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Review

Biotic and abiotic stress roles in drugs production through *in vitro* approaches in plants – a review

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Abstract. *Plant metabolic engineering is a modern discipline that promises to create opportunities in pharmaceutical industries to produce and biomedicine. Over the long period natural and synthetic plant hormones have had tremendous implications in callus/cell culture /suspension/ for secondary metabolites production (SMs). Generally, SMs plays a vital fundamental role in protecting the plant from biotic and abiotic attacks to which it may be subjected. This review article focused on the relationship between various factors related to the drug production. In medicinal plants, in vitro studies, based on biotic factors such as fungal/endo-phytic fungal elicitors/microbe-derived exogenous elicitor yeast extract (YE) were cross checked with the abiotic six factor groups, including auxins and cytokinins, gamma radiation, lights, temperature, carbon sources, photoperiods, precursor chemicals and plant metabolic enzymes. Moreover, key enzymes and gene networks can serve as a resource to selected potential targets for specific SMs production. This is the first review to describe the light factors needed for the SM production, which has favorable role for SMs. We envisage that the researcher can design how to modulate the stress factors in terms of drug improvement from medicinal plants.*

Keywords: medicinal plants, stress factors, enzymes, gene networks, secondary metabolites production

1. Introduction

Medicinal plants have been an exemplary source of drugs. The drug discovery from medicinal plants is time-consuming for screening, isolation, identification, quantification and biological studies must be employed (Koehn and Carter, 2005). Plant secondary metabolites (PSMs) started about 200 years ago (Hartmann, 2007) and drugs have been used in medicines and preservation of foods for long times (Patra and Saxena, 2010). More than 200000 defined structures of PSMs have been identified (Hartmann, 2007). These SMs are drugs produced by plants for which no role has yet been found in growth, photosynthesis, reproduction and other primary functions. These chemicals are extremely diverse; environment is directly regulating the drug constituents of medicinal plants, often leading to unpredictable changes at both the SM level and plant growth development (Colling et al., 2010). In terms of ecology, interaction between plants and insects has delivered the color pigments. It has attracted pollinators followed by dispersed seeds.

PSMs are commercially exploited as drugs, dyes, flavors, fragrances, insecticides, etc. It is quite expensive because of low abundance in the intact plant and very limited storage in dedicated cells or organs. For many years, *in vitro* approaches have been an attractive source of tools (*In vitro* callus culture, cell suspension culture, cell culture, hairy root culture, etc.) for developing biologically active compounds, which helps to increase the accumulation of biomass as a source of independent climatic conditions (Verpoorte et al., 1998). Plant SMs are mostly involved in the organism and environment, e.g. as signal or plant defense mechanisms, synthesis of SMs has been induced by different kinds of biotic and/or abiotic stresses (Amdoun et al., 2009). *In vitro* techniques are very useful in ensuring

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sustainable optimized sources of plant-derived natural products. However, *ex situ* cultivation and optimization are specific target SMs in advancement of plant biotechnology. They have delivered the valuable secondary metabolites, including pharmaceuticals, pigments, and other chemicals (Razdan et al., 2016). In this paper, we reviewed the PSMs studies of biotic and abiotic factors with medicinal plants for the last 25 years, with a particular emphasis on the abiotic (physical and chemical) factors and biotic factors (elicitors) that possibly influence SMs and compared with field grown plants through chromatography techniques. In addition, we have emphasized, model drug production (gymnemic acid) via metabolic pathway enzymes, precursors, the abiotic stress factors.

The aim of this review is to provide readers with useful information for understanding biotic and abiotic stress with medicinal plants for secondary metabolites production (SMs) production. This review will also discuss the existing problems in research and human applications for pharmaceutical drug production.

2. Influence and mechanism of genes and enzyme in SMs production (biotic and abiotic)

Biosynthesis of metabolites could be improved effectively through genetic engineering, which requires full information of all the genes/enzymes involved in biosynthetic pathway. Utilization of genes and biosynthetic pathway enzyme studies are very limited. Gene, enzyme as well as metabolites of the respective metabolic pathway show differential pattern expression according to the plant part, age, season, physical and chemical factors. Isoprenogenesis is known to proceed through two different independent pathways; mevalonic acid (MVA) and methylerythritol phosphate pathways (Pandey et al., 2017).

