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Recent advancements in legumes: Next generation sequencing and omics approaches

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ABSTRACT

Legumes are important food crops in sustaining food security globally along with improving physio-chemical soil properties by mechanism of biological nitrogen (N_2) fixation. Different types of abiotic stresses (especially their intensity, duration, and magnitude) such as drought, salt, cold and heat affect crop yield negatively and threaten overall food security. As the world population is expanding rapidly on the limited agricultural resources, sustainable management of the same is the need of the hour. Legumes are major nitrogen fixers that are enriched with metabolites, which provide second line of defence against several biotic as well as abiotic stresses. In past years genome sequence information of several grain legumes has been well documented. Due to genome sequencing, re-sequencing and RNA sequencing (RNA Seq.) of grain legumes, information associated to legume development, structural variation, differentially expressed genes and functional genomics was made available. Regulation of entire plant physiology and nitrogen fixation in grain legumes during abiotic stress is multifaceted and only some pathways have been revealed. This review is focussed on exploring the genetic variations analysed through omics approaches to enhance crop yield and productivity under drought, salt, cold and heat stress of grain legumes. Therefore this reviewis a compilation of recent biotechnological advancements on grain legumes using omics approaches for better understanding of their abiotic stress tolerance.

Keywords: Abiotic stress, Climate change, Grain, Legume, Technological advancements

Legumes belong to the family Fabaceae/Leguminosae with about 700 genera and 18000 species. Legume crops are group of plants having inherent ability to fix atmospheric nitrogen (N_2). They are essential source of nitrogen, therefore play an important role in crop fertilization and sustainable agriculture. Legumes are cultivated mainly for human food, cattle feed, forage and as an alternative natural nitrogen fertilizer source. Some of the examples of primary legumes are, peanuts, chickpeas, beans, lentils and lupins, are most versatile and wholesome food available (Foyer *et al.* 2019). World population is projected to be ~10 billion by 2100 and current food productivity is insufficient to feed the same (Ray *et al.* 2013, Gerland *et al.* 2014). Legumes are among the most easily available economical source of proteins, vitamins and other key micronutrients necessary for overall human growth and development. Legumes are only second to cereal crops worldwide in providing food for consumption. The main legume crops are chickpea, french bean or kidney bean, black-eye pea, broad bean, groundnut, pulses, peas, congopea and sojabean, etc (Dwivedi *et al.* 2018). Several climatic factors are affecting crop production, such as higher CO_2 concentration, rising sea level, increasing earth temperature along with population ballooning. The unpredicted heat and cold waves along with other uncertain weather changes further worsen the problem. These stressful environmental incidences severely limits the legume crop production globally. Altogether, such incidences of unpredictable weather conditions increase the risk of global epidemic, pandemic and other parasitic and predator infestation and thus affecting crop performance negatively (Araujo *et al.* 2015, Myers *et al.* 2017). Substantial reduction in crop yield has been observed under different abiotic stresses in legumes such as in soyabean by 40% (Hoyos-Villegas *et al.* 2016), in chickpea by 50% (Sabaghpour *et al.* 2006) and in groundnut by 25% respectively (Girdthai *et al.* 2010).

Abiotic stresses are multigenic in nature, meaning they affect several vital cellular, biochemical, physiological and molecular pathways simultaneously (Udawat *et al.* 2014). Salinity and desiccation disturb normal biosynthesis of DNA, RNA, proteins and other pathways such as glycolysis, tricarboxylic acid cycle (TCA), hormone biosynthesis, and photosynthesis, etc (Udawat *et al.* 2016). Normal cellular

