lines, optimizing the culture conditions and improving cell permeability (Amdoun et al. 2021). Decoding the mechanism of induction of plant secondary metabolite production in response to environmental stresses and increased resistance against herbivores would help to optimize the targeted gene manipulation for achieving enhanced crop yield (Kessler and Kalske 2018).

5 Conclusion

Secondary metabolites play a significant role in plant growth and affect crop production in various ways. Biosynthesis and the function of plant secondary metabolites are tightly regulated by several genes and transcription factors that are involved in complex crosstalks between them. Biotic and abiotic elicitors stimulate the biosynthesis of secondary metabolites by different molecular mechanisms. Overexpression of these transcription factors by differential expression of these genes, modification at the transcriptional and translational level, induction and maintenance of polyploidy, production of new transgenic crops, use of nanoparticles, etc. are considered effective engineering approaches in increasing secondary metabolite production for crop improvement. Although there are some concerns regarding the sensitivity and specificity of these techniques, several reports are available citing successful implementations of genetic engineering approaches towards crop improvement. Future scope lies in a detailed understanding of the signaling cascade of the secondary metabolites and their specific molecular interaction with the other components responsible for plant growth and development.

References

- Abbas F, Ke Y, Yu R et al (2017) Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta* 246:803–816. <https://doi.org/10.1007/s00425-017-2749-x>
- Abbas BH, Stiles AR, Saxena PK, Liu CZ (2012) Gibberellic acid increases secondary metabolite production in *Echinacea purpurea* hairy roots. *Appl Biochem Biotechnol* 168:2057–2066. <https://doi.org/10.1007/s12010-012-9917-z>
- Abenavoli MR, Santis CD, Sidari M et al (2001) Influence of coumarin on the net nitrate uptake in durum wheat. *New Phytol* 150:619–627. <https://doi.org/10.1046/j.1469-8137.2001.00119.x>
- Abenavoli MR, Sorgonà A, Albano S, Cacco G (2004) Coumarin differentially affects the morphology of different root types of maize seedlings. *J Chem Ecol* 30:1871–1883. <https://doi.org/10.1023/B:JOEC.0000042407.28560.bb>
- Abid G, Ouertani RN, Jebara SH et al (2020) Alleviation of drought stress in faba bean (*Vicia faba* L.) by exogenous application of β -aminobutyric acid (BABA). *Physiol Mol Biol Plants* 26:1173–1186. <https://doi.org/10.1007/s12298-020-00796-0>
- Ahamed GJ, Gantait S, Mitra M et al (2020) Role of ethylene crosstalk in seed germination and early seedling development: a review. *Plant Physiol Biochem* 151:124–131. <https://doi.org/10.1016/j.plaphy.2020.03.016>
- Ahmed E, Arshad M, Zakriyya Khan M et al (2017) Secondary metabolites and their multidimensional prospective in plant life. *J Pharm Phytochem* 6:205–214
- Akhi MZ, Haque M, Biswas S (2021) Role of secondary metabolites to attenuate stress damages in plants. In: Waisundara V (ed) *Antioxidants—benefits, sources, mechanisms of action*, InTech Open, pp 1–15. <https://doi.org/10.5772/intechopen.95495>
- Alagöz 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-Cas 9 genome editing technology. *Sci Rep* 6:1–9. <https://doi.org/10.1038/srep30910>
- Amdoun R, Benyoussef EH, Benamghar A et al (2021) Approaches for modeling and optimization of the secondary metabolite production by plant biotechnology methods. In: Ramawat KG, Ekiert HM, Goyal S. (eds) *Plant cell and tissue differentiation and secondary metabolites*. Reference series in phytochemistry. Springer, Cham, pp 803–836. https://doi.org/10.1007/978-3-030-30185-9_37
- Amir R, Munir F, Khan M, Iqbal T (2019) Use of plant hormones for the improvement of plant growth and production under salt stress. In: Akhtar MS (ed) *Salt stress, microbes, and plant interactions: Causes and solution*, Springer Nature Singapore Pte Ltd, pp 59–90. https://doi.org/10.1007/978-981-13-8801-9_3
- Asl KR, Hosseini B, Sharafi A, Palazon J (2019) Influence of nano-zinc oxide on tropane alkaloid production, h6h gene transcription and antioxidant enzyme activity in *Hyoscyamus reticulatus* L. hairy roots. *Eng Life Sci* 19:73–89. <https://doi.org/10.1002/elsc.201800087>
- Batool SU, Javed B, Sohail, et al (2021) Exogenous applications of bio-fabricated silver nanoparticles to improve biochemical, antioxidant, fatty acid and secondary metabolite contents of sunflower. *Nanomaterials* 11:1750. <https://doi.org/10.3390/nano11071750>
