Chapter

# Unlocking Pharmacological and Therapeutic Potential of Hyacinth Bean (*Lablab purpureus* L.): Role of OMICS Based Biology, Biotic and Abiotic Elicitors

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## Abstract

Hyacinth bean also known as Indian bean is multipurpose legume crops consumed both as food by humans and as forage by animals. Being a rich source of protein, it also produces distinct secondary metabolites such as flavonoids, phenols and tyrosinase which not only help strengthened plant's own innate immunity against abiotic/biotrophic attackers but also play important therapeutic role in the treatment of various chronic diseases. However, despite its immense therapeutic and nutritional attributes in strengthening food, nutrition and therapeutic security in many developing countries, it is still considered as an "orphan crop" for unravelling its genetic potential and underlying molecular mechanisms for enhancing secondary metabolite production. Several lines of literatures have well documented the use of OMICS based techniques and biotic and abiotic elicitors for stimulating secondary metabolite production particularly in model as well as in few economically important crops. However, only limited reports have described their application for stimulating secondary metabolite production in underutilised crops. Therefore, the present chapter will decipher different dimensions of multi-omics tools and their integration with other conventional techniques (biotic and abiotic elicitors) for unlocking hidden genetic potential of hyacinth bean for elevating the production of secondary metabolites having pharmaceutical and therapeutic application. Additionally, the study will also provide valuable insights about how these advance OMICS tools can be successfully exploited for accelerating functional genomics and breeding research for unravelling their hidden pharmaceutical and therapeutic potential thereby ensuring food and therapeutic security for the betterment of mankind.

**Keywords:** Hyacinth bean, OMICS, biotic and abiotic elicitors, therapeutic, secondary metabolites, miRNAs

## 1. Introduction

Evolution, expansion and transformation of several wild crops via domestication and breeding have blessed the humans and animals with never ending wide varieties of plant-based foods around the globe [1]. Nevertheless, ~ 1 billion population around the world combat with hunger and malnutrition as they are unable to consume important vitamins/minerals thus affecting food and nutritional security in many developing countries [2]. These nutritional deficiencies could be due to the increased inclination towards consumption of specific crops as majority of the peoples relies on wheat, rice and maize for their food [1]. Several lines of literatures have documented that around 50% of total world population relies on above crops for catering while other crops like legumes are cultivated and consumed by marginal communities [3]. The crops cultivated by marginal communities are referred as neglected and underutilised crop species (NUCS) and rich source of vitamins, minerals and secondary metabolites having pharmaceutical properties [4]. These NUCS have the potential to counteract malnutrition by ensuring health/nutritional security, alleviate poverty by increasing resilience and sustainability to the farming systems [1]. However, compare to mainstream crops, less focused have been given towards the genetic improvement of NUCS.

Hyacinth bean (*Lablab purpureus* L.) which comes under the NUCS category is also an underutilised legume crops with great potential. The notable properties of hyacinth bean are (i) great adaptability under wide range of environmental conditions, (ii) rich source of protein, fibre and secondary metabolites like tyrosinase which is used in the treatment of various chronic diseases (iii) being stress tolerant, it require low cultivation/maintenance cost which could help smallholder farmers and their communities to generate more income [5, 6]. In Asia and Africa, hyacinth bean is also extensively consumed both as pulse and vegetable and is also exploited as green manure, forage/fodder for livestock, ornamental or medicinal herb [6]. The crop is ancient origin of which dates back to 1500 B.C. in Africa and later on introduce to India where it is mainly cultivated in tropical and subtropical regions and commonly known as “Sem” [7]. Besides, hyacinth bean possesses remarkable therapeutic potential for pharmaceutical application therefore, also considered as medicinal legume [5–7]. Consequently, hyacinth bean is tremendously used by therapeutic/pharmaceutical industries like for the large-scale production of drugs and skin ointments as it is rich source of aloe-emodin, rhein, chrysophanol, alkaloids, flavonoids and tyrosinase with broad spectrum pharmacological activities [8].

Furthermore, the seeds of hyacinth bean are also abundant in carbohydrate myoinositol that are exceptional in ovarian function in women by controlling oligomenorrhea and polycystic syndrome [8]. Additionally, the seeds also contain brassinolide which is a steroid which is clinically proven to cure prostate cancer in humans. The alkaloid spermidine found in hyacinth bean seeds is comprehensively used as a biomarker for the perception of skin cancer where as another alkaloid spermine is commonly used in the treatment of cancer/tumours [9]. Trigonelline another alkaloid found in its seed has demonstrated its role in the treatment of diabetes mellitus and also possess antimicrobial property against *Salmonella enterica* [8, 10]. Further, pantothenic acid obtained from hyacinth bean leaf have shown potential to combat aponeurosis thereby by stimulating fibroblast content in early postoperative period in rabbits [10, 11]. Flt3 receptor interacting lectin (FRIL) isolated from hyacinth bean seeds are exclusively employed as preservatives and has been used for preserving human cord blood for 1–2 months [12]. The hyacinth bean is also a rich source of flavonoids like isoflavone, kievitone and genistein which are phenomenal in reducing breast cancer progression in humans [12].

Despite of its pharmaceutical/therapeutic importance and catering food requirements of both humans and animals, hyacinth bean still lack focused research for its genomic improvement as compared to other mainstream crops (wheat, rice maize etc.). The genomic improvement through state-of -the art tools and techniques will not only reform its architectural growth but will also pave the way for rewiring the biosynthesis of imperative metabolites which will significantly impact its growth, yield and therapeutic potential. Therefore, this chapter provides valuable insights about the different state-of -the art tools and techniques that can be employed for the genetic improvement of hyacinth bean and how they can be exploited to inspire its therapeutic potential. Further, role of biotic and abiotic elicitors in stimulating the production of important metabolites in hyacinth bean has also been critically reviewed.

## 2. Medicinal/therapeutic properties of hyacinth bean

Being sessile in nature, plants have to withstand against various adverse climatic conditions to maintain their growth and developmental architecture. The plants are able to survive stressful conditions by synthesising diverse range of secondary metabolites and protease inhibitors that improve their adaptability [13]. Hyacinth bean for example, produces higher level of trypsin inhibitor (14–27 unit/mg) which is a unique property of this orphan legume crop compared to any other major legumes [14]. Like other serine inhibitors, trypsin inhibitor could also function as antifeedant or could also be responsible for strengthening growth, development and productivity by efficiently modulating proteolytic events with in hyacinth bean, mechanism of which has yet to be revealed [15]. Besides this, hyacinth bean also contains wide range of alkaloids, phenols and flavonoids which can be used in treatment of various chronic diseases essentially arthritis, nephritis sepsis as well as skin diseases thus significantly contributing towards human and animal health. All these nutritional and therapeutic properties make hyacinth bean a prime source of food, forage and cash crop in arid and semi-arid areas. However, till date the genes encoding these secondary metabolites are still ambiguous as the crop it self is considered as "orphan crop" for its genome revolution [1]. Further, both conventional and molecular breeding techniques also have been futile in the identification/linking of quantitative trait loci (QTLs) with production of these imperative secondary metabolites [4]. Therefore, all the above information's have reinstated the need to implement advance omics technology for unleashing the genetic constituent of hyacinth bean and to identify genes/proteins involved in the biosynthesis of important secondary metabolites.

### 2.1 Anti-inflammatory and analgesic properties

The phytochemicals or secondary metabolites synthesised by various underutilised crops have the potential to boost innate immune response in humans as well as in animals thus providing immunity against infection, injury and irritation [7]. Several lines of literatures have strongly substantiated that various fruits, vegetables and food legumes synthesise various phytochemicals which are effectively exploited for the treatment of anti-inflammatory disorders, however their mechanism of action is still vague and needs to explored [13]. Various legumes such as soybean, mung bean, moth bean including hyacinth bean have diverted the attention of plant science community due to the presence of functional biological compounds which not only have health benefits and can also be simultaneously used for the treatment of various chronic diseases [8]. Researchers have analysed, tested and confirmed that the crude extracts of mung bean, hyacinth bean and soybean checks the synthesis of nitric oxide (NO) which is an inflammatory mediator thus significantly reducing the ear edema in mice caused by up-accumulation of arachidonic acid [16]. Likewise, another researcher evaluated crude extract of *Phaseolus vulgaris* and hyacinth bean was also effective in controlling the expression of 15-lipooxygenase (15-LOX) thus supressing the release of NO and prostaglandin $E_2$ ($PGE_2$). The limited release of NO and $PGE_2$ further downregulated the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) with in the macrophages thus preventing the inflammatory disorder [17]. Overall, the result indicated that ethanolic extract of the above-mentioned beans can effectively modulate anti-inflammatory response by regulating the expressions of anti-inflammatory enzymes and transcription factors [17].

