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Grain Legumes (Soybean, Chickpea, and Peanut): Omics Approaches to Enhance Abiotic Stress Tolerance

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Legumes rank third in world crop production, and abiotic stress is the major constraint to crop productivity. Biotechnological applications including all "omics" have been the direct and potential approaches for improving abiotic stress tolerance in grain legumes and requires knowledge of stress response at molecular level, which includes gene expression to protein or metabolite and its phenotypic effects. Genome-wide expression profiling studies have been carried out in the legumes to identify the candidate genes and regulatory networks among abiotic stress responses. Among the grain legumes, although soybean has been more intensively studied, more recently, sensitive and tolerant varieties of chickpea and peanut have been characterized under abiotic stress conditions. Nevertheless, proteomic studies in response to abiotic stress in legumes are still very limited with only *Medicago truncatula* and soybean protein reference maps available. Some of the major QTL controlling abiotic stress tolerance in legumes have been mapped for a major QTL for salt tolerance in soybean and drought tolerance-related traits in peanut. Although, *Agrobacterium*-mediated gene transfer has been reported in all the major legume crops, so far only one legume, that is, soybean, has been commercialized. Transgenic technologies for improved abiotic stress tolerance involving regulatory genes have proved more efficient than using single or multiple functional genes involved in stress tolerance. Hence, the current advances in "omics" technologies and availability of the genome sequences of model legumes and soybean offer great potential to improve the stress tolerance of the legume crops. This chapter attempts to provide a detailed discussion about the different "omics" approaches and their applications for abiotic stress research on major legumes.

39.1 Introduction

Legumes represent the most utilized plant family with 20 000 species and are among the most important crops worldwide, having major impacts on agriculture, the

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environment, and human/animal nutrition and health [1]. Legumes rank third behind cereals and oilseeds in world production [2] that accounts for 27% of the world's primary crop production [1]. Grain legumes constitute an important dietary constituent for humans and animals and these alone contribute 33% of the dietary protein nitrogen (N) needs of humans [3] besides being a source of income and livestock feed. These perfectly match the requirements of small-scale, low-income farmers in the developing countries where they accounted for 61.3 million hectares in 2002, compared to 8.5 million hectares in developed countries [2]. In order of rank, common beans (*Phaseolus* spp.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), broad bean (*Vicia faba* L.), pigeonpea (*Cajanus cajan* L.), cowpea (*Vigna unguiculata* L.), and lentil (*Lens esculentum* L.) constitute the primary dietary legumes [4]. Moreover, grain legumes, predominantly soybean (*Glycine max* L.) and peanut (*Arachis hypogeae* L.), are also a major source for vegetable oil, providing more than 35% of the world's processed vegetable oil.

Abiotic stress is the major constraint to crop productivity in the semiarid tropics (SAT) that include parts of 55 developing countries, populated by about 1.4 billion people, where grain legumes are mainly cultivated. Abiotic stress, which includes multiple stresses such as drought, salinity, waterlogging, high temperature, chilling, and so on are the primary causes of crop losses worldwide, reducing average yields for most major crop plants by over 50% [5, 6]. Only 10% of the global arable land can be classified under the nonstress category, which implies that crops grown on the other 90% of arable lands experience one or more environmental stresses [7]. Furthermore, crops under abiotic stress are usually more susceptible to weeds, insects, and diseases, which considerably increase the losses [8].

The grain legumes constitute important food and oilseed crops of the SAT, are mostly grown in low-input, rain-fed agriculture, and suffer from drought due to insufficient, untimely, and erratic rainfall in these climates that becomes major constraints to crop productivity. Several of the abiotic stresses associated with legume crops also directly affect symbiotic interactions and therefore limit their growth. Water deficits continue to be the major abiotic factor that affect crop yields globally [9] and are likely to worsen with the projected rapid expansion of water-stressed areas of the world encompassing 3 billion people by 2030 [10]. Moreover, in legumes such as peanut (*A. hypogaea*), Brazil nuts (*Bertholletia excelsa*), and faba bean (*V. faba*), aflatoxin contamination is a common occurrence during preharvest drought stress [11, 12]. In addition to drought, soil salinity is another major problem affecting the total nitrogen uptake and soil nitrogen contribution [13] resulting in reduced yields. Hence, there is a crucial need to increase the abiotic stress tolerance in legumes, which is a major challenge in crop improvement programs for enhancing yield stability. Although conventional plant breeding and enhanced management strategies have addressed several constraints that limit crop productivity or quality, there are situations where the existing genetic resources lack the required traits. Yield losses due to constraints like drought are highly variable in nature depending on the stress timing, intensity, and duration. Moreover, location-specific environmental stress factors such as high irradiance and temperature make breeding for drought

tolerance difficult through conventional approaches. Cutting-edge, knowledge-based breeding practices complemented adequately by genomics and genetic transformation technologies could lead to simpler and more effective gene-based approach for improving abiotic stress tolerance in the grain legumes. Application of biotechnological approaches has a potential to contribute efficiently to solve or reduce these problems in the grain legumes, thereby contributing to sustainable agriculture, especially in the SAT.