Precursors of chemicals are giving the signal to the cell signal generators followed by receptors, messengers, and intercellular targets as well as plant biosynthetic pathway. The extracellular signals are involved in different stages in plant cells; i) synthesis; ii) signal transmission to the target cell; iii) detection of the signal by specific receptor protein; iv) change in cellular metabolism; v) function or development trigger by receptor signal complex; and vi) termination of cellular complex (George et al., 2007). Plant cells are constantly bombarded with signal transduction, and whereby it has been defined in Figure 1. Precursor binds with the activating gene in meristematic cells by altering the second and tertiary messengers of a cellular cascade. Precursor may indirectly control gene expression through biosynthetic pathway, enzymes acting transcription, mRNA processing, mRNA stability, translational and post-translation modification (Gaspar et al., 2003; George et al., 2007).

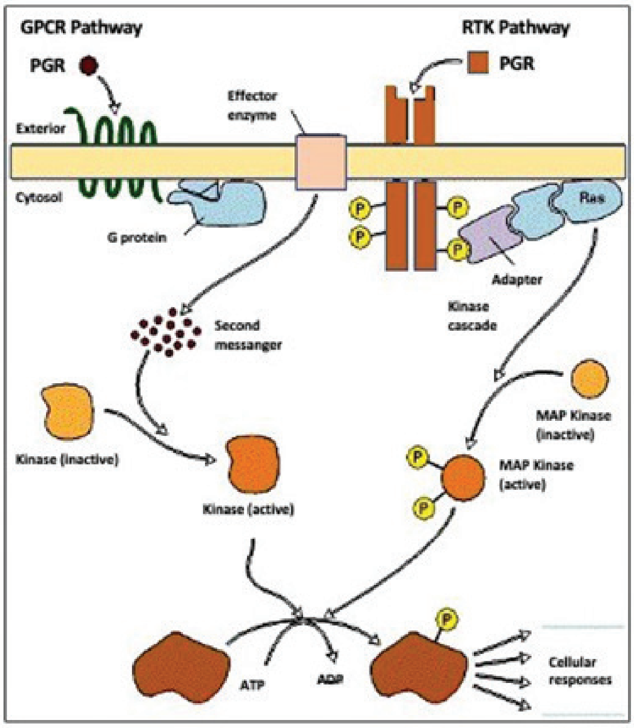

Figure 1. Schematic diagram representing an overview of generalized signaling pathways of cellular response (Image obtained from the "NCBI Bookshelf" /Lodish et al., 2000/ and modified moderately with regard to our illustrations of mechanism of PGR)

In general, the plant cell involves after the first division, the apical daughter cell of the hypothesis remains to maintain the phosphorelay activity of cytokinins signaling and is the precursor for activating quiescent center (QC), whereas the basal daughter cell responds to cytokinin signaling. In many studies SMs production was successful by the treatment of auxins with cytokinins and regulates the gene expression and causes DNA to become more methylated and might be necessary for re-programming of differentiated cells. Its activation of several defense-related genes (or) activation of non-related genes, transient phosphorylation/ dephosphorylation of protein, expression of the specific enzyme, which regulate the biosynthetic pathway (Rao and Ravishankar, 2002). An enzymatic step of MVA and MEP pathways gives the transcriptome details. Genes encoding DXS, DXR and HMGR enzymes expressed their importance by catalyzing the key regulatory step or the isoprenoid biosynthesis. These genes revealed tissue specific, chemotype specific and modulated expression while exposed to Salicylic acid, methyl jasmonate (Pandey et al., 2017).