The phenols present in the dry seeds of legumes such as hyacinth bean have also been implicated to exaggerate anti-inflammatory response upon their adequate consumption [18]. A plethora of research have well indicated that seed and other ethanolic extract of food legumes is rich source of polyphenols and natural antioxidants capable of stimulating anti-inflammatory activity by suppressing the expression of 15-LOX as well as modulating the expression of cyclooxygenase −1 (COX-1) and COX-2 [19]. Similar findings have also been reported by Zhu et al. [16] in pinto bean, black bean and common bean where seed extract was effective in regulating the expression of interlukin-6 (IL-6), interferon-γ (IFN- γ) and IL-17A thus effectively ameliorating acute colitis in mice. In addition to phenolic compounds, these legumes also contain lectins which is protein capable of showing anti-inflammatory response after binding reversibly to carbohydrates [20]. For example, lectins isolated and purified from *Clitoria fairchildiana* showed enhanced anti-inflammatory activity against paw edema and reducing it up to 70% in affected mice [21]. Nonetheless, these phytochemicals have also been identified and documented in hyacinth bean which are capable of inducing multitude of immune response against chronic diseases, however, the functional genes/proteins responsible for their synthesis, transport and mechanisms by which they induce immune response needs an in-depth investigation.

### 2.2 Anti-diabetic properties

The flavonoids such as flavanones, flavanols, anthocyanidins, flavones and isoflavones present in fruits and vegetables have delineated themselves as key players in the treatment of cardiovascular diseases, diabetes and cancers [22]. Various legumes are also a rich source of dietary flavonoids that can regulate carbohydrate digestion, glucose uptake and insulin signalling via various signalling pathways [23]. Among all the flavonoids, dietary isoflavones *per se.,* daidzein and genistein are abundantly found in leguminous plants [24] found exclusively in soy foods. Increasing evidences have well suggested and evaluated the anti-diabetic properties of both the isoflavones i.e. daidzein and genistein in cell culture studies [24]. Dietary intake of both the isoflavones have been shown to modulate glucose metabolism and insulin levels in Type 1 & 2 diabetes thus exerting anti-diabetic effect by increasing lipid plasma composition and accordingly insulin sensitivity [25]. Concomitantly, another study used soy supplemented diet to control blood glucose level in mice [26]. The result of the study successfully demonstrated that soy food significantly improves lipid profile and glucose metabolism in the mice as direct result of increased phosphorylation of AMP activated protein kinase (AMPK) which in turn have caused favourable metabolic changes upon activation of genes/proteins involved in fatty acid oxidation pathway in peroxisome [27]. This report is consistent with the previous findings attributing exceptional role of soy food in the modulation of genes/proteins of AMPK pathway thus efficiently controlling blood glucose levels similar to other flavonoids [27].

Some researchers have also reported neutral to moderate effect of soy food and methanolic extract of hyacinth bean rich in isoflavones in controlling plasma lipid profile thereby confirming anti-diabetic effect of isoflavones could act differentially under *in-vitro* and *in-vivo* conditions [28]. For instance, dietary intake of isoflavones prevented the onset of diabetes in rats and improved blood glucose homeostasis by stimulating the function of pancreatic β-cells. Further, the researchers also noticed a sharp increase in insulin/glucagon ratio and C-peptide level in affected mice as compared to normal healthy rats [29]. The possible reason behind improved insulin/glucagon ratio in affected mice that consumed isoflavones could be due to the downregulation of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase beta oxidation. Furthermore, researchers have also indicated that genistein could modulate pancreatic β-cells via distinct metabolic pathways, $Ca^{2+}$ signalling and calmodulin kinase II pathway [30] thus effectively regulating insulin synthesis in target cells/tissues. The isoflavones such as genistein and daidzein have also been shown to modulate Janus kinase/Signal transduce and activation of transcription (JAK/STAT), ERK-1/2 (serine/threonine protein kinase) and nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) pathways thus stimulating the synthesis cytokinin's in response to pathological alterations [31].

Genistein have also been documented to stimulate the expression of protein kinase A and cAMP cascade which play important role in the proliferation of INS1 and pancreatic β-cells thus efficiently regulating glucose metabolism in mice [31]. However, in addition to isoflavones, anthocyanidin found in soybean seeds rich in cyanidin, delphinidin and petunidin have also demonstrated anti-diabetic effect in streptozotocin induced diabetic rats [32]. Researchers have used methanolic extract of anthocyanidin to diabetic mice and observed that the anthocyanidin effectively raised serum insulin concentration and glucose metabolism in rats. The possible reason behind the anti-diabetic effect of anthocyanidin could be due to the enhance translocation of GLUT4 (glucose transporter) which in turn have stimulated glucose uptake or anthocyanidin could have improved insulin signalling by causing phosphorylation of insulin receptor [33]. Similarly, in another study, researchers have also documented the beneficial effect of anthocyanidin by analysing it on diabetic animal model where they observe that diabetic animal treated with soybean anthocyanidin showed enhanced plasma insulin levels and low triglyceride content [34]. Furthermore, researchers continued their observation up to 12 weeks and observed that the diabetic mice exhibited reduced body weight, blood glucose level, triglyceride levels as revealed by lower expression of lipogenic gene expression in liver and fat [35]. Although, various studies have demonstrated the anti-diabetic effect of both isoflavones and anthocyanidin on animal system, there effect on controlling diabetes in humans are still limited. Therefore, efforts are needed to expand the dimension of research involving structural, biochemical and molecular characterisation of important therapeutic compounds obtained from underutilised legume crops for their efficient use in the human and animal welfare.

### 2.3 Anti-cancerous/tumour properties

The bioactive peptides found in certain legumes and cereals crops has been implicated to regulate growth and development of crops plants by imparting biotic and abiotic stress tolerance [36]. Further, researchers have also isolated and purified some of the plant bioactive peptides and demonstrated their pivotal impact on human health and immune response [37]. Lunasin, a 43 amino acid peptide initially identified and isolated from soybean has shown its tremendous competency in inhibiting cell division in tumour/cancer cells and protect DNA damage by delaying histone acetylation in mammalian cells under oxidative stress [38]. Later, lunasin was also identified in cereals and pseudo-cereals such as rice, wheat, barley and amaranth, however, its present in extremely low quantity as compared to soybean [39]. Being a rich source of lunasin, soybean has been extensively investigated in order to get valuable insight into its structure and function properties, mode action in preventing cancer and the ecological factors that can influence its biosynthesis and transport [37]. Initially, lunasin was identified as chemo-preventive agent but in-depth investigations by several researchers demonstrated that lunasin can effectively suppress skin tumorigenesis in mouse by delaying foci formation in DMBA NIH/3 T3 cells [40].

In addition, researchers have also well documented the chemo-preventive property of lunasin in breast cancer affected mice where they observed significant reduction (30–40%) in tumour cells after treating the mice with lunasin for two months [37]. However, not much research has been focused on lunasin therapeutic properties in soybean as well as in other underutilised legumes still researchers have hypothesised its broad-spectrum role in the treatment of lung cancer, colon cancer and leukaemia [36]. One of the possible mechanisms by which lunasin block cell division in cancer cells could be due to its ability inhibits cell cycle at G2 phase thereby inducing apoptosis in the affected cells [40]. Initial studies on lunasin's mode of action revealed that it can bind to hypoacetylated histone cores in cancer cells and inhibit acetylation in breast cancer cells and prostrate cancer cells [37]. Recently, researchers have made striking discovery claiming that lunasin binding can suppress the integrin signalling in cancer/tumour cells thereby inhibiting focal adhesion kinase/protein kinase B (FAK/AKT) and extracellular signal-regulated kinase 1 (ERK1) signalling in cancer cells [41]. Certain plant protease inhibitors such as Bowman-Birk inhibitors and flavonoids such as flavon-3-ols found in soybean and other legumes have also demonstrated their role in controlling breast and colon cancer [41]. However, detailed characterisation of their structural and functional properties in many legume crops is still ambiguous and need extensive research by employing advance omics technology for their potential application.

### 2.4 Anti-hypertension properties

Hypertension is one of the most important factors (apart from diabetes and high cholesterol level) causing cardiovascular disease in humans which is characterised by the increase in systolic/diastolic arterial pressure [42]. Studies have well documented that healthy diet/lifestyle i.e. reduce sodium intake and physical exercise are important factors controlling blood pressure, hypertension and ultimately risk of cardiovascular disease [42]. Various major and underutilised legumes are rich source of secondary metabolites, fibres and micronutrients thus forming an important framework of plant's bioactive compounds for healthy diet [43]. For example, some bioactive peptides from food as well as grain legumes have demonstrated their potential to combat both hyper and hypotensive effects. Peptides having Glu-Phe, Ile-Arg and Lys-Phe dipeptides identified form legume crops have shown anti-hypertensive effect by inhibiting the activity of Angiotensin-I- Converting Enzyme (ACE) [36, 44]. Similarly, proteins like tyrosinase and lupin present in legume crops have also shown their remarkable ability control both systolic/diastolic blood pressure in peoples suffering from hypertensive disorder [44].

