

*Chapter 9***ENHANCING ABIOTIC STRESS TOLERANCE IN
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Institute of Science, Banaras Hindu University, Varanasi, India²ICAR-Indian Institute of Vegetable Research, Varanasi, India**ABSTRACT**

Legumes Play an important role in safeguarding global nutritional food security and also contribute to the improvement of soil fertility by fixing biological nitrogen in agriculture. However, the production of legumes is adversely affected by several abiotic stresses. Therefore, it has become imperative to understand molecular mechanisms of plant response to adverse environmental cues by discovering candidate genes involved in stress tolerance. Recent developments in “omics” technology has witnessed advancements in legume genomics by providing reference genome of various crops such as chickpea, pigeonpea, cowpea, soybean etc. that has accelerated the genetic gains in these crops in order meet the supply demand gap of ever-increasing global populations. Hyacinth bean, an Indian origin crop of great economic value serving both as food crop and medicinal plant is still categorized as orphan crop at genomic level. With the availability of draft genomes of various important legume crops it has now become possible to identify structural variations at large scale. Advancement in genomic resources and with the low-cost ultra-throughput sequencing technology marker trait association studies and QTL mapping in hyacinth bean can be done with higher resolution and efficiency. More importantly, deployment of molecular breeding approaches can result in the development/identification of tolerant/resistant cultivar in hyacinth bean as it has been deployed for other legume crops such as chickpea, pigeonpea and cowpea. Once this crop is characterized at its genomic level, other realm of functional genomics such as proteomics, metabolomics and epigenomics can be used in the understanding hyacinth bean stress tolerance.

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INTRODUCTION

Among various food and fibre crops, the crop legumes are the key sources of protein, minerals, vitamins, iron, zinc, calcium, and magnesium, as well as omega-3 fatty acids. The importance of these legumes is higher where a large section of the society depends on vegetarian food such as in India. The unique ability to fix atmospheric nitrogen by the legume crops play a crucial role in sustaining the farming system by making available the residual nitrogen to the non-legume crops. Legumes also serve as an excellent source of high quality and nutritious feed to livestock leading to ~20% increase in animal productivity (Tarawali and Ogunbile 1995). The human civilization has a long association with legume cultivation in the last 6000 years which has contributed significantly toward economical, nutritional, and improving the livelihood of subsistence farmers across the world.

Indian bean or hyacinth bean (*Dolichos lablab* L. or *Lablab purpureus* L.) is a twining herbaceous crop legume with $2n = 22$ chromosomes belonging to family Fabaceae which is being maneuverer for various purposes such as a green vegetable, pulse, soil improvement and protection, forage and weed control (Rai et al. 2016). The wild forms of Indian bean are believed to have originated in India and were introduced into Africa from South East Asia during the eighth century. Outside India, hyacinth bean is prevalent in Africa, Zimbabwe, Cameroon, Ethiopia, Sudan, Uganda and Tanzania (Maass et al. 2010) and have been called by various common names such as poor man bean and tange bean in England, rongaidolichos and lablab bean in Australia, betas in Philippines, lubiaafin in Sudan, hyacinth bean in Brazil and in India (Gibbs et al. 2008).

TAXONOMY

The genus lablab refers to the hyacinth bean, Bonavist bean, Sem, Waby Salad bean, Egyptian bean, Field bean, Australian pea, Indian butter bean belongs to the family leguminosae (Fabaceae), sub family papilionaceae and varying chromosomes $2n = 20, 22, 24$. In Flora Indica, Roxburgh (1832) exemplify “Dolichos” in *Dolichos lablab* as Greek word that symbolize long pod and “lablab” as Arabic or Egyptian word implying shallow vessel enclosing seed. He also distinguishes hyacinth bean into seven varieties, demarcating them into five cultivated and in two wild varieties. Later, Backer (1984) on the basis of shape, texture and angle of attachment of seeds to the suture of the pods

categorised all the five cultivated varieties in two cross compatible botanical groups i.e., *Lablab purpureus* var. *typicus* and *Lablab purpureus* var. *lignosus* (Figure 1).

Lablab purpureus L. var. *typicus* (Syn. *Lablab niger* var. *typicus*) is widely cultivated as garden type bean with soft pods that has long axis in which seeds are arranged parallelly to the suture and *Lablab purpureus* var. *lignosus* (Syn. *Lablab niger* var. *lignosus*) where the seeds are arranged at right angle to the suture, is mainly cultivated as field bean and most commonly used as pulse containing more protein (20.9-29.2%) than *Lablab purpureus* var. *typicus*. Pods of *Lablab purpureus* var. *lignosus* is highly preferred by farmers and consumers as they radiate certain oily substances that gives pods their characteristic fragrance. This distinctive aroma of *Lablab purpureus* var. *lignosus* pods is due the presence of two dominant fatty acids i.e., trans-2-dodecenoic acid and trans-2-tetradecenoic acid (Uday Kumar et al. 2017).

BOTANY AND FLORAL BIOLOGY

Hyacinth bean plant is a tropical, dicotyledonous, herbaceous and temperature sensitive flowering plant with annual growth habit. Plants are robust twiner with woody stem, having large trifoliate heart shaped leaves along with broad ovate-rhomboid shape leaflets measuring 7 to 15 cm long and 4.5 -15 cm wide. The dorsal side of the leaf is smooth with the ventral side being hairy. Plant consists of tap root system and main root has several lateral roots distributed uniformly along the main axis. Flower is usually bisexual, hypogenous and pentamerous varies from white, pink to purple in colour with axillary, erect and raceme inflorescence (Figure 1). Stamens are usually arranged in 9+1 configuration, with uniform anther, having sessile ovary with incurved style and terminal glabrous stigma. Seeds are variable in colour such as white, ochreous with black's dots or black with white dots, uniformly brown or black.

The flowers generally open two days after anther dehiscence. The presence of superfluous floral nectarines at the bottom of the corolla attract bees, flies and ant, however, heavy insect pressure may suppress the development of wing petals that could lead to stamen and stigma exertion. In hyacinth bean, flowers bloom between 6.30 hr. to 7.00 hr and are predominantly a self-pollinated crop (Kukade and Tidke 2014). However, insect mediated cross pollination occurs up to an extent of 6-13%. Anthesis in hyacinth bean occurs between 9.00-17.00 hr and anther dehiscence occurs between 5.00-14.00 hr. The optimum time for emasculation of hyacinth bean flower ranges between 16.30-17.30 hrs and for hand pollination 7.00-9.00 hrs, the next day in the morning. Pollens are fertile on the day of anthesis which remains viable for 42 hrs at room temperature of 28.5°C with 85-90% relative humidity. The pollens can remain viable for much longer duration i.e., up to 50-60 hrs if they are stored under cold environment in refrigerator.

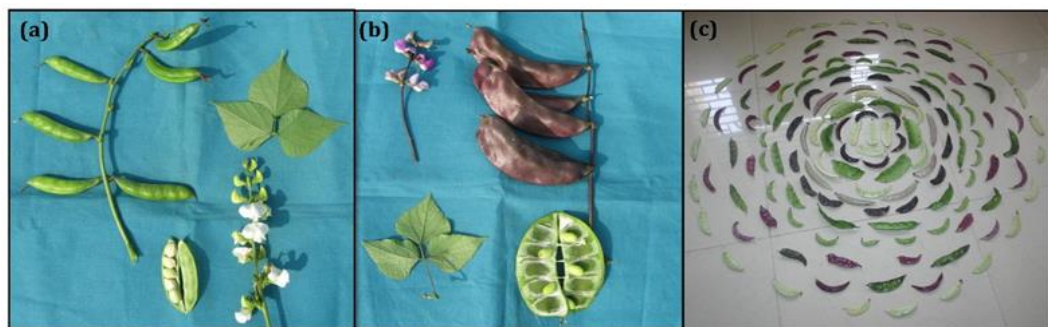


Figure 1. Phylogeny and pattern of floral biology in *Lablab purpureus* L. var. (a) typicus (b) lignosus (c) pod diversity.

NUTRITIVE AND MEDICINAL VALUE

Proteins are crucial macronutrient for growth as well as maintenance of human body and it is well known that a minimum protein intake of 0.8, 1.5 and 1.0 g protein/kg body weight/day is recommended for adults, children, and adolescents, respectively (Kafatos and Hatzis 2008). Among other crop legumes such as soybean, common bean, winged bean, cowpea, mung bean, pea, moth bean, urd bean, chickpea, pigeon pea and rice bean, hyacinth bean also serve as an excellent source of high quality and nutritious source of protein, minerals and vitamins and is richer than common bean in nutritive value. The nutritional composition of hyacinth bean pods varies greatly depending upon the genotypes and pod maturity stages. In general, 100 g of mature green pods contain 86.1 g moisture, 3.8 g protein, 0.7 g fat, 0.9 g minerals, 1.8 g fibres, 6.7 g carbohydrates, 34 mg magnesium, 68 mg phosphorus, 55.4 mg sodium, 0.13 mg copper, 210 mg calcium, 74 mg potassium, 1.7 mg iron, 40 mg sulphur, 0.06 mg riboflavin, 1 mg oxalic acid, 0.7 mg nicotinic acid, 0.1 mg thiamine, 312 I.U. vitamin A and 9 mg vitamin C.

