E-ISSN 2549-8703 I P-ISSN 2302-7282

BIOTROPIKA *Journal of Tropical Biology*

https://biotropika.ub.ac.id/

Vol. 9 | No. 3 | 2021 | DOI: 10.21776/ub.biotropika.2021.009.03.08

REVIEW: THE ROLE OF PHYTOHORMONES AND ENVIRONMENTAL STRESSES TO INCREASE THE PRODUCTION OF PLANT SECONDARY METABOLITES

REVIEW: PERAN FITOHORMON DAN CEKAMAN LINGKUNGAN UNTUK MENINGKATKAN PRODUKSI METABOLIT SEKUNDER TANAMAN

Selis Meriem[1)*]

Received : September, 6 2021

Accepted : October, 13 2021

Authors affiliation:

[1)] Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Alauddin, Gowa 92113, Indonesia

Correspondence email:

*selis.meriem@uin-alauddin.ac.id

ABSTRACT

Secondary metabolites are synthesized by plants when subjected to abiotic and biotic stresses as a defense mechanism and play an ecologically vital role both as pollination attractants and as repellents. These compounds are not essential for the process of growth and development but provide a significant impact on human welfare in various aspects as pharmaceuticals and therapeutics, aroma and taste, biopesticides, and agrochemicals. This natural product has high economic and commercial value, so it is important to explore it along with the increasing needs of consumers and industry at a large scale. Various extensive studies were carried out to improve and increase the production and accumulation of secondary metabolites. One of the strategies adopted in this review is the role of exogenous phytohormones in triggering the production of important secondary metabolites based on their classification, namely terpenoids, phenols, and flavonoids, as well as alkaloids and nitrogen-containing compounds. Bioregulators work as elicitors to induce the production of secondary metabolites through up-regulation of specific gene expression. In some cases, the application of phytohormones combined with exposure to extreme abiotic stresses showed overexpression of secondary metabolite synthesis compared to treatment with only those subjected to phytohormone. The abiotic stress of irradiation, UV-B, temperature, drought, ultrasonic, salinity, and metal stress was discussed in this study. Oxidative conditions under environmental changes trigger the plant to produce reactive oxygen species (ROS), which induce various secondary metabolites as part of the regulation of antioxidant systems. Micropropagation of medicinal plants by the addition of exogenous growth regulators could enhance the production and accumulation of important secondary metabolites.

Keywords: *alkaloids, phenols, phytohormones, terpenoids, stress*

ABSTRAK

Metabolit sekunder disintesis oleh tanaman ketika tercekam pada kondisi abiotik maupun biotik sebagai mekanisme pertahanan dan secara ekologis memainkan peran penting baik sebagai atraktan polinasi maupun sebagai repelen. Senyawa ini tidak esensial bagi proses pertumbuhan dan perkembangan tetapi memberikan manfaat signifikan bagi kesejahteraan manusia dalam berbagai bidang sebagai obat-obatan dan terapeutik, aroma dan cita rasa, biopestisida dan agrokimia. Produk alami ini sangat bernilai ekonomi tinggi dan komersil sehingga menjadi penting untuk dieksplor seiring dengan peningkatan kebutuhan konsumen dan industri pada skala yang lebih luas. Berbagai studi ekstensif dilakukan untuk memperbaiki serta meningkatkan produksi dan akumulasi metabolit sekunder. Salah satu strategi yang diangkat dalam review ini adalah peran fitohormon eksogen dalam memicu produksi metabolit sekunder penting berdasarkan klasifikasinya yaitu terpenoid, fenol dan flavonoid, serta alkaloid dan senyawa yang mengandung nitrogen. Bioregulator bekerja sebagai elisitor dalam memicu produksi metabolit sekunder melalui up-regulasi ekspresi gen spesifik. Pada beberapa kasus, aplikasi fitohormon yang dikombinasikan dengan paparan cekaman ekstrem menunjukkan over ekspresi sintesis metabolit sekunder dibandingkan dengan penambahan fitohomon saja. Stres abiotik yaitu iradiasi, UV-B, suhu, kekeringan, ultrasonik, salinitas, dan cekaman logam berat dibahas dalam studi ini. Kondisi oksidatif menghasilkan reactive oxgyen species (ROS) yang menginduksi berbagai senyawa metabolit sekunder sebagai bagian dari regulasi sistem antioksidan. Mikropropagasi tanaman obat dengan menambahkan regulator pertumbuhan secara eksogen mampu meningkatkan produksi dan akumulasi metabolit sekunder penting.