The hypertensive property of both lupin and tyrosinase have also been extensively investigated under in vivo conditions in Goto-Kakizaki rats suffering from hypertension due to excessive consumption of Na rich diet. The researchers fed the hypertensive rats with both lupin and tyrosinase for two weeks and then observed significant reduction in the systolic/diastolic pressure in both the groups [45]. However, lupin treatment also significantly improved endothelium-dependent vasodilation in hypertensive rats more efficiently as compared to tyrosinase [45]. A large body of literatures have also indicated that these bioactive peptides/proteins do not only possess hypertensive and ACE inhibitory effect but are also actively involved in lowering cholesterol and lipid levels [44]. Researchers have also extensively studied hypocholesterolaemia by using bioactive peptides and proteins and identified that the peptide Ile-Ala-Val-Pro-Gly-Glu-Val-Ala was compellingly involved in lowering cholesterol and triglycerides levels by stimulating the activity of bile salts [46]. Furthermore, other studies have also well documented the role of soybean peptides/proteins in efficiently controlling high cholesterol and lipid levels by efficiently modulating ratio of low density/high density lipo-proteins and expression of beta-very-low-density lipoprotein (β-VLDL) receptors thus minimising risk factors for cardiovascular disease [46]. Researchers have identified and evaluated several of these bioactive peptides from other legumes crops as well, however efforts are needed for in-depth characterisation of their function and mode of action in other underutilised legumes such as in hyacinth bean.

### 2.5 Antioxidative properties

Reactive oxygen species (ROS) generated as a consequence of oxidative stress are concomitantly involved in the onset and progression of various chronic diseases. Increased level of ROS has been shown to instigate severe damage to nucleic acids, cause membrane damage via lipid peroxidation and inhibit protein synthesis thus causing cell death or apoptosis [47]. Several crop plants including legumes contains various bioactive compounds such as flavonoids, phenols and some peptides that can efficiently scavenge ROS thus ameliorating stress induced oxidative damages [48]. Flavonoids such as flavanones and flavon-3-ols present in the seeds of certain leguminous plants such soybean and hyacinth bean have been reported to have antioxidative effect as demonstrated by both animal and cell culture studies [49]. In a study conducted by Babu et al. [50] oral infusion of flavanones and flavon-3-ols to an alloxan induced insulin dependent diabetic mouse, significantly enhanced the activity of hepatic catalase, superoxide dismutase and glutathione reductase enzymes thereby confirming their function as antioxidants. Similarly, in another study, researchers orally administered a flavonoid rich compound apigenin to streptozotocin-induced diabetic rats that significant reduction in the triglyceride levels which could be due the antioxidative effect of apigenin that effectively maintained ion/osmotic homeostasis [51]. Moreover, like apigenin, researchers also used anthocyanidin and luteolin treatment to diabetic rats which ultimately protected rat cells from oxidative damage via controlling the synthesis of iterlukin-1β and interferon-γ [52]. Like other flavonoids, anthocyanidin is also extensively present in legume plants which have received significant recognition owing to their health benefits and potential antioxidative properties [52].

Antioxidant peptides like His-Trp-Tyr-Lys have demonstrated to play exceptional role in ameliorating stress induce oxidative damage by efficiently regulating the scavenging of ROS [53]. Moreover, several studies have shown that thiol group of Cys residue can efficiently chelate metallic ions thus lowering the effect of oxidative stress and minimising the onset of chronic disease [53]. A study conducted by Morales-Medina et al. [54] reported that Val and Leu residues present at N-terminus of a peptide and Tyr and Trp residues present at C-terminus of same peptide have antioxidative properties that were effective in minimising lipid peroxidation and strengthening ion homeostasis. Furthermore, it is also well documented that seeds and leaves of legume plants are rich source of other bioactive compounds such as anthocyanins, polyphenols with antioxidative properties and are also capable of performing metal sequestration and stimulate the expression of other stress responsive genes [55]. Additionally, Zhu et al. [56] evaluated various other peptides from soybean and wheat having Leu-Leu-Pro-His-His repeat for its antioxidative activity by using distinct experimental procedure and conditions. The results indicated that the peptide was effective in stimulating 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity, expression of enzymatic and non-enzymatic antioxidants such as catalase, superoxide dismutase, peroxidase and ascorbate thus controlling the level of ROS generation and minimising the chance of severe disease. Lunasin peptide found in soybean and other legume has also been extensively investigated for its antioxidative properties where the researchers documented that lunasin was effective in scavenging both hydrogen peroxide and superoxide anion thereby protecting cell from oxidative damage [42].

### 2.6 Cytotoxic properties

Since ancient times legumes have been ascribed to have pharmaceutical/therapeutic values far beyond than providing essential nutrition in the form of amino acids [57]. In recent years, various proteins/peptides form several legume species have been included in the category of nutraceuticals i.e., food or products derived from them having medicinal or therapeutic role in the prevention of disease along with nutritional benefits [57]. Various legume-based bioactive proteins/peptides have been isolated and characterised for their functional role such as Bowman-Birk inhibitors (BBIs), Kunitz inhibitors (KIs) and alpha amylase inhibitors (AAIs) which are also commonly known as anti-nutritional compounds [58]. Several researchers during their early epidemiological studies observed that the protease such as Bowman-Birk inhibitors isolated from soybean seeds were highly effective in the counteracting tumour growth under both *in vitro* and *in vivo* conditions [58]. Later, these inhibitors also demonstrated their involvement in the treatment of hypocholesteraemia, cell toxicity, lowering of blood glucose level and pressure. The BBIs are distributed across many plant species including fruits and vegetables which are characterised by the presence of conserved pattern of 14 cysteine residues forming disulphide linkages having multigene origin. An exceptional property of these protease inhibitors is that they are structurally and functionally stable under changing environment conditions and can effectively bind to IgE thus embarking their anti-proliferative effect in gut mucosa and colon cancer thereby keeping cellular toxicity at a bay [59]. Additionally, researchers have isolated and purified these inhibitors from liver, kidney and lungs to understand their mechanism of action however, their course of action is still under debate [60].

Furthermore, AAIs have demonstrated themselves has a suitable candidate for controlling triglyceride levels thus keeping obesity under check whereas lectins obtained from the seeds of legumes have also shown immense therapeutic potential displaying cytotoxic and anti-cancer activity [60]. For example, concanavalin lectin obtained from the seeds of *Canavalia ensiformis* L. are structurally stable and are highly resistant to denaturation and in vivo proteolysis displaying strong antihepatoma activity under acidic conditions [61]. The cytotoxic and anti-cancer activity of lectins have also been demonstrated using animal model where internalisation of lectins in small intestine showed stimulation in immune and hormonal activity thus confirming their role as therapeutic agents. Researchers in last few years have identified and characterised AAIs from soybean and *Phaseolus vulgaris* for clinical studies and obtained interesting outcome as AAIs were effective in controlling obesity and blood glucose level in hypercholesterolemic rat model [60]. Similarly, α-subunit of soybean 7S globulin protein has been well ascribed to stimulate the transcription of HepT9A4 hepatic cells thus increasing low density lipoprotein (LDL) uptake in HepG2 cells in hypercholesterolemic rat model [62]. However, the involvement of these protease inhibitors and lectins in the treatment of various chronic diseases are mainly confined to in vitro studies or animal model, therefore, efforts are needed to increase the dimension of their application by performing more human clinical trials [63]. Furthermore, efforts are also being diverted towards the identification of these medicinally important bioactive compounds in underutilised legumes such as hyacinth bean for increasing the bioavailability of these bioactive compounds for the benefit of mankind.

### 2.7 Anti-microbial properties

Several major and underutilised legumes are rich source of bioactive phenolic compounds or polyhydroxylated compounds with immense anti-nutritional and therapeutic potential [46]. These phenolic compounds also play significant role in the stimulation of immune response, protect cells from oxidative damage and other pathogenic diseases [64]. Several studies have documented that some phenolic compounds isolated from seeds of legume crops are indispensably involved in the treatment of cancer disease, microbial/pathogenic attack, inflammatory disease thus providing potential health benefits [46]. Phenolic compounds are large group of compounds comprising phenolic acids, flavonoids, tannins and stilbenes [64]. Several recent studies have well documented the anti-microbial activity of phenolic compounds obtained from Faba bean, broad bean, adzuki bean and Dolichos bean in their crude methanolic extract of leaf and seeds [65]. The total antioxidant activity (TAA) of methanolic extract of various phenolic compounds and tannins obtained from adzuki bean and lectins have been shown to exhibit anti-microbial activity against several bacterial strains showing average zone of inhibition of 8–20 mm [66]. In addition, these methanolic extract have also shown potential anti-fungal activity against *Saccharomyces cerevisiae, Candida albicans* and *Aspergillus niger* [65]. Plethora of research have well documented that phenolic compounds are actively involved in the termination of ROS signalling as well as metal sequestration thus strengthening anti-microbial activity against various pathogenic micro-organisms [66].