Among the legumes, hyacinth bean constitutes an important source of therapeutic agents used in the modern as well as traditional systems of medicine (Morris 2003, 2009). The beans, including hyacinth bean, are also stack of flavonoids and isoflavones such as genistein, phaseollidin and isovestinol that can act both as signal molecule for pigments or co-pigments in mediating flower colour by modulating auxin distribution and as an armour for plants against UV damage and pathogenic microbes (Kan et al. 2016). Despite of its distinct performance as vegetables and fodder, it is also conceivable as medicinal legume and performs an indispensable role as therapeutic agents directly in the interception of discrete forms of cancer and various chronic diseases, most commonly sparring against breast cancer (Morris 2009). All these chemo-preventive proprietaries of hyacinth bean against various chronic and cancer diseases (Takagi et al. 2015) have interlineated it among important medicinal plants.

THE CHALLENGE OF MULTIPLE ENVIRONMENTAL STRESSES ON AGRICULTURE

Most of the agricultural crop plants thrive in substandard environments that preclude them from acquiring their full genetic capacity for their growth and reproduction thus imposing severe repercussion on their economic yield (Ray et al. 2013). For instance, in India, the most favourable season for hyacinth bean yield is November-January and in this period the yield can be up to 6-8 times high compared to their average yield under adverse environmental condition (www.lablablab.org).

Global Scenario

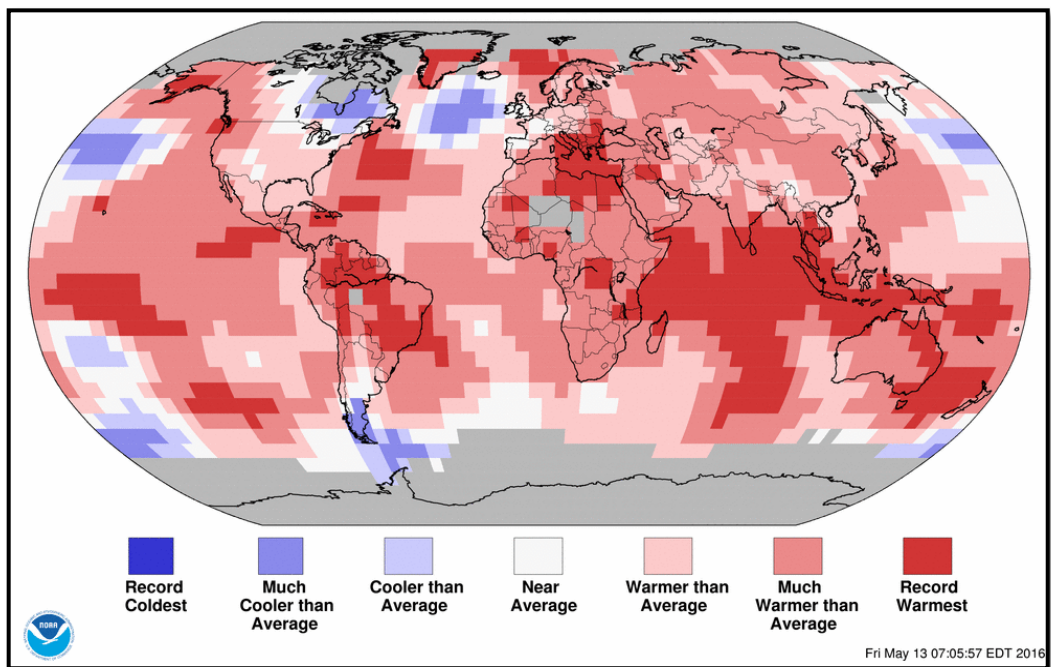


Figure 2. Global climate report of high temperature stress in August 2016 (Source: National Centre for Environmental Information, <https://www.ncdc.noaa.gov/sotc/>).

Current reports on climate change (Figure 2) simulates that mean surface temperatures will increase by 3-5°C in the next 50-100 years through reiteration of the heat waves, which will have drastic effect on global agriculture systems (Vereecken et al. 2016). Inconsistencies of temperature and in rainfall may impoverish nutritional quality of vegetables crops as well as can cause huge deflation in their yield and may also alter the inclination of insects and pests thereby expediting pathogen spread. Crop plants are more likely to meet a wide extent of biotic and environmental stresses, which when transpire,

can have severe damaging effect on their usual corporal growth (Okazaki and Saito 2016). Several recent models for climate change have predicted that the probability of plants to stumble upon different biotic and abiotic stresses and their combinations is likely to be increased in the near future than previously anticipated. Climate change together with growing loads on global food security due to increasing population will compound the demand for stress-resistant/tolerant crop varieties which is still a milestone to cover.

Various diversified legumes such as soybean (*Glycine max*), pea (*Pisum Sativum*), chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), peanut (*Arachis hypogea*), faba bean (*Vicia faba*), lupin (*Lupinus luteus*) and mung bean (*Vigna radiata*) have indeed strengthen global food, nutrition security and had ensured environmental sustainability around the globe (Varshney et al. 2013). Recently, several underutilized tropical crop legumes essentially lima bean (*Phaseolus lunatus*), jack bean (*Canava liaensiformis*), winged bean (*Psophocarpus tetragonolobus*) and hyacinth bean (*Lablab purpureus* L.) are now being exploited as forage legumes in livestock production. However, most of these legume crops including hyacinth bean are cultivated in minimal environments, where plants are constantly threatened by various abiotic (heat, drought, salinity) and biotic stresses (white mould, anthracnose, angular leaf spot, yellow mosaic virus, thrips, bacterial blight), which cramp their growth and eventually their productivity (Rodziewicz et al. 2014).

Indian Scenario

In India, mainstream, production of beans, transpires in low level farms via using minimal agriculture by poor farmers which are much more prone to be attacked by abiotic stresses (high temperature, drought) and biotic stresses (insects and pests, diseases) as compared to high level farms as they have assets to fight such stresses by applying fertilizers, pesticides and proper irrigation (Ramalingam et al. 2015). Use of such protective measures, however, can reduce the profit and can have a negative impact on the environment, and majority of them are not even effectively controlled with chemicals. Thus, in agriculture, abiotic and biotic stresses are progressively becoming the major constraints on productivity and economic yield of hyacinth bean. Due to ever increasing global population, increase in global mean temperature (Figure 3) with decreasing land and water resources especially in India where people are largely dependent upon legume vegetables for their protein requirements, will lead to the protein unavailability in the near future, if the productivity of the legumes crops are not met with the protein requirement of the future population.

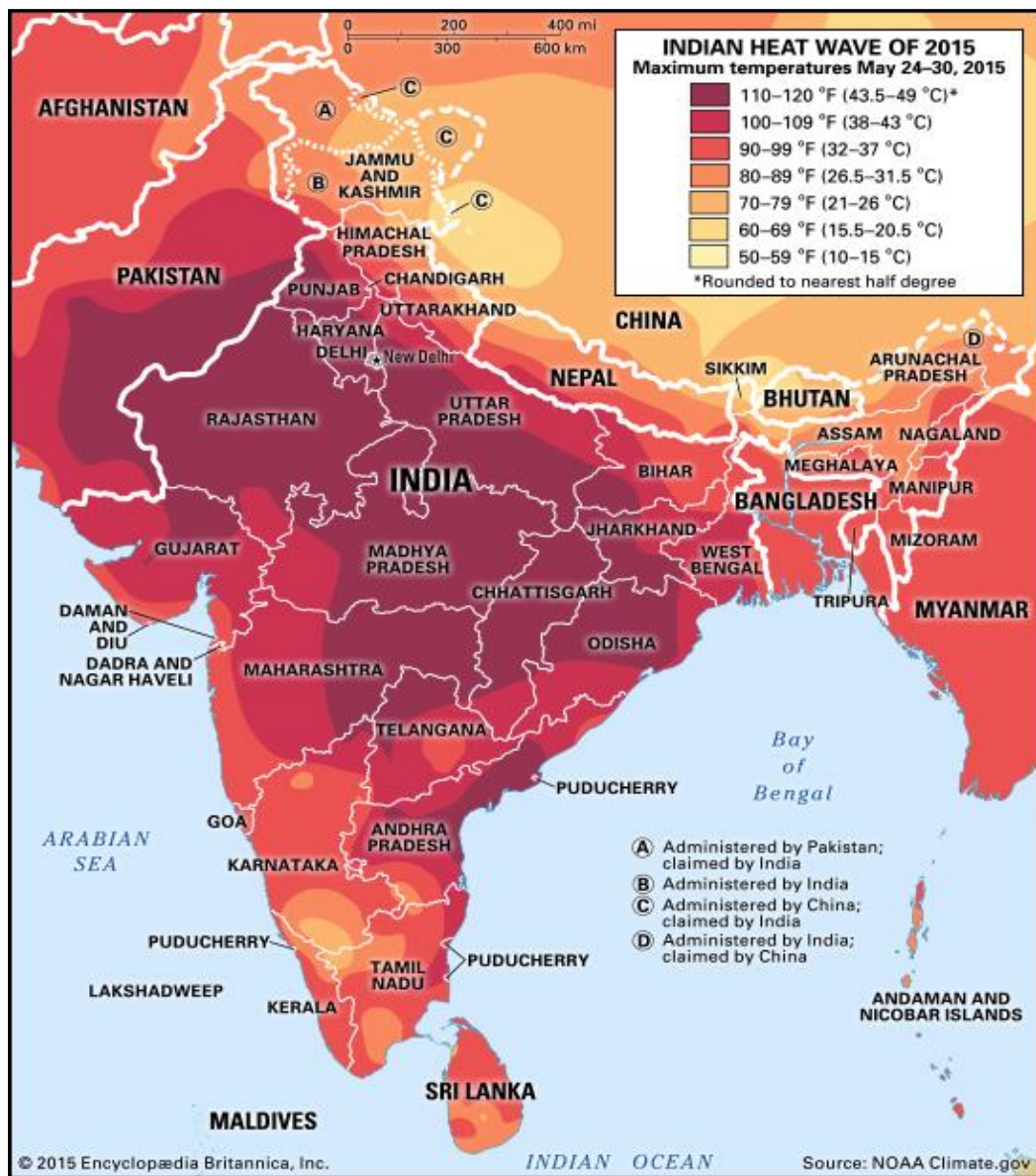


Figure 3. Geographical area under high temperature stress in May 2015 (Source: "India-Pakistan heat wave of 2015". Encyclopedia Britannica. Encyclopedia Britannica Online).