Kata kunci: *alkaloid, cekaman, fenol, fitohormon, terpenoid*

How to cite:
Meriem S. 2021. Review: The role of phytohormones and environmental stresses to increase the production of plant secondary metabolites. *Journal of Tropical Biology* 9 (3): 237-245.

INTRODUCTION

Plants encounter various environmental stress conditions that could not be obviated either as a result of changes in abiotic and biotic factors. Plants naturally have defense mechanisms and also resistance systems to overcome the detrimental effects of stress. The protection strategy is indicated by the accumulation of natural products, i.e., secondary metabolites. The synthesis of these compounds is highly prominent for defense regulation but does not provide an essential role for plant survival. Protection mechanisms from abiotic stresses such as high temperature, heavy metals, light (UV-B), salinity, and drought have been reported to act as elicitors in modulating the biochemical and physiological content of plants through the increase of secondary metabolites such as ginsenoside [1], polyphenols [2], anthocyanins [3], and bacoside [4].

Secondary metabolite compounds provide demand and use for humans because of their high biological activity as the main ingredients of drugs, fragrances, flavoring agents of food, also defensive agents as antimicrobials and herbicides. Nevertheless, direct extraction of natural products from plants is synthesized in limited quantities in cells. In addition, it is necessary to consider several types of slow-growing plants that take an extended time to produce secondary metabolites. Cultivation through in vitro plant propagation is the most effective technique for increasing and harvesting secondary metabolites to increase demand on an industrial scale. Understanding to enhance and improve metabolites through a study of hormones and stress is needed to be explored to provide regulation of metabolite production at a large scale.

Phytohormones also play a fundamental role in response to stress conditions that lead to an increase the secondary metabolites. These molecules or elicitors act as chemical messengers, in which at low concentrations have been able to give a positive effect on cell metabolism, growth, and development processes, including cellular activities of secondary metabolites. These plant growth regulators induce the up-regulation of secondary metabolite biosynthetic gene expression. Abscisic acid (ABA), auxin (IAA), cytokinins (CK), ethylene (ET), gibberellins (GA), brassinosteroids (BR), jasmonic acid (JA), salicylic acid (SA), and strigolactone (SL) hormones are important messengers as a plant defense response to abiotic stress [5]. There is an important signal communication with the action mechanism of hormones under stress conditions. Environmental stress and herbivore attacks, as well as an infection caused by pathogens, trigger plants to generate high reactive oxygen species (ROS) that consequently lead to oxidation processes of lipids, proteins, and DNA [6]. Adverse effects of oxidative stress cause imbalance of metabolisms, therefore, reduce plant mechanism against stress.

The increase in free radicals is responded to by the cells to induce the synthesis of stress hormones. These bioregulators stimulate the productions of secondary metabolites, organic acids, and amino acids that play a crucial role in antioxidant defense systems such as osmoregulator and osmoprotectant mechanisms to prevent ROS-induced photooxidation [7]. Different types of stress and species showed variations in the response of phytohormones. This review encompasses how secondary metabolites of terpenoids, phenols, and alkaloids in plants can be induced by elicitors from phytohormones and stress.

Terpenoids, phenols, and alkaloids. The chemical structure of terpenoids is a five-carbon isoprene skeleton. This hydrocarbon molecule is a precursor of terpenoid biosynthesis through the mevalonate pathway and 2-C methyl-D-erythritol-4-phosphate (MEP) or deoxy-xylulose-phosphate (DOXP). Repeated incorporation of isoprene units and enzymatic modification produces other polyisoprenoids. These compounds are dominantly present in nature, namely as much as 55% of the total secondary metabolites [8].

Phenolic compounds consist of an aromatic ring binding to a hydroxyl group. The biosynthesis of polyphenols is formed through the shikimate-phenylpropanoid-flavonoid pathway in chloroplasts or the malonic acid pathway from the acetyl Co-A precursor. Flavonoid compounds are the largest class of phenols consisting of six categories, namely flavones, isoflavones, flavonols, flavanols, flavonones, and anthocyanins.