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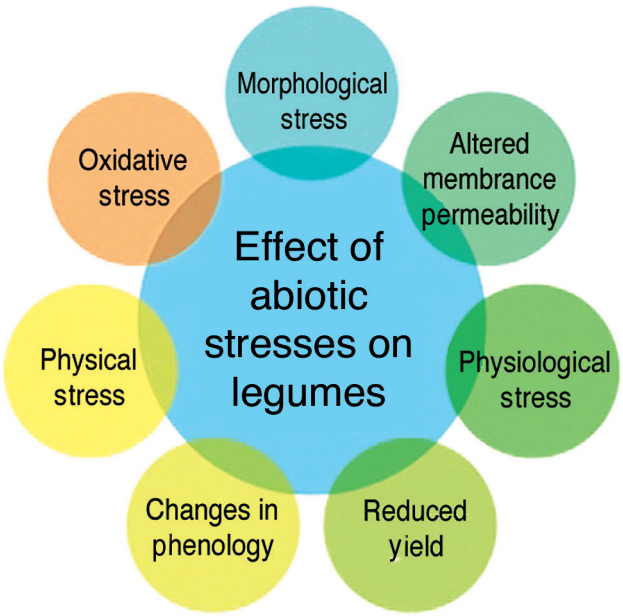

Fig 1 Effect of abiotic stresses on grain legumes at different level.

growth and development is retarded under stressful condition due to adverse impact on several vital physiological and biochemical processes like, inhibition of chlorophyll biosynthesis, stomatal regulation, leaf senescence, and free radical generation, etc. Furthermore, these stresses negatively affect seed germination, seed number, yield and vigour (Fig 1). The most sensitive stage to salt and desiccation stress is reproductive stage, i.e. pollen fertility, anthesis, germination, and seed setting, etc (Pang *et al.* 2017). Subsequently, abiotic stresses also adversely affect the process of symbiotic nitrogen (N_2) fixation by limiting root nodule formation and legume-rhizobium symbiosis.

Plants receive changes in environmental conditions through the opening or closing of channels and beginning of signalling cascade which requires changes in calcium and other ion fluxes, alteration in hormonal level, protein phosphorylation and ROS induced PTMs (Matamoros and Manuel 2021). Nonetheless, the procedures by which alterations in the cytosolic redox state are transduced into particular response are ambiguous in nature. Main determining factors are the amount, duration of exposure, type and sub-cellular location of ROS in every cellular compartment (Sita *et al.* 2017). In recent years, several studies have focussed on understanding of the physiological, molecular and genetic basis of stress tolerance in grain legumes. The nutritional aspect and benefits of legumes are shown in Fig 2. Moreover, application of biotechnology based approaches in addition to molecular assisted breeding in order to improve abiotic stress tolerance in grain legumes are mainly emphasised in this review (Kaushal *et al.* 2013, Kaushal *et al.* 2016. To increase agricultural yield under changing climatic scenario is one of the vital concern towards achieving the food security and agricultural sustainability (Varshney *et al.* 2019, Matamoros and Manuel 2021).

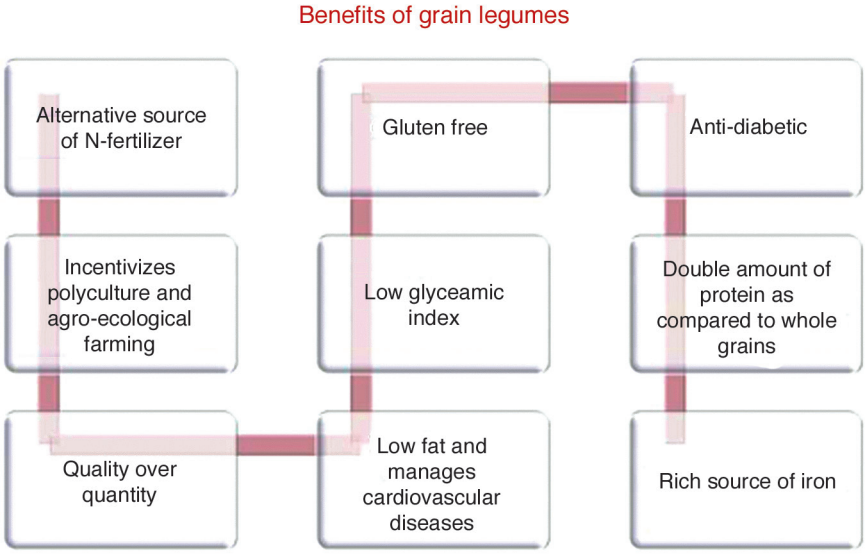

Fig 2 Benefits of grain legumes associated to human growth and development.