In a study Therefore, it has become imperative to unravel the issue of low productivity, stress tolerance and improved nutritional quality in some underutilized legumes perse., hyacinth bean, which is not happening at desired rate through conventional breeding approaches. Most of the elite crop varieties that we used today have been produced through traditional plant breeding approach. So, in order to comprehend the nature of multiple stress responses and to develop avenues to create varieties with enhanced stress tolerance together with high yields, an understanding of stress response mechanisms in legumes

through genomics, transcriptomics, proteomics, metabolomics and recently epigenomic has become an indispensable biotechnological tool for their improvement.

MAJOR ABIOTIC STRESSES AFFECTING HYACINTH BEAN

Climate extremes such as high temperature, extreme frost, salinity and drought have massive impact on world agriculture that can cause deflation in the total yield of economically important crops by >50% for most of the commercial crops. This section deals with the major abiotic stresses that severely affect hyacinth bean growth and developmental processes.

High Temperature (HT) Stress

HT stress has symbolic repercussions on plant growth and reproductive processes which may limit their metabolism and productivity worldwide. HT stress increases metabolic toxicity in plants via generating excess reactive oxygen species (ROS) that stimulate oxidative stress (Siebers et al. 2015). Being sparsely susceptible, high temperature stress significantly alters various biological processes that occur in hyacinth beans. Most productive habitat for beans at the time of planting, flowering and fruiting is 18-29, 25-30, 25-32° C and temperature above can significantly affect their growth and reproduction (Rai et al. 2015). It has been documented that increase in temperature decreased the pod yield and reduced growth of *Lablab purpureus* L genotypes (Rai et al. 2015). HT stress drastically alters the expressions of stress induced genes, regulatory proteins, metabolites and antioxidant defence system which are involved in the re-establishment of cellular redox balance and ion homeostasis.

Plant response to HT stress varies dynamically depending upon the severity/duration of stress which in turn is dependent upon the plant types and other surrounding factors and till date none of the traits have been identified and confirmed that can confer tolerance to HT stress in plants. Scientists involved in the plant abiotic stress tolerance research are aspiring to discover the mechanisms behind the plant response that induces HT tolerance. Alteration in the expression of certain genes alters competency of antioxidant defence system, metabolites and proteins stability that triggers some of the physiological and biochemical processes in plants that may lead to fatal disruption of cellular organization and metabolic imbalance (Ahuja et al. 2010). Plant tolerance (a highly specific trait) to HT stress is predominantly interpreted as their ability to grow and reproduce economic yield. This ability varies significantly in closely related species and may even show distinct variation in tissues/organs of the same plant. To thrive under HT stress, plants have evolved several mechanisms such as enhanced functioning of ion transporters, expression of late

embryogenesis abundant (LEA) proteins and upregulation of antioxidant defence related genes are one of the essential mechanisms adopted by plants to overcome stress effects.

Only recently, applications of exogenous protectants in the form of phyto-hormones ABA (abscisic acid), JA (jasmonic acids), SA (salicylic acid) etc, signalling molecules (NO: nitric oxide), osmo-protectants (proline, GB: glycine betaine etc.) have been found effective in mitigating HT stress-induced damage in plants (Shahzad et al. 2015). However, limited information is available on the use of phytohormones or plant growth regulators in the management of heat stress in hyacinth bean plants. Unfolding of new crop cultivars that are tolerant to high temperature stress is becoming a major constraint for plant scientists, who are continuously involved in research exclusively on high temperature stress are striving to conceive plant responses to heat tolerance and discretion used by the plants in HT environments. Overall, further insights on role of phytohormones in regulating defence networks in hyacinth bean plants can be a field worth further investigation, which can be uncovered by adopting an integrated approach of genomics, proteomics and metabolomics.

Drought Stress

Reciprocal and inconsistent climatic condition such as high temperature, salinity and drought are the main factors that limits plant growth and productivity. Due to ever-changing climatic conditions, drought is considered as one of the most devastating adverse environmental condition around the globe, and with the inflation in the CO₂ levels in the current atmosphere and aerial changes, drought prone areas will be contemplated as more stressful areas for agricultural crops in foreseeable future (Adams et al. 2016). In crop legumes per se, cowpea, soya bean and hyacinth bean, severe drought may reduce growth, photosynthesis and can significantly reduce their yield up to 40%. The period during anthesis and following anthesis are considered as one of the most pivotal periods for drought stress in legume plants. In general, on the basis of drought tolerance nature, the defensive response of plants to cope with drought stress can be grouped into following: drought escape, drought avoidance and drought tolerance (Basu et al. 2016).

Lablab purpureus L is believed to grow well under rain fed conditions is well adapted to drought and have better drought tolerance capacity than major legume crops such as common bean or cowpea (Maass et al. 2010). In the area experiencing heavy rainfall, hyacinth bean can be used as forage, once plant has attained its vegetative growth. In India, due to its nutritional and therapeutic property, hyacinth bean is exploited as pulse as well as a vegetable not only in the main season and this demand is expected to increase in the foreseeable future as therapeutic uses of legumes are now better understood. However, in the off-season, the growth of hyacinth bean is restrained recurrently in the month of April and May, especially in Uttar Pradesh which a drought prone region. Some of the early

maturing varieties of hyacinth bean are sensitive to drought if the drought occurs between anthesis and reproductive phase. Therefore, understanding physiological and genetics of early maturing hyacinth bean lines is most important for the enhancement of growth, survival and drought tolerance for sustainable growth and yield under changing climates. So, a unifying approach including breeding and biotechnology is need to be integrated in breeding program so that the breeders can move from “chance breeding” to “knowledge-based breeding” for abiotic stress tolerance in hyacinth bean. In recent years, several drought related genes (*BhGRP1*) through subtraction subtractive hybridization (SSH) transcription factors (*R2R3-MYB*) through SSH library (Yao et al. 2016) and two miRNA genes (*miRNA 156* and *miRNA 172*) have been isolated and confirmed from hyacinth bean (Thilagavathy and Devaraj 2016). These advances made in hyacinth bean genomics will foster trait mapping, gene discovery and their functional characterization in the development of functional markers and their introgression through marker assisted selection (MAS).

Salinity Stress

Salinity stress is one of the major limiting factors for plant productivity. Based on the type and causes, salinity stress has been characterized as (1) Natural Salinity-It is also called as primary salinity stress that results from the accumulation of salts through natural process in the soil or groundwater. It is mainly caused by the weathering process that breaks down rocks that releases various mineral salts and to some extent sulphates and carbonates in to the soil. Salinity stress may also result from the periodic deposition of ocean salts that travel inland through wind and deposited by rainfall, and (2) Secondary salinization or human-induced salinization are caused by irrigation through salt-rich water or replacement of perennial crops by annual crops (Iseki et al. 2016).

Salinity stress causes rapid decrease in the osmotic potential of soil thereby causing hyper-ionic/hyperosmotic stress that reduces the availability of water to roots and this leads to reduction in their growth rate. The salinity factors like evaporation (increase salt concentration in soil and precipitation (decrease salt concentration in soil) are three times more severe as compared to other abiotic stresses as it adversely affects seed germination, photosynthetic pigment content, disturbed ion homeostasis, reduces water uptake, thereby limiting plant growth and productivity.

Most of the bean plants including hyacinth bean have shown remarkable flexibility/adaptivity to adverse environmental factors such as drought, high temperature under different belts and there are reports delineating about the salt tolerance nature of this crop (Rai et al. 2015). In hyacinth bean, with the advancement in the ‘Omics’ techniques considerable amount of expressed sequence tags (ESTs), miRNAs and transcription factors has been identified under various abiotic stresses (Table 1) including salt stress (Yao et al.

2016). However, till date no significant effort has been made to analyse hyacinth bean transcriptome/proteome level and to map QTLs for different abiotic stress tolerance in this plant. With the developments in genome sequencing technology, transcriptomics, and protein profiling together with metabolomics has emerge as a promising tool that can be successfully used to unravel different components concerning plant biotic and abiotic stress tolerance and simultaneously can identify functional which can be used to engineer plants with increase tolerance/resilience to adverse situations to meet the increasing demand of food for ever increasing populations.

ROS as Key Players in Plant Stress Signalling

Reactive oxygen species (ROS; O_2^- , H_2O_2 , OH^\bullet , 1O_2) are moderately charged forms of atmospheric oxygen which are deliberately produced in photosynthetic organisms that causes cellular damage by reacting with biomolecules and simultaneously also function in cells as signalling molecules as well as in responses to adverse environmental conditions (Mittler 2017; Rai et al. 2017). In plants, ROSs (Figure 4) are mainly produced as unavoidable toxic by-products of aerobic metabolism that take place in different cellular compartments. A large body of literature data supports that under normal physiological conditions these reactive compounds are promote several basic biological processes including cellular growth and differentiation whereas under the situation of oxidative stress these reactive compounds are actively scavenged by distinctive enzymes antioxidant defence system. The equilibrium concerning generation and scavenging of free radicals varies greatly under adverse environmental conditions such as high temperature, drought and salinity that may result in the inflation of the intracellular levels of free radicals. Among ROSs, superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and per-hydroxyl radical (HO_2), singlet oxygen (1O_2), ozone (O_3), peroxides and alcoxides are non-radicals. Some free radicals (like hydroxyl radical, HO^\bullet and the hydrogen radical, H^\bullet) are extremely reactive, whereas some free radicals, like ions of transition metals exhibit weak reactivity.