3. Biotic stresses (Fungal/Endo-phytic fungal) on PSMs production

Biotic stress of microbes plays a crucial role in plant development and SMs production through *in vitro* studies. Endophytic fungus has a role in affecting drug production in terms of quality and quantity. Specific fungus-host interaction in medicinal plants requires promotion of drug production (Razdan et al., 2016). Ideally, the drugs production through bioengineering method, the selected medicinal plants with endophytic fungus under certain cultural conditions followed by metabolite production and fungus-host relationships are clearly understood (Kumaran et al., 2009). Besides that, the nutrient factors, genetic information, ecology habitats are important to standardize the fungus growth (Rodriguez and White, 2009). Most of the endophytic fungi are promoting the PSMs production from medicinal plants (Chen et al., 2016). Many endophytic fungi interact with the host of the medicinal plants and produce valuable SMs such as *Tubercularia* sp. strain TF5 (Wang et al., 2006); *Metarhizium anisopliae* produced taxol (Liu et al., 2009) and *Coetotrichum gloesporides* could induce the production of Artemisinin (He et al., 2009). Entophytic fungi are helpful to improve the PSMs welfare for the human health.

4. Abiotic stresses

For plants to produce certain bioactive substances are largely influenced by the physical and chemical environments in which they grow. Some studies declared that PGRs with light is an important factor affecting the growth, organogenesis and the formation of plant products including both primary and secondary metabolites. In addition, PGRs were used for callus growth, and modifying the metabolite level, nature through shaking speed, temperature, carbon sources, ammonium nitrate concentrations, pH and medium plays an important role in the production of PSMs. The present review elicits that biotic and abiotic treatments are effective for improving the following phases - log phase, lag phase, exponential phase, stationary phase and decline biomass as well as SMs content in medicinal plant.

4.1. Influence on plant growth regulators

PGR promotes or inhibits plant growth and SMs level. Individual or a combination of PGRs could be a crucial factor for SM production. It has provided changes in sensitivity of plant cells for the SMs production. Biotic and abiotic stresses are important factors to increase the metabolites and have shown pharmacological activities. Precursors acts depend on the synergistic interaction of the plant parts, apoplastic (or) symplastics transport; changes in target sensitivity with time, and other factors (He et al., 2009).

4.2. Influence on auxins and cytokinins

Auxins have characteristic features of polar translocation, root and shoot growth as well as delayed root induction, delay in abscission and differentiation of xylem elements (Ahmed et al., 2011). *In vitro* induced auxins seem to be capable of erasing the genetically programmed physiology of whole plant tissue, which had previously determined their differentiated state. The effect of auxins depends on the magnitude of dedifferentiating effect of the auxins applied. Strong auxins have specific inhibitory effect in which the secondary product accumulates in tandem with a special mechanism via direct influence on enzymatic activities and influences transcription. Tissue specific programmes, associated with cell differentiation would be eradicated by hypermethylation, perhaps with a small fraction of the cells reaching an ultimate state of dedifferentiation, which leads to plant cell morphogenesis. Auxins are directly modifying the growth or indirectly by inducing cell differentiation and SMs. In addition, a balanced combination of PGR plays an important role in regulating cellular and subcellular differentiation (Rao and Ravishankar, 2002). Hence, auxins are capable of producing or increasing their secondary compounds constantly at their low concentration (Machakova et al., 2008).

PGRs have the specific *in vitro* responses at very low concentrations, whereas, high concentrations may cause non-physiological effects to exert multiple functions in plants (Figure 2).

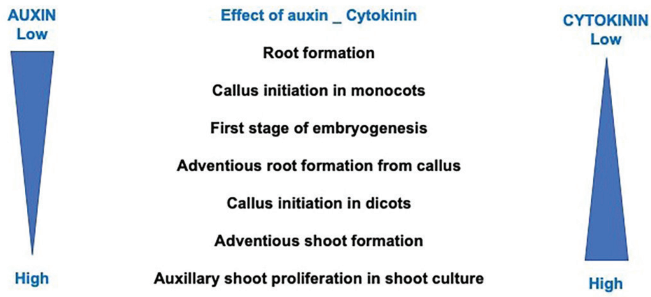

Figure 2. The relative concentration of auxins and cytokinins for callus growth and secondary metabolites production from Medicinal plants (Gaspar et al., 2003)

The concentrations of auxin or cytokinin ratio alter dramatically both the cell growth and SMs formation in cultured plant cells; it inhibits photosynthesis and induces isofavonoids from *Pueraria lobata* (Mathkowski, 2004) and gymnemic acid from *Gymnema sylvestre* (Ahmed et al., 2008; 2009; 2010). Optimization of PGRs plays a major role in large scale SMs production with significant biological applications (Figure 3). Cytokinins appear to be necessary for plant cell division. Cytokinins lead to the considerable protraction in metaphase, but not the prophase of mitosis, which suggested that cytokinins might be required for the regulation and synthesis of proteins formation and function of the mitotic spindle.