- Blažević I, Montaut S, Burčul F et al (2020) Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. *Phytochemistry* 169. <https://doi.org/10.1016/j.phytochem.2019.112100>
- Bleeker PM, Diergaarde PJ, Ament K et al (2011) Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. *Phytochemistry* 72:68–73. <https://doi.org/10.1016/j.phytochem.2010.10.014>
- Bleeker PM, Mirabella R, Diergaarde PJ et al (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *Proc Natl Acad Sci USA* 109:20124–20129. <https://doi.org/10.1073/pnas.1208756109>
- Boehm R, Sommer S, Li SM, Heide L (2000) Genetic engineering on shikonin biosynthesis: Expression of the bacterial ubiA gene *Lithospermum erythrorhizon*. *Plant Cell Physiol* 41:911–919. <https://doi.org/10.1093/pcp/pcd013>
- Bruno L, Talarico E, Cabeiras-Freijanes L, et al (2021) Coumarin interferes with polar auxin transport altering microtubule cortical array organization in *Arabidopsis thaliana* (L.) heynh. root apical meristem. *Int J Mol Sci* 22:7305. <https://doi.org/10.3390/ijms22147305>
- Brzycki CM, Young EM, Roberts SC (2021) Secondary metabolite production in plant cell culture: A new epigenetic frontier. In: Malik S (ed) *Exploring plant cells for the production of compounds of interest*, Springer Nature, Switzerland, pp 1–37
- Bulgakov VP, Shkryl YN, Veremeichik GN (2010) Engineering high yields of secondary metabolites in *Rubia* cell cultures through transformation with rol genes. *Methods Mol Biol* 643:229–242. https://doi.org/10.1007/978-1-60761-723-5_16
- Blanco-Ulate B, Hopfer H, Figueroa-Balderas R et al (2017) Red blotch disease alters grape berry development and metabolism by interfering with the transcriptional and hormonal regulation of ripening. *J Exp Bot* 68:1225–1238. <https://doi.org/10.1093/jxb/erw506>
- Caliskan O, Radusiene J, Temizel KE et al (2017) The effects of salt and drought stress on phenolic accumulation in greenhouse-grown *Hypericum pruinatum*. *Ital J Agron* 12:271–275. <https://doi.org/10.4081/ija.2017.918>
- Cara N, Piccoli PN, Bolcato L, et al. (2020) Variation in the amino acids, volatile organic compounds and terpenes profiles in induced polyloids and in *Solanum tuberosum* varieties. *Phytochemistry* 180:112516. <https://doi.org/10.1016/j.phytochem.2020.112516>
- Campos FV, Oliveira JA, Pereira MG, Farnese FS (2019) Nitric oxide and phytohormone interactions in the response of *Lactuca sativa* to salinity stress. *Planta* 250:1475–1489. <https://doi.org/10.1007/s00425-019-03236-w>
- Caser M, Chitarra W, D'Angiolillo F et al (2019) Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* Codd. *Ind Crops Prod* 129:85–96. <https://doi.org/10.1016/j.indcrop.2018.11.068>
- Chandra S, Chandra R (2011) Engineering secondary metabolite production in hairy roots. *Phytochem Rev* 10:371–395. <https://doi.org/10.1007/s11101-011-9210-8>
- Charlet S, Gillet F, Villarreal ML et al (2000) Immobilisation of *Solanum chrysostrichum* plant cells within Ca-alginate gel beads to produce an antimycotic spirostanol saponin. *Plant Physiol Biochem* 38:875–880. [https://doi.org/10.1016/S0981-9428\(00\)01196-7](https://doi.org/10.1016/S0981-9428(00)01196-7)
- Chaudhuri K, Das S, Bandyopadhyay M et al (2009) Transgenic mimicry of pathogen attack stimulates growth and secondary metabolite accumulation. *Transgenic Res* 18:121–134. <https://doi.org/10.1007/s11248-008-9201-8>
- Chen BX, Peng YX, Yang XQ, Liu J (2021) Delayed germination of *Brassica parachinensis* seeds by coumarin involves decreased GA4 production and a consequent reduction of ROS accumulation. *Seed Sci Res* 20:1–11. <https://doi.org/10.1017/S0960258521000167>
- Chen S, Wang Q, Lu H et al (2019) Phenolic metabolism and related heavy metal tolerance mechanism in *Kandelia obovata* under Cd and Zn stress. *Ecotoxicol Environ Saf* 169:134–143. <https://doi.org/10.1016/j.ecoenv.2018.11.004>
- Chodiseti B, Rao K, Gandi S et al (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. <https://doi.org/10.1007/s11627-014-9655-8>
- Cosme M, Fernández I, Declerck S et al (2021) A coumarin exudation pathway mitigates arbuscular mycorrhizal incompatibility in *Arabidopsis thaliana*. *Plant Mol Biol* 106:319–334. <https://doi.org/10.1007/s11103-021-01143-x>
- Cuny MAC, La Forgia D, Desurmont GA et al (2019) Role of cyanogenic glycosides in the seeds of wild lima bean, *Phaseolus lunatus*: defense, plant nutrition or both? *Planta* 250:1281–1292. <https://doi.org/10.1007/s00425-019-03221-3>
- Czerniewicz P, Sytykiewicz H, Durak R et al (2017) Role of phenolic compounds during antioxidative responses of winter triticale to aphid and beetle attack. *Plant Physiol Biochem* 118:529–540. <https://doi.org/10.1016/j.plaphy.2017.07.024>