Recently, different studies conducted on plants have confirmed that reactive oxygen species are good for stimulating normal growth and physiological processes contrary to having toxic effect on their survival. High production of ROS in chloroplast and peroxisome during photosynthesis and photorespiration may cause cell death as a result of oxidative stress, which is now contemplated as ROS triggered physiological response that lead to cell death. Furthermore, the findings have also depicted that generation of ROSs below a threshold level may suppress cellular growth, differentiation and immunity (Diebold and Chandel 2016), which clearly pinpointed that maintaining basal level of ROS i.e., above cytostatic level, but below cytotoxic level is essential for normal life. Thus, ROS level either too low or too high can impair plant growth and development, whereas when ROS are maintained in adequate level stimulate plant health and when the level ROS break

the boundary between cytostatic and cytotoxic levels, impairs the normal functioning of plants.

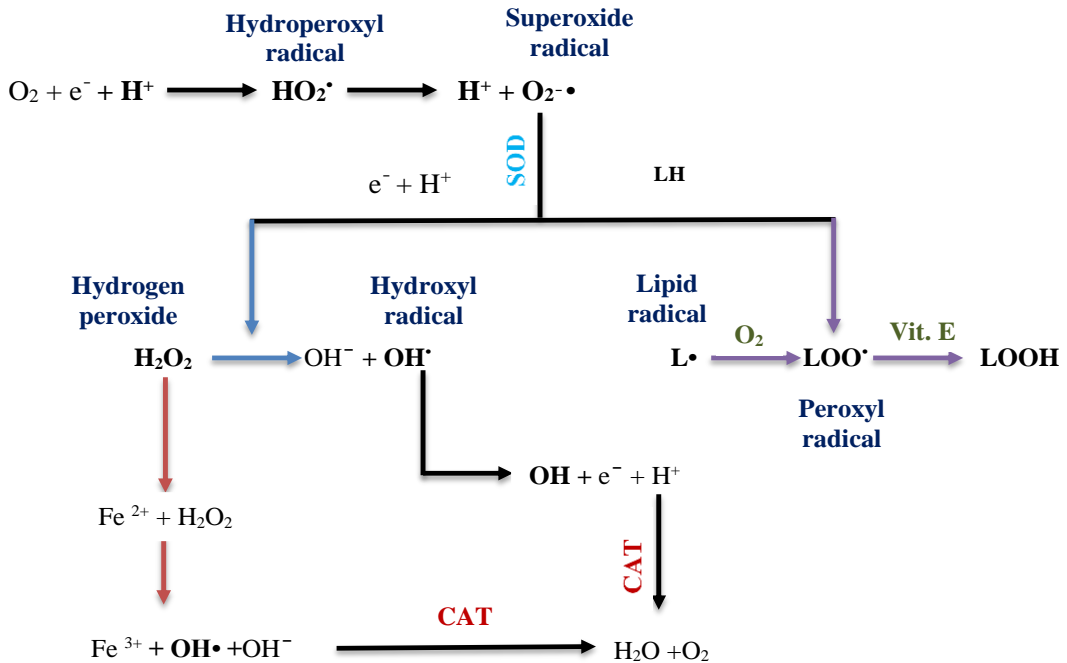


Figure 4. Overview of the reactions involved in generation of ROS. Purple arrows represent lipid peroxidation. Blue arrows represent the Haber-Weiss reactions and the red arrows represent the Fenton reactions. The bold letters represent reactive oxygen species (ROS). SOD and CAT refers to the enzyme superoxide dismutase and catalase.

Enzymatic and Non-Enzymatic Defence System

Plants must protect themselves from unfavourable environmental conditions such as temperature extremes, salinity and drought *via* adaptation or acclimation process in order to foster under different climate adversities. In crop plants, enhanced tolerance to oxidative stress is often associated with improved functioning of enzymatic and non-enzymatic antioxidants. Plants that shows the tendencies to over-come damaging effects of ROS induced oxidative stress are often associated with the enhanced production of ROS scavenging and detoxification enzymes which are described below.

Enzymatic Antioxidants

ROS scavenging enzymatic antioxidants in plants include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR). The metalloenzymatic SODs is the highly effective omnipotent antioxidant enzyme that act as the first line of defence and is found in all aerobic organisms

that dismutase superoxide anion to H_2O_2 (Figure 5). Catalase, one of the largest heme containing tetrameric enzymes has maximum turnover rates among all the enzymes i.e., one catalase molecule can transform up to 6 million molecules of H_2O_2 to H_2O and O_2 per minute and is vital for scavenging of free radicals under stressed conditions. Apart from catalases, APX is believed to play a crucial role in scavenging of H_2O_2 thereby protecting cells from oxidative damage via ascorbate-glutathione (ASH-GSH) cycles that utilizes ASH as the electron donor. APX has high affinity for H_2O_2 as compared to CAT and POX. Catalytic action of APX involve oxidation of ascorbate to Malone dialdehyde (MDA), that result in the formation of monodehydroascorbate-reductase (MDHAR) and dehydroascorbatereductase (DHAR), using NADPH as reducing equivalents and by transforming reduced glutathione (GSH) to oxidized glutathione (GSSG) (Xin et al. 2014). Finally, a flavo-protein oxidoreductase enzyme *viz.*, glutathione reductase (GR), NADPH dependent regeneration of GSH from GSSG, plays an imperative role in strengthening of defence mechanism by scavenging free radicals and by maintaining glutathione in its reduced form. Target oriented roles for different antioxidant enzymes have been explored in many plant species using transgenic approaches (Shafi et al. 2015).

Non-Enzymatic Antioxidants

The cellular osmolyte and compounds like proline, flavonoids alkaloids tocopherol, carotenoids, ascorbate and glutathione are some the major redox buffers that are collectively characterized under the heading of non-enzymatic antioxidants (Figure 5). Commonly, ascorbic acid and tocopherols are known by their generic names i.e., Vitamins C and E that play an imperative role in the scavenging of reactive oxygen species (Boubakri et al. 2016). Ascorbic acid is one of the most generous, influential and water-soluble antioxidants that comprehends L-ascorbic acid and L-dehydroascorbic acid, two compounds with antioxidant activity. The concentration of ascorbic acid has been reported to maximum in mature leaves with fully developed chloroplast. Vitamin E (tocopherol) is a lipid soluble antioxidant with four isoforms *viz.*, α -tocopherol, β -tocopherol, γ -tocopherol and δ -tocopherol and tocotrienols per se., α -tocotrienol, β -tocotrienol, γ -tocotrienol. Vitamin E is one of the most exuberant antioxidants found in bio-membranes which is actively involved in the arrest of lipid peroxidation by contributing phenolic hydrogen to the hydroxyl radicals and converting it to tocopheroxyl radicals which is an unreactive radical which is unable to initiate oxidative chain reaction. It is the only major chain breaking lipid-soluble antioxidant present in RBCs, plasma and tissues that protect the integrity of lipid structures and primary membranes.

Flavonoids, an antioxidant metabolite commonly occur in leaves, floral parts and pollens are composed of isoflavonoids, flavanones and flavones flavonols, flavanols and anthocyanins that share the same diphenylpropane ($C_6C_3C_6$) skeleton (Rai et al. 2015).