In addition to phenolic compounds, several bioactive peptides have also been instigated to play important role in regulating various biological activities along with antimicrobial and anti-inflammatory effects [57]. Studies have well reported that several of the ACE-inhibitory peptides containing Arg-Lys residues at C-terminus have shown enhanced anti-microbial activities against pathogenic microorganism [44]. Similarly, peptide containing Leu-Leu-His-His residues also have been shown to posses anti-microbial and anti-oxidative properties. Moreover, a group of researchers working on legume bioactive proteins attempted to used bioactive peptides in conjunction with phenolic compounds and ascertain that the amalgamation of both stimulate the defence mechanisms of plants against pathogenic attack [44]. Similarly, a protein dolichin extracted from hyacinth bean exhibited strong anti-microbial activity against *Fusarium oxysporum, Rhizoctonia solani and Coprinus comatus* [10]. Likewise, a 36 KDa AAIs from hyacinth bean showed significantly inhibited conidial germination and hyphal growth of *A. flavus* [10]. In addition, several studies have well documented to inhibit the progression of human immunodeficiency virus (HIV) by regulating the expression of reverse transcriptase and alpha/beta glucosidase enzyme as well as it also demonstrated to have low ribonuclease and translational inhibitory activities [44].

## 3. Integrated "OMICS" techniques for enhancing its therapeutic potential

Plants act as factories that synthesises wide array of nutritional and secondary metabolites with complex structure and functions. Essentially, therapeutic/pharmaceutical secondary metabolites are often extensively isolated and purified from wild plant species or under-utilised crops as compared to cultivated species. However, the chemical synthesis of these medicinally important metabolites is a daunting challenge and is not economically feasible. Recent advancement in the system biology tools have pave the way to exaggerate their synthesis in tissue culture plants, but still their applications are limited to certain plant species because of the complex nature of technological standardisation in respective crops and lack of proper understanding of biosynthetic pathway. In this section, we will be discussing recent advancements made in the system and synthetic biology tools to provide detailed glimpse of the biosynthetic pathways and to explore the unprecedented possibilities of their application for the human welfare. These cutting-edged technologies can be successfully exploited for the improvement/enhancement of secondary metabolites production or could also help in the identification of novel metabolites in cultivated plants as well.

### 3.1 Phenomics based imaging and analytical toolkits

The phenotype exhibited by plants at certain stages of growth/developments are the function of gene × environment interaction that govern a peculiar trait of interest expressed from the plant's genome [67]. The term "phenotype" corresponds to precise and rigorous recording of the distinct phenotypic parameters from single cell to whole plant level, which if conducted explicitly can help facilitate identification/classification of novel traits in several plant species. Phenomics is a sub-discipline of plant biology that deals with phenotyping under controlled green-house conditions as well as field experimentation using advanced imaging technologies and imaging tools [67]. Phenomics study is a three-step process involving (i) setting up experimental plot, light intensities, nutrition acquisition and temperature (ii) rigorous monitoring/phenotyping such as growth, stress response, photosynthesis, chlorophyll and secondary metabolite contents etc. using advanced imaging tools and (iii) computer-assisted data visualisation, interpretation and storage [68]. Recent technological advancements have paved the way for the development of high-resolution imaging platforms aided with advanced bio-informatic tools for the phenotyping several important traits in plants for cellular and functional analysis [69]. Therefore, phenomics has now been recognised as an indispensable tool that can provide valuable insights into plant's morphology and physiology which can be further integrated with functional genomics data for analysing key traits such as secondary metabolites production and other economically important traits [68].

Several informatorily databases and analytical toolkits have been developed to facilitate phenomics and taxonomic studies in various cultivated and under-utilised crops at a greater pace. For example., PlantCLEF (2019) is an online repository that contain wide variety of images of plant's organs with the sole purpose to facilitate identification and classification of underutilised crop plants having distinct features [70]. PlantCLEF act like a real-life computerised program that can identify and classify plant species using raw images by extracting similar traits/characteristics and matching them defined plant species and family [70]. Similarly, MPID (Medicinal plant images database) which is a premium database maintained by Hong Kong Baptist University that is known to accommodate vast range of phenotypic data related to medicinal and therapeutically important plants [71]. Furthermore, in addition to phenotypic data, it also acts as a repertoire of scientific/botanical names, therapeutic values, physiological and ecological parameters of more than 1000 medicinal plants. Likewise, MPDB (Medicinal plant database of Bangladesh) database is also specifically dedicated to store phenotypic and physiological data associated with medicinal and aromatic plants found in Bangladesh [72].

Apart from databases, several computer-based analytical tools and techniques have also been developed and implemented for recording high-resolution images and morpho-physiological parameters in selected plants [70]. Plant computer vision (PlantCV) is a freeware software package written explicitly in python language that provide valuable algorithms for analysing phenotypic data [71]. It can analyse phenotypic data for multiple plant species and compare them with in the database for identification of novel traits/characteristics in genetically un-explored crops [67]. Similarly, ImageJ is a Java based program equipped with various algorithms such as image enhancer, graphic correction, segmentation and measurement to facilitate accurate phenotyping of plant species [73]. HTPheno is an algorithm of ImageJ that allows monitoring of plant's growth and development in terms colour spectrum. It captures image related to growth and fitness by various angles, time period and temperature/light conditions in the form high-resolution images [74]. However, despite of these technological breakthroughs, the implantation of these state-of-the art techniques are limited certain plant species. Therefore, efforts are needed to establish, standardise and implement these advanced phenomics techniques in various under-utilised medicinally important crops in order to facilitate comprehensive analysis of their physiological, morphological and cellular functions.

### 3.2 Functional genomics approach

Identification of hereditary determinants governing morphological, physiological and biochemical properties are of astute importance to uncover genetic potential of plant species. With the advent of next-generation sequencing techniques it has now become possible to perform in-depth studies on economically/therapeutically important under-utilised crops [75]. Till date whole genome sequencing projects has led to the development of draft genomes and chloroplast genomes of various medicinally important plants which can be efficiently exploited in-conjunction with advanced bio-informatic tools to obtain information about gene families, gene regulatory networks, miRNA and non-coding RNAs involved in gene regulation in those plants whose genome sequence is not available [76]. Furthermore, it can also result in the development of DNA markers for DNA fingerprinting and DNA barcoding to facilitate efficient taxonomic identification of plant under study using specific region of DNA [77]. Several DNA fingerprinting/barcoding primers such as 18-S-rRNA, 5S-rRNA, rupture of the cranial cruciate ligament (rccl), maturase K (matK), internal transcribed spacer (ITS), intergenic spacer (trnH-psbA) have been successfully implemented for identification and classification of medicinal plants. In addition, several dominant and co-dominant markers such as single nucleotide polymorphism (SNP), sequence characterised amplified region (SCAR), amplified fragment length polymorphism (AFLP), inter simple-sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) have also facilitated identification and authentication of medicinal plants [76].

Transcriptome-wide profiling of genes of regulatory pathways can help researchers gain valuable insight into the functional mechanisms of plant's biosynthetic pathways. In the recent years, researchers have exploited expressed sequence tags (ESTs) for transcriptome wide analysis of important medicinal plants [77]. Later, the scientists began to use microarray which is probe hybridization-based technique for studying regulation of gene expression and candidate gene discovery [78]. Recently, various transcriptome-wide analysis studies have been conducted in several medicinally important plants and their sequencing and expression profiling data are available in various online databases such as GarlicESTdb (garlic EST database), GEO (gene expression omnibus), ArrayExpress, RASP (RNA atlas of structure probing), AgriSeqDB (RNA sequence database), EGENES

(EST database) that can help expedite transcriptomic research in those plants in which transcriptome wide analysis has yet not been completed [79]. Likewise, several toolkits have also been designed that explicitly analyse microarray data and can also be used in conjunction with other phenomics, transcriptomics, proteomics and epigenomics for the identification of functional biological pathways liked with secondary metabolite synthesis [79]. Notably used toolkits are iArray, BRB-Arraytools, KEGG (Kyoto encyclopaedia for genes and genomes), GENEVESTIGATOR, PLEXdb, ExPath are the ones which offers various features for microarray data analysis, visualisation, interpretation and annotation in the form of heat map, graph and tables [80].

