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Application Of Plant Growth Regulators To Enhance Secondary Metabolite Production

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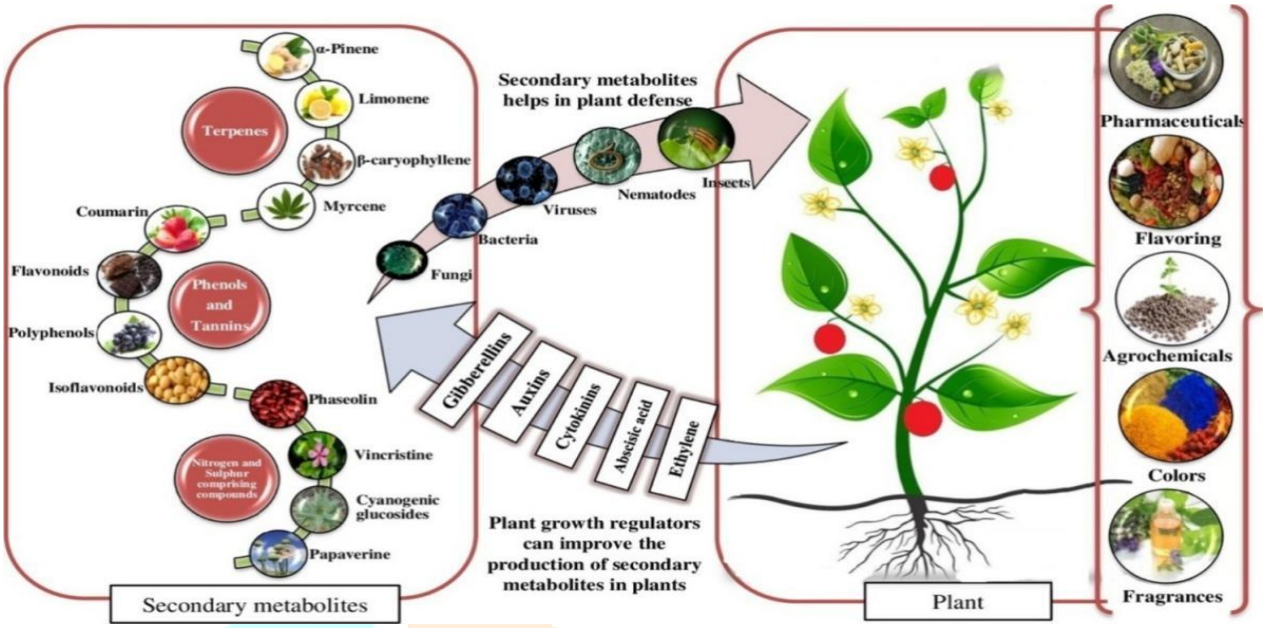
Abstract: Secondary metabolites not only perform important roles in plant defense against herbivory and other interspecies defense but also utilized by mankind as medicines, flavorings, pharmaceuticals, agrochemicals, fragrances, colors, biopesticides, food additives and drugs. Most of the drugs sold today are mere synthetic alterations of the naturally derived substances. The increasing commercial status of secondary metabolites in the past decade has meant much interest in secondary metabolism. Various strategies have been researched widely with the aim of enhancing the yield of secondary metabolites in plants. From this spotlight, the intention of the current review is to investigate the use of plant growth regulators towards the yield of certain critical plant secondary metabolites. Plant growth regulators (PGRs) are applied to induce production of secondary metabolites in plants by playing the role of "elicitors," more or less eliciting a stress reaction in the plant, and so inducing biosynthesis of the special compounds that often act as protective mechanisms against stressors from the environment; this is made possible through alteration of the plant's hormonal status, specifically under controlled conditions such as tissue culture, in order to maximize production of sought-after secondary metabolites of interest due to potential pharmacological or economic value.

Graphical Abstract:



Keywords: Secondary Metabolite Auxins, Cytokinins, Salicyclic Acid, Ethylene.

Introduction: The biosynthesis of secondary metabolites (SMs) in plants comes in different forms and quantities, and their applications in contemporary medicine are prevalent. Fifty percent of human needs are obtained from plant commodities, such as fuel, cosmetics, pharmaceuticals, building materials, and food staples. Numerous medicinal plant species have applications due to their medicinal activities and their content of SMs, but naturally occurring SMs in plants in quantities are not many. Hence, a number of traditional and new strategies are used to improve the production of bioactive constituents from plants. Conventional approaches are economical, simple, and quicker, but yield fewer metabolites, whereas advanced strategies like metabolic pathways engineering, genome editing, metabolic transformation, biotechnology-based replacements, and synthetic biology are more costly but provide useful tools for improving SMs content. Optimization of culture media and signaling pathways involved in SMs production offers a promising chance to enhance the amount of bioactive compounds naturally occurring. This review consolidates the various cultivation techniques employed to improve the class and amount of metabolites produced and emphasizes the advantages of strategies for enhancing SMs and synthesizing metabolites through various methods.

Secondary metabolites (SMs) are natural products synthesized by plants that play varied roles in their ecological interactions and possess wide-ranging applications in pharmaceutical, cosmetic, and agriculture industries. SMs possess a variety of biological activities, including antimicrobial, antioxidant, and anticancer activities, which render them extremely useful for human health and well-being. The therapeutic use of medicinal plants in the treatment of diseases is due to the presence of varied variety of SMs. These biologically valuable substances are biosynthesized in unfavorable conditions of the environment as a

mutable tolerance mechanism. Biosynthesis of SMs in plants may be controlled by several factors, such as genetic characteristics, environmental factors, and nutritional availability. Plants face several types of environmental stresses like physical (salinity, temperature, drought, metal, UV) or pathogen (viruses, nematodes, fungi, and insects). Upon encountering these stress situations, the receptors on the plant plasma membrane trigger a series of reactions resulting in the biosynthesis of SMs.