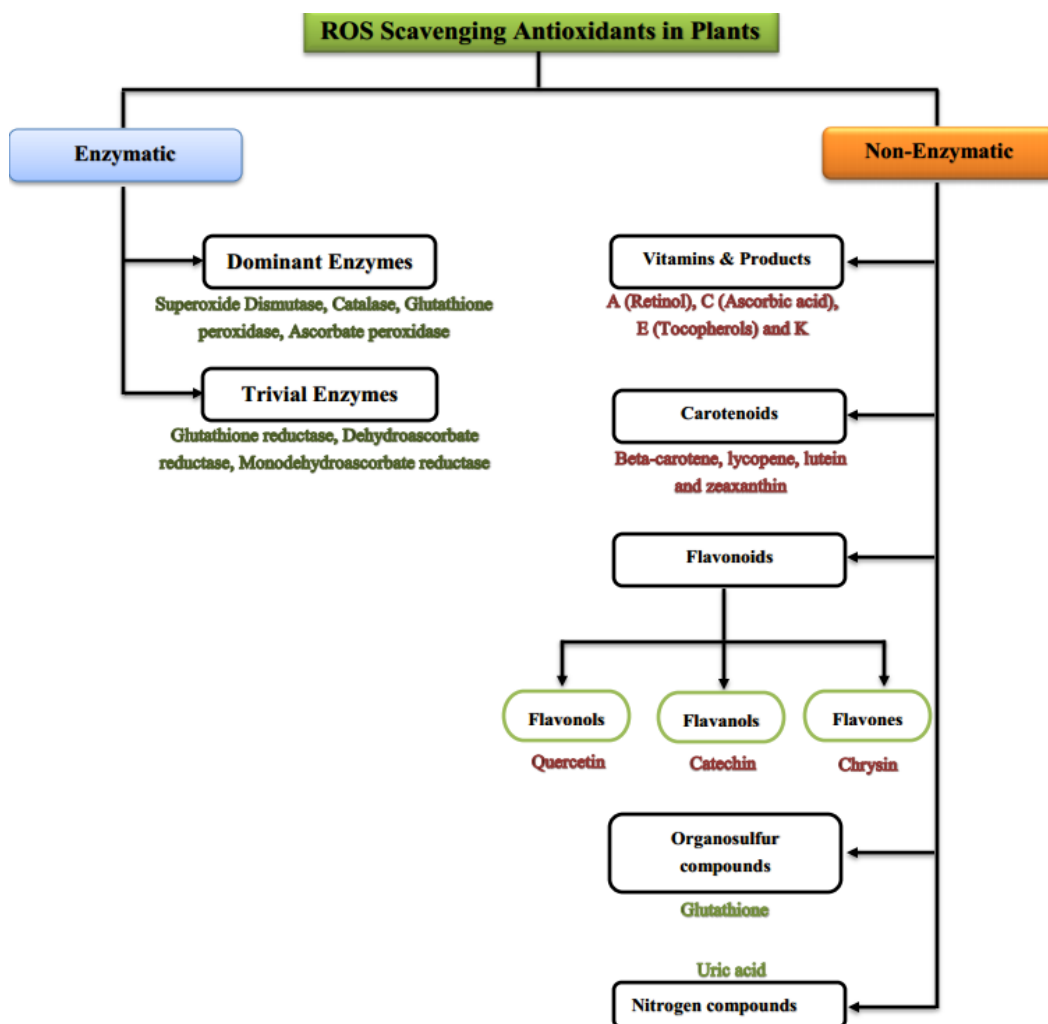


Figure 5. Natural antioxidants separated in classes.

Flavonoids are reported to be involved in myriad of function such as flower/fruits development, protection against UV damage and phyto-pathogens and also act as signalling molecule. Flavonoid and anthocyanin also function as hydrogen donors, reducing agents, singlet oxygen quenchers, superoxide radical scavengers and even as metal chelators. They also activate enzyme of halliwel-asada pathway, reduce α -tocopherol radicals (tocopheroxyls), absorb UV light thereby inhibiting oxidases and mitigating oxidative stress. Some of the most important flavonoids are catechin, catechin-gallate, quercetin and kaempferol. Carotenoids are a group of natural photosynthetic pigments which are synthesized by plants and microorganisms which serve as an important protective role. They are characterized into two broad categories: the carotenes containing lycopene, b-carotene; and the oxygenated xanthophyll, like zeaxanthin, lutein (Zou et al. 2015). The carotenoids are mainly involved in the quenching of singlet oxygen and triplet sensitizer (Chl^3) thereby directing PSI assembly and the stability of light harvesting

complex proteins along with thylakoid membrane stability, thus returning to the unexcited state and allowing them to quench more radical species.

USE OF EXOGENOUS PROTECTANTS IN MITIGATING ABIOTIC STRESSES

Plant growth regulators serve dual function in plant that offers one of the ways to cope with climatic adversities and are also involve in the regulation of plant growth, developmental and signalling processes. In recent decades, exogenous applications of various plant growth regulators (SA; salicylic acid, ABA; abscisic acid and JA; Jasmonic acid), osmo-protectants (Pro; proline and GB; glycine betaine), signalling molecules (SNP; sodium nitroprusside) have shown to have constructive effect on plant growth as these molecules play significant role in mitigating abiotic stress induced oxidative damages in plants (Khan et al. 2013).

Salicylic Acid (SA)

Salicylic acid, a naturally occurring seven-carbon containing phenolic compound plays significant role in the modulation of plant growth and reproduction and is also believed to be effective in conferring abiotic stress tolerance by decreasing the content thiobarbituric acid reactive substances (TBARS), protecting photosynthetic machinery and stimulating antioxidant enzymes and metabolites (Zhang et al. 2011). Several researchers have pinpointed toward the involvement of SA in plants defence against major abiotic stress particularly drought, HT stress and salinity either through seed soaking, foliar spraying, or supplementing it with nutrient solution. In plants, SA exert these functions in a dose dependent manner, where induced or inhibited plant functions has been observed with high/low doses of SA, respectively.

In a study, foliar application of SA @ 0.1 and 0.5 mM have been shown to enhanced photosynthesis and growth, however an inhibited growth was evidenced with 2.0 mM of SA in *vigna radiata* (Nazar et al. 2011). Apart from dose, age, duration and nutritional stage of treated plant species also regulate the SA effects in plants. Wang and Li (2006) reported that exogenous application of 0.1 mM of SA has shown ameliorative effect by decreasing thiobarbituric acid reactive substance (TBARS) and increasing water content in young grape leaves subjected to heat stress, thus mitigating heat stress induced oxidative stress in grape vines. Treatment of plants with SA have also shown to mediate proline accumulation under abiotic stress. Foliar application of SA (at 0.5 mM) significantly increased the activity of proline biosynthesis enzyme (pyrroline-5-caboxylate reductase) under salinity stress that resulted in the increased proline content in *Lens esculenta*, (Misra

and Saxena 2009). Recently, molecular studies have confirmed that SA can modulate the plant function at gene level, by regulating genes involve in secondary metabolites production, antioxidants, and heat shock proteins, thereby refining plant-abiotic stress tolerance.

Abscisic Acid (ABA)

Abscisic acid is considered as a signalling molecule involved in many physiological processes and play an important role in regulating plant defence against diverse environmental stresses such as heat, drought, salinity and flooding (Ding et al. 2010). An unprecedented increase in the endogenous level of the ABA under different stress conditions, has been associated with the enhancement of thermotolerance, suggesting its involvement in the initiation and regulation of defence related genes and signalling pathways which are essential for the survival of plants under climate extremes (Ding et al. 2010). Various studies have confirmed that foliar application of ABA is effective in ameliorating abiotic stress induced oxidative damage in crop plants by interacting and stimulating with other signalling molecules like nitric oxide (NO). In a study, ABA (2.5 μ M) stimulated the growth of chickpea seedlings at 40-45°C by stimulating of endogenous level of ABA with osmolytes and by inducing the regulation of several heat shock proteins and heat shock transcription factors (Rojas et al. 1999) and this effect of ABA in conferring heat tolerance has been extensively studied in maize (Gong et al. 1998). Even though, a large body of literature have confirmed defensive role of ABA in conferring abiotic stress tolerance in plants, till date comparably less is known about physiological and biochemical mechanisms of ABA underlying HT stress tolerance in plants. Furthermore, the cooperative involvement of ABA with other stress induced phytohormones and osmolyte need to observed in plants under different stress condition.

Sodium Nitroprusside (SNP)

Nitric oxide (NO) is a highly diffusible bioactive molecule that plays a significant role in several key metabolic processes both as a signalling as well as protective molecule, that play a vital role in imparting abiotic stress tolerance in plants (Hasanuzzaman et al. 2012). Sodium nitroprusside (SNP), one of the most influential NO donar, supposed to have a wide range of function in plant growth and developmental processes such as osmotic balance, fruit ripening, germination, floral development, apoptosis due to which it is now being considered as a new member of phytohormones. Several researchers have shown that foliar application of nitric oxide improves the growth and development of various plants under the changing environmental conditions such as salinity, drought, UV as well as HT

stress (Gill et al. 2015). In plants, exogenous application of NO donor SNP has been well documented to confer tolerance to severe drought and heat stress conditions by increasing water retention capacity and lowering membrane damage (Hasanuzzaman et al. 2010). SNP treated heat stress seedlings showed upregulation in the activities antioxidant defence systems of catalase, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase thus protecting seedlings from heat stress induced oxidative damage (Hasanuzzaman et al. 2013). Results of several findings have indicated that exogenous application of SNP is effective in regulating plant growth and development processes under stress conditions via modulating antioxidant defence system. However, till date, not much study has been conducted regarding the effect of exogenous application NO on antioxidant metabolism and detoxification system of plants under stress conditions.

Proline (PRO)

Proline are osmolytes/compatible solutes found in a wide range of organisms from bacteria to plants and animals (Alcazar et al. 2006). They also serve as one of the main adaptive mechanism by which plants protect themselves against abiotic stresses via adjusting their osmotic level. Proline perform myriad of functions from detoxification of excess ROS, protects biological membranes to stabilizing enzymes/proteins thus providing protection to plants from damaging effects of various stresses (Hossain et al. 2014). Iqbal et al. (2014) in their study reported that proline provide protection against heat stress by regulating cellular homeostasis. Exogenous application of proline (10-20 μ M) are able to maintain thermostability of thylakoid membranes thereby reducing protein oxidation, lipid peroxidation and increasing photosynthetic efficiency (Banu and Hoque 2010). Several studies have shown that proline has the ability to enhance antioxidant defence system by regulating enzymatic and non-enzymatic antioxidants thus improving stress tolerance in plants (Hossain et al. 2014). A study conducted by Okuma et al. (2004) concluded that exogenous application of proline stimulates intracellular proline content that prevent oxidative cell death in tobacco cultured cells via regulating antioxidant defence systems. Furthermore, researchers have shown that exogenous application of proline is effective in improving abiotic stress tolerance in plants by improving ion homeostasis and regulating the activities of antioxidant enzymes. However, in many of agriculturally important crops per se., hyacinth bean, the protective effect of exogenous application of proline in response to abiotic stress remain to be elucidated.