In addition, few databases have also been developed such as CroFGD (*Catharanthus roseus* functional genomic database), TeaCon (database of gene co-expression network), PlaNet (plant co-expression network), AraNet (*Arabidopsis* co-expression network) for functional analysis and study of co-expression networks to identify functional biosynthetic pathways [81–83]. Furthermore, several non-coding RNAs (ncRNAs) such as small interfering RNAs (siRNA) and microRNAs (miRNAs) have also been discovered and are thought to play pivotal role in the regulation of secondary metabolite synthesis in medicinal and crop plants [84]. Intriguingly, several transcriptome-wide analyses in medicinal plants have well indicated that these ncRNAs whether siRNA or miRNA indeed have therapeutic properties which if harnessed systematically can help in the prevention of various chronic diseases such as cancer and influenza A virus infection [84]. In this context, a group of researchers have developed a miRNA database (MepmiRDB; medicinal plant microRNA database) devoted specifically for medicinal plants that provide plethora of information regarding gene sequence, expression levels and target miRNA for 30 different medicinal plants [85]. Besides, several software packages have also been developed such as sRNA-Seq-data, NATpipe, PLncPRO and CNIT that can greatly facilitate the identification ncRNAs, siRNAs and miRNAs in various medicinal plants as well as in crop plants that specifically involved in the regulation of secondary metabolites of therapeutic importance [85].

Several protein-coding genes have also been qualitatively and quantitatively analysed for their corresponding products to generate a profile of their proteome to help researchers gain valuable insights into the mechanisms underlying cellular and metabolic pathways in medicinal plants [86]. Fewer studies have been conducted to develop a complete proteome map in the medicinal plants describing the proteins involved in the regulation of secondary metabolite synthesis. For example, a study conducted by Jacobs et al. [87] identified various proteins involved in alkaloid biosynthesis in *C. roseus* using 2D gel electrophoresis and mass spectrometry. Likewise, Chin [88] also performed in-depth proteomic study using Matrix-Assisted-Laser Desorption and Ionisation (MALDI) Time of Flight (TOF) analysis to unravel proteins involved in the secondary metabolite production in the germinating seeds of orchid plants. In addition, several online toolkits such as STRING (search tools for retrieval of interacting genes), PAIR (predicted Arabidopsis interactome resource), UniProt, Pfam (protein families), IntAct (molecular interaction database) can also be exploited in non-model crop plants such as hyacinth bean to gain functional insight into proteins involved in the secondary metabolite productions [79]. A list of putative genes/TFs involved in the regulation of bioactive metabolites in legumes are presented in **Table 1**.

### 3.3 Metabolomics approach

Metabolomics is also a functional genomics tool with the sole purpose to provide in-depth understanding of different cellular and metabolic pathways in various

| **S. No** | **Legumes** | **Genes/Transcription factors (TFs)** | **Secondary metabolites** | **Pathway involved** | **Technique used** | **References** |
|---|---|---|---|---|---|---|
| 1. | *Glycine max* | Fatty acid desaturase 2 (FAD2) | Linoleic acid | Octadecanoid pathway | Generation of mutant followed by LC–MS analysis | Liu et al. [89] |
| 2. | *Medicago truncatula* | Cytochrome 72A67 (CYP72A67), lateral organ boundaries domain TFs | Saponins | Isoprenoid pathway | Generation of mutant followed by GC–MS | Biazzi et al. [90] |
| 3. | *Lupinus angustifolius* L. | Apetella 2/ ethylene responsive factor (AP2/ ERF TF) | Quinolizidine alkaloids | Decarboxylation of lysine | Transcriptome de-novo assembly and QTL mapping | Kroc et al. [91] |
| 4. | *Lupinus angustifolius* L. | 13-hydroxylupanine O-tigloyltransferase (HMT/HLT), Lysine/ornithine decarboxylase (LDC) and 4-hydroxy-tetrahydrodipicolinate synthase (DHDPS) | Quinolizidine alkaloids | Decarboxylation of lysine | Transcriptome de-novo assembly and QTL mapping | Kroc et al. [91] |
| 5. | *Trifolium repens* and *Medicago sativa* | R2R3-MYB TF (TaMYB14) | Proanthocyanidin | Flavonoid biosynthetic pathway | Gene silencing followed by LC–MS analysis | Hancock et al. [92] |
| 6. | *Medicago truncatula* | MYB TF and MtLAR and MtANR | Proanthocyanidin | Flavonoid biosynthetic pathway | Targeted mutagenesis by HPLC analysis | Cañas and Beltrán [93] |
| 7. | *Medicago truncatula* | Uridine diphosphate glucosyltransferases (UGT73K1 and UGT71G1) | Saponins and isoflavonoids | Triterpenoid saponins biosynthetic pathway | Localization through prokaryotic expression system followed by microarray analysis | Achnine et al. [94] |
| 8. | *Leucaena leucocephala* | Hyp 1 – Hyp 7 | β-amyrin and mimosine | Triterpenoid saponins biosynthetic pathway | Microarray analysis | Honda and Borthakur [95] |
| 9. | *Lotus japonicus* | TM1624.23 | Phenylpropanoid derivatives and pro-anthocyanidin metabolism | Phenylpropanoid pathway | Gas chromatography coupled to electron impact ionisation/time-of-flight mass spectrometry | Sanchez et al. [96] |
| 10. | *Cicer arietinum* | CaUGT | Isoflavonoids | methylerythritol phosphate pathway | Next generation sequencing followed by marker assisted breeding | Jha et al. [97] |

| S. No | Legumes | Genes/Transcription factors (TFs) | Secondary metabolites | Pathway involved | Technique used | References |
|---|---|---|---|---|---|---|
| 11. | *Medicago truncatula* | Trc genes | Trigonelline, | Tryptophane-kynurenine pathway. | Gene cloning and mutagenesis | Boivin et al. [98] |
| 12. | *Medicago truncatula* and *Glycine max* | mtPAR, isoflavone synthase (IFS), mtTT8 and mtWD40 1 | proanthocyanidin | Flavonoid biosynthetic pathway | Cloning, gene expression and microarray analysis | Li et al. [99] |
| 13. | *Glycine max* | GmF3H1, GmF3H2 and GmFNSII-1 | Isoflavones | Flavonoid biosynthetic pathway | CRISPR/Cas9-mediated metabolic engineering | Zhang et al. [82, 83] |
| 14. | *Glycine max, Cicer arietinum* | No apical meristem-Arabidopsis transcription activator factor-Cup shaped cotyledon (NAC TF) NAC 4, NAC 29, NAC 25 and NAC 72 | Abscisic acid and secondary metabolite synthesis | Biosynthetic pathway | Multi-OMICS platform | Jha et al. [100] |
| 15. | *Glycine max* | GmCHS1–GmCHS9 | Flavonoids and isoflavonoids | Flavonoid biosynthetic pathway | Cloning, gene expression and microarray analysis | Dastmalchi and Dhaubhadel [101] |
| 16. | *Glycyrrhiza uralensi* | 2-hydroxyisoflavanone synthase (CYP93C), 2,7,4 0-trihydroxyisoflavanone 4 0-O-methyltransferase/isoflavone 4 0-O-methyltransferase (HI4OMT) and isoflavone-7-O-methyltransferase (7-IOMT) | Flavonoids and isoflavonoids | Flavonoid biosynthetic pathway | Whole genome sequencing, assembly and gene expression | Mochida et al. [102] |

**Table 1.**
*List of putative genes/transcription factors and functional genomics tools involved in regulating biosynthesis of secondary metabolites in legumes.*

organisms. Metabolomics is an advanced system biology tool with improved analytical methodologies, sensitivity and resolution that has been successfully exploited to understand biosynthesis of important metabolites in various plant species [103]. Several researchers have used this technique to discover candidate genes/proteins involved biosynthesis of specialised metabolites [104]. Furthermore, it has also provided great depth of understanding about the structural properties and diversity that exists among different metabolites as well as has facilitated to gain valuable insight into the type active ingredients that gives each metabolites its specific nutritional and medicinal properties [103]. Recent decades have witnessed the detailed characterisation of various medicinally important metabolites such as paclitaxel, artemisinin, vincristine, vinblastine, camptothecin and accuminata etc. from Pacific yew tree, *Artemisia annua*, *C. roseus, Camptotheca acuminata* and *Papaver somniferum* having anti-cancer and anti-malarial properties using this approach [104]. The identification of these medicinally important metabolites in above mentioned plants has served as model for studying the biosynthesis of specialised metabolites in other crop plants as well, which however, could not be possible by phenomics, genomics, transcriptomics and proteomics studies [105]. Metabolite profiling studies have been conducted in various transgenic plants by generating over-expression and gene-insertion based mutants to track the regulation of flavonoid biosynthesis (Nguyen et al. 20). In addition, metabolomics-based reverse genetic approach has also led to the identification of putative genes involved in the regulation of flavonoid synthesis driven by the conjugative action of post-translational modifications such as acetylation, phosphorylation, methylation, ubiquitination and biotinylation [105].