Alkaloids are a group of heterocyclic nitrogenous bases derived from amino acid precursors and their derivatives such as lysine, ornithine, tyrosine, tryptophan, anthranilic acid, histidine and amination reactions producing terpenoid alkaloids and ephedra alkaloids. Alkaloids are classified into three categories that comprise of (1) true alkaloids, which are amino acid derivatives and have an N-heterocyclic structure, (2) protoalkaloid, which are synthesized from amino acid derivatives without a heterocyclic ring, and (3) pseudoalkaloids with a carbon skeleton source derived from amination and transamination reactions [9].

Effect of phytohormones and environmental stresses on the accumulation of secondary metabolites. Abiotic stress, e.g., high irradiation, high temperature, drought, ultrasonic stress, nitrogen deficiency, high salinity, metal stress (Pb, Cd, and $AgNO_3$), and hormones act as elicitors as well as inductors of active phytochemical synthesis in plant tissues. Upregulation of secondary metabolites and their accumulation at higher concentrations was positively correlated with the increase in stress hormones such as ABA and jasmonic acid when plants were stressed. Table 1 showed the increase in terpenoids regulated by exogenous growth hormones of auxin, salicylic acid, ABA, GA, and ethylene in coping with adverse environments. The relationship between stress and endogenous hormones on the accumulation of metabolites can be explained by the tendency of changes in metabolic activity to the production of synthesized bioactive natural compounds. Various stressed conditions such as light, nitrogen deficiency, saline, ultrasonic, and high temperature enhance ROS compounds in plant cells. Although this condition certainly reduces productivity, plants activate the expression of stress-related genes that synthesize antioxidants of terpenoids such as carotenoids to control free radical damage and promote the formation of multiple protections against oxidation at the cellular and tissue level.

Table 1. Terpenoid compounds induced by phytohormones and environmental stress

Phytohormones	Stress factor	Secondary metabolites	Responses	Plants	References
Indole propionic acid (IPA) and indole acetic acid (IAA)	High light irradiation and low nitrogen	carotenoid (astaxanthin)	Auxin triggered astaxanthin synthesis as much as 89.9 mg/L under high light irradiation and low nitrogen	*Chromochloris zofingiensis*	[10]
Salicylic acid (SA)	40 mM NaCl	Monoterpenes (trans-pinocarveol, trans-carveol, isopinocarvone) Sesquiterpenes (β-cubebene, γ-muurolene)	Increase in volatile compounds after applied exogenous SA	*Egletes viscosa*	[11]
		Monoterpenes (isopinocarvone, β-phellandrene) Sesquiterpenes (β-cubebene, β-caryophyllene, γ-muurolene, caryophyllene oxide, γ-amorphene)	Increase in volatile compounds under stress of 40 mM NaCl		
	Ultrasonic stress	Ginsenoside of Rb1, Rb2, Rc, Rd, Rg3, Rh2	Addition of elicitor SA and ultrasonic stress induced an increase of ginsenoside	*Panax quinquefolius (L.)* dan *Panax sikkimensis (Ban.)*	[12]
	High temperature	Steroid of cardenolide triterpenoid (digoxigenin, gitoxigenin, lanatoside C, digitoxin)	Increase in cardenolide after application of SA at high temperature	*Digitalis trojana*	[13]
ABA and GA_3	-	Monoterpenes and sesquiterpenes. In roots (nerolidol dan α-tocopherol), berries (γ-terpinene, terpinolene, nerodilol, pinene), leaves (cymene, eucalyptol, γ-terpinene, terpinolenen, α-farnesene, β-farnesene, bergamotene, nerolidol, farnesol, α-tocopherol)	Application of ABA and GA_3 as elicitors to increase the percentage of terpenoid metabolites in plant tissues	*Vitis vinifera* L. *cv. Malbec*	[14]

Phytohormones	Stress factor	Secondary metabolites	Responses	Plants	References
Jasmonic acid (JA), Salicylic acid (SA), abscisic acid (ABA) and ethephon (ETH)	-	Glucosinolate (GSL) and anthocyanin	Addition of SA, ABA and ETH exogen increased the gene expression of phytochemical production	*Brassica rapa* spp. *pekinensis*	[15]
SA dan ABA		Lutein and β-carotene			
ABA		Chlorophyll and carotenoids			