Plant SMs are generally classified into phenolics, alkaloids terpenes, steroids, and flavonoids which exhibit varied functions in deterring herbivores, in symbiosis signaling and modifying the structure of microbial communities. The naturally occurring SMs are in limited quantities since they are only secreted for survival under biotic or a biotic stress conditions. Presently, the increasing use of medicinal plants in the treatment of diseases has led to a worldwide reduction in plant populations. Most medicinal plants are now classified as protected species as a result of their wide use. In addition, the current environmental factors, such as global warming and the fast increase in the human population, have also played a part in the continuous overexploitation and possible extinction of these precious medicinal plants. To accomplish the growing demand for these medically important natural products, additional methods like suspension culture, elicitation, hairy root culture , shoot culture, genetic engineering, micro propagation, and callus culture are considered as an alternate process for higher production of metabolites. All these methods involve controlling different environmental and physiological parameters to develop favorable conditions to trigger the biosynthesis of a particular secondary metabolite. By knowing and utilizing these methods of growth, researchers seek to maximize the yield, diversity, and quality of the secondary metabolites, in the process broadening their uses in all sectors.

The information regarding the utilization of various strategies to acquire plants with elevated levels of metabolites is found to be new to agriculture and horticulture based on low-cost biomass resources. Further, various methods like genetic transformation, tissue culture, greenhouse conditions are used today for better control over the amount of metabolites when used separately or collectively. The unique plant growth practices utilized to increase SM synthesis include a broad variety of approaches. Environmental conditions like temperature, light intensity and quality, humidity, and water availability have been found to be significant determinants of SM production. For example, it was observed in a study that short-day conditions adversely impacted the growth, flower initiation, and alkaloid accumulation of Papaver somniferous when compared to long-day conditions. Enhancing light intensity under long photoperiods improved growth, development, and alkaloid biosynthesis. Besides environmental manipulations, the application of plant hormones, elicitors, and stress-inducing agents has proven to be a potent method to induce SM production. Exogenous chemicals have the ability to mimic natural signaling molecules or elicit stress responses in plants, which activates biosynthetic pathways and results in enhanced SM synthesis. In addition, genetic engineering methods have been utilized to alter plant genetic traits and manipulate the gene expression of SM biosynthetic genes, providing a direct and specific method

for increasing their production. Although several studies have investigated the impacts of different plant growth practices on SM synthesis, there is a requirement for a systematic review integrating the available knowledge in this regard. Hence, this article proposes to offer an exhaustive review of the different plant growth practices utilized to increase the synthesis of SMs. By critiquing the current literature, filling knowledge gaps, and signaling directions for future research, this review will be of benefit in advancing knowledge within the field and informing the establishment of innovative approaches to the sustainable production of precious SMs.

Techniques for Enhancing Secondary Metabolites Production:

***In-vitro* strategies for sustainable production of secondary metabolite:**

For the unceasing production of commercially valuable secondary metabolites and designed molecules, in-vitro metabolic engineering is envisioned as a powerful biological tool. In-vitro approaches possess the potential to synthesize unprecedented levels of target chemicals that can be utilized to discover and trigger new natural molecules for pharmacological or alimentary purposes. Tremendous potential for the content increase of SMs can be carried out by navigating the metabolic spectrum of plants. The bioactive compounds obtained from medicinal, floral, and perfumed plants through undifferentiated organogenic and callus culture via in-vitro cultures produce thousands of SMs purified in industries for medicinal and culinary uses. Stress induction and manipulation of the metabolic profile with respect to the elicitor application is a significant application of elicitation for metabolite improvement in in-vitro cultures in various species of plants.