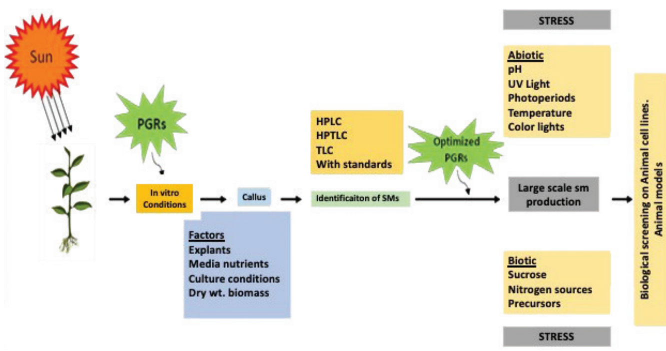

Figure 3. Schematic diagram representing the PGR induced development and large-scale production of secondary metabolites (SMs) under various stress conditions

4.3. Influence on physical stress

SMs were found in cultured cells at a higher biomass through optimization of culture conditions, than field plants. The optimum

PGR levels in a culture plant are very important in inducing rather than simply maintaining the SM production under the plant stress conditions *viz.*, light, temperature, photoperiod, carbohydrates, nitrogen, etc. (Figure 3). In plant cell culture, various approaches such as illumination and changing nutrient supply can improve the yield of the cell biomass and regulate the SMs (Ho et al., 2010).

4.3.1. Influence of light

This is first review to describe the light factors needed for SM production and could be used for the large-scale production. Cytokinins are a light dependent process. It has been activating RNA synthesis and stimulates protein synthesis and enzyme activity. Light is also involved in regulating PSMs. In process, the small amount of energy in each quantum of lights is inversely proportional to the wavelength of radiation. The action of light on higher plants occurs mainly in two aspects. First, light provides the energy source required by plant through photosynthesis. Second, light is a signal received by photoreceptor to regulate the growth, differentiation and metabolism (Wang et al., 2001).

Influence and mechanism of color lights (blue, green, red and white): Light source, which can create stresses on the callus, and may act as an elicitor of plant defense response so as to stimulate the SMs synthesis. In regard to the mode of action of light in callus culture, three photo sensors of the wavelength are involved: Phytochorme (>520 nm: red/ far red), cryptochrome (340-520 nm, blue /UV-A) and UV-B photo sensor (290-350nm). Phytochrome appears in two forms: P_r and P_{fr} under the influence of light. P_r changes into the physiologically active form P_{fr}, which can affect the activation of enzymes and gene expression. In specific, blue light (cryptochrome) stimulates chlorophyll, carotenoid synthesis, activation of gene expression and increases phenylalanine ammonium lyase activity. Cryptochrome has a top peak at 450 nm, which is also close to the wavelength peak value of blue light. Light absorption in cryptochrome and UV-B photoreceptor determines the sensitivity of the signal reaction chain of individual responses towards P_{fr} (Mohr and Schopfer, 1995) and has made it possible to get PSMs in recent years. Beside that, the production of ROS such as H_2O_2 in the callus known as the oxidative burst is an early event of plant defense response to different stress and also acts as a secondary messenger to signal subsequent defense reactions in plants. Light treatment processes, the formation of H_2O_2 is the important phenomenon of signal transduction induced by stress conditions, which changes the content of redox agents and the redox state of cells. H_2O_2 may function as a signal for the induction of defense systems and could enhance SMs production (Jabs et al., 1997). Cryptochrome is most sensitive to blue light and it is closest to UV-B in wavelength. Under blue light, more P_r was transformed to P_{fr} than under white light, and the phenylalanine ammonium lyase activity in the cultures was higher than that under white light (Ouyang et al., 2003). Shohael et al. (2006) reported that total phenolic, total flavonoid and chlorogenic acid accumulation significantly increased compared with red light. In addition, blue light enhanced the maximum accumulation of eletheroside B; however, red light produced higher amount the eleutheroside E and E1 than blue and white lights. Controversy, the triterpene biosynthesis was successful in less light treatment. Thus, darkness produced triterpene from leaf and favourable for triterpene purification by reducing the amount of chlorophyll in the culture by dark treatment (Ho et al., 2010).