BIOTECHNOLOGICAL TOOLS FOR EMPOWERING ABIOTIC STRESS TOLERANCE

Major abiotic stresses influencing cool season food legumes has been a matter comprehensive discussion (Singh and Saxena 1993). Numerous stresses are known to affect hyacinth bean such as cold, drought, heat salinity, nutrition deficiency/toxicity, of these heat is considered as the most significant stress worldwide. Susceptibility of hyacinth bean to high temperatures has a major constrain on its productivity in tropical and temperate areas of the world. Though, pervasive germplasms are available that can withstand/tolerate high temperature stress that provide us the opportunity to breed summer hardy cultivars that can planted in late winter with a practical probability of growth and reproduction the summer (Yao et al. 2016). Breeding for abiotic stress tolerance in vegetable crops is mainly incarcerated due to, (i) variation in time, strength and period of abiotic stress tolerance; (ii) dissemination of lethal genes along with desirable traits (iii) limited transfer of suitable alleles due to reproductive barricade from diverse genetic resources (Varshney et al. 2013). Integrated 'omics' techniques have emerged as indispensable tools for improving genotypes/lines that can enroot growth and development of plants under astringent conditions, especially for (ii) and (iii) above. Deployment of molecular breeding approaches can help in the identification of superior genes and alleles that can be transferred into elite genotypes. Furthermore, with the emerging breeder friendly genomics along with decision support tools will play a critical role in breeding programs in developing countries.

Advanced Genomic Tools Applicable to Hyacinth Bean Genomics

Decoding of plant genome sequence has enabled excavation of entire genomic sequences in model plants per se., in *Arabidopsis*, *Lotus japonicus* and *Medicago truncatula*. Although, the same approach does not seem to be applicable to other crops species including hyacinth bean not only due to its large genomic size compared to model species but also due to limitation in financial and human resources. With the advancement in modern genomic tools such issues can be avoided in an efficient way. By the virtue of sequencing-based trait mappings techniques has facilitated trait mapping at sequence level that has enabled the selection of plants at nucleotide levels. Two of these techniques viz., genome wide association studies (GWAS) and genotyping by sequencing (GBS) has led to the identification of large-scale genome-wide SNPs that can facilitate high resolution trait mapping in plants of diverse mapping populations. GBS allows quick identification of vast number of SNPs that can be used for trait mapping, GWAS has been found promising in several crops (He et al. 2014). Both of these techniques i.e., GBS and GWAS has received immense recognition as they do not require any prior genome information, low cost

genotyping, provide high-density genotyping data, and provide advantage over other sequencing based-methods as it allows substantial degree of false positive detection.

In legumes, with the advent of next generation sequencing technologies (NGS) several new genotyping platforms such as GBS and GWAS are currently available and have been deployed extensively for construction of genetic maps, genetic diversity/purity analysis and marker assisted selection (Varshney et al. 2014). The utilization of both GBS and GWAS for the improvement of horticultural crops has been extensively reviewed by Kim et al. (2016). Similarly, in hyacinth bean, GBS approach may emerge as most powerful tool as compared to other sequencing techniques for targeting candidate genes from diverse germplasm. It can also facilitate the understanding of the role of candidate genes for targeted traits by sequencing of protein coding regions and in the identification of SNPs. Similar techniques have also been used in rice for the identification of candidate genes against biotic stress.

Transcriptomics for Genome Annotation and Marker Development

Discovery of genes and characterization of their regulatory pathways that are stimulated under various abiotic stresses could play an important role in improving the productivity of legumes under adverse environmental conditions. In this context, transcriptome sequencing has emerged as an indispensable tool for trait specific gene sequencing.

In legumes, with the help of transcriptome sequencing various efforts has been made on the development of cDNA libraries, gene expression analysis, *in-silico* mining of expressed sequence tags (ESTs) before the genome sequence of crop became available (Morozova and Marra 2008). Significant amount of work has been done in legume transcriptomics and a considerable number of ESTs and transcription factors (Table 1) have been developed in several legume plants challenged by different stresses such as in soybean (1.5 million ESTs), *Medicago truncatula* (280,000 ESTs), *Lotus japonicus* (242,000 ESTs), chickpea (20,162 ESTs) and pigeon pea (9,888 ESTs) has been generated through Sanger sequencing approach (Varshney et al. 2015). So, in hyacinth bean, deployment of transcriptomics techniques needs to be focused on the generation of cDNA libraries, expressed sequence tags (ESTs), gene expression analysis together with in silico mining of ESTs data sets to obtain functional annotation and discovery of novel genes to understand the molecular basis of gene function under climate extremes.

Recently, numerous sequencing platforms are readily available at low cost that can be used for the generation of wide range of stress responsive transcript reads in different crop legumes. In the case of peanut, differentially expressed genes and mRNA transcripts, have been identified under drought stress and well-watered condition by using differential

Table 1. List of different transcription factors with a potential role in the abiotic stress signalling pathways and regulation gene expression in plants

Stress condition	Transcription factors	Sequence	Mode of action	Gene regulated	References
Drought	bZIP	PyACGTGGC	Promotion of salicylic acid biosynthesis by regulating drought-responsive genes	<i>Em, RAB16, Osem, RD29B</i>	Zhu (2002)
Drought, Cold	ERF/AP2	TGCCACCGG	Positively regulate dehydration responsive element binding protein (DREB/CBF) and stimulate freezing/drought tolerance pathways	<i>HVA1, RD29A, Cor15A, BN115</i>	Lee et al. (2004)
Drought	MYB	TGGTTAG	Regulates ABA-and JA biosynthetic genes overexpression which confers resistance to cold, drought, salt, ozone stresses	<i>RD22</i>	Schenke et al. (2011)
Drought	bHLH	CACATG	Activation of ABA and JA signalling response	<i>RD22</i>	Abe et al. (2003)
Drought	NAC	ACACGCA-TGT	An inducer of abiotic stress responses	<i>ERD1</i>	Seo et al. (2010)
Drought	ZFHD	CACTAAA-TTG	When over-expressed confers tolerance to both biotic and abiotic stresses	<i>ERD1</i>	Tran et al. (2004)
Salinity	ERF	GCCGCC	Regulation of tolerance to abiotic stresses	<i>BIERF1-4</i>	Park et al. (2001)
Salinity	AP2/ERFBP	AGCCGCC	Overexpression enhances tolerance salt stress.	<i>OPBP1</i>	Guo et al. (2004)
Drought, salinity	ABF	PyACGTG-G/TC	ABA-mediated abiotic stress tolerance	<i>AREB1</i>	Orellana et al. (2010)
Heat, cold, salinity, drought	WRKY	TTGACCT	Regulation of abiotic stress response inducing ABA-responsive abiotic stress tolerance genes.	<i>WRKY45/82</i>	Peng et al. (2011)
Heat, salinity	Zinc-finger	CCCH/AAAG	Regulation of genes involved in heat and oxidative stress tolerance	<i>ZAT7/12</i>	Davletova et al. (2005)
Heat	HSF	AGAATTCT	Regulation of antioxidant defence related genes under abiotic stresses and maintains growth	<i>HSFA1b, HSFA2</i>	Mullineaux et al. (2011)
Drought, heat, salinity	DREB	GCCGAC	Mediate positive and negative regulation of ABA and sugar responses	<i>RD/COR</i>	Lee et al. (2004)

display reverse transcriptase PCR (DDRT-PCR) (Qiao et al. 2012). In addition, ESTs libraries has also been developed using suppression subtractive hybridization (SSH) techniques which has led to the development of 480 abiotic stress responsive ESTs in chickpea. Furthermore, SSH approach has also been used to strengthen the EST libraries in in chickpea and pigeon pea by generating 3,062 and 182 ESTs in response to drought and salinity stress (Qiao et al. 2012). Hyacinth bean, taxonomically one of the closest crops to the model legume *Medicago truncatula* is the most important food legume of semi-arid tropics and. In hyacinth bean, both sensitive and tolerant varieties have been characterized under different abiotic stress conditions, although very little is known about the genes involved in these responses. Though, transcriptome assemblies of genes involved in the differential behaviour and profiling of regulatory genes from developed transcriptome can facilitate in generating highly dense genetic map with well distributed markers and Hiz et al. (2014), identified 441 transcription factors from salt-stressed common bean and classified them under 59 different transcription factor families.

Proteomics Analysis

Proteomic analyses of plants under adverse environmental cues can provide valuable insight on proteins involved in the abiotic stress response which can lead to the identification cellular and molecular mechanisms that are linked between different abiotic stresses. Proteomics involve high-throughput analysis of (1) total cellular proteomes of a species that are expressed in specific cells/tissues/organs at distinct developmental stages (2) several post transcriptional modifications to find out how proteins get modified (3) protein-protein interactions to know how proteins interact with other biomolecules (4) deciphering protein structure and functional group attached to proteins (Jorin-Novo et al. 2015). Advancement in proteomics approach has made complex biological questions to answer more efficiently due to the availability of wide range of proteome coverage that have facilitated the interpretation of protein structure and modulation/modifications of stress induced enzymatic and regulatory functions of specific proteins (Table 2) encoded by specific genes (Katam et al. 2010). However, proteomics in crop research still have to travel a milestone to study response mechanism of several stress specific traits as obtaining good sample quality is a bit challenging owing to the fact that plant tissues are often enriched with several proteases and oxidases which make it quite difficult to obtain stable protein mixtures.

Several lines of experiment have been conducted on model legumes *per se.*, *Medicago*, *Lotus* and in soybean (Hossain and Komastu 2014) and substantial load of protein reference map providing detailed proteomic information have made available which can be exploited in other legume crops to better understand abiotic and biotic stress responses. Earlier studies on hyacinth bean have shown that limited information is available on proteomics-

based datasets which can be enhanced by increasing the availability of transcriptome data sets and legume genome sequences (Thilagavathy and Devaraj 2016). Such techniques have been successfully employed in other crop legumes such as chickpea, pea and groundnut under biotic and abiotic stresses that has facilitated the protein research in these crops by enhancing the availability of protein reference maps (Katam et al. 2010). Comparative proteomics together with differential expression studies can significantly contribute toward understanding of stress specific protein response in crop legumes such as response of dehydrin proteins in drought/osmotic stress, salt stress (Kottapalli et al. 2008). Therefore, in hyacinth bean, future investigation on these areas can improve our understanding by augmenting the use of comparative proteomics approach from other legume crops.