- Danova K, Motyka V, Todorova M et al (2018) Effect of cytokinin and auxin treatments on morphogenesis, terpenoid biosynthesis, photosystem structural organization, and endogenous isoprenoid cytokinin profile in *Artemisia alba* Turra *in vitro*. *J Plant Growth Regul* 37:403–418. <https://doi.org/10.1007/s00344-017-9738-y>
- Davies K, Espley R (2013) Opportunities and challenges for metabolic engineering of secondary metabolite pathways for improved human health characters in fruit and vegetable crops. *N Z J Crop Hortic Sci* 41:154–177. <https://doi.org/10.1080/01140671.2013.793730>
- Deguchi M, Kane S, Potlakayala S, et al (2020) Metabolic engineering strategies of industrial hemp (*Cannabis sativa* L.): a brief review of the advances and challenges. *Front Plant Sci* 11. <https://doi.org/10.3389/fpls.2020.580621>
- Delgoda R, Murray JE (2017) Evolutionary perspectives on the role of plant secondary metabolites. In: Badal S, Delgoda R (eds) *Pharmacognosy: fundamentals, applications and strategies*. Academic Press, pp 93–100. <https://doi.org/10.1016/B978-0-12-802104-0.00007-X>
- Dong NQ, Sun Y, Guo T et al (2020) UDP-glucosyltransferase regulates grain size and abiotic stress tolerance associated with metabolic flux redirection in rice. *Nat Commun* 11:1–16. <https://doi.org/10.1038/s41467-020-16403-5>
- Dubois M, Van den Broeck L, Inzé D (2018) The pivotal role of ethylene in plant growth. *Trends Plant Sci* 23:311–323. <https://doi.org/10.1016/j.tplants.2018.01.003>
- Eom SH, Baek SA, Kim JK, Hyun TK (2018) Transcriptome analysis in chinese cabbage (*Brassica rapa* ssp. *Pekinensis*) provides the role of glucosinolate metabolism in response to drought stress. *Molecules* 23. <https://doi.org/10.3390/molecules23051186>
- Erb M, Kliebenstein DJ (2020) Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The blurred functional trichotomy. *Plant Physiol* 184:39–52. <https://doi.org/10.1104/PP.20.00433>
- Essoh AP, Monteiro F, Pena AR et al (2020) Exploring glucosinolates diversity in Brassicaceae: a genomic and chemical assessment for deciphering abiotic stress tolerance. *Plant Physiol Biochem* 150:151–161. <https://doi.org/10.1016/j.plaphy.2020.02.032>
- Farvardin A, Ebrahimi A, Hosseinpour B, Khosrowshahli M (2017) Effects of growth regulators on callus induction and secondary metabolite production in *Cuminum cyminum*. *Nat Prod Res* 31:1963–1970. <https://doi.org/10.1080/14786419.2016.1272105>
- Fazal H, Abbasi BH, Ahmad N, Ali M (2016) Elicitation of medicinally important antioxidant secondary metabolites with silver and gold nanoparticles in callus cultures of *Prunella vulgaris* L. *Appl Biochem Biotechnol* 180:1076–1092. <https://doi.org/10.1007/s12010-016-2153-1>
- Ferrari S (2010) Biological elicitors of plant secondary metabolites: mode of action and use in the production of nutraceuticals. *Adv Exp Med Biol* 698:152–166. https://doi.org/https://doi.org/10.1007/978-1-4419-7347-4_12
- Fouad AS, Hafez RM (2018) Effect of cobalt nanoparticles and cobalt ions on alkaloids production and expression of CrMPK3 gene in *Catharanthus roseus* suspension cultures. *Cellular and Molecular Biology* 64:62–69. <https://doi.org/10.14715/cmb/2018.64.12.13>
- Frank L, Wenig M, Ghirardo A et al (2021) Isoprene and β -caryophyllene confer plant resistance via different plant internal signalling pathways. *Plant, Cell Environ* 44:1151–1164. <https://doi.org/10.1111/pce.14010>
- Gantait P, Mukherjee E (2021) Induced autopolyploidy—a promising approach for enhanced biosynthesis of plant secondary metabolites: an insight. *J Gene Eng Biotechnol* 19. <https://doi.org/10.1186/s43141-020-00109-8>
- Gantet P, Memelink J (2002) Transcription factors: Tools to engineer the production of pharmacologically active plant metabolites. *Trends Pharmacol Sci* 23:563–569. [https://doi.org/10.1016/S0165-6147\(02\)02098-9](https://doi.org/10.1016/S0165-6147(02)02098-9)
- Ghassemi-Golezani K, Hassanzadeh N, Shakiba MR, Esmaeilpour B (2020) Exogenous salicylic acid and 24-epi-brassinolide improve antioxidant capacity and secondary metabolites of *Brassica nigra*. *Biocatal Agric Biotechnol* 26. <https://doi.org/10.1016/j.cbab.2020.101636>
- Gupta S, Chaturvedi P (2019) Enhancing secondary metabolite production in medicinal plants using endophytic elicitors: A case study of *Centella asiatica* (Apiaceae) and asiaticoside. In: Hodkinson TR, Doohan FM, Saunders MJ, Murphy BR (eds) *Endophytes for a growing world*, Cambridge University Press, pp 310–327. <https://doi.org/10.1017/9781108607667.015>
- Habash SS, Könen PP, Loeschcke A et al (2020) The plant sesquiterpene nootkatone efficiently reduces *Heterodera schachtii* parasitism by activating plant defense. *Int J Mol Sci* 21:1–17. <https://doi.org/10.3390/ijms21249627>