4.3.2. Influence and mechanism of temperature

It is known that exposure of plants to abiotic factors including low and high temperature causes oxidative stress in which increased production of reactive oxygen species (ROS) is evident. Temperature may influence the chemical modification of the substance that was synthesized, e.g. the degree of saturation of fatty acids. Thus, saturated fatty acids increased in response to increased temperature, but unsaturated fatty acids increased in decreased temperature (Suri and Ramawat, 1995). It has been shown that the optimal temperature treatment was required even in suspension cultures for accumulation of biomass and production of eleutherosides (Shohael et al., 2006). Plants exposed to either low or high temperatures suffer injury, exhibiting reduction or stop of growth and the degree of injury can vary with plant species, stage of crop development and with the treatment conditions such as nitrogen and phosphorus sources are useful to improve ginsenoside by bioreactor (Kochan et al., 2016). In Asclepiadaceae species of *Cynanchum wilfordii*, the biomass was higher at 31°C than 25°C, but the gamaminine production was higher at 25°C than 31°C temperature (Shin et al., 2003). According to Zhao et al. (2001) in callus culture, temperature regulates various secondary metabolites from medicinal plants, the maximum temperature of 25°C found to induce the jaceosidin production from *Saussurea medusa*. The 30°C temperature significantly increased the callus biomass and gymnemic acid.

4.3.3. Influence and mechanism on photoperiod

Photoperiod induces CO_2 concentration inside the vessel and the daily exchange, in turn affects the growth of callus under CO_2 non-enriched condition. Generally, the growth of photoautotrophic culture requires 1-2% of CO_2 concentration. Light is usually considered an important factor that affects the growth, organogenesis, and the formation of primary and secondary compounds (Liu et al., 2002). MS medium supplemented with 2.4-D and KN with different photoperiods (e.g., 4, 8, 12, 20, 24 hrs and dark) showed tremendous influence on callus initiation and proliferation, and gymnemic acid production in leaf explants of *Gymnema sylvestre* (Zhao et al., 2001). In addition, similar effect was found to induce harringtonine, homoharringtonine and isoharringtonine in 12h photoperiod solid cultures of *Cephalotaxus fortune* (Zhang et al., 1995); gagaminine production in *Cynanchum wilfordii* was better in dark conditions than in light conditions (Shin et al., 2003). It has been reported that different photoperiods and LED lights were used to induce the phenolic acid, flavonoids and myricetin synthesis (Cioc et al., 2018).

4.4. Influence and mechanism of sucrose

In plant cell culture, cells are usually grown with simple sugars as carbon sources in the medium for the energy resource. The fact is that the level of sucrose has been shown to affect the productivity of secondary metabolites in many plants (Ho et al., 2010). These elements influence the cell growth and division by regulating protein, gene expression and other pathways (Zheng, 2009). Sucrose concentration above 3% often enhances the biosynthesis of SMs by heterotrophic plant cells. High levels of sucrose could be related to osmotic stress conditions, which cause the cells to die (Mekky et al., 2018). In addition, the considerable amount of decline in the callus growth and chlorophyll content has been observed in a medium without sucrose. The negative impact of exogenous sugars in photosynthesis was most probably attributable to the effects exerted by sucrose on a series of biochemical reactions, leading to the down regulation as feedback inhibition of ribulose biophosphate carboxylase activity. MS medium supplemented with optimum growth regulators 2.4-D and kinetin (KN) along with 5% sucrose induced SM production in *Gymnema sylvestre* (Ahmed et al., 2010).

On the other hand, sucrose has played an important role in callus cell growth on batch culture of *Gymnema sylvestre*. A similar result was observed in *Cynanchum wilfordii* (Asclepiadaceae), where the gamaminine synthesis was enhanced when 5% sucrose was observed to be superior to 3% sucrose concentration (Shin et al., 2003). MS medium supplemented with growth regulators (2.4-D and BA) containing 4% sucrose induced trachelogenin production in *Ipomoea cairica*, 5% sucrose induced SMs production in *Morinda citrifolia*. MS medium supplemented with NAA and 2.4-D containing 5% sucrose induced SMs production from *Ocimum basilicum* (Meira et al., 2017).