Next Generation Sequencing (NGS) approaches

The recent sequencing approaches are mainly directed by next generation sequencing (NGS) technologies, that resulted into collection of a large numbers of reference genomes and re-sequencing of several germplasms. It helps in generating high density genomic maps and identification of novel genes and their associated families in different grain legumes (Singh *et al.* 2017). The transcript sequencing approach involves sequencing of cDNA obtained from entire content of RNA produced in an organism at given time and under given set of conditions. These advanced genomic tools may step up the identification of valuable quantitative trait loci (QTLs) responsible for enhanced abiotic stress tolerance in wild legumes, which can be incorporated into cultivable varieties (Abdelrahman *et al.* 2018). For instance, *de novo* sequencing of soybean W05 facilitated the discovery of novel *cation/H^+ exchanger1* responsible for salt-stress tolerance (Qi *et al.* 2014). Furthermore, a relative transcriptome analysis under salinity and drought stress provided significant insight into the transcriptional regulation in chickpea at early and late reproductive stages (Garg *et al.* 2016). Similarly, genome wide NAC transcription factors have been identified in *Medicago sativa* under different abiotic stresses by Min *et al.* (2020).

Modern development in plant based omics approaches has aided in identification of QTLs, genes, gene families, they also benefited in deciphering the associated network and regulatory mechanisms involved in providing abiotic stress tolerance to grain legumes (Varshney *et al.* 2014).

Molecular mapping of legumes

QTL analysis of bi-parental lines of legumes has facilitated in identification of vital candidate genes responsible for providing abiotic stress tolerance (Mir *et al.* 2012). Since, abiotic stresses adversely affect multiple plant development stages with varying intensity, magnitude and severity. Therefore, identification and understanding of growth phase specific QTLs for specific functional traits are essential for enhancing salt and drought stress tolerance of grain legumes (Varshney *et al.* 2014). QTLs responsible for drought stress tolerance have been identified in chickpea for example, *CaLG04* (Sivasakthi *et al.* 2018) and also in common bean (Mukeshimana *et al.* 2014). Moreover, as roots are primarily the initial plant part which gets exposed to various abiotic stresses, hence developing strategies for studying plant root architecture could be an important aspect to enhance crop yield under stressful conditions by augmenting crop resilience (Ye *et al.* 2018). In several legumes, such as chickpea, common bean and soybean, QTLs associated to root length, root volume, harvest index, seed weight, pods per plant, grain yield and root biomass have been identified under limiting water conditions or drought stress (Asfaw and Blair 2012, Varshney *et al.* 2014, Kale *et al.* 2015, Manavalan *et al.* 2015). QTL analysis of different physiological traits in legumes has led to identification of signature sequences related to stomata regulation (Rehman *et al.* 2011), canopy development, wilting (Hwang *et al.* 2015, Sivasakathi *et al.* 2018), relative water content (Vu *et al.* 2015), transpiration effectiveness (Ravi *et al.* 2011), and delayed senescence (Muchero *et al.* 2013). Altogether these physiological traits and associated QTLs facilitate a plant to mitigate different abiotic stresses and thus complete normal life cycle under adverse conditions. All these information were made available by uniting high density genotype and phenotype data of different legumes across locations and seasons. An outstanding example is ~ 14 cM long QTL hotspot stretch in chickpea that has 13 vital-cause QTLs regulating ~ 12 water deficit associated traits responsible for 58% phenotypic discrepancies (Varshney *et al.* 2014) which was further sequenced and divided into QTL-hotspot_a and QTLhotspot_b (Kale *et al.* 2015). As shown by recent developments in grain legumes, high efficiency of latest genotyping technologies laid the basis for functional validation of genes associated to abiotic stress tolerance along with marker assisted selection for trait emergence (Kale *et al.* 2015).