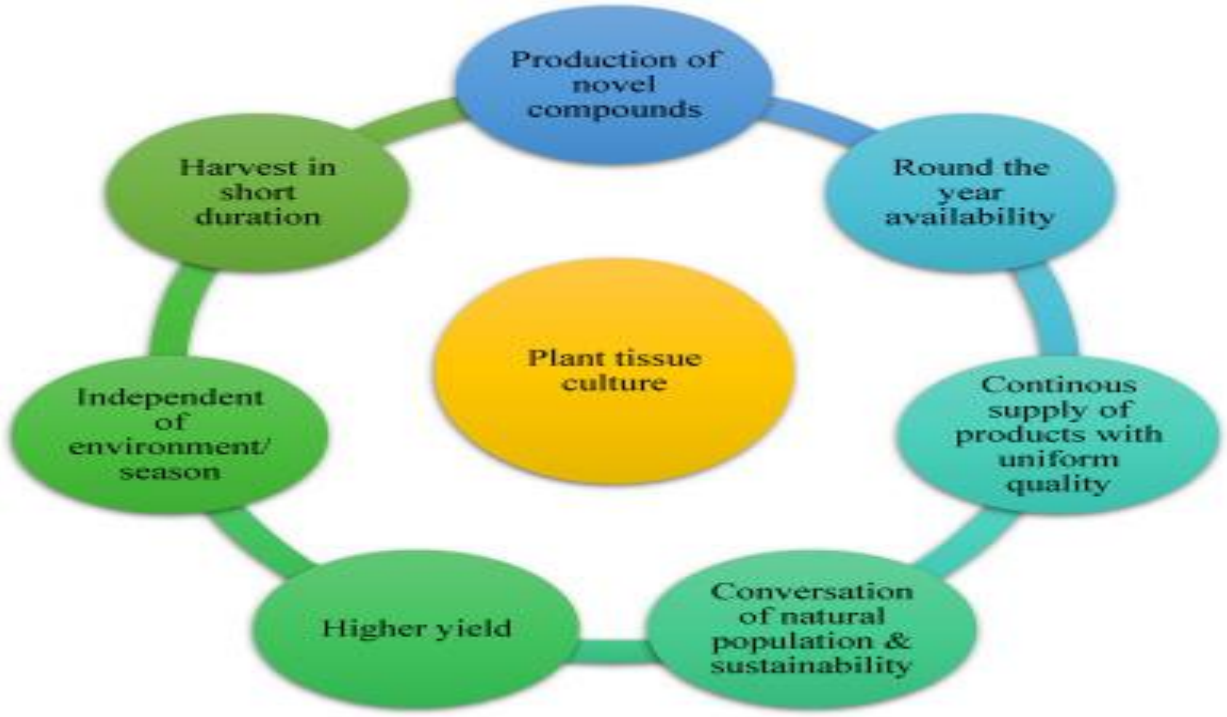


Fig-1. Advantage of tissue culture on the biosynthesis of secondary metabolites.

Tissue Culture: Tissue culture methods revolutionize plant SM production by providing maximized nutrient composition, genetic and epigenetic manipulations, stress imposition, and renewable culture systems. With the specific regulation of growth conditions, such as nutrient levels and hormone contents, tissue culture triggers the biosynthesis of SMs. Genetic engineering and epigenetic manipulation facilitate the reactivation of silent biosynthetic pathways, tapping into the potential for enhanced metabolite yields. By exposing plant tissues to stressors, i.e., a biotic or biotic stresses, SM synthesis is additionally boosted as a defence reaction. The maintenance of cell suspension cultures or organ cultures in bioreactors guarantees a perpetual and scalable manufacture of secondary metabolites. In sum, tissue culture methods provide flexible strategies to enlarge the manufacture of useful plant molecules for diverse purposes.

The potential of biotechnology-based breeding methods to be cost-effective as well as eco-friendly makes them suitable for the enhancement of bioactive compounds. Plant tissue culture methods employ controlled aseptic conditions and optimization of nutritive culture media for tissue, cell, and organ growth. In-vitro cultures are independent of external complications like alterations in seasons, geographical or environmental conditions and hence found effective in mediating conditions needed for developmental phases of plant. Different in-vitro culture techniques, such as callus, hairy-roots, shoot, and suspension cultures can be employed to promote the production of certain metabolites for commercially significant plants. Out of which, callus cultures are seen as a potential approach of biosynthesizing bioactive compounds in endangered species of most medicinal plants.

The interaction of plant secondary metabolism and defence reactions has been widely recognized. Defence reactions are result of herbivores, insects, pathogen infection or other a biotic stresses. These in-turn triggers a cascade of events leading to the activation of biosynthesis of secondary metabolites like phytoalexins, lignin deposition, hypersensitive reactions, and structural defensive barriers. The signaling process begins with reception of signals via receptors localized on plasma membrane and organelles. Plant receptors activate the signaling processes by varying number of molecules indicating various elicitors responding to same receptors. Perception of elicitor signals by receptors results in initiation of elicitation process and the signals are transduced in the form of secondary messengers. Downstream signals result in initiation of various actions required to adapt and survive based on the changes in the surrounding environment. Both categories of signaling molecules take up varying pathway and eventually change the physiological and biochemical constitution of the cells. Though the elicitor response starts from the plasma membrane, but each elicitor's pathway is different and a number of responses can occur to different targets through several pathways. A representation encompassing the series of events as recognition of signaling molecules, binding, and activation of transduction cascades culminating in the biosynthesis of SMs are illustrated is displayed.

Effect of fungal growth on secondary metabolites:

The growth culture of fungi has a significant impact on the synthesis of secondary metabolites in plants. Through endophytic interactions, mycorrhizal association, elicitation, or nutrient modulation, the activity and presence of fungi have the ability to induce changes in the secondary metabolite profiles of plants. Understanding and exploiting these effects provide means for manipulating secondary metabolite production for different applications. Of late, microbe-derived biotic elicitors have gained prominence owing to their ability to simulate disease reaction noted in natural settings and commence the signaling pathways towards biosynthesis of SMs. Fungal elicitation has been reported in great detail in cell suspension cultures, as it can be monitored with ease in bioreactors. In plants, arbuscular mycorrhizal fungi (AMF) are regarded as crucial for growth and development by increasing water uptake and defense against pathogenic bacteria, fungi, and nematodes which infect the plant roots. AMF are regarded to be involved in secondary metabolite production. The elicitation by Agrobacterium rhizogenes under controlled conditions differentiate into hairy roots. These cultures exhibit the increased rate in physiological characters like unlimited branching, biochemical and genetic stability. The hairy roots do look like regular roots and demonstrates the ability to produce and increase the production of useful SMs compared to regular roots. The study elaborates on the biosynthesis of SMs in relation to tissue differentiation degree. The utilization of fungal elicitors for SM production have already been established with different levels of success in terms of number of species. Such an example is the employment of fungal elicitors in Catharanthus roseus for enhancing the yield of SMs like vinblastine and vincristine through somatic embryogenesis.