Metabolic Fingerprinting in Legumes

Metabolic fingerprinting (Table 2) allow contemporaneous analysis of all or a set of metabolites which have diverse functions and are manufacture at relatively large scale (Genovese et al. 2015). The advantage of metabolic fingerprinting is that it identifies certain metabolic signatures per se., specific pattern associated with particular stress response without prior information about the metabolites involved. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) have facilitated the identification of large number distinct metabolites in plants (Sumner et al. 2003). Different analytical techniques such as liquid chromatography-MS (LC-MS), gas chromatography-MS (GC-MS) and GC-time of flight MS (GC-TOF-MS) may also be used for metabolic fingerprinting (Johnson et al. 2003). On the contrary, GC-MS is more advantageous over NMR spectroscopy because of its low resolving power, as GC-MS allow quantification of large number of primary metabolites. Furthermore, GC-TOF-MS has also emerged as more rapid and robust technique for identification of secondary metabolites that are expressed at certain developmental stages of plants and have better resolution and provide better peak compared to GC-MS (Fernie and Schauer 2009).

However, metabolic profiling technique has not been extensively used in crop legume but this approach has been successfully used in model legumes. As for example, in a study in drought tolerant Lotus genotypes, comparative metabolomic approach coupled with GC-TOF-MS lead to the profiling of rhizobia Nod factors to visualize metabolic variation among different symbionts. Similarly, using GC coupled to electron impact ionization (EI)-TOF-MS (GC-EI-TOF-MS), a comprehensive and advanced understanding of drought induce metabolic re-programming of metabolites were made in response to water stress (Sanchez et al. 2012). However, limited metabolic fingerprinting studies has been implicated in other forage legumes. For example, profiling of chickpea in response to biotic stress, pea in response to abiotic stress and in common bean under heavy metal stress

(Pandey et al. 2016). In addition, metabolite profiling in Lotus have also been done to analyse flavonoids under abiotic stress (Zhang et al. 2012). Hyacinth bean one of the rich source of flavonoids which characterize it as medicinal therapeutic plant (Rai et al. 2015), deployment of metabolite profiling techniques can facilitate the identification of candidate metabolites that are expressed in response to abiotic and biotic stresses.

Table 2. Application of proteomics/metabolomics for the identification of key protein/metabolites in model and crop legumes under abiotic stresses conditions

Legume	Stress conditions	Techniques used	Differentially expressed proteins and metabolites	References
Model legume				
Medicago	<ul style="list-style-type: none"> • Salt • Drought 	<ul style="list-style-type: none"> • ESI-LC-MS/MS • 2D-GE • MALDI-TOF-MS • LC-MS/MS • GC/MS • LC-ESI-TOF-MS • HPLC 	<ul style="list-style-type: none"> • Regulation of photosynthesis • Protein metabolism and chaperones metabolism 	Staudinger et al. (2012)
Lotus	<ul style="list-style-type: none"> • Salt • Drought 	<ul style="list-style-type: none"> • GC-EI-TOF-MS • GC-MS • LC-MS 	<ul style="list-style-type: none"> • Storage proteins, • Sucrose synthase • Regulation of nod genes 	Sanchez et al. (2011, 2012)
Crop legume				
Pea	<ul style="list-style-type: none"> • Salt • Drought • Osmotic 	<ul style="list-style-type: none"> • 2D-GE • MALDI-TOF-MS • LC-ESI-TOF • ESI-TOF-MS • NMR 	<ul style="list-style-type: none"> • Regulation of glycolysis, Flavonoid and sulphur metabolism • Antioxidant metabolites 	Brosowska-Arendt et al. (2014)
Soybean	<ul style="list-style-type: none"> • Salt • Drought • Osmotic • Cold • Heat 	<ul style="list-style-type: none"> • 2D-GE • MALDI-TOF-MS • LC-MS/MS • ESI-TOF-MS • NMR • UPLC-QTOF-MS • HPLC-UV-ESI-MS • GC-MS • CE-TOF-MS 	<ul style="list-style-type: none"> • Regulation of defence related genes and proteins • Apoptotic proteins • Photosynthetic proteins • Secondary metabolites 	Swigonska and Weidner (2013)
Chickpea	<ul style="list-style-type: none"> • Drought • Cold 	<ul style="list-style-type: none"> • 2D-GE • MALDI-TOF-MS • LC-MS/MS • ESI-TOF-MS 	<ul style="list-style-type: none"> • Regulation of defence related metabolites • Molecular chaperones • Cell wall modification proteins 	Jaiswal et al. (2014)
Common bean	<ul style="list-style-type: none"> • Drought 	<ul style="list-style-type: none"> • 2D-DIGE • LC-MS/MS 	<ul style="list-style-type: none"> • Regulation of photosynthesis • Defence related genes • Protein synthesis and metabolism 	Zadraznik et al. (2013)

EPIGENETIC CHANGES IN RESPONSE TO ABIOTIC STRESS

Alteration in histone modification and DNA methylation due to regulation of stress-responsive genes have been shown to be involve plant growth and development thereby increasing the ability of them to adapt under stringent abiotic stress condition (Figure 6). Several histone modifications (Table 3) such as phosphorylation, methylation, acetylation and SUMOylation (small ubiquitin-related modifier) are known to influence abiotic stress responses in plants (Luo et al. 2012). In angiosperms, histone modifiers are well conserved as for example, histone deacetylase, histone methyltransferase, histone acetyltransferase, histone demethylases have been identified and phylogenetically classified in many of the agriculturally important crops such as tomato, rice, *Arabidopsis* (Cigliano et al. 2013). In drought stress, transcriptional fortitude of drought responsive genes shows strong association with DNA methylation and histone modification where To and Kim (2014) reported that modification of H3K4me3 and H3K9ac upregulated the expression of *RD20* and *RD29A* genes in severe drought condition as compared to moderate drought stress.

A body of literatures have suggested that stress responsive genes uses histone modification as one of the tools to memorize abiotic stress response and in this context H3K4me3 act as one of the potential markers (Table 4) for gene activation and stress memory (Ding et al. 2012). Acetylation of histone is mainly associated with activation of stress responsive genes which is under tight regulation of histone acetyltransferase and histone deacetylase. In *Arabidopsis*, gene *ADA2b*, which is known to regulate function of histone acetyltransferase modifier, have shown to activated in response to salt stress (Kaldis et al. 2011), thereby suggesting the defensive role of histone acetyltransferase modifier in imparting salinity tolerance. Several researchers in their study have confirmed positive connection between changes in histone modification and DNA methylation with changes in gene expression. In an interesting study was conducted by Sani et al. (2013) with the aim to decipher changes in DNA methylation in response to salinity stress. They reported that genes viz., H3K27me3 and *HKT1* that show high affinity for K^{+} transporter was induce under salinity stress, thus playing a major role in salinity tolerance.

SUMOylation i.e., small ubiquitin-related modifier is also a type of histone modification that is predominantly found under thermal stress response. SUMOylation was previously identified as reversible post-translational modification with the ability to regulate protein-protein interaction in eukaryotes (Lu et al. 2009). Study conducted in model plant *Arabidopsis* have confirm that SUMOylation of core histone in particular H2A and H3 histone play significant role in the assembly of centromeric chromosomes, DNA repair pathways and is also able to regulate gene expression under heat stress (Coleman-Derr and Zilberman 2012). The researchers have showed that both *H4K16ac* and *H3K23ac* showed distinct expression patterns in association with particular developmental stages and

also contribute to stress memory in plants (Chinnusamy and Zhu 2009). The techniques viz., NGS-based whole genome bisulphite sequencing (WGBS) and chromatin immunoprecipitation-sequencing (ChIP-Seq) are being currently deployed to comprehend DNA methylation mechanism and heterosis in model plants under stress conditions. These techniques can also be used in other legume crops for understanding DNA methylation and histone modifications to study these epigenetic changes under biotic and abiotic stresses for crop improvement and management (Pandey et al. 2016).