Hormones, abiotic stresses, including metal stress of Pb, Cd, and $AgNO_3$, as well as drought stress, salinity, UV-B and heat shock, also affect the production of phenolics (Table 2) and alkaloid compounds (Table 3) in several plants. The similar stress hormone discovered in terpenoid accumulation was also promoted in inducing phenols and alkaloids. The effectiveness of jasmonic acid as an elicitor can be demonstrated in the stress signal transduction pathway [16]. Jasmonic acid has evoked a biochemical response that enhanced the defense and physiological response induced by secondary metabolism. The free hydroxyl group in the chemical structure of phenol acts as an electron acceptor of hydrogen peroxide [17], which can ward off free radicals and absorb singlet oxygen due to stress in the form of excess radiation, heavy metals, and drought. Hormones of ABA, ethylene, and GA increased the activity of phenolic acid synthesis enzymes, namely tyrosine aminotransferase and phenylalanine ammonia-lyase [18]. In stress-tolerant plants to biotic factors caused by *P. cinnamomi* infection, there was an increase in stress hormones and phenolic compounds. This study shows that stress-resistant plants have a high potential of increased secondary metabolites that provide resistance systems.

In other metabolites, drought stress also increases the alkaloid biosynthesis. More studies have been carried out on medicinal plants with high alkaloid potential as medicines. An increase in vinblastine was followed by vindoline and catharanthine (precursor) in low light irradiation through activated up-regulation of terpenoid indole alkaloids (TIAs) biosynthesis in *Catharanthus roseus* (L.) [30]. These compounds are known as anti-cancer compounds that have high concentrations of TIAs. Several studies report the application of growth regulators in the induction of pharmacological bioactive compounds. It is noted in this study that salicylic acid and jasmonic acid proved a positive effect on the increase of alkaloids.

Table 2. Phenol compounds induced by phytohormones and environmental stress

Phytohormones	Stress factor	Secondary metabolites	Responses	Plants	References
Indole-3-acetic acid (IAA) and kinetin	-	Isoflavonoids: Glycoside (genistein 7-O-diglucoside, daidzin, apigenin 7-O-glucoside, genistin) and aglicones (daidzein, genistein, apigenin)	Accumulation of isoflavonoids in roots after application of IAA under light condition. Accumulation was also recorded in stem after adding kinetin	*Genista monspessulana*	[19]
Jasmonic acid (JA)	0.25-0.75 mM Pb	Total of phenols, poliphenols, flavonoids and anthocyanins	Up-regulation of chalcone synthase and phennylalanine ammonia lyase under Pb-stressed condition (0.25-0.75 mM)	*Solanum lycopersicum*	[20]
Absicic acid (ABA)	50-400 µM Cd	Phenylethanoid glycoside (echinacoside and acteoside)	Increase in endogenous ABA triggered an increase of phenylpropanoid enzymes under Cd stress (50 - 400 µM)	*Scrophularia striata*	[21]
	Moderate water stress (field capacity of	Total flavonoids	Increase in endogenous ABA and total flavonoids under	*Stellaria dichotoma* L. *var. lanceolata* Bge	[23]

Phytohormones	Stress factor	Secondary metabolites	Responses	Plants	References
	60-70% or 80-90%)		moderate stress elevated root biomass		
IAA, Indole 3 butyric acid (IBA) and Naphthalene acetic acid (NAA)	-	Total flavonoids	Seed application with IAA, IBA and NAA increased total flavonoids	*Solanum lycopersicum*	[24]
Salicylic acid (SA)	Drought stress	Total phenols (flavonol, isoflavone and glycosides)	Increase in total phenolic content acts ass radical scavenging capacity	*Ammi visnaga* L.	[25]