Effective in vitro elicitation for secondary metabolites (SMs) enhancement is always regarded as a demanding task. The elicitation of the content relies on numerous factors, such as fungal species, elicitor application mode and type, preparation of elicitor, culture age, and conditions like medium composition and growth regulators. Utilization of living fungal cells and other fungal elicitors such as mannan has also been noted to induce metabolite content. Strikingly, one fungal elicitor can induce varying responses in the same plant species towards SM production. For example, elicitation with Aspergillus flavus in cell suspension cultures of Hypericum perforatum enhanced anthocyanin production but did not affect the accumulation of hypericin. Nevertheless, the hypericin content can be improved using different fungal species in cell suspension cultures, i.e., Fusarium oxysporum, indicating the involvement of other elicitors in the same plant species. In other studies, the elicitation method is found to be vital for success. In the scenario of Lantana camara, the use of elicitors through two methods, that is, filter-sterilized and autoclaved, resulted in different outcomes. Surprisingly, cell-suspension cultures given filter-sterilized elicitors showed greater biosynthesis of SMs than cultures receiving autoclaved culture media. This method has been proven effective in various plant species.

S.No.	Plant	Method used	Enhanced property/SMs	Application
1.	Artemisia spp.	Plant cell suspension, hairy root cultures and tissue culture	Increases density of glandular trichomes in flowers and leaves	Treatment of malaria, parasitic infections such as schistosomiasis.
2.	Nicotiana tabacum	Gene cloning	Increase in artemisinin production	Anti-malarial
3.	Isatis indigotica	Methyl jasmonate (MeJA) elicitation in Hairy root cultures	Increase the production of lignans	Anti-inflammatory and anti-viral activities.
4.	Coffea arabicaL. (Arabica type coffee)	Cell suspension cultures	Significant increase in caffeine synthase activity was detected under Al-treatment	Reduces wrinkle, decreased blotchy redness and tactile roughness.
5.	Artemisia. AnnuaL.	Callus cultures	Increases the trichome sizes the production of artemisinin.	Antimalarial, anti-inflammatory and immunoregulatory
6.	Swertia chirayita	*In-vitro* plantlets and callus cultures	Number of SMs was found significantly higher.	As expectorant, laxative, antispasmodic, antioxidant, antidiabetic, antipyretic, antitussive, stomachic, anthelmintic, and antidiarrhea.
7.	Rauwolfia serpentina	Callus and hairy roots	Production of SMs *in-vitro*	Treatment of dysmenorrhea
8.	Celastrus paniculatus	Homogenous cell suspension culture	Established for multi-fold production of SMs	Antitumor, cytotoxic, and antimicrobial
9.	Gymnema sylvestre	Suspension cultures	Improving gymnemic acid production	Anti-microbial and anti-inflammatory
10.	Hypericum perforatum	*Rhizobacterium* elicitor treatment	Leads to a slow expansion of hypericin and pseudohypericin in plantlets	Wound healing and burn injury
11.	Taxus	Cell cultures	Taxane production	Anti-cancer

			was reported to be enhanced by accumulation of coronatine	
12.	Vitis vinifera	Cell suspension culture	Coronatine triggered the synthesis of viniferins	Anticancer, and anti-microbial
13.	Taverniera cuneifolia	Root culture	Increase the production of glycyrrhizic acid.	Anti-microbial and Anti-tumor
14.	Gingko biloba	Cell suspension cultures	Enhanced production of ginkgolide and bilobalide biosynthesis	Anti-aging agent

Table.1- Secondary metabolite enhancement properties.

Hydroponics secondary metabolite modulation: The high demand for medicinal and aromatic plant products, in terms of freshness, crispness, and uniformity, presents a major challenge to manufacturers. Plants have developed elaborate systems for nutrient uptake from the soil to nourish their growth and development. Plant physiology treats mineral nutrition as an elementary topic owing to its efficiency and selectivity

Recent developments in plant science have centered on the comprehension of secondary metabolism since plant commodities are the final products of such biochemical pathways. As a result, several studies have examined the impact of the growth medium on plant production. This has prompted producers and the scientific community to seek alternative approaches that provide controlled nutrient availability and environmental conditions, including hydroponics. Hydroponics involves an agricultural system characterized by low pests and pathogen occurrence, a high nutritional profile, and yield enhancement over the other agricultural systems. Additionally, hydroponics enables the possibility of off-season crop production using a shorter production period. In a study related to Carlina acaulis, the impact of different cultivation methods such as soil culture, hydroponics and in-vitro cultivation was noticed focusing on significant SMs and antioxidant activity of plant. The result signifies the enhancement in Ursolic acid (UA), oleanolic acid (OA) and chlorogenic acid (CA) and other phenolic compounds. The chromatographic investigation disclosed the presence of two valuable compounds- ChA & 3,5QA in every system except that CA occurs in soil culture and hydroponic system but in varying amount only. The triterpenoid content was seen increased in the leaves of the plants in hydroponics and field culture but the triterpenoid content was significantly low in in-vitro cultures. In addition to this, metabolite content of