A high-resolution study in different legumes was made possible due to availability of large number of DNA markers (Xu *et al.* 2017). For instance, in soybean genome wide association study (GWAS) of 373 genotypes in different environmental conditions have shown effective relation of 39 single nucleotide polymorphism (SNPs) with carbon isotope ratio ($\Box^{13}C$) which is an essential trait associated to improved water use efficiency (Dhanapal *et al.* 2015). Similarly, GWAS analysis of 345 soybean accessions have also revealed considerable association of 52 SNPs with canopy temperature, which is considered to be an essential physiological trait of drought tolerance (Kaler *et al.* 2018). Likewise, GWAS study of 96 accessions in common bean has led to the discovery of several water stress related parameters, viz. leaf elongation and shoot biomass under both control and drought stress conditions and led to the discovery of one of the aquaporin gene *Phvul. 011G102700* on LG11 chromosome (Hoyos-Villegas *et al.* 2016). Similarly, GWAS analysis of 86 wild type genotypes of common bean helped in identification of two important signature genes i.e. ankyrin repeat rich protein and photo-reactive *NPH3* genes (Cortes and Blair 2018).

Transcriptomic under abiotic stress

Transcriptome is the complete set of transcripts of an organism expressed under certain set of conditions for example specific developmental stage or physiological conditions, etc. Transcript profile helps in analysing the expressed part of a genome under certain set of conditions. Transcriptome analysis can be carried out using several contemporary most advanced molecular techniques like polymerase chain reaction (PCR), real-time quantitative PCR (RT qPCR), microarray, serial analysis of gene expression (SAGE), expressed sequence tag analysis (EST), suppression subtractive hybridisation and RNA sequencing (RNA seq) (Udawat *et al.* 2016). Transcriptome analysis of legumes vis-a-vis different abiotic stresses has provided insight into genes and their related network, such as DREB, AP2/ERF, NAC, bZIP, and MYB/MYC transcription factors (TF) (Garg *et al.* 2015, Xu *et al.* 2018). Some grain legumes have comparable genomes but have exceptionally diverse gene expression profile, indicating extensive biochemical, physical and developmental differences. Therefore, developing and analysing relative expression profile of diverse tissues or cell types under different abiotic stresses, the interconnection between genome vis-a-vis gene function can be deciphered (Udawat *et al.* 2017). Varshney *et al.* (2009) has developed transcriptome profile of chickpea accessions namely, ICC 4958 and ICC 1882 under drought stress and generated 5982 and 5922 ESTs respectively. Relative expression profile of a groundnut genotype Huayu 25 using suppression subtractive hybridisation technique revealed a total of 111 differentially expressed genes (DEGs) and their associated ESTs (Ding *et al.* 2014). Functional studies of these genes provided vital information about their role in reactive oxygen species (ROS) scavenging, osmolyte accumulation. Triggering several stress induced signal transduction pathways, encoding several TFs, protein kinases, phosphatases, hormone signalling and other metabolic pathways under different abiotic stresses in groundnut (Govind *et al.* 2009, Udawat *et al.* 2017), and soybean (Rodrigues *et al.* 2015). Similarly, a transcriptome profile was developed by using qRT PCR in common bean with drought sensitive and tolerant cultivar, which led to the identification of stress responsive 22 NAC TFs belonging to 20 NAC gene families (Wu *et al.* 2016). Likewise, global transcriptome analysis of water stressed root tissues in several legumes, viz. chickpea, groundnut and lentil led to the identification

of DEGs associated to different biochemical pathways and several essential TFs belonging to WRKY, bZIP, bHLH, NAC, TFIIB, AP2/ERF, ZOS, NAC/NAM and MYB domain containing family (Brasilerio *et al.* 2015, Singh *et al.* 2017, Kumar *et al.* 2019). In a similar way, RNAseq analysis of chickpea desi and kabuli variety was studied under water deficit condition and led to discovery of genes associated to ion homeostasis, hormone signalling, several biochemical pathways, cell wall biosynthesis and several TFs such as NAC, MYB/MYC, bHLH, bZIP, WRKY, ZOS and AP2 (Garg *et al.* 2016, Mahdavi Mashaki *et al.* 2018). Using these transcriptome data sets, numerous candidate genes associated to drought, salt, cold and heat stress adaptation have been discovered in these grain legumes.