4.5. Influence on nutrient level

Nutrient level alteration influences the expression of many SM pathways (Suri and Ramawat 1995, Costa et al., 2008). Ammonium/nitrate-nitrogen ratio has been shown to markedly affect the production of SMs. Medium with PGRs and NH_4/NO_3 ratio induce antharaquinones production in callus cultures of *Rheum ribes* (Sepehr and Ghorbanli, 2002). Modifications in the nitrogen source of the culture media may lead to biomass and SMs production (Costa et al., 2008). From our findings, the sucrose level and ammonium nitrate concentrations induced the SMs in *Gymnema sylvestre* (Ahmed et al., 2010) and *Sutherlandia frutescens* (Colling et al., 2010). Mineral nutrition is more important for increasing this metabolite than water and salinity stress for canavanine biosynthesis in *Sutherlandia frutescens* (Colling et al., 2010).

Ethylene is essential for plant defense and is not common for induction of PSMs. In some case, ethylene treatment enhances the flavonoid, anthocyanin and stillbenoid production via downstream up-regulating their biosynthetic genes in grape (*Vitis vinifera*) cell cultures (Ishiai et al., 2017). Whereas the high concentration of ethylene inhibits SMs production and low concentration helpful for elicitor production. Abscisci acid (ABA) acts as an important signal molecule to regulate expression of sets of defense genes. ABA is also reported to regulate biosynthesis of SMs in some plant cell cultures. It is osmotic stress-induced agent, it regulates the osmotic stresses from sorbitol, mannitol, sucrose, and salt stress in a culture medium. For example, it can stimulate production of indole alkaloids in *C. roseus* (Zhao et al., 2000); taxol in *Taxus* spp. (Luo et al., 2001). On the other hand, ABA negatively regulates the elicitor induced synthesis of capsidiol in wild tobacco (Mialoundama et al., 2009).

4.6. Influence on other chemical stresses (precursors)

PSMs could be possible to enhance through applying different abiotic stresses; it has proved the active compounds (volatile sulphur compounds, ascorbic acid, carbonyl compounds, vitamins and flavonoids) are useful in pharmaceutical industry. External phytohormone, shaking speeds, pH of the medium played very crucial and important roles in SMs production (Devi et al., 2006). Along with the sucrose, inoculums density, auxins, aeration also plays an important role in cell growth of bioreactor cultures for secondary metabolites production (Zimare et al., 2017). *In vitro* suspension cultures, the active compounds of gymnemic acid and gymnemagenin in the cultured undifferentiated cells (Zimare et al., 2017).

Elicitors are chemicals or bio-factors from various sources that can induce physiological and morphological changes of the plant cells. It may include abiotic elicitors such as inorganic compounds. The chemical perception can induce the elicitor signals, plant receptors are activated, and then in turn activate their effectors, such as ion channels, GTP binding proteins (G-proteins), protein kinases. Activated effects transfer the elicitor signals to second messengers, which further amplify the elicitor signal to other downstream reactions (Blume et al., 2000). Methyl jasmonate as a chemical elicitors could induce the xanthone in *Gentiana dinarica*; whereas the salicylic acid and chitosan treatment increased the norswertianin. In *Salvia virgata* shoots contains phenolic acid when MS medium was treated with Ag^+ ions, yeast extract and methyl jasmonate (Attaran et al., 2017).

It has been recently reported that protoberberine alkaloids were induced from suspension culture of *Tinospora cordifolia*; when the cells were treated with methyl jasmonate (250 μm) (Kumar et al., 2017) and Cannabinoids from *Cannabis sativa*; it has increased in abiotic treatments (Gorelick and Bernstein, 2017).

5. *In vitro* drugs and health care perspectives

In vitro approaches for bioactive compounds have been used in pharmacological activities and industrial product formation and pharmaceutical aspects, which need to increase large scale. However, the SM production is merely dependent on the timely eliciting effect of the biotic and abiotic stresses. Hence, the bioactive compounds are available in the world market in the form of food additives, pigments, dyes, insecticides, cosmetics and perfumes and fine chemicals. The medicinal plants are the most exclusive sources of life saving drugs for the majority of the world's population (Mulabagal and Tsay, 2004).