ChA, 3,5 QA and antioxidant activity was found very alike in hydroponic and soil system. Considering other metabolites under study, antioxidant metabolites of leaf extracts were found most in soil culture. The superior differentiation concerning extracts and compounds were limited highly in culture medium and found most in in-vitro cultures. In comparison, the level of ChA is greater in certain samples and 3,5 QA was even greater in field systems concluding callus culture is the efficient source of 3,5QA and field systems are superior for other phenolic and antioxidant compounds prioritizing high raw material & biomass. The yield of experiment indicating the advantages of soil culture or field cultivation as hydroponics can never compete with the biomass yield even performing numerous cycles per annum. An experiment on lettuce has been made to deduce the growth patterns in comparison with the strength of nutrient medium utilized in hydroponic system as lettuce has been ranked highest valued vegetable salad as an excellent source of antioxidants, phenols, and vitamins. The findings were notable as regards positive modulation of phenolic profile, antioxidant activity and amylase inhibiting activity. Nevertheless, the clear significance was attached to recognizing the augmentation of individual classes of phenolics, for example, anthocyanins, flavones, lignans, and phenolic acids, owing to nutritional eustress. The findings point towards the interaction and emphasis on specific responses, as opposed to an overall plan for the production of phenolic compounds. Nevertheless, the modulation in nutritional potency did not influence tyrosinase and cholinesterase. The implications of altering nutritional strength in lettuce offer a motivating and useful method to create products with excellent nutrient qualities. In addition, this method operates both ways by supporting future food needs in agriculture for consumers and producers and preserving the quality of healthy food as well

Another case is the plant Passiflora quadrangularis L., family Passifloraceae, and a great source of phytochemicals. Intentional differences in the content of phytochemicals of the plant represent the impact of different cultivation methods. The investigation indicated differences in the content of phytochemicals of the plant when produced conventionally and organically. Among all the plant parts, the leaves of Passiflora were found to contain the most antioxidants when the crop was grown organically as opposed to conventional farming. In the other edible parts of the plant, including the fruit, the levels of antioxidants were increased in the organically farmed culture as opposed to the conventional process of farming, and with high results seen in the mesocarp of the fruit and higher flavonoid concentration in the pulp. The general secondary metabolite profile indicated that mesocarp was rich in phenolics, pulp had high levels of flavonoids, and leaves were rich in flavonoids, terpenoids, and phenolics. The research implies the significance and diversification of organically produced Passiflora, where there is a rise in metabolites coupled with the shift in the mode of cultivation. Numerous reports have been documented which exhibit that elicitors or other biotechnological techniques increases the level of SMs in plant cell suspension cultures.

Role of plant-microbe interactions in enhancing secondary metabolites: Plant SMs are engaged in multifaceted functions like protection response to environmental stresses, facilitation of organism interactions and defense against pathogens, herbivores, and pests. Plant micro biomes are also thought to be involved in regulation plant secondary metabolite metabolism. Plant micro biomes have been reported to play a significant role in regulating plant characters, including growth and development, resistance against the infectious diseases, alleviating a biotic stress along with increased biosynthesis of plant growth hormones. The mechanism of the effects caused by phyto-microbiome has not been fully comprehended, yet a general perception can be attained by studying the interactions involved between the two. Recently, the action of Variovorax bacteria on Arabidopsis root growth was shown. The host plant-bacteria interaction causes the increase in plant auxin and ethylene content which ultimately affects overall growth and development. The primary benefit of interaction between microbes and plants are exemplified by plant phenotype change, soil micro-biome, and plant RNA expression. Understanding of the important facts of the association between the two results in improved knowledge of the plant and soil metagenomes. The majority of the genes that are part of the signaling pathways are shared between plants and microbes. A proposed evolutionary idea is to account for the conservation of plant genomic sequences compared to sequences that are part of microbial function identified in soil profiles. The plants grown in various regions might produce different SMs, with some differences attributed to their respective microorganisms at each region. More researches of the plant microbiomes influence on medicinal crops confirms the enhanced productivity in respect of enhancement in alkaloids, terpenoids and steroids. Microbiome consists of a pool of genes connected to the production of forterpenoid backbone and other chemical compounds, transferring host plants new metabolic capabilities. Furthermore, experiments in Echinacea purpurea demonstrated microbiome's impact on the formation of alkamides, phenylpropanoid and volatile compounds. Apart from medicinal plants, microbiomes also exhibit interesting interactions in economic crops like Cannabis sativa and Papaver somniferum.

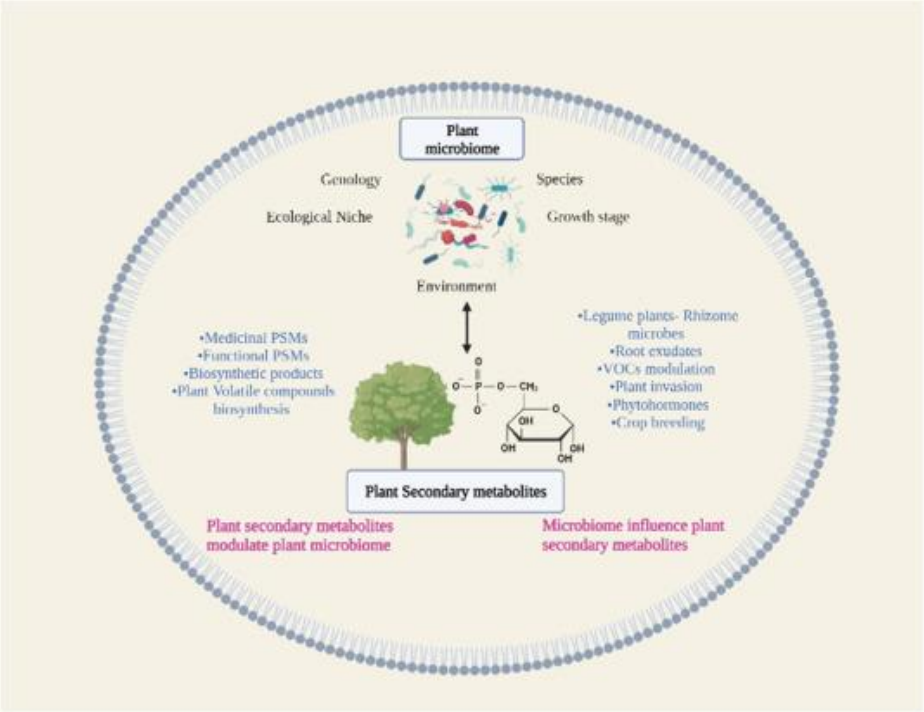


Fig-2. Secretion of certain metabolites.