Proteomics under abiotic stress

Proteomics is studying the protein content both quantitatively and qualitatively of an organism under stressful condition and at a particular or different growth stages. Since last two decades the area of proteomics has witnessed regime change in the identification and characterization of plant proteins associated in response to abiotic stresses. Functional proteins obtained from translated part of the genome play a vital role in various plant processes including, plant response against different abiotic stresses. These studies are very useful as they suggest a global outlook of plant adaptation to unfavourable conditions. It has revealed role of different stress responsive proteins involved in regulating signal transduction, antioxidant scavenging mechanism and hormonal signalling to help acclimatize plant under stressful conditions (Dwivedi *et al.* 2006, Gruber *et al.* 2009). In legumes a substantial number of proteomic studies have been conducted to decipher the mechanism of abiotic stress adaptation. For example, a protein profile was studied using two dimensional gel electrophoresis (2-D) and matrix assisted laser desorption ionisation – time of flight (MALDI-TOF/TOF) in groundnut, lead to identification of 42 and 20 distinct proteins in the drought tolerant and sensitive cultivars, respectively. However, the study revealed that these unique proteins were found to be involved in abiotic stress responsive biochemical pathways, cellular defence, molecular chaperons, signal transduction and hormonal signalling (Katam *et al.* 2016, Thangella *et al.* 2018, Saxena *et al.* 2019, Saxena *et al.* 2021). Similarly, protein profile of soyabean at seedling stage was generated for root tips under water stress indicated the abundance of glycoprotein glucosyl transferase which is involved in carbohydrate metabolism for better tolerance against abiotic stress (Wang *et al.* 2016). Wang *et al.* (2017) further studied the protein profile of soyabean leaf and roots, and discovered role of β-glucosidase and β-amylase 5 in biochemical pathways to cope with drought stress. Interestingly the study revealed higher activity of aldehyde dehydrogenase during drought stress and elevated activity of peroxidase during recovery stage. A comparison of drought-sensitive and drought-tolerant genotypes of chickpea showed differential expression of numerous proteins engrossed in ROS homeostasis, enhanced seed germination and early seedling growth (Subba *et al.* 2013). Previous studies have also recommended a significant role of ROS in stress adaptation, tolerance was ascribed mainly to modified expression of proteins associated to ROS catabolism. According to the proteomics of salt-tolerant and salt-sensitive cultivar of soybean, it was assumed that tolerant cultivar had a better ability to provide energy supply, to regulate ROS equanimity, photosynthesis rate and ethylene production (Ma *et al.* 2012). Likewise, in context of proteins involved in vital cellular processes i. e. carbon fixation and oxidative phosphorylation, recently in a study conducted by Gayen *et al.* (2019) have shown considerable variation in mitochondrial protein profile under drought stress. Several post-translational modifications such as methylation, phosphorylation, sulphonation and glycosylation affects protein structure and function. However, several knowledge gaps are still there such as effect of these modifications on protein function during stressful conditions is still not exactly known. Therefore, it is necessary to understand the underlying molecular mechanism that makes PTMs discerning towards an explicit purpose. However, in legumes, further studies are required at cellular, molecular, and physiological level to enhance yield and to secure food supply with a lower fertilizer input (Matamoros and Manuel 2021).