Table 3. Alkaloid compounds induced by phytohormones and environmental stress

Phytohormones	Stress factor	Secondary metabolites	Responses	Plants	References
Salicylic acid (SA)	Drought stress	Vinblastine and vincristine	Increase in alkaloids and antioxidants under drought stress	*Catharanthus roseus*	[27]
	100 mM NaCl	Indole alkaloids	Increase of alkaloids in roots under SA and NaCl ameliorate oxidative stress by elevated antioxidant enzymes	*Catharanthus roceus*	[28]
	-	Total alkaloids and atropine	Increased synthesis of total alkaloids and atropine after application of 5 and 20 μM SA	*Arthrospira platensis*	[29]
	-	Isopteropodine, speciophylline, mit-raphylline, isomitraphylline, rhynchopylline, dan isorhynchopylline	55% increase in Monoterpenoid Oxindole Alkaloids (MOA) by SA due to accumulation of free radicals	*Uncaria tomentosa*	[30]
Methyl jasmonate (MJ) dan salicylic acid (SA)	-	Reserpine and ajmalicine	Accumulation of indole alkaloids in plant roots after application of MJ and exogen SA	*Rauvolfia serpentina* Benth. ex Kurz	[31]
Methyl jasmonate (MJ)	50 and 100 µM Ag NO_3	Vincristine, vinblastine, ajmalicine, vindoline and catharanthine	Upregulation of TIAs biosynthetic genes after application of 100 µM MJ and $AgNO_3$	*Catharanthus roseus*	[33]
-	UV-B	Vinblastine, vindoline, and catharanthine	Increase in alkaloids as photoprotectans under UV-B stress provided by elevated nitrate supply	*Catharanthus roseus*	[32]
-	Heat stress of 40^0C	Brachycerine	Accumulation of brachycerine was induced by heat shock, followed by biosynthesis of tryptophan and tryptamine precursor	*Psychotria brachyceras* Müll. Arg	[34]

Cellular communication to induce secondary metabolites under stress conditions and its connecting network with hormones. Increased stress levels trigger a high response of endogenous hormone synthesis. This affects the accumulation of terpenoids, phenols and alkaloids in roots, stems, leaves, and fruits. The strong correlation between stress, hormones and secondary metabolites can be explained based on the hypothesis of carbon-nutrient balance and the hypothesis of the effect of stress on metabolite biosynthesis [23]. Abiotic stress conditions such as high temperature, excess irradiation, salinity, and water stress cause drought conditions, leading to the reduction of root ability to absorb water and essential nutrients for plants. Although stress inhibits and reduces the growth index, plants synthesize more carbon-based molecules, which comprise the basic hydrocarbon constituents in secondary metabolites.

Environmental stresses induce the synthesis of stress hormones in which then modulate the defense systems in plants. Hormones activate signal transduction cascades to adjust metabolism fluxes as a response to stress. Therefore, these hormones generate regulated expression of biosynthetic genes for secondary metabolic enzymes. In these signaling networks, the cellular antioxidant system is enhanced to cope with stress. It has been reported that regulation by application of methyl jasmonate doubles phenylpropanoid biosynthesis, transcription factors of defense biosynthetic enzymes and also mediates cell communication with other hormones such as auxins [35]. Expression of SQS and bAS genes also increased during drought stress [36]. These genes encode precursors and enzymes for the synthesis of the triterpenoid saponin glycyrrhizin in *Glycyrrhiza glabra*. Based on the results by review from several studies, phytohormones do not work alone in the signal transduction pathway of the defense system, but they interact synergistically with other bioregulators. For example, ABA and jasmonic acid increase phenol and flavonoid levels [22]; exogenous ABA, salicylic acid, jasmonic acid and ethylene increase glucosinolates and anthocyanins [15].

The elevation of ROS production induces the signaling in response to stressed conditions that is shown to increase the accumulation of antioxidant secondary metabolites. Peroxidation of PUFA (Poly Unsaturated Fatty Acid) by hydroxyl ROS can be neutralized by flavonoid and terpenoid compounds by adding hydrogen ions which can reduce free radical reactivity [37]. Excitation of hormones, for instance, methyl jasmonate, upregulates the activity of strictosidin synthase and TIA biosynthetic genes that stimulate the metabolism of indole, particularly reserpine in *Rauvolfia serpentina* L. [38].

Modification of terpenoid metabolites acts as a growth regulator such as zeatin, gibberellins, and ABA. Hormone of ABA is an isoprenoid and carotenoid precursor which is synthesized in plastids through the MEP metabolic pathway. Carotenoids and their derivatives work as photo protectors to encounter with oxidative damage of chlorophyll. For humans, carotenoids are an important nutrient for health, thereby this antioxidant could be increased by applying hormone elicitors at the right dose in industrial production.