Table 3. List of various histone post-translational modifications and their relationship with gene expression in plants under abiotic stress conditions

Histone modification	Modified histone residue	Mode of action	Major enzyme involved	Regulation of gene expression	References
Phosphorylation	Hydroxyl groups of serine and threonine in H2A, H2B, H3, and H4	Protein kinases of aurora family are phosphorylated	Phosphatase 1 (PP1) family enzymes	Transcription activation	Houben et al. (2007)
Acetylation	H2A (K 4, 5, 7) H2B (K 5, 11, 12, 15, 16, 20) H3 (K 4, 9, 14, 18, 23, 27) H4 (K 5, 8, 12, 16)	Acetyl group from histone acetyltransferases (HATs) is transfer to the ε-amino group of lysine	Histone deacetylases (HDACs)	Activation of transcription through acetylation	Peterson and Laniel, (2004)
Methylation	H3 (K4, 9, 27, 36) H4 K20 H3 R3	Methyl group from S-adenosyl-L-methionine is transfer to ε-amino group of lysine and guanidino group of arginine	Lysine-specific demethylase1 (LSD1/ KDM1) and Jumonji C (JmjC) domain-containing proteins	Methylation of H3K4 and H3K36 activate geneexpression. Methylation of H3K9, H3K27 and H4R3 repress gene expression.	Couture and Trievel (2006)
Ubiquitination	H2A H2B (monoubiquitination)	Ubiquitination of Brefeldin A-sensitivity protein1, histone monoubiquitination1, and ubiquitin carrier protein1	Ubiquitin-specific thiol proteases, Utubain-like deubiquitinase (OTLD1)	Ubiquitination of H3K4me3 activate transcription	Deal and Henikoff, (2011)
SUMOylation	H2A.Z H2B H4	Small ubiquitin-like modifier (SUMO) E3-ligases	SUMO-specific proteases	SUMOylation of Histone deacetylases represses transcription	Garcia-Dominguez and Reyes (2009)

Table 4. Effects of different abiotic stresses and exogenous protectants on histone modification and DNA methylation in plants. The table depicts genes regulated, methylation status, duration of stress and techniques used to decipher epigenetic marks (ChIP-Seq: Chromatin immunoprecipitation sequencing, RT-qPCR: Real time quantitative PCR, RNA-Seq: RNA sequencing, Western blot: Western blotting)

Stress condition	Duration	Plant species	Histone modification	Genes regulated	Methylation status	Techniques used	References
Drought	1, 2, 4, 5h	<i>Arabidopsis</i>	H3K9ac, H3K1ac, H3K23ac, H3K4me3, H3K4me2, H3K4me1	<i>RD29A, RD29B, RD20, RAP2.4, AtGOLS2, ProDH</i>	Methylation	ChIP-Seq, RT-qPCR	Fang et al. (2014)
	35 h	<i>Oryzasativa</i>	H3K18ac, H3K27ac, H4K5ac, H3K9ac, H3K4me3	<i>OsHAC703, OsHAG703, OsHAM701, OsHAF701</i>	Methylation	RT-qPCR, Western blot	
Salinity	5, 20 days	<i>Arabidopsis</i>	H3K9K14ac, H3K4me3, H3K9me2	<i>ABII, ABI2, RD29A, RD29B, KATI, KAT2, DREB2A, ERF4</i>	Methylation	ChIP-Seq, RT-qPCR	Zhao et al. (2016)
	5-14 days	<i>Oryzasativa</i>	H3K18ac, H3K27ac, H4K5ac, H3K9ac, H3K4me3	<i>OsGA20ox2, OsGA20ox3, OsGA3ox1</i>	Methylation	RT-qPCR, Western blot	
Cold	6 days	<i>Arabidopsis</i>	H3K27me3	<i>COR15A, ATGOLS3</i>	Methylation	ChIP-qPCR	Roy et al. (2014)
	16 h	<i>Oryzasativa</i>	H3K9ac, H3K9K14ac, H3K27ac	<i>OsDREB1</i>	Methylation	ChIP-Seq, RNA-Seq, Microarray	
	3-6 weeks	<i>Zea mays</i>	H3K9ac, H4K5ac, H4K4ac	<i>ZmDREB1, ZmICE1, ZmCOR413</i>	Methylation/De-methylation	ChIP-qPCR, RT-qPCR, Western blot	Hu et al. (2012)
Heat	8 days	<i>Arabidopsis</i>	H3K27me3, H3K4me2, H3K9ac	<i>APX2, HSP18.2, HSP22.0, HSP70, WRKY53, FRK1, NHL10</i>	Methylation/De-methylation	ChIP-qPCR, RT-qPCR, Western blot, RNA-Seq	Gan et al. (2014)
Submergence	24 h	<i>Oryzasativa</i>	H3K4me3, H3K4me2, H3K9/14ac	<i>OsADH1, OsPDC1</i>	Methylation	ChIP-qPCR, RT-qPCR	Tsuji et al. (2006)

Stress condition	Duration	Plant species	Histone modification	Genes regulated	Methylation status	Techniques used	References
Phytohormone priming							
Salicylic acid + Drought	100 μ M	<i>Hordeumvulgare</i>	-	<i>HvHDAC2-1, HvHDAC2-2</i>	Methylation	ChIP-qPCR, RT-qPCR, Western blot,	Demetriou et al. (2009)
Abscisic acid + drought + salt	0.5-2.0 μ M	<i>Arabidopsis</i>	H3K9K14ac, H3K4me3, H3K9me2	<i>ABI1, ABI2, RD29A, RD29B, KAT1, KAT2, DREB2A</i>	Methylation	ChIP-Seq, RT-qPCR	Chen et al. (2010)
Jasmonic acid + drought	100 μ M	<i>Hordeumvulgare</i>	-	<i>HvHDAC2-1, HvHDAC2-2</i>	Methylation	RT-qPCR	Demetriou et al. (2009)
	100 μ M	<i>Hordeumvulgare</i>	-	<i>HvHDAC2-1, HvHDAC2-2</i>	Methylation	ChIP-Seq, RT-qPCR	

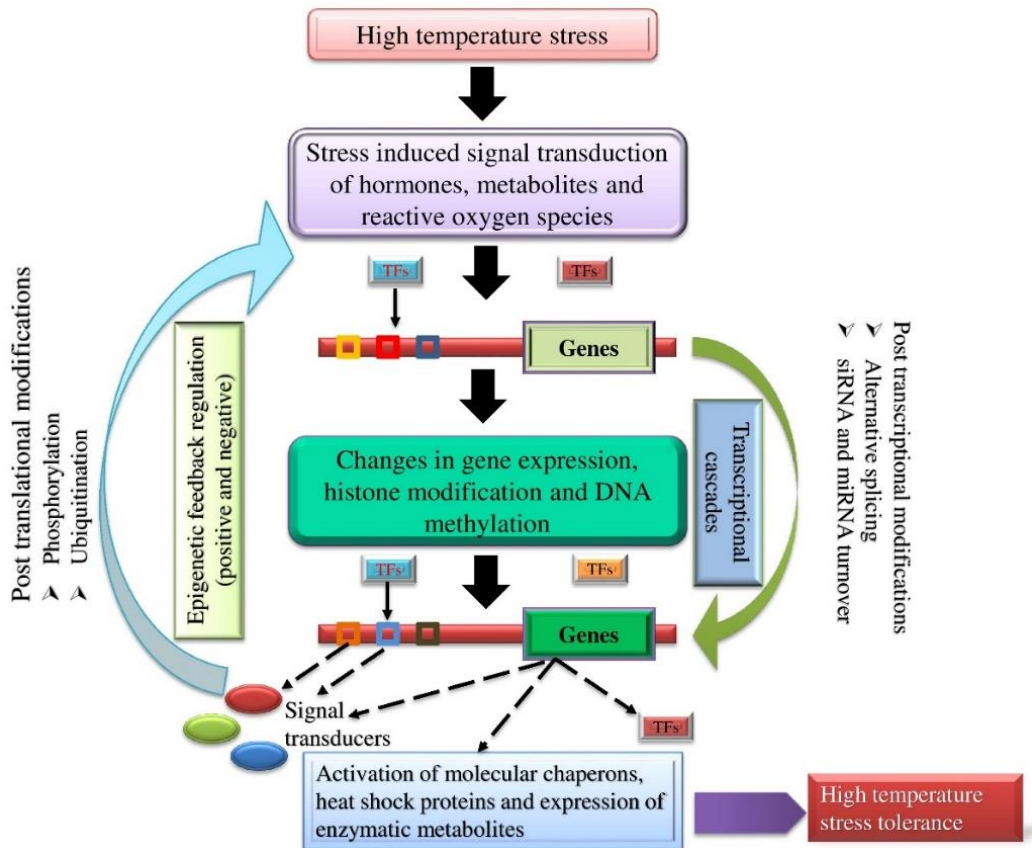


Figure 6. Overall model for high temperature stress tolerance in terms of post-transcriptional, post translational and epigenetic regulation. Plant cell perceive and transduce stress signal via various signalling pathway, plant hormones and secondary metabolites which in turn regulate stress inducible genes and transcription factors (TFs) thereby expressing functional metabolites and proteins that helps the plants in combating stress condition.

CONCLUSION AND FUTURE OUTLOOK

The era of “omics” technology has empowered the development in the area of transcriptome, proteome and metabolome that has enhanced our possibility of studying regulation of plant growth and development at all the three levels. The effect of stress and their response are not straightforward as they involve series of physiological, biochemical and molecular changes that instigate severe repercussion on plants. For instance, to decipher quantitative relation between different proteins and metabolites that are regulated under various abiotic stress conditions, identification of bio-markers is an utmost obligation. With the advancement of “omics” techniques, several genomics and protein databases has been made available in model legume crops such as Medicago and Lotus and in crop legumes as well such as soybean and common bean, which can facilitate high

throughput identification and validation of stress responsive proteins and metabolites in other crop legumes *per se.*, hyacinth bean.

Legumes including hyacinth bean are cultivated widely in the tropical and sub-tropical part of the world where plants are affected not by one stress but also a combination of stress at a same point under natural field condition that severely effect their growth and productivity. The only option by which legume production can be sustained under these variable environments is to develop high yielding/resistant/tolerant cultivars by incorporating integrated “omics” technology into breeding program that can pinpoint those genes that responsible for desired traits and thus facilitate the selection plants on the basis of their genotyping information. Few of these techniques have been successfully employed in some legume crops such as chickpea, soybean and pea that has significantly contributed towards the understanding of stress related traits in these crops leading to implication of effective strategy for achieving higher yield. With the ever-increasing global population, the demand-supply gap for the legumes is widening day by day. Therefore, in order to meet that gap in near future, hyacinth bean need to be advanced at all the three “omics” levels for better understanding of physiological, biochemical and molecular pathways involved in stress response to sustain its cultivation under adverse environmental cues.

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