SMs Production and Bioreceptors: Bioreactors have proven useful in the manufacturing of secondary metabolites (SMs) from plants. They are large vessels used for growth of organisms or tissues submerged in liquid nutrient medium under controlled environments. These are used in conversions with microorganisms, enzymes, or plant cells. The common design of bioreactors is to grow the tissues under conditions that are controlled, converting or transformed by targeted reactions. Unlike traditional reactors, the bioreactors are made to place emphasis on specific biological entities and metabolic pathways in particular. Bioreactors systems need to be designed to allow more control over process disturbances and contaminations by sustaining desired activity and removing unwanted ones. The bioreactor can be operated in batch, fed-batch, chemostat, or continuous perfusion modes depending on the need of tissue to be developed. All the nutrients are supplied at the beginning of the culture using a batch method. The fed-batch is started at a small volume and the culture is supplemented with concentrated feed solution and no quantity of nutrient medium is removed. The culture in a chemostat is constantly supplied with fresh medium, and spent media and cells are removed simultaneously. Fresh medium is added at the same rate as culture lost in perfusion culture. The cells are grown up to the time when growing nutrient media become limiting and ready for harvest. Cell densities of up to 5 × 106 cells/mL can be achieved or perhaps higher subject to culture time. Continuous culturing is regarded as the most promising approach for enhancing bioreactor productivity. For instance- Biotechnological study employing continuous cell culture of Taxus cuspidata and semi-continuous culture of Taxus canadensis enhanced paclitaxel production respectively with 100% recycling of the cells. The aim of bioreactor is to sustain and regulate biological reactions favorably considering two area of parameters like reactor limitations for necessary biological, physical or

macrokinetic and chemical system. Microbial growth and metabolite production are included in the macrokinetic system. Other region consists of bioreactor conditions including optimal pH and temperature, substrate (carbon source), consistent water supply, vitamins, oxygen, gas formation and byproduct removal. Bioreactors are hinted as biological factory, yielding high-grade products along with several other benefits, such as standalone supply of plant availability, improved control of environment and growth factors, excellent regulator of mechanism stirring mechanism, reproducible utilization of end product, improved nutrient absorption, rapid and easy cell harvesting with stimulating multiplication rates and higher concentration of production of secondary metabolites.

In general, bioreactors have transformed the production of SMs in plants. They provide a flexible and effective platform for the optimization of growth conditions, scale-up of production, and manipulation of genetic factors to improve the synthesis of target metabolites. With continued development of bioreactor technology and genetic engineering, we can anticipate even more significant advances in the cost-effective and sustainable production of SMs with potential uses in pharmaceuticals, nutraceuticals, and other industries. For the purpose of augmenting secondary metabolites production, the application of Agrobacterium rhizogenes have been popular in recent decades compared to cell suspension cultures based on genetic stability and rapid growth. Commercialized mass production of secondary metabolites through hairy root cultures are of utmost significance. Employment of bioreactors as gas and liquid phase reactors for large-scale production of SMs have been utilized for the same. Numerous attempts until now have been directed to achieve the highest biomass, opportunities still exist to upgrade technologies for achieving the best suitable bioreactor that could deliver improved growth features, homogenous culture medium, high oxygen transfer and lower shear stress.

Research on plant metabolite production with bioreactors has shown encouraging results. They have been able to enhance the production of several metabolites, including pharmaceuticals, nutraceuticals, and specialty chemicals. Bioreactor systems have been engineered to maximize the accumulation of target compounds, such as alkaloids, flavonoids, terpenoids, and polyphenols. Furthermore, improvements in bioreactor design and technology, such as better agitation, aeration, and nutrient delivery, have made metabolite production more efficient and economical.

These researches have implications in various industries such as pharmaceuticals, agriculture, and cosmetics in which plant metabolites have applications. Bioreactor-based production provides a green and controlled method of addressing the increased demand for these precious compounds. It also mitigates the dependency on conventional cultivation practices, which are often resource- and environmentally intensive. In summary, research on the production of plant metabolites in bioreactors has yielded encouraging outcomes in terms of increased yield, quality, and sustainability. By employing bioreactors, scientists are able to maximize growth conditions, engineer genetic aspects, and produce at large scales to accommodate

the rising demand for plant-based compounds. Further research and development in this area promise increased advances in the production of plant metabolites as well as new uses for these important compounds.