The prospect of biotechnology, such as micropropagation in bioreactors, is an effort to increase secondary metabolites. The application of a single hormone is able to provide a plant adaptation system under extreme environmental changes and also enhances plant biomass and secondary metabolite productions [39]. The application of physiological elicitor bioregulators and the combination of various stress levels with appropriate regulation might be a good strategy for improving the phytochemical quality of targeted medicinal plants.

CONCLUSION

The compilation of this review was composed based on literature studies. The review provides several clues that phytohormones and controlled stress increase the production of secondary metabolites in significant amounts. These elicitors can be applied singly or in appropriate combinations to trigger the expression of genes involved in the metabolism of terpenoids, phenols, and alkaloids in medicinal plants. Intensive research is needed to produce a suitable method for the commercial use of phytochemicals that play an important biological role in preventing oxidative stress and various types of diseases.

ACKNOWLEDGEMENT

We thank the Department of Biology of UIN Alauddin Makassar for the motivation in increasing academic capacity.

REFERENCES

[1] Yu K, Murthy HN, Hahn E-J, Paek K-Y (2005) Ginsenoside production by hairy root cultures of *Panax ginseng:* influence of temperature and light quality. Biochemical Engineering Journal 23 53–56. doi: 10.1016/j.bej.2004.07.001.

[2] Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C (2007) Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiology and Biochemistry 45 244–249. doi: 10.1016/j.plaphy.2007.02.001.

[3] Chan LK, Koay SS, Boey PL, Bhatt A (2010) Effects of abiotic stress on biomass and anthocyanin production in cell cultures of *Melastoma malabathricum*. Biological

Research 43 (1): 127–135.

[4] Sharma M, Ahuja A, Gupta R, Mallubhotla S (2015) Enhanced bacoside production in shoot cultures of *Bacopa monnieri* under the influence of abiotic elicitors. Natural Product Research : Formerly Natural Product Letters 29 (5): 37–41. doi: 10.1080/14786419.2014.986657.

[5] Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. The Crop Journal 4 (3): 162–176. doi: 10.1016/j.cj.2016.01.010.

[6] Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012 : 1–26. doi: 10.1155/2012/217037.

[7] Thakur M, Bhattacharya S, Khosla PK, Puri S (2019) Improving production of plant secondary metabolites through biotic and abiotic elicitation. Journal of Applied Research on Medicinal and Aromatic Plants 12 1–12. doi: 10.1016/j.jarmap.2018.11.004.

[8] Ramawat KG, Mérillon J-M (2013) Natural Products. New York, Springer-Verlag Berlin Heidelberg.

[9] Takshak S, Agrawal SB (2019) Defense potential of secondary metabolites in medicinal plants under UV-B stress. Journal of Photochemistry & Photobiology, B: Biology 193 51–88. doi: 10.1016/j.jphotobiol.2019.02.002.

[10] Chen J, Wei D, Lim P (2020) Enhanced coproduction of astaxanthin and lipids by the green microalga *Chromochloris zofingiensis*: Selected phytohormones as positive stimulators. Bioresource Technology 295 122242. doi: 10.1016/j.biortech.2019.122242.

[11] Batista VCV, Pereira IMC, Paula-Marinho SO, Canuto KM, Pereira RCA, Rodrigues THS, Daloso DM, Gomes-Filho E, de Carvalho HH (2019) Salicylic acid modulates primary and volatile metabolites to alleviate salt stress-induced photosynthesis impairment on medicinal plant *Egletes viscosa*. Environmental and Experimental Botany 167 103870. doi: 10.1016/j.envexpbot.2019.103870.

[12] Biswas T, Mathur A, Gupta V, Singh M, Mathur AK (2018) Salicylic acid and ultrasonic stress modulated gene expression and ginsenoside production in differentially affected *Panax quinquefolius* (L.) and *Panax sikkimensis* (Ban.) cell suspensions. Plant Cell, Tissue and Organ Culture (PCTOC) 136 575–588. doi: 10.1007/s11240-018-01538-7.