- Han J, Liu H tao, Wang S chang, et al (2020) A class I TGA transcription factor from *Tripterygium wilfordii* Hook.f. modulates the biosynthesis of secondary metabolites in both native and heterologous hosts. *Plant Science* 290:110293. <https://doi.org/10.1016/j.plantsci.2019.110293>
- Iqbal N, Khan NA, Ferrante A et al (2017) Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Front Plant Sci* 8:1–19. <https://doi.org/10.3389/fpls.2017.00475>
- Isah T, Umar S, Mujib A et al (2018) Secondary metabolism of pharmaceuticals in the plant *in vitro* cultures: Strategies, approaches, and limitations to achieving higher yield. *Plant Cell, Tissue Organ Cult* 132:239–265. <https://doi.org/10.1007/s11240-017-1332-2>
- Jabeen M, Akram NA, Ashraf M et al (2021) Thiamin stimulates growth and secondary metabolites in turnip (*Brassica rapa* L.) leaf and root under drought stress. *Physiol Plant* 172:1399–1411. <https://doi.org/10.1111/ppl.13215>
- Jadaun JS, Sangwan NS, Narnoliya LK, et al (2017) Over-expression of DXS gene enhances terpenoidal secondary metabolite accumulation in geranium and *Withania somnifera*: active involvement of plastid isoprenogenic pathway in their biosynthesis. 159:381–400. <https://doi.org/https://doi.org/10.1111/ppl.12507>
- Jain M, Rajput R, Mishra A (2013) Enhancement of secondary metabolite biosynthesis in *Bacopa monnieri*: An *in vitro* study. *Res J Recent Sci* 2:13–16
- Jan R, Asaf S, Numan M et al (2021) Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy* 11:1–31. <https://doi.org/10.3390/agronomy11050968>
- Jiao X, Li Y, Zhang X et al (2019) Exogenous dopamine application promotes alkali tolerance of apple seedlings. *Plants* 8:580. <https://doi.org/10.3390/plants8120580>

- Jurić S, Sopko Stracenski K, Król-Kilińska Ż et al (2020) The enhancement of plant secondary metabolites content in *Lactuca sativa* L. by encapsulated bioactive agents. *Sci Rep* 10:1–12. <https://doi.org/10.1038/s41598-020-60690-3>
- Karalija E, Neimarlija D, Cakar J, Paric A (2016) Elicitation of biomass and secondary metabolite production, antioxidative and antimicrobial potential of basil and oregano induced by BA and IBA application. *Euro J Med Plants* 14:1–11. <https://doi.org/10.9734/ejmp/2016/26121>
- Karimi N, Behbahani M, Dini G, Razmjou A (2018) Enhancing the secondary metabolite and anticancer activity of *Echinacea purpurea* callus extracts by treatment with biosynthesized ZnO nanoparticles. *Adv Nat Sci Nanosci Nanotechnol* 9. <https://doi.org/10.1088/2043-6254/aaf1af>
- Keller CP, Stahlberg R, Barkawi LS, Cohen JD (2004) Long-term inhibition by auxin of leaf blade expansion in Bean and Arabidopsis. *Plant Physiol* 134:1217–1226. <https://doi.org/10.1104/pp.103.032300>
- Kessler A, Kalske A (2018) Plant secondary metabolite diversity and species interactions. *Annu Rev Ecol Evol Syst* 49:115–138. <https://doi.org/10.1146/annurev-ecolsys-110617-062406>
- Kim YC, Kim YH, Lee YH et al (2013) B-Amino-N-Butyric acid regulates seedling growth and disease resistance of kimchi cabbage. *Plant Pathol J* 29:305–316. <https://doi.org/10.5423/PPJ.OA.12.2012.0191>
- Kumar A (2015) Improving secondary metabolite production in tissue cultures. In: Bahadur B, Venkat Rajam M, Sahijram L, Krishnamurthy K (eds) *Plant biology and biotechnology*. Springer, New Delhi, pp 397–406. https://doi.org/10.1007/978-81-322-2283-5_20
- Kumar SR, Shilpashree HB, Nagegowda DA (2018) Terpene moiety enhancement by overexpression of geranyl(Geranyl) diphosphate synthase and geraniol synthase elevates monomeric and dimeric monoterpene indole alkaloids in transgenic *Catharanthus roseus*. *Front Plant Sci* 9:1–16. <https://doi.org/10.3389/fpls.2018.00942>
- Kumari A, Parida AK (2018) Metabolomics and network analysis reveal the potential metabolites and biological pathways involved in salinity tolerance of the halophyte *Salvadora persica*. *Environ Exp Bot* 148:85–99. <https://doi.org/10.1016/j.envexpbot.2017.12.021>
- Li B, Fan R, Guo S et al (2019) The Arabidopsis MYB transcription factor, MYB111 modulates salt responses by regulating flavonoid biosynthesis. *Environ Exp Bot* 166. <https://doi.org/10.1016/j.envexpbot.2019.103807>
- Li B, Fan R, Sun G et al (2021) Flavonoids improve drought tolerance of maize seedlings by regulating the homeostasis of reactive oxygen species. *Plant Soil* 461:389–405. <https://doi.org/10.1007/s11104-020-04814-8>
- Li FX, Jin ZP, Zhao DX, et al (2006) Overexpression of the *Saussurea medusa* chalcone isomerase gene in *S. involucrata* hairy root cultures enhances their biosynthesis of apigenin. *Phytochemistry* 67:553–560