Metabolomics under abiotic stress

Metabolomics is studying the complete metabolome or metabolites of an organism under particular set of conditions. Plants produce both primary and secondary metabolites which are significantly affected by environmental conditions including stressful situations. Moreover, metabolites production varies with different set of conditions. Therefore, understanding of metabolic profiling can provide comprehensive assessment of plant responses under abiotic stress conditions and may help to develop better drought tolerant legume varieties (Handa *et al.* 2021). Under different abiotic stresses, considerable variation has been observed in different metabolites related to vital cellular pathways such as carbohydrate metabolism, hormonal pathways, signal transduction and protein metabolism (Khan *et al.* 2018). Legumes are the major source of protein and also produce primary and secondary metabolites which play an important role in induction of abiotic stress tolerance in crops. The key metabolites that undergo changes under several abiotic stresses are proline, glucose, sucrose, galactinol, quercetin, γ-aminobutyric acid (GABA), sugar alcohols and phenolic compounds (Ullah *et al.* 2017). Metabolomic profile of groundnut drought tolerant cultivar has shown elevated nitrogenase activity in root nodule thus demonstrated higher availability of sucrose, asparagine and glutamine (Furlan *et al.* 2017). Similarly, higher accumulation of metabolites such as aspartate, sucrose, alanine, pinitol, histidine, choline, GABA, phenylalanine, and myo-inositol in soyabean and chickpea helped plant acclimatise under abiotic stresses (Silvente *et al.* 2012, Khan *et al.* 2018). Therefore, understanding the interplay of transcriptomics,

proteomics and metabolomics will offer a better picture of plant homeostasis and response to multiple uncertain stresses. Hence, metabolomics should become an integral part of a scientific study while understanding plant adaptation to abiotic stresses.

Modern breeding tools

Modern breeding tools such as multi-parent mapping by multi-parent advanced generation intercross (MAGIC tool), rapid breeding along with nested association mapping (NAM) has provided better opportunities to comprehend drought tolerant parameters in grain legumes (Li *et al.* 2018). Speed breeding involves flexible and cost-effective breeding protocols that have been standardized for long-day and day-neutral legumes to accelerate trait detection and introgression. So far several genes have been successfully integrated for improving root and nodule traits (Du *et al.* 2016) such as a photoperiod related gene *GmFT2a*, *GmDrb2a*and *GmDrb2b* in soybean (Cai *et al.* 2018, Curtin *et al.* 2018) and *SPL9* in *Medicago sativa* respectively (Gao *et al.* 2018). Therefore, advantages of these modern breeding techniques must be explored for developing abiotic stress tolerant varieties of grain legumes to improve yield and ensure global food security and sustainability (Li *et al.* 2017, Watson *et al.* 2018).

High-throughput integrated platforms

The draft genome and whole genome sequencing data of legumes have provided insight into the genomic sequences associated to evolution, domestication, population structure and their response to a variety of abiotic stresses. In addition, whole genome re-sequencing data has offered information related to diversity of signature genomic segments, unique alleles, markers and candidate genes associated to different stresses. Recently, next generation sequencing (NGS) approaches have been implemented in grain legumes that have huge merits over previous genotyping techniques. By delightfully taking benefit of advanced NGS technologies, draft genome has been prepared for pigeonpea (Varshney *et al.* 2012), chickpea (Varshney *et al.* 2013) and peanut (Chen *et al.* 2016). For instance, sequencing based approaches have been undertaken for mapping traits associated with flowering time, seed setting and pod dehiscence in pigeonpea, water stress related traits in chickpea (Kale *et al.* 2015), and early and late leaf spot in groundnut (Agarwal *et al.* 2020). Progresses in NGS-based sequencing technologies have shown transition from sanger sequencing of ESTs or microarray interpretation in legume expression studies. For instance, Illumina HiSeq was lately used to decipher trnascriptome changes associated to adaptation of desiccation tolerant PDL-2 and desiccation sensitive JL-3 lentil (*Lens culinaris* Medikus) seedlings to water stress (Singh *et al.* 2017).

Conclusion

Legumes are key source of proteins and provide food and nutritional security to a large segment of people. Research on legumes is lagging behind in terms of amalgamation of breeding programs with genomic approaches. Developing abiotic stress tolerant cultivars presents a big challenge to breeders due to complex inheritance of traits. This review has emphasized on advanced techniques such as genomics, transcriptomics, proteomics, metabolomics and modern breeding techniques for crop specific genome improvement. In conclusion, there is a big possibility for merger of conventional breeding with genomic and other multi-omics approaches along with several advancements, not only in research but also on farm and real time delivery of resources to the farmers and scientific community around the globe. At last, growing public concern of the wellbeing and nutritional advantages of leguminous crop based diet is crucial while considering the farming of legume crops for improving soil biological N_2 content and food security.

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