Plant metabolites synthesis basis of molecular: Abiotic and biotic stressors are major environmental factors that reduce agricultural productivity drastically. Plants are continuously subjected to various stressors, producing intricate response interactions; however, agricultural production is greatly diminished. With a perspective of future gradual changes in scenarios and weather conditions, there remains a pressing necessity to more precisely elucidate plants' intricate responses to single and combination stressors for enhancing crop tolerance to climatic changes. Plant stress response processes include alterations at the cellular, molecular, physiological, and biochemical levels. A number of key genes that encode transcription activators and repressors regulating downstream stress-induced genes and pathways regulate these varied responses. Comprehensive research in the past decade has helped identify key variables participating in abiotic and biotic stress responses. Transcription factors (TFs) are pivotal modulators of gene expression, and consequently, they control fundamental aspects of plant function including responses to hormones and environmental stimuli as well as cell differentiation and organ development. The operation and overexpression of transcription factors in biotic and abiotic stress conditions are shown in. In conditions of biological stress, TFs get associated with local and distal cis-elements of a specific gene in order to regulate gene expression. The ability of TFs to recognize specific DNA sequences and interact with a wide variety of proteins within transcriptional complexes that control the expression of hundreds of genes is essential to their activity. Plant transcription factor database plant has identified 134 WRKY, 180 NAC, 145 MYB, 172 ERF, and 166 bZIP genes. TFs are fascinating genetic engineering targets since they function as master regulators for multiple stress-related genes. Numerous TF families have played roles in stress responses, such as WRKY, MYB, NAC, and bZIP, and numerous TF genes are associated with higher tolerance in model and crop plants. Because numerous TF genes are stress-responsive and control a large set of downstream genes, increasing plant stress tolerance by altering TF gene expression has been a hot research topic. Consequently, there is potential to develop crops that are more stress-resistant. There has been considerable success in this area by the overexpression of numerous TF genes.

Conclusion: It can be seen from the above list that plant growth regulators control secondary metabolite production in considerable quantity. They work well when applied in mixtures as well as individually. Both in-vitro and in-vivo conditions have been utilized by scientists for the improvement of different secondary metabolites in various crops, but cell cultures give control over environment, production, quality and are economical. They are capable of producing a particular medicinal product at a rate.

References:

1. Seca A.M.L., Pinto D.C.G.A. Biological potential and medical use of secondary metabolites. Medicines. 2019;6:66. doi: 10.3390/medicines6020066. [DOI] [PMC free article] [PubMed] [Google Scholar]
2. Tiwari R., Rana C.S. Plant secondary metabolites: A review. Int. J. Eng. Res. Gen. Sci. 2015;3:661–670. doi: 10.5511/plantbiotechnology.14.1002a. [DOI] [Google Scholar]
3. Gaspar T., Keveks C., Penel C., Greppin H., Reid D.M., Thorpe T.A. Plant hormones and plant growth regulators in plant tissue culture. In Vitro Cell. Dev. Biol. Plant. 1996;32:272–289. doi: 10.1007/BF02822700. [DOI] [Google Scholar]
4. **Hamill, J.D., and A.J. Lidgett (1977)**. *Hairy root cultures—Opportunities and key protocols for studies in metabolic engineering*. In: P.M. Doran (ed.), Hairy Roots. Harwood Academic Publishers, Australia.
5. https://link.springer.com/article/10.1007/BF03028631
6. **Uozumi, N., and T. Kobayashi (1994)**. *Application of hairy root and bioreactors*. In: D.D.Y. Ryu and S. Furusaki (eds.), Advances in Plant Biotechnology. Elsevier, New York, NY, USA. Link
7. **Jeong, G.T., and D.H. Park (2005)**. Comparative evaluation of modified bioreactors for enhancement of growth and secondary metabolite biosynthesis using Panax ginseng hairy roots. *Biotechnol. Bioprocess Eng. 10: 528–534. Link*
8. **Rao, S.R., and G.A. Ravishankar (2002)**. *Plant cell cultures: Chemical factories of secondary metabolites*. Biotechnol. Adv. 20: 101–153. Link
9. Moubayidin L., Mambro R.D., Sabatini S. Cytokinin-auxin crosstalk. Trends Plant Sci. 2009;14:557–562. doi: 10.1016/j.tplants.2009.06.010. [DOI] [PubMed] [Google Scholar]
10. Lee Z.H., Hirakawa T., Yamaguchi N., Ito T. The roles of plant hormones and their interactions with regulatory genes in determining meristem activity. Int. J. Mol. Sci. 2019;20:4065. doi: 10.3390/ijms20164065. [DOI] [PMC free article] [PubMed] [Google Scholar]
11. **Rao, S.R., and G.A. Ravishankar (2002)**. *Plant cell cultures: Chemical factories of secondary metabolites*. Biotechnol. Adv. 20: 101–153. Link
12. Shin J., Bae S., Seo P.J. De novo shoot organogenesis during plant regeneration. J. Exp. Bot. 2020;71:63–72. doi: 10.1093/jxb/erz395. [DOI] [PubMed] [Google Scholar]
13. Pernisová M., Klíma P., Horák J., Válková M., Malbeck J., Souček P., Reichman P., Hoyerová K., Dubová J., Friml J., et al. Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux. Proc. Natl. Acad. Sci. USA. 2009;106:3609–3614. doi: 10.1073/pnas.0811539106. [DOI] [PMC free article] [PubMed] [Google Scholar]