[13] Cingoz GS, Gurel E (2016) Effects of salicylic acid on thermotolerance and cardenolide accumulation under high temperature stress in *Digitalis trojana* Ivanina. Plant Physiology et Biochemistry 105 145–149. doi: 10.1016/j.plaphy.2016.04.023.

[14] Murcia G, Fontana A, Pontin M, Baraldi R, Bertazza G, Piccoli PN (2017) ABA and GA_3 regulate the synthesis of primary and secondary metabolites related to alleviation from biotic and abiotic stresses in grapevine. Phytochemistry 135 34–52. doi: 10.1016/j.phytochem.2016.12.007.

[15] Thiruvengadam M, Kim S, Chung I (2015) Exogenous phytohormones increase the accumulation of health-promoting metabolites, and influence the expression patterns of biosynthesis related genes and biological activity in Chinese cabbage (*Brassica rapa* spp . pekinensis). Scientia Horticulturae 193 136–146. doi: 10.1016/j.scienta.2015.07.007.

[16] Ramakrishna A, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants Akula. Plant Signaling & Behavior 6 (11): 1720–1731. doi: 10.4161/psb.6.11.17613.

[17] Simic SG, Tusevski O, Maury S, Delaunay A, Joseph C, Hagège1 D (2014) Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response in *Hypericum perforatum* L. shoot cultures. The Scientific World Journal Article ID 1–10.

[18] Liang Z, Ma Y, Xu T, Cui B, Liu Y, Guo Z, Yang D (2013) Effects of abscisic acid, gibberellin, ethylene and their interactions on production of phenolic acids in *Salvia miltiorrhiza* Bunge hairy roots. Public Library of Science 8 (9): e72806. doi: 10.1371/journal.pone.0072806.

[19] Meza A, Rojas P, Cely-Veloza W, Guerrero-Perilla C, Coy-Barrera E (2020) Variation of isoflavone content and DPPH scavenging capacity of phytohormone-treated seedlings after in vitro germination of cape broom (*Genista monspessulana*). South African Journal of Botany 130 64–74. doi: 10.1016/j.sajb.2019.12.006.

[20] Bali S, Jamwal VL, Kohli SK, Kaur P, Tejpal R, Bhalla V, Ohri P, Gandhi SG, Bhardwaj R, Al-Huqail AA, Siddiqui MH, Ali HM, Ahmad P (2019) Jasmonic acid application triggers detoxi fi cation of lead (Pb) toxicity in tomato through the modifications of secondary metabolites and gene expression. Chemosphere 235 734–748. doi: 10.1016/j.chemosphere.2019.06.188.

[21] Beshamgan ES, Shari M, Zarinkamar F (2019) Crosstalk among polyamines, phytohormones, hydrogen peroxide, and phenylethanoid glycosides responses in *Scrophularia striata* to Cd stress. Plant Physiology and Biochemistry 143 129–141. doi: 10.1016/j.plaphy.2019.08.028.

[22] Camisón Á, Martín MÁ, Sánchez-bel P, Flors V, Alcaide F, Morcuende D, Pinto G, Solla A (2019) Hormone and secondary metabolite profiling in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomi*. Journal of Plant Physiology 241 153030. doi: 10.1016/j.jplph.2019.153030.

[23] Zhang W, Cao Z, Xie Z, Lang D, Zhou L, Chu Y, Zhao Q, Zhang X, Zhao Y (2017) Effect of water stress on roots biomass and secondary metabolites in the medicinal plant *Stellaria dichotoma* L. var. *lanceolata* Bge. Scientia Horticulturae 224 280–285. doi: 10.1016/j.scienta.2017.06.030.

[24] Olaiya CO, Adigun AA (2010) Chemical manipulation of tomato growth and associated biochemical implications on flavonoid, lycopene and mineral contents. African Journal of Plant Science Vol 4 (6): 167–171.

[25] Osama S, El Sherei M, Al-Mahdy DA, Bishr M, Salama O (2019) Effect of salicylic acid foliar spraying on growth parameters, γ - pyrones, phenolic content and radical scavenging activity of drought stressed *Ammi visnaga* L. plant. Industrial Crops & Products 134: 1–10. doi: 10.1016/j.indcrop.2019.03.035.

[26] Liu Y, Zhao D.-M, Zu Y.-G, Tang Z (2011) Effects of low light on terpenoid indole alkaloid accumulation and related biosynthetic pathway gene expression in leaves of *Catharanthus roseus* seedlings. Botanical Studies 52: 191–196.