The isolated SMs derived from callus/plant cell extracts having various pharmacological activity, the *Centaurea ragusina* callus extract contains polyphenols and shows antibacterial, cytotoxic effect, thermal denaturation and circular dichroism (CD) for DNA/RNA interactions (Vuicic et al., 2017); callus derived from *Rosa damascene* has therapeutic effect as anti-inflammatory to suppresses the activated T-cells from human blood; anti-oxidant activity of *Crataegus monogyna* (Valls et al., 2007), anticarcinogenic activity from *Linum* species (Ionkova et al., 2003), antinociceptive effects of *Phyllanthus* species (Catapan et al., 2000), antiplasmodial activity of *Phyllanthus niruri* (Luyindula et al., 2004) and anti-oxidant activity reduce the diabetes plants of *Cassia fistula* callus (Bahorun et al., 2005), *Aegle marmelos* (Arumugam, et al., 2008), *Gymnema sylvestre* (Ahmed et al., 2008) and this methanolic callus extract was more suitable in pancreatic β cell regeneration and anti-diabetes (Ahmed et al., 2010). Perhaps, in various cellular animal models, plant SMs have displayed various pharmacological and medicinal activities such as neuroprotective, antihypertensive, cardioprotective, anticancer, anti-inflammatory, vasorelaxant, antiparasitic, anti-bacterial, antifungal, antiprotozoal, antipyretic, abortifacient, antidiabetic, and antitussive activities (Dey and Mukherjee, 2017).

6. *In vitro* gymnemic acid production from *Gymnema sylvestre* under abiotic stress

In vitro optimization is quite a long process, we have selected the explants (mature, young and juvenile) with different media such as MS, SH, WPM, and B5 media with six PGRs (auxins and cytokinins). Our results showed that Juvenile explants, MS media, auxin and cytokinins are suitable for callus biomass production (Ahmed et al., 2010). We have optimized the callus and gymnemic acid production through callus growth curve. The maximum biomass was observed in stationary phase than lag, log, exponential and decline phase. The calluses were screened with TLC, HPTLC and HPLC with standard Gymnemic acid. The bioactive compound (gymnemic acid) is significantly increased in intact leaf explants than in vitro treatment callus. Explants were treated with different abiotic stress conditions (light, temperature, photoperiods, carbon sources, ammonia nitrate treatments. Blue light enhanced the gymnemic acid accumulation up to 4.4-fold of that found under white fluorescent light and 2.8-fold of that found in intact leaves. Present findings concluded that blue light can be used as a tool for enhancing gymnemic acid in batch culture of *G. sylvestre* (Ahmed et al., 2008). Gymnemic acid content callus extracts were used in anti-diabetic studies; the results declared that blue light induced callus extract significantly increased pancreatic beta cells and showed anti-diabetic effect in Wistar rats. On the basis of the available literature it can be concluded that in plant biosynthetic pathway many enzymes, fungal elicitors and precursors could regulate gymnemic acid biosynthetic pathway.

7. Conclusion

This review highlights the role of biotic and abiotic stress factors involved in the plant defense mechanisms and their mode of action favorable (or) against numerous plant secondary metabolites production (SMs) in medicinal plants. It was concluded that such an approach could be used as a useful tool to screen the other abiotic and biotic stress factors. The finding data recommended that the studied plant material can positively and significantly affect biomass and yield respective SMs. We argue here that SMs optimization increases through industries scale, *in vitro* propagation and gene transformation studies to be developed with abiotic and biotic stress. To the best of our knowledge, the plant metabolic engineering studies are of great importance to the next generations for the purpose of producing valuable SMs in plants in the pharmaceutical industry and could be improved through using transcription factors, introducing biosynthetic genes, improving metabolic flux, Genomic and proteomic tools. Finally, we hope this review will give an in-depth understanding of the important roles of abiotic and biotic treatment in various plants to enhance the metabolites to the human needs with respective clinical trials.

Conflict of interest

The authors have no conflict of interest.

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