Several metabolomic studies have been conducted in model as well as crop legumes such as *Medicago truncatula*, *Lotus japonicus*, *Glycine max* and *Pisum sativum* to identify functional metabolites that are involved in the imparting biotic and abiotic stress tolerance for thus improving their growth and productivity [106]. However, fewer studies have conducted for the identification of medicinally important metabolites in legume plants compared to other model and medicinal plants [93]. Nonetheless, efforts are being made to revamp, standardise and implicate these advanced system biology tools for the identification, characterisation and quantification of important metabolites in various under-utilised crops as well [106]. The techniques like gas-chromatography mass spectrometry (GC–MS), liquid chromatography-mass spectrometry (LC–MS), nuclear magnetic resonance (NMR), capillary electrophoresis-mass spectrometry (CE-MS) and high-performance liquid chromatography-time of flight-mass spectrometry (HPLC-TOF-MS) have been successfully used for the assessment of medicinal constituents of functional metabolites [106]. With the advent of technological breakthroughs, metabolomics has also facilitated the generation of various protein reference maps of various model plant species and legume crops which can expedite the functional genomic analysis of genes/proteins in those plant species whose genome sequence is not available [93]. Nevertheless, efforts are needed to generate more protein reference maps to unravel cellular and biochemical signalling pathways and to identify novel genes and their product through comparative proteomic approach. The integrative analysis of "OMICS" datasets is crucial for the implementation of system biology tools for identification and mapping of secondary metabolite pathways in medicinal plants as well as legume crops and mechanism by which it can be achieved is depicted in **Figure 1**. Therefore, it has now become imperative to generate resourceful OMICS database that can help in the advancement integrative omics technology for precise understanding of molecular mechanisms and their possible application in legume improvement through breeding programs.

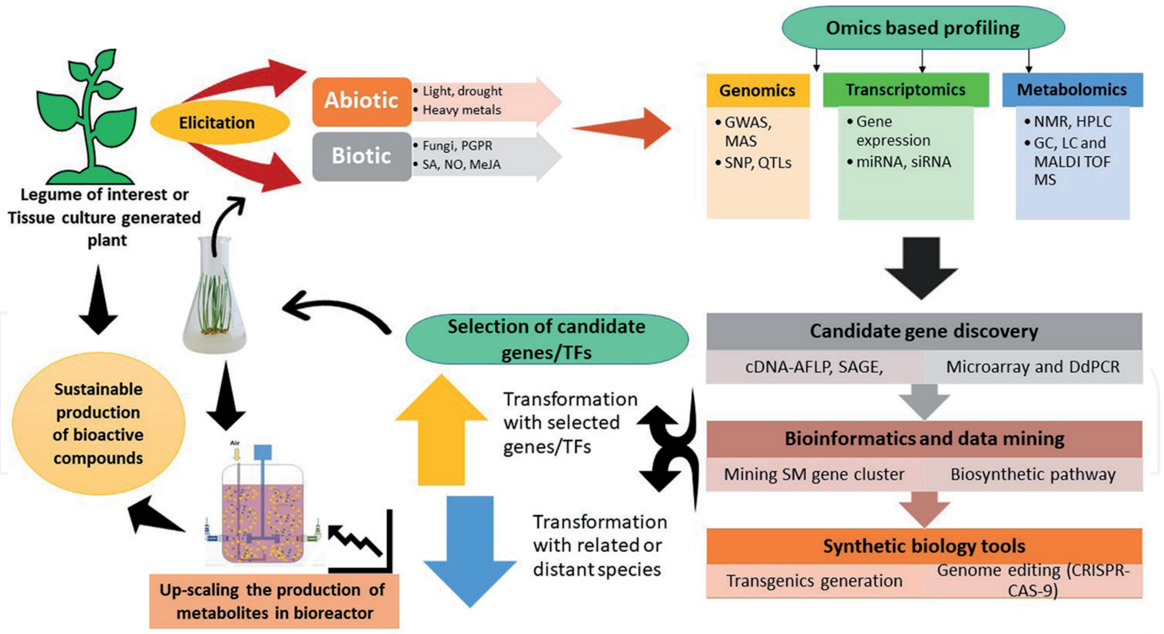

**Figure 1.**

*Systemic workflow depicting the application of integrated OMICS tools as well as role of different biotic/abiotic elicitors for improving the biosynthesis known bioactive compounds and identification of novel therapeutic metabolites in legume crops. In this process, tissue culture plants or plants grown in field are treated with different biotic or abiotic elicitors either independently of in combination. The plants are then analysed for the differential expression of genes involved in the regulation of secondary metabolites using integrated OMICS techniques. Candidate genes are discovered using various techniques such as cDNA-AFLP, SAGE, analysed by bioinformatics tools and are rewired using synthetic biology tool. The transformed plants are then exploited for sustainable production of important bioactive metabolites. GWAS: Genome wide association studies; MAS: Marker assisted selection; SNP: Single nucleotide polymorphism; QTLs: Quantitative trait loci's; miRNA: microRNA; siRNA: Small interfering RNA; NMR: Nuclear magnetic resonance; HPLC: High performance liquid chromatography; GC: Gas chromatography; LC: Liquid chromatography; MALDI-TOF-MS: Matrix assisted laser desorption ionisation-time of flight-mass spectrometry; cDNA AFLP: Complementary DNA amplified fragment length polymorphism (RNA finger printing technique); SAGE: Serial analysis of gene expression; DdPCR: Differential display PCR; SM: Secondary metabolites; CRISPR-CAS 9: Clustered regulatory interspaced short palindromic repeat, CRISPR associated protein 9; TFs: Transcription factors.*

## 4. Role of biotic and abiotic elicitors for enhancing its therapeutic potential

In plants, increase synthesis and accumulation of secondary metabolites occur upon their exposure to adverse climatic conditions which not only strengthen their growth but also revamp their innate immune response [107]. Several studies have indicated that distinct physical, chemical and microbial factors could act as abiotic/biotic elicitors for stimulating genes of metabolic pathways which will in turn result in the increase production important/specialised metabolites [108]. Now a day's elicitation is extensively used as a biotechnological tool to induce the biosynthesis of medicinally important metabolites in various tissues and organs of tissue cultured plants. The functional mechanism behind elicitor's elicitation involves signal perception by the receptors designed specifically to initiate signal transduction of the genes/transcription factor involved in the biosynthetic pathway (**Figure 2**) which in turn enhance the production and accumulation of different metabolites [107]. This section briefly describes different biotic/abiotic elicitors that can be employed for enhancing the production of secondary metabolites in medicinal as well as crop plants. A list of different biotic/abiotic elicitors involved in the regulation of bioactive metabolites in legumes are presented in **Table 2**.

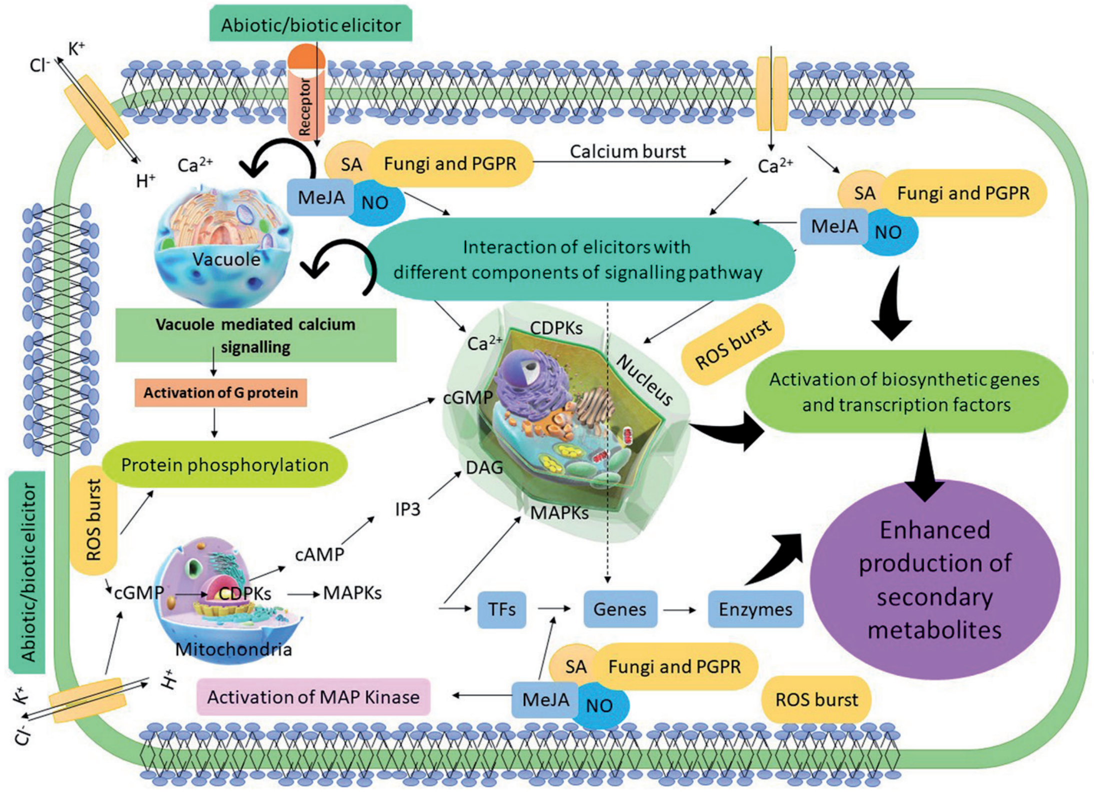

**Figure 2.**
*Schematic representation of mechanisms by which biotic/abiotic elicitors signalling pathways involved in the biosynthesis of important bioactive metabolites in plants. The elicitors are perceived by the receptors which then interacts with various components of signal atransduction pathway. This interaction activates certain transcription factors which in turn regulate the expression of various biosynthetic genes/proteins thus stimulating enhanced production of important therapeutic metabolites. SA: Salicylic acid; NO: Nitric oxide; MeJA: Methyl jasmonate; ROS: Reactive oxygen species; CDPKs: Calcium dependent protein kinases; MAPKs: Mitogen activated protein kinases; IP3: Inositol triphosphate; DAG: Diacyglycerol; TFs: Transcription factors.*