[27] Ababaf M, Omidi H, Bakhshandeh A (2021) Changes in antioxidant enzymes activities and alkaloid amount of *Catharanthus roseus* in response to plant growth regulators under drought condition. Industrial Crops & Products 167 (1): 113505.

[28] Misra N, Misra R, Mariam A, Yusuf K, Yusuf L (2014) Salicylic acid alters antioxidant and phenolics metabolism in *Catharanthus roceus* grown under salinity stree. African Journal of Traditional, Complementary and Alternative Medicines 11 (5): 118–125.

[29] Hadizadeh M, Ofoghi H, Kianirad M, Amidi Z (2019) Elicitation of pharmaceutical alkaloids biosynthesis by salicylic acid in marine microalgae *Arthrospira platensis*. Algal Research 42 101597. doi: 10.1016/j.algal.2019.101597.

[30] Sánchez-Rojo S, Cerda-García-Rojas CM, Esparza-García F, Plasencia J, Poggi-Varaldo HM, Ponce-Noyola T, Ramos-Valdivia AC (2015) Long-term response on growth, antioxidant enzymes, and secondary metabolites in salicylic acid pre-treated *Uncaria tomentosa* microplants. Biotechnology Letter 37 2489–2496. doi: 10.1007/s10529-015-1931-0.

[31] Dey A, Nandy S, Nongdam P, Tikendra L, Mukherjee A, Mukherjee S, Pandey DK (2020) Methyl jasmonate and salicylic acid elicit indole alkaloid production and modulate antioxidant defence and biocidal properties in *Rauvolfia serpentina* Benth. ex Kurz. in vitro cultures. South African Journal of Botany 135 1–17. doi: 10.1016/j.sajb.2020.07.020.

[32] Guo X, Chang B, Zu Y, Tang Z (2014) The impacts of increased nitrate supply on *Catharanthus roseus* growth and alkaloid accumulations under ultraviolet-B stress. Journal of Plant Interactions 9 (1): 640–646. doi: 10.1080/17429145.2014.886728.

[33] Paeizi M, Karimi F, Razavi K (2018) Changes in medicinal alkaloids production and expression of related regulatory and biosynthetic genes in response to silver nitrate combined with methyl jasmonate in *Catharanthus roseus* in vitro propagated shoots. Plant Physiology and Biochemistry 132 623–632. doi: 10.1016/j.plaphy.2018.10.015.

[34] da Silva Magedans YV, Matsuura HN, Tasca RAJC, Wairich A, Junkes CFO, de Costa F, Fett-Neto AG (2017) Accumulation of the antioxidant alkaloid brachycerine from *Psychotria brachyceras* Müll. Arg. is increased by heat and contributes to oxidative stress mitigation. Environmental and Experimental Botany 143 185–193. doi: 10.1016/j.envexpbot.2017.09.008.

[35] Chen L, Chen Y, Lee C, Lin T (2007) MeJA-induced transcriptional changes in adventitious roots of *Bupleurum kaoi*. Plant Science 173 12–24. doi: 10.1016/j.plantsci.2007.03.013.

[36] Nasrollahi V, Mirzaie-asl A, Piri K, Nazeri S, Mehrabi R (2014) The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (*Glycyrrhiza glabra*). Phytochemistry 103 32–37. doi: 10.1016/j.phytochem.2014.03.004.

[37] Mithöfer A, Schulze B, Boland W (2004) Biotic and heavy metal stress response in plants: evidence for common signals. FEBS Letters 566 1–5. doi: 10.1016/j.febslet.2004.04.011.

[38] Nurcahyani N, Solichatun S, Anggarwulan E (2008) The reserpine production and callus growth of Indian Snake Root (*Rauvolfia serpentina* (L.) Benth. Ex Kurz) culture by addition of Cu2+. Biodiversitas 9 (3): 177–179. doi: 10.13057/biodiv/d090305.

[39] Stirk WA, Staden J Van (2020) Potential of phytohormones as a strategy to improve microalgae productivity for biotechnological applications. Biotechnology Advances 44 107612. doi: 10.1016/j.biotechadv.2020.107612.