- Liang J, He J (2018) Protective role of anthocyanins in plants under low nitrogen stress. *Biochem Biophys Res Commun* 498:946–953. <https://doi.org/10.1016/j.bbrc.2018.03.087>
- Liu Q, Gao T, Liu W et al (2020) Functions of dopamine in plants: a review. *Plant Signal Behav* 15:1827782. <https://doi.org/10.1080/15592324.2020.1827782>
- Liu S, Zhang P, Li C, Xia C (2019) The moss jasmonate ZIM-domain protein PnJAZ1 confers salinity tolerance via crosstalk with the abscisic acid signalling pathway. *Plant Sci* 280:1–11. <https://doi.org/10.1016/j.plantsci.2018.11.004>
- Ma YR, Wang KF, Wang WJ et al (2019) Advances in the metabolic engineering of *Yarrowia lipolytica* for the production of terpenoids. *Biores Technol* 281:449–456. <https://doi.org/10.1016/j.biortech.2019.02.116>
- Malekzadeh P, Khosravi-Nejad F, Hatamnia AA, Sheikhhakbari Mehr R (2017) Impact of postharvest exogenous γ -aminobutyric acid treatment on cucumber fruit in response to chilling tolerance. *Physiol Mol Biol Plants* 23:827–836. <https://doi.org/10.1007/s12298-017-0475-2>
- Manjili FA, Sedghi M, Pesarakli M (2012) Effects of phytohormones on proline content and antioxidant enzymes of various wheat cultivars under salinity stress. *J Plant Nutr* 35:1098–1111. <https://doi.org/10.1080/01904167.2012.671411>
- Martinez-Esteso MJ, Martínez-Márquez A, Sellés-Marchart S et al (2015) The role of proteomics in progressing insights into plant secondary metabolism. *Front Plant Sci* 6:1–8. <https://doi.org/10.3389/fpls.2015.00504>
- Matthews D, Jones H, Gans P et al (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. *J Agric Food Chem* 53:7766–7776. <https://doi.org/10.1021/jf050589r>
- McLay ER, Pontaroli AC, Wargent JJ (2020) UV-B induced flavonoids contribute to reduced biotrophic disease susceptibility in lettuce seedlings. *Front Plant Sci* 11:1–13. <https://doi.org/10.3389/fpls.2020.594681>
- Mekawy AMM, Abdelaziz MN, Ueda A (2018) Apigenin pretreatment enhances growth and salinity tolerance of rice seedlings. *Plant Physiol Biochem* 130:94–104. <https://doi.org/10.1016/j.plaphy.2018.06.036>
- Memelink J, Kijne JW, van der Heijden R, Verpoorte R (2001) Genetic modification of plant secondary metabolite pathways using transcriptional regulators. *Adv Biochem Eng Biotechnol* 72:103–125. https://doi.org/10.1007/3-540-45302-4_4
- Mishra J, Srivastava R, Trivedi PK, Verma PC (2020) Effect of virus infection on the secondary metabolite production and phytohormone biosynthesis in plants. *3 Biotech* 10:1–16. <https://doi.org/10.1007/s13205-020-02541-6>
- Moustaka J, Tanou G, Giannakoula A et al (2020) Anthocyanin accumulation in poinsettia leaves and its functional role in photo-oxidative stress. *Environ Exp Bot* 175. <https://doi.org/10.1016/j.envexpbot.2020.104065>
- Moyano E, Jouhikainen K, Tammela P et al (2003) Effect of *pmt* gene overexpression on tropane alkaloid production in transformed root cultures of *Datura metel* and *Hyoscyamus muticus*. *J Exp Bot* 54:203–211. <https://doi.org/10.1093/jxb/erg014>
- Munné-Bosch S, Alegre L (2003) Drought-induced changes in the redox state of α -tocopherol, ascorbate, and the diterpene carnosic acid in chloroplasts of Labiateae species differing in carnosic acid contents. *Plant Physiol* 131:1816–1825. <https://doi.org/10.1104/pp.102.019265>
- Myrans H, Vandeger RK, Henry RJ, Gleadow RM (2021) Nitrogen availability and allocation in sorghum and its wild relatives: Divergent roles for cyanogenic glucosides. *J Plant Physiol* 258–259. <https://doi.org/10.1016/j.jplph.2021.153393>
- Nagegowda DA, Gupta P (2020) Advances in biosynthesis, regulation, and metabolic engineering of plant specialized terpenoids. *Plant Sci* 294. <https://doi.org/10.1016/j.plantsci.2020.110457>
- Nakabayashi R, Yonekura-Sakakibara K, Urano K et al (2014) Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. *Plant J* 77:367–379. <https://doi.org/10.1111/tpj.12388>
- Narayani M, Srivastava S (2017) Elicitation: a stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production. *Phytochem Rev* 16:1227–1252. <https://doi.org/10.1007/s11101-017-9534-0>
- Nascimento NC, Fett-Neto AG (2010) Plant secondary metabolism and challenges in modifying its operation: an overview. *Methods Mol Biol* 643:1–13. https://doi.org/10.1007/978-1-60761-723-5_1

- Navarro-León E, López-Moreno FJ, Atero-Calvo S et al (2020) CAX1a tilling mutations modify the hormonal balance controlling growth and ion homeostasis in *Brassica rapa* plants subjected to salinity. *Agronomy* 10:1–12. <https://doi.org/10.3390/agronomy10111699>