### 4.1 Biotic elicitors

Biological materials such as proteins, carbohydrates, inactivated enzymes, and polysaccharides etc. whether of plant, fungi or bacterial origin either in crude or purified form is used to induce the synthesis of secondary metabolites are termed as biotic elicitors [120]. Researchers have well indicated that proteins/enzymes are being explicitly used to stimulate the defence system of plants by increasing the synthesis of secondary metabolites involved in the regulation of stress responsive genes [121]. In tissue culture generated plants, several glycoprotein elicitors have been shown to elicit the production of phytoalexin, lectins and agglutinins that tremendously ameliorate the stress-induced oxidative damage [122]. Similarly, various fungal elicitor proteins such as PebC and PevD1 from *Botrytis cinerea* from and *Ss*Cut from *Sclerotinia sclerotiorum* elicited multiple defence response tomato and cotton plants in response to various biotic and abiotic stresses [123]. Furthermore, these elicitors have also been shown to activate G-proteins which in turn can also act as elicitor in stimulating secondary metabolite synthesis in plants such as stimulation of flavonoids and isoflavanones in soybean, benzophenanthridine alkaloids in bloodroot and β-thujaplicin in *Cupressus lusitanica* [122]. $Ca^{2+}$ signalling also play pivotal role in the activation of various protein kinases such as calcium dependent protein kinase (CDPKs) and mitogen activated protein kinases (MAPKs) that have stimulated the sesquiterpenes biosynthesis in tobacco and French bean plants [120].

Polysaccharides such as xyloglucans, oligogalacturonides, hemicellulose and pectin derived from plant, bacterial or fungal cell wall could also be exploited as an

| S. No. | Legumes | Abiotic/biotic elicitor used | Secondary metabolite elicited | Pathway involved | Probable role | References |
|---|---|---|---|---|---|---|
| 1. | *Lupinus luteus* | Chitosan (0.12%), Salicylic acid (800 μM) and potassium cyanide (400 μM) | Isoflavonoid genistein | Phenylpropanoid pathway | Treatment of cancer, osteoporosis, and ischemic heart disease | Kneer et al. [109] |
| 2. | *Vicia faba* | UV light (30-50 W for 5, 10 and 15 hr) | Phenolics and L-Dopamine | Pentose phosphate pathway | Act as neuromodulator and used for treatment of Parkinson's disease | Shetty et al. [110] |
| 3. | *Medicago truncatula* | UV light (5.5 min at 8000 J $m^{-2}$), Methyl jasmonate (50 mM), Yeast | Triterpene saponins and other primary metabolites | Phenylpropanoid pathway | Act as anti-tumour, anti-mutagenic, anti-inflammatory, anti-viral and cardiac activities | Broeckling et al. [111] |
| 4. | *Glycine max* | Methyl jasmonate (at 0.1 kg/$m^3$) | Genistein and Daidzein, and β-glycosidic type isoflavonoids | Phenylpropanoid pathway | Treatment of cancer, osteoporosis, and ischemic heart disease | Gueven and Knorr [112] |
| 5. | *Lupinus luteus* | cadmium (at 10 mg/l) and lead (at 150 mg/l) | 2′-hydroxygenistein glucoside and 2′-hydroxygenistein 7-O-glucoside | Phenylpropanoid pathway | Treatment of cancer, osteoporosis, and ischemic heart disease | Pawlak-Sprada et al. [113] |
| 6. | *Lupinus angustifolius* | Fungal spore suspension (2 × $10^6$ spores/ml, approximately 5 ml/plant) | Isoflavone phytoalexins or their precursors | Phenylpropanoid pathway | Treatment of cardiovascular disease, osteoporosis, hormone-dependent cancer and loss of cognitive function | Wojakowska et al. [114] |
| 7. | *Phaseolus vulgaris* | Ascorbic acid (500 μM) Folic acid (50 μM) and glutamic acid (5 mM) | Phenolic composition and angiotensin I converting enzyme (ACE) | — | Treatment of hypertension and cardiovascular disease, inhibition of cholesterol synthesis | Dueñas et al. [115] |
| 8. | *Phaseolus vulgaris* | Sucrose, gibberellins and proline | Quercetin-3O-glucoside, malvidin-3O-glucoside, and soyasaponins | Phenylpropanoid pathway | Act as anti-tumour, anti-mutagenic, anti-inflammatory, anti-viral and cardiac activities | Díaz-Sánchez et al. [116] |

| **S. No.** | **Legumes** | **Abiotic/biotic elicitor used** | **Secondary metabolite elicited** | **Pathway involved** | **Probable role** | **References** |
|---|---|---|---|---|---|---|
| 9. | *Glycine max* | $AgNO_3$ and $H_2O_2$ | Glyceollin and Isoflavones | Phenylpropanoid pathway | Act as anti-tumour, treatment of cardiovascular disease and other chronic diseases | Kalli et al. [117] |
| 10. | *Glycine max* | *B. subtilis and Rhizopus* | 6-Prenyl daidzein and phaseol | Phenylpropanoid pathway | Menopausal relief, treatment of osteoporosis, blood cholesterol, and lowering the risk of some hormone-related cancers, and heart disease | Kalli et al. [117] |
| 11. | *Trigonella foenum* | Arbuscular mycorrhizal fungal inoculum and exogenous methyl jasmonate | Trigonelline and diosgenin | Acetyl coenzyme A through the mevalonate pathway | A novel multitarget based chemo-preventive or therapeutic agent neuroprotective, anti-diabetic | Irankhah et al. [118] |
| 12. | *Lens culinaris* | Sodium silicate | Flavonoids and phenolic acids | Phenylpropanoid and shikimic acid pathway | Act as anti-tumour, anti-mutagenic, anti-inflammatory, anti-viral and cardiac activities | Dębski et al. [119] |
| 13. | *Trigonella foenum* | Sodium silicate + Fe EDTA | Flavonoids and phenolic acids | Phenylpropanoid and shikimic acid pathway | Act as anti-tumour, treatment of cardiovascular disease and other chronic diseases | Dębski et al. [119] |
| 14. | *Medicago sativa* | Sodium silicate | Flavonoids and phenolic acids | Phenylpropanoid and shikimic acid pathway | Act as anti-tumour, anti-mutagenic, anti-inflammatory, anti-viral and cardiac activities | Dębski et al. [119] |

**Table 2.**
*List of different abiotic/biotic elicitors used for eliciting secondary metabolites production in legume crops.*

elicitor to stimulate secondary metabolite synthesis in plants [124]. For instance, a polysaccharide derived from *Trichoderma atroviride* D16 was successfully regulated the genes involved in the production of tanshinone diterpene in *Salvia miltiorrhiza* and also increased the production of hairy roots up to 60% compared to control plants [125]. Likewise, oligosaccharide derived from *Fusarium oxysporum* efficiently stimulated the production of artemisinin in *Artemisia annua* and flavonoids in *Fagopyrum tataricum* plants suggesting definitive role of biotic elicitors in the stimulation of therapeutically important metabolites [126]. In another study, researchers also successfully exploited oligogalacturonides as biotic elicitor to stimulate the synthesis of phytoalexins in soybean plants [125]. The oligogalacturonides was also further utilised efficiently to stimulate stress defence response in *Nicotiana tabacum* plants by stimulating the biosynthesis of nutraceuticals [126]. Furthermore, the oligogalacturonides based elicitation of phytoalexins was also confirmed by Ferrari et al. [127] in soybean cell cultures.