14. Schaller G.E., Bishopp A., Kieber J.J. The yin-yang of hormones: Cytokinin and auxin interactions in plant development. Plant Cell. 2015;27:44–63. doi: 10.1105/tpc.114.133595. [DOI] [PMC free article] [PubMed] [Google Scholar]
15. Hamany Djande C.Y., Steenkamp P.A., Steenkamp A., Piater L.A., Madala N.E. Habituated Moringa oleifera callus retains metabolic responsiveness to external plant growth regulators. Plant Cell. Tissue Organ Cult. 2019 doi: 10.1007/s11240-019-01565-y. [DOI] [Google Scholar]
16. Malik S., Zia M., Rehman R., Chaudhary F. In vitro plant regeneration from direct and indirect organogenesis of Momordica charantia. Pakistan J. Biol. Sci. 2007;10:4118–4122. doi: 10.3923/pjbs.2007.4118.4122. [DOI] [PubMed] [Google Scholar]
17. Yu J., Liu W., Liu J., Qin P., Xu L. Auxin control of root organogenesis from callus in tissue culture. Front. Plant Sci. 2017;8:1–4. doi: 10.3389/fpls.2017.01385. [DOI] [PMC free article] [PubMed] [Google Scholar]
18. Alagarsamy K., Shamala L.F., Wei S. Influence of media supplements on inhibition of oxidative browning and bacterial endophytes of Camellia sinensis var. sinensis. 3 Biotech. 2018;8:1–7. doi: 10.1007/s13205-018-1378-9. [DOI] [PMC free article] [PubMed] [Google Scholar]
19. Murata M., Nishimura M., Murai N., Haruta M., Itoh Y. A Transgenic apple callus showing reduced polyphenol oxidase activity and lower browning potential. Biosci. Biotechnol. Biochem. 2014;65:383–388. doi: 10.1271/bbb.65.383. [DOI] [PubMed] [Google Scholar]
20. Chaudhary G., Dantu P.K. Evaluation of callus browning and develop a strategically callus culturing of Boerhaavia diffusa L. J. Plant Dev. 2015;22:47–58. [Google Scholar]
21. Gaspar T., Kevers C., Bisbis B., Franck T., Crevecoeur M., Greppin H., Dommes J. Special symposium: In vitro plant recalcitrance loss of plant organogenic totipotency in the course of in vitro neoplastic progression. In Vitro Cell Dev. Biol. Plant. 2000;36:171–181. doi: 10.1007/s11627-000-0033-3. [DOI] [Google Scholar]
22. Zhang T.Q., Lian H., Tang H., Dolezal K., Zhou C.M., Yu S., Chen J.H., Chen Q., Liu H., Ljung K., et al. An intrinsicmicroRNA timer regulates progressive decline in shoot regenerative capacity in plants. Plant Cell. 2015;27:349–360. doi: 10.1105/tpc.114.135186. [DOI] [PMC free article] [PubMed] [Google Scholar]
23. Ikeuchi M., Ogawa Y., Iwase A., Sugimoto K. Plant regeneration: Cellular origins and molecular mechanisms. Development. 2016;143:1442–1451. doi: 10.1242/dev.134668. [DOI] [PubMed] [Google Scholar]
24. **Hooker, T.S., and T.A. Thorpe (1998)**. Effects of fluridone and abscisic acid on lateral root initiation and root elongation of excised tomato roots cultures in vitro. *Plant Cell* Tissue Organ Culture 52: 199–203. Link

25. **Bais, H.P., G. Sudha, J. George, and G.A. Ravishankar (2001)**. Influence of exogenous hormones on growth and secondary metabolite production in hairy root cultures of Cichorium intybus L. cv. Lucknow Local. In Vitro Cell. Dev. Biol. Plant. 37: 293–299. Link
26. **Rhodes, M.J.C., A.J. Parr, A. GiuLietti, and E.L.H. Aird (1994)**. Influence of exogenous hormones on the growth and secondary metabolite formation in transformed root cultures. Plant Cell Tissue Organ Culture 38: 143–151.
27. **Ford, Y.Y., J.M. Taylor, P.S. Blake, and T.R. Marks (2002)**. *Gibberellin A3 stimulates adventitious rooting of cut
28. **Yamaguchi, T., and H.E. Street (1977)**. Stimulation of the growth of excised cultured roots of soya bean by abscisic acid. Annals of Botany 41: 1129–1133. Link
29. **Mak, Y., and P.M. Doran (1993)**. Effect of cell-cycle inhibition on synthesis of steroidal alkaloids by Solanum aviculare plant cells. Biotechnol. Letters 15: 1031–1034. Link
30. E. Shahinasi, F. Brahushi, A. Devolli, M. Kodra. (2017). The ecotoxicology of pesticides group of triazole and their use to control apple scab (Venturia inaequalis). Journal of Hygienic Engineering and Design. 18: 36-42.
31. M. Naeem, M.M.A. Khan, Moinuddin. (2012). Triacontanol: a potent plant growth regulator in agriculture. Journal of Plant Interactions. 7(2): 129-142.
32. https://www.iscientific.org/wp-content/uploads/2020/05/14-IJCBS-19-15-14.pdf
33. https://www.google.com/search?q=application+of+plant+growth+regulators+to+enhance+secondary+metabolite+production&oq=&gs_lcrp=EgZjaHJvbWUqCQgAECMYJxjqAjIJCAAQIxgnGOoCMgkIARAjGCcY6gIyCQgCECMYJxjqAjIJCAMQIxgnGOoCMgkIBBAjGCcY6gIyCQgFECMYJxjqAjIJCAYQIxgnGOoCMgkIBxAjGCcY6gLSAQs0MjIxMDMwajBqN6gCCLACAfEFtEV9a-_0iAPxBbRFfWvv9IgD&sourceid=chrome&ie=UTF-8
34. M. Hamayun, S.A. Khan, A.L. Khan, J.-H. Shin, B. Ahmad, D.-H. Shin, I.-J. Lee. (2010). Exogenous gibberellic acid reprograms soybean to higher growth and salt stress tolerance. Journal of Agricultural and Food Chemistry. 58(12): 7226- 7232.
35. https://www.sciencedirect.com/science/article/abs/pii/S2214786117303285#:~:text=Accumulation%20of%20secondary%20metabolites%20normally,production%20of%20plant%20secondary%20metabolites.
36. Ibrahim, M.; Xin, D.; Manjree, A.; Giles, H.; Muslim, A.; Yonglin, R. Influence of benzyladenine on metabolic changes in different rose tissues. *Plants* **2018**, *7*, 95. [**Google Scholar**] [**CrossRef**] [**PubMed**]