- Nguyen DTP, Lu N, Kagawa N, Takagaki M (2019) Optimization of photosynthetic photon flux density and root-zone temperature for enhancing secondary metabolite accumulation and production of coriander in plant factory. *Agronomy* 9. <https://doi.org/10.3390/agronomy9050224>
- Niro E, Marzaioli R, De Crescenzo S et al (2016) Effects of the allelochemical coumarin on plants and soil microbial community. *Soil Biol Biochem* 95:30–39. <https://doi.org/10.1016/j.soilbio.2015.11.028>
- Nomura T, Yoneda A, Ogita S, Kato Y (2021) Activation of cryptic secondary metabolite biosynthesis in bamboo suspension cells by a histone deacetylase inhibitor. *Appl Biochem Biotechnol* 193:3496–3511. <https://doi.org/10.1007/s12010-021-03629-2>
- Parvin K, Hasanuzzaman M, Mohsin SM et al (2020) Coumarin improves tomato plant tolerance to salinity by enhancing antioxidant defence, glyoxalase system and ion homeostasis. *Plant Biol* 23:181–192. <https://doi.org/10.1111/plb.13208>
- Perkowska I, Potrykus M, Siwinska J et al (2021) Interplay between coumarin accumulation, iron deficiency and plant resistance to *Dickeya* spp. *Int J Mol Sci* 22:6449. <https://doi.org/10.3390/ijms22126449>
- Petridis A, Therios I, Samouris G, Tananaki C (2012) Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environ Exp Bot* 79:37–43. <https://doi.org/10.1016/j.envexpbot.2012.01.007>
- Pilankong P, Suska-Ard P, Wannakrairoj S (2017) Effects of colchicine and oryzalin on polyploidy induction and production of capsaicin in *Capsicum frutescens* L. *Thai J Agric Sci* 50:108–120
- Poveda J, Eugui D, Velasco P (2020) Natural control of plant pathogens through glucosinolates: an effective strategy against fungi and oomycetes. *Phytochem Rev* 19:1045–1059. <https://doi.org/10.1007/s11101-020-09699-0>
- Priya M, Sharma L, Kaur R et al (2019) GABA (γ -aminobutyric acid), as a thermo-protectant, to improve the reproductive function of heat-stressed mungbean plants. *Sci Rep* 9:1–14. <https://doi.org/10.1038/s41598-019-44163-w>
- Ptak A, Simlat M, Morańska E et al (2019) Exogenous melatonin stimulated Amaryllidaceae alkaloid biosynthesis in *in vitro* cultures of *Leucojum aestivum* L. *Ind Crops Prod* 138. <https://doi.org/10.1016/j.indcrop.2019.06.021>
- Rajam MV (2020) RNA silencing technology: A boon for crop improvement. *J Biosci* 45:118. <https://doi.org/10.1007/s12038-020-00082-x>
- Sabzehzari M, Zeinali M, Naghavi MR (2020) CRISPR-based metabolic editing: next-generation metabolic engineering in plants. *Gene* 759. <https://doi.org/10.1016/j.gene.2020.144993>
- Saleh AM, Madany MMY, González L (2015) The effect of coumarin application on early growth and some physiological parameters in faba bean (*Vicia faba* L.). *J Plant Growth Regul* 34:233–241. <https://doi.org/10.1007/s00344-014-9459-4>
- Sami F, Siddiqui H, Hayat S (2019) Interaction of glucose and phytohormone signaling in plants. *Plant Physiol Biochem* 135:119–126. <https://doi.org/10.1016/j.plaphy.2018.11.005>
- Sarashgi A, Puschenreiter M, Baune M et al (2021) Does the exudation of coumarins from Fe-deficient, soil-grown Brassicaceae species play a significant role in plant Fe nutrition? *Rhizosphere* 19:2–4. <https://doi.org/10.1016/j.rhisph.2021.100410>
- Shahhoseini R, Azizi M, Asili J et al (2020) Effects of zinc oxide nanoelicitors on yield, secondary metabolites, zinc and iron absorption of Feverfew (*Tanacetum parthenium* (L.) Schultz Bip.). *Acta Physiol Plant* 42:1–18. <https://doi.org/10.1007/s11738-020-03043-x>
- Shakya P, Marslin G, Siram K et al (2019) Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of *Hypericum perforatum*. *J Pharm Pharmacol* 71:70–82. <https://doi.org/10.1111/jphp.12743>
- Shi J, Habben JE, Archibald RL et al (2015) Overexpression of ARGOS genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both Arabidopsis and maize. *Plant Physiol* 169:266–282. <https://doi.org/10.1104/pp.15.00780>
- Siegień I, Filoc M, Staszak AM, Ciereszko I (2021) Cyanogenic glycosides can function as nitrogen reservoir for flax plants cultured under N-deficient conditions. *Plant, Soil Environ* 67:245–253. <https://doi.org/10.17221/573/2020-PSE>
- Simic GS, Tusevski O, Maury S, et al (2014) Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response in *Hypericum perforatum* L. shoot cultures. *Sci World J*. <https://doi.org/10.1155/2014/609649>
- Singh RK, Muthamilarasan M, Prasad M (2021) Biotechnological approaches to dissect climate-resilient traits in millets and their application in crop improvement. *J Biotechnol* 327:64–73. <https://doi.org/10.1016/j.jbiotec.2021.01.002>
- Sita K, Kumar V (2020) Role of Gamma Amino Butyric Acid (GABA) against abiotic stress tolerance in legumes: a review. *Plant Physiol Rep* 25:654–663. <https://doi.org/10.1007/s40502-020-00553-1>