Various phytohormones/signalling molecules such as salicylic acid (SA), nitric oxide (NO) jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) which can serve as an elicitor to elicit secondary metabolites production and stress-induced defence response in various plant species [127]. Among all, the role of SA, NO and JA have been extensively investigated for the elicitation of secondary metabolites synthesis and imparting resistance against biotic/abiotic stress induced oxidative damage in plants [128]. Methyl-jasmonate a derivative of jasmomic acid precisely activated the production of indole glucosinolate, β-thujaplicin and terpenes indole alkaloids in *Arabidopsis*, *C. roseus* and *C. lusitanica* plants [129, 130]. Similarly, studies have also indicated that both methyl jasmonate and salicylic acid either alone or in combination significantly enhanced the therapeutic attributes of *Hemidesmus indicus* by stimulating the synthesis of 2-hydroxy 4-methoxy benzaldehyde [131]. Moreover, Gai et al. [132] also observed SA and methyl jasmonate based elicitation of pharmacologically active alkaloids in the hairy root cultures of *Isatis tinctoria* L. Apart from plants, researchers have also widely used SA and methyl jasmonate to elicit the pharmaceutical alkaloids biosynthesis in microalgae *Arthrospira platensis* suggesting their robust application in prokaryotic system [133].

Various plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungal inoculum in conjunction with methyl jasmonate have been shown to enhance production of various secondary metabolites in *Rauvolfia serpentine,* fenugreek and *Solanum khasianum* [118, 134]. Bacterial and fungal based elicitation of therapeutically important compounds is being commonly used by various researchers because apart from increasing secondary metabolites production they also significantly improve growth and developments of plant exposed to various biotic and abiotic stress conditions [118]. In a study, Gorelick and Bernstein [135] observed differential effect of fungal elicitation in *Cannabis sativa* plants where fungal elicitation increases the production of cannabinoid and 3-deoxyanthocyanidin but causes significant reduction in anthocyanin content. Furthermore, researchers have also used arbuscular mycorrhizal fungal inoculum along with foliar application of chitosan that significantly boosted the biosynthesis of menthol and essential oils in *Mentha* × *Piperita* L. [136].

### 4.2 Abiotic elicitors

Elicitation of secondary metabolite synthesis by using substance of non-biological such as inorganic salts of heavy metals ($VOSO_4$, $NiSO_4$, $CdCl_2$, $AgNO_3$, $CuCl_2$), UV-radiation, heat, light etc. is known as abiotic elicitation and the substance used are known as abiotic elicitors. Abiotic elicitors such as high temperature, salt, drought, light and heavy metals etc. have also been successfully used as physical and chemical stimuli to elicit the biosynthesis of medicinally important metabolites in various plants [122]. These abiotic elicitors have been successfully used either independently or in combination either by foliar spray, irrigation or as hydroponics under both open field or controlled conditions for secondary metabolite production in medicinally important plants [122]. Present section deciphers the functional mechanism by which these different abiotic based elicitors elicit the production of therapeutically important compounds.

Drought is one of the most prevalent abiotic stress that alter plant growth and productivity around the globe [108]. Researchers have also indicated that in order to cope up with drought induced oxidative stress, plants synthesise certain metabolites such as glycine betaine and proline as mean to strengthen their defence system [122]. Based on this notion, researchers are using mannitol, calcium chloride and polyvinyl pyrrolidone (chemical which are used to induce drought stress) as a physical elicitor to induce the production of terpene indole alkaloids up to 2-fold in treated *C. roseus* plants compared to non-treated control [107]. Likewise, researchers have also observed increased synthesis of anti-inflammatory metabolite saikosaponins in *Bupleurum chinense* plants exposed to mild drought stress [137]. Furthermore, exposure of drought stress also significantly enhanced the production of rosmarinic ursolic and oleanolic acid in *Prunella vulgaris* [122]. Similarly, researchers have also observed sharp increase in the glycyrrhizic acid and betulinic acid content in *Glycyrrhiza uralensis* and *Hypericum brasiliense* plants upon exposure to drought stress conditions [137].

Salinity is also known to affect wide array of physiological and biochemical properties in plants thus affecting their growth and development [137]. Prolong exposure to salinity stress causes cellular dehydration and generation of oxidative stress in plants thus limiting their ion/osmotic homeostasis [122]. However, in order to withstand to salinity stress, plants synthesised various secondary metabolites like phenols, alkaloids and terpenes as an ameliorative mechanism to overcome oxidative damage. For instance, researchers observed significant increase in the biosynthesis of terpene indole alkaloids (TIAs) in *C. roseus* plants exposed to mild salt stress as compared to control plants. Similarly, another group of researchers also enhanced production of vincristine alkaloids and anthocyanin in *C. roseus* and *Grevillea ilicifolia* plants exposed to salt stress [137]. Salinity induced elicitation of secondary metabolites are also reported in *Datura innoxia*, *Oryza sativa*, *Triticum aestivum* and *Trifolium repens* where researchers have observed enhance synthesis of various alkaloids, polyamine and glycine betaine using NaCl as an elicitor [138].

High light intensity and temperature are also able to alter the course of secondary metabolites production in plants [138]. Prolong exposure of both high light and temperature can induce oxidative stress in plants that can have adverse effect growth, ontology and development. High temperature can also lead to the induction of premature leaf senescence, stomatal closure and can stimulate transpiration rate to a greater extent [137]. Nonetheless, despite affecting plant's growth these physical factors have also been reported to stimulate the biosynthesis of important secondary metabolites in the root of *Panax quinquefolius* [138]. Likewise, researchers have stimulated the production of gingerol and zingiberene metabolites by culturing *Zingiber officinale* explants under high light conditions. Moreover, exposing plants to short-term UV-B radiations have also been reported to stimulate the secondary metabolite synthesis. For example., Klein et al. [139] observed increase biosynthesis of betacyanin and betaxanthin metabolites in *Alternanthera sessilis* and *Alternanthera brasiliana* by exposing them to 10–40 J cm$^{-2}$ of UV-B radiation.

Similarly, UV-B radiation (up to 30–90 min) and low temperature treatment significantly improved hypericin biosynthesis in *Hypericum perforatum* adventitious roots and enhances the synthesis of total hydroxycinnamic acids (HCAs) and some sesquiterpenes in *Crepidiastrum denticulatum* [140]. Furthermore, exposure of both high/low temperature have also been shown to improve biosynthesis of ginsenoside, hypericin and hyperforin metabolites in *Panax ginseng* and *Hypericum perforatum* plants [137].

Increasing bioaccumulation of heavy metals such as As, Cd, Cu, Ni, Co and Ag have significantly impacted the agricultural lands and productivity. These heavy metals when presence in excess amount adversely affects plant growth and development [137]. However, at low levels these heavy metals act as co-enzymes/co-factors in various cellular and metabolic pathways thus stimulating secondary metabolite production in plants [122]. Several researchers have well documented the role of heavy metals in stimulating oil content, shikonin/digitalin levels in *Brassica juncea* and production of betalains in *Beta vulgaris* [107]. Likewise, stimulatory effect of $Cu^{2+}$, $Co^{2+}$, $CdCl_2$ and $AgNO_3$ in stimulating lepidine in cultures of *Lepidium sativum*, betacyanins in callus of *Amaranthus caudatus*, tanshinone in root culture of *Perovskia abrotanoides* and various sesquiterpenoids in *Datura stramonium* [122]. In the recent years, researchers have synthesised various nanoparticles using various metals that have induce compelling impact on the plant, secondary metabolite production. For example., CdO nanoparticles not only induced the biosynthesis of phenolic compounds but also significantly improve growth and productivity of barley plants [141]. Likewise, Tripathi et al. [142, 143] also reported development of silver nanoparticles in *Withania coagulans* possess strong antibacterial, cytotoxic and antioxidative properties and was also able to enhance the production of withanolides. A large body of literature have well characterised the functional mechanism by which the nanoparticles can induce secondary metabolite synthesis is by stimulating ROS bursts [122]. These ROS act as signalling molecules at lower levels but can impose severe repercussion on the growth and development of plants when their level reaches beyond their antioxidative defence system. Thus, these nanoparticles presented themselves as most efficient mean to strengthen secondary metabolite production that will not only improve plant performance under stress conditions but can also improve the therapeutic/pharmacological potential of plants.

## 5. Conclusion

In the present era, hyacinth bean has been recognised as an omnipotent legume crop which has the ability to conquer malnutrition, food/hunger index and several chronic diseases all around the globe. Being rich source of genetic and genome resources, the information's reviewed here can significantly contributes towards unravelling its structural, biochemical and molecular genomics which can lead to the identification of signalling pathways involve in the biosynthesis of important therapeutic metabolites/compounds. Furthermore, the implementation of multi "OMICS" techniques are the need of the hour which can transform hyacinth bean and other underutilised legume crops from being "orphan" to "model crop" by exploiting them in the breeding programs. These underutilised legumes hold the potential for developing sustainable agriculture which can lead to hunger and disease-free world in the era of global warming/pandemic. Therefore, synergistic use of multi OMICS tools are of ultimate requirement for expanding the current horizons of underutilised legume crops to address important problems relevant to Nations be it on health, nutrition and environment.

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