- Sivanandhan G, Kapildev G, Rajesh M et al (2013) Increased production of withanolide A, withanone, and withaferin A in hairy root cultures of *Withania somnifera* (L.) Dunal elicited with methyl jasmonate and salicylic acid. *Plant Cell, Tissue Organ Cult* 114:121–129. <https://doi.org/10.1007/s11240-013-0297-z>
- Sohail MN, Blomstedt CK, Gleadow RM (2020) Allocation of resources to cyanogenic glucosides does not incur a growth sacrifice in *Sorghum bicolor* (L.) moench. *Plants* 9:1–15. <https://doi.org/10.3390/plants9121791>
- Song N, Ma L, Wang W, et al (2019) Capsidiol, a defensive sesquiterpene produced by wild tobacco in response to attack from the fungal pathogen *Alternaria alternata*, is regulated by an ERF2-like transcription factor. *bioRxiv* 1–42. <https://doi.org/10.1101/573675>
- Stringlis IA, Yu K, Feussner K et al (2018) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc Natl Acad Sci USA* 115:E5213–E5222. <https://doi.org/10.1073/pnas.1722335115>
- Sultana R, Abbasi MW, Adnan MY, Azeem M (2020) exogenously applied coumarin-induced salt tolerance in a multipurpose crop *Sorghum bicolor* under saline conditions. 17:177–184
- Sun J, Qiu C, Ding Y et al (2020) Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. *BMC Genomics* 21:1–13. <https://doi.org/10.1186/s12864-020-06815-4>
- Tao H, Hu S, Xia C et al (2021) Involvement of glucosinolates in the resistance to zinc oxide nanoparticle-induced toxicity and growth inhibition in Arabidopsis. *Environ Sci Process Impacts* 23:1040–1049. <https://doi.org/10.1039/d1em00134e>
- Tattersall DB, Bak S, Jones PR et al (2001) Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science* 293:1826–1828. <https://doi.org/10.1126/science.1062249>
- Teoh ES (2016) Medicinal orchids of Asia. Springer, Cham. <https://doi.org/10.1007/978-3-319-24274-3>
- Thanh NT, Murthy HN, Yu KW et al (2005) Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble bioreactors. *Appl Microbiol Biotechnol* 67:197–201. <https://doi.org/10.1007/s00253-004-1759-3>
- Tiago O, Maicon N, Ivan RC et al (2017) Plant secondary metabolites and its dynamical systems of induction in response to environmental

- factors: A review. *Afr J Agric Res* 12:71–84. <https://doi.org/10.5897/ajar2016.11677>
- Tiwari R, Rana CS (2015) Plant secondary metabolites: a review. *Int J Eng Res Gen Sci* 3:661–669
- Vardhan PV, Shukla LI (2017) Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. *Int J Radiat Biol* 93:967–979. <https://doi.org/10.1080/09553002.2017.1344788>
- Venugopalan A, Srivastava S (2015) Endophytes as *in vitro* production platforms of high value plant secondary metabolites. *Biotechnol Adv* 33:873–887. <https://doi.org/10.1016/j.biotechadv.2015.07.004>
- Verma N, Shukla S (2015) Impact of various factors responsible for fluctuation in plant secondary metabolites. *J Appl Res Med Arom Plants* 2:105–113. <https://doi.org/10.1016/j.jarmap.2015.09.002>
- Wang A, Zhu M, Luo Y et al (2017) A sweet potato cinnamate 4-hydroxylase gene, *IbC4H*, increases phenolics content and enhances drought tolerance in tobacco. *Acta Physiol Plant* 39. <https://doi.org/10.1007/s11738-017-2551-1>
- Wang F, Park YL, Gutensohn M (2020) Glandular trichome-derived sesquiterpenes of wild tomato accessions (*Solanum habrochaites*) affect aphid performance and feeding behavior. *Phytochemistry* 180. <https://doi.org/10.1016/j.phytochem.2020.112532>
- Wang J, Hou B (2009) Glycosyltransferases: key players involved in the modification of plant secondary metabolites. *Front Biol China* 4:39–46. <https://doi.org/10.1007/s11515-008-0111-1>
- Wang Q, Reddy VA, Panicker D et al (2016) Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor MsYABBY5 from spearmint (*Mentha spicata*). *Plant Biotechnol J* 16:1619–1632. <https://doi.org/10.1111/pbi.12525>
- Wang X, Wang X, Peng C et al (2021) Exogenous gamma-aminobutyric acid coordinates active oxygen and amino acid homeostasis to enhance heat tolerance in wheat seedlings. *J Plant Growth Regul*. <https://doi.org/10.1007/s00344-021-10474-4>
- Wany A, Nigam VK, Pandey DM (2014) Understanding new paradigm of plant metabolomes through secondary metabolite enrichment. *Res J Biotechnol* 9:90–98
- Weremczuk-Jeżyna I, Hnatuszko-Konka K, Lebelt L, Grzegorzczak-Karolak I (2021) The protective function and modification of secondary metabolite accumulation in response to light stress in *Dracocephalum forrestii* shoots. *Int J Mol Sci* 22:7965. <https://doi.org/10.3390/ijms22157965>
- Woith E, Guerriero G, Hausman JF et al (2021) Plant extracellular vesicles and nanovesicles: Focus on secondary metabolites, proteins and lipids with perspectives on their potential and sources. *Int J Mol Sci* 22:1–20. <https://doi.org/10.3390/ijms22073719>
- Wu CH, Popova EV, Hahn EJ, Paek KY (2009) Linoleic and α -linolenic fatty acids affect biomass and secondary metabolite production and nutritive properties of *Panax ginseng* adventitious roots cultured in bioreactors. *Biochem Eng J* 47:109–115. <https://doi.org/10.1016/j.bej.2009.07.011>
- Wu X, Jia Q, Ji S et al (2020) Gamma-aminobutyric acid (GABA) alleviates salt damage in tomato by modulating Na⁺ uptake, the GAD gene, amino acid synthesis and reactive oxygen species metabolism. *BMC Plant Biol* 20:1–21. <https://doi.org/10.1186/s12870-020-02669-w>
- Xing SH, Guo SB, Wang Q, et al (2011) Induction and flow cytometry identification of tetraploids from seed-derived explants through colchicine treatments in *Catharanthus roseus* (L.) G. Don. *Journal of Biomedicine and Biotechnology* 2011:793198. <https://doi.org/10.1155/2011/793198>
- Xu B, Long Y, Feng X et al (2021) GABA signalling modulates stomatal opening to enhance plant water use efficiency and drought resilience. *Nat Commun* 12:1952. <https://doi.org/10.1038/s41467-021-21694-3>
- Xu L, Yue Q, Bian F et al (2017) Melatonin enhances phenolics accumulation partially *via* ethylene signaling and resulted in high antioxidant capacity in grape berries. *Front Plant Sci* 8:1–12. <https://doi.org/10.3389/fpls.2017.01426>
- Xue Y, He Q (2015) Cyanobacteria as cell factories to produce plant secondary metabolites. *Front Bioeng Biotechnol* 3:57. <https://doi.org/10.3389/fbioe.2015.00057>
- Yadav AK, Singh S, Yadav SC et al (2013) Induction and morpho-chemical characterization of *Stevia rebaudiana* colchicoids. *Ind J Agric Sci* 83:159–165
- Yang D, Huang Z, Jin W et al (2018) DNA methylation: A new regulator of phenolic acids biosynthesis in *Salvia miltiorrhiza*. *Ind Crops Prod* 124:402–411. <https://doi.org/10.1016/j.indcrop.2018.07.046>
- Yang T, Lian Y, Wang C (2019) Comparing and contrasting the multiple roles of butenolide plant growth regulators: Strigolactones and karrikins in plant development and adaptation to abiotic stresses. *Int J Mol Sci* 20:6–11. <https://doi.org/10.3390/ijms20246270>
- Yavas I, Unay A, Ali S et al (2020) UV-B Radiations and Secondary Metabolites. *Turkish J Agric Food Sci Technol* 8:147–157. <https://doi.org/10.24925/turjaf.v8i1.147-157.2878>
- Ye H, Huang LL, De CS, Zhong JJ (2004) Pulsed electric field stimulates plant secondary metabolism in suspension cultures of *Taxus chinensis*. *Biotechnol Bioeng* 88:788–795. <https://doi.org/10.1002/bit.20266>
- Yu B, Liu Y, Pan Y et al (2018) Light enhanced the biosynthesis of terpenoid indole alkaloids to meet the opening of cotyledons in process of photomorphogenesis of *Catharanthus roseus*. *Plant Growth Regul* 84:617–626. <https://doi.org/10.1007/s10725-017-0366-0>
- Zaim M, Verma RK, Pandey R, Lal RK (2014) Genotype-dependent response of an RNA virus infection on selected pharmaceutically important alkaloids in *Papaver somniferum*. *J Herbs Spices Med Plants* 20:124–131. <https://doi.org/10.1080/10496475.2013.840817>
- Zeng W, Hassan MJ, Kang D et al (2021) Photosynthetic maintenance and heat shock protein accumulation relating to γ -aminobutyric acid (GABA)-regulated heat tolerance in creeping bentgrass (*Agrostis stolonifera*). *S Afr J Bot* 141:405–413. <https://doi.org/10.1016/j.sajb.2021.05.028>
- Zhang F, Guo H, Huang J et al (2020a) A UV-B-responsive glycosyltransferase, OsUGT706C2, modulates flavonoid metabolism in rice. *Sci China Life Sci* 63:1037–1052. <https://doi.org/10.1007/s11427-019-1604-3>
- Zhang Y, Deng T, Sun L, et al (2020b) Phylogenetic patterns suggest frequent multiple origins of secondary metabolites across the seed plant “tree of life”. *Nat Sci Rev* 8:nwaa105. <https://doi.org/10.1093/nsr/nwaa105>
- Zhang K, Sun Y, Li M, Long R (2021) CrUGT87A1, a UDP-sugar glycosyltransferases (UGTs) gene from *Carex rigescens*, increases salt tolerance by accumulating flavonoids for antioxidation in *Arabidopsis thaliana*. *Plant Physiol Biochem* 159:28–36. <https://doi.org/10.1016/j.plaphy.2020.12.006>
- Zhao T, Li S, Wang J et al (2020) Engineering tropane alkaloid production based on metabolic characterization of ornithine decarboxylase in *Atropa belladonna*. *ACS Synth Biol* 9:437–448. <https://doi.org/10.1021/acssynbio.9b00461>
- Zhou Z, Tan H, Li Q et al (2018) CRISPR/Cas9-mediated efficient targeted mutagenesis of RAS in *Salvia miltiorrhiza*. *Phytochemistry* 148:63–70. <https://doi.org/10.1016/j.phytochem.2018.01.015>
- Zwenger S, Basu C (2008) Plant terpenoids: Applications and future potentials. *Biotechnol Mol Biol Rev* 3:1–7