39.2 "OMICS" in Legumes and Abiotic Stress

Biotechnological approaches such as tissues culture, *in vitro* mutagenesis, marker-assisted breeding, and genetic transformation can speed up and overcome major bottlenecks of classical plant breeding due to the lack of natural sources of resistance and sexual incompatibility. However, successful application of biotechnology to abiotic constraints requires a good biological knowledge of both the target species and the mechanisms underlying tolerance to these stresses. Mechanisms of responses to stress can be measured at many different levels from the whole plant to the molecular level. The type, length, and severity of the stress have more influence on the plant response to stress [14]. Since responses are controlled by the plant genome, recent efforts have focused on the molecular response of the plant to water deficits [15]. Until a few years, the research on plant stress responses was focused on model plants such as *Arabidopsis*, and not much work was done on the legumes. However, since substantial similarities exist between the two crops, the knowledge on stress responses of *Arabidopsis* were used as source of information for legume research. Nevertheless, there are also significant fundamental differences like all physiological processes that differ and must be exploited to unravel the specific mechanisms involved in abiotic stress tolerance in the legumes [16]. Since the large genome size and the polyploidy of some legumes have hampered this goal, recent progress in legume biology has been greatly enhanced by the development of model systems to investigate the genetics of nodulation and other important processes such as resistance or tolerance to stresses. The two model legume plant systems, *Lotus japonicus* and *Medicago truncatula*, due to their small and diploid genomes, autogamous nature, short generation times, and prolific seed production were the obvious choices [17, 18]. Since then, powerful genetic and genomic tools have been developed that include genome sequencing [19], isolation of expressed sequence tags (ESTs) [20, 21], and establishment of genetic and physical maps for each model species [22, 23]. The increasing wealth of genetic and genomic data and the high degree of synteny between legume genomes [24, 25] make these two species valuable models for the molecular genetic study of the biotic and abiotic constraints that hamper legume crop yields. Furthermore, the soybean genome sequence and the high synteny between soybean and the model legumes have a potential to facilitate positional cloning and other genetic procedures for these studies.

While sequence information is invaluable and a necessary starting point, it is insufficient to answer questions concerning gene function, regulatory networks, and the biochemical pathways activated in response to stresses. To address these questions, more comprehensive approaches, including quantitative and qualitative analyses of gene expression products are necessary at the transcriptomic, proteomic, and metabolomic levels. This comprehensive knowledge about the genes involved in stress response and tolerance will further allow a more precise use of marker-assisted selection (MAS) and transgenics [7]. Since the "omics" involves genomics and functional genomics, genetic engineering, transcriptome profiling, proteomics, and metabolomics describing an organism's genome contribution to its overall phenotype, the recent progress made in these areas has considerably contributed to better understanding of the molecular and genetic basis of stress response that has been an important bottleneck for molecular and transgenic breeding. So far, a significant progress has been made in research on the abiotic stress tolerance of major legumes including soybean, chickpea, and peanut as discussed in the following sections.

39.3 Transcript "OMICS"

A eukaryotic cell contains ~15 000–30 000 distinct mRNAs with a prevalence ranging from one to several thousands in a total mass of ~100 000 mRNAs [26]. About 50% of the transcript population is made up of a relatively small number (some hundreds) of abundant transcripts representing only 1% of the different mRNA species, and the other half contains the "rare" mRNAs [27]. The set of all the messenger RNAs (mRNAs) in a cell/tissue/organism is referred to as the transcriptome and investigation of populations of mRNAs is thus called "transcriptomics." A genome-wide expression profiling is a powerful tool for studying genes involved in various biological phenomena, identifying the candidate genes, and revealing the molecular crosstalk of gene regulatory networks among abiotic stress responses.

Plants undergoing abiotic stresses in general face dehydration at the cellular level and hence almost 50% of the genes activated by these stresses including drought, salinity, or ABA treatment are common. Cellular water deficit in a plant stress triggers many changes in gene expression that in turn define its response to a particular environmental condition. The induced genes in response to cellular water deficit stress constitute different functional categories such as metabolism, transport, signaling, transcription, hydrophilic proteins, and the unknown, including the repression of genes involved in plant growth and development, such as photosynthesis-related genes. Broadly, the genes responding to abiotic stress can be categorized into two classes based on their response in terms of timescale or based on their involvement in tolerance; some respond immediately within seconds or minutes, while others respond later, in hours, days, or even weeks [28]. This allows for the speculation that the early responsive genes may provide initial protection and

regulate gene expression by being involved in amplification of signals and signal transduction. These include various protein kinases and genes encoding transcription factors, whereas the genes that respond later may be involved in adaptation to stress conditions, such as heat shock proteins, LEA proteins, ROS scavenger proteins, and so on [14, 28].

The genomic approaches allow changes induced by abiotic stresses on a global scale to be analyzed at the level of the whole organism. Much more extensive gene expression studies have been performed in *Arabidopsis*, and the resulting knowledge can also be used in legumes through comparative genomics. For example, Ishitani *et al*. [29] selected 100–200 genes from the *Arabidopsis* database and showed that at least 3 DREB-like genes, thought to be key transcriptional regulators of drought and/or cold tolerance, were present in common bean. Similarly, in *Arabidopsis*, analysis of the transcriptome changes occurring during cold, drought, and salt stress in a survey of 7000 genes showed a shared response for a majority of cold and drought stress-regulated genes, supporting the hypothesis that a common set of signal transduction pathways are triggered during different stress responses [30]. Around 11% of the stress-inducible genes are potential transcription factors further confirming the relevance of gene regulation in stress adaptation [31].

The *Arabidopsis* model is likely to be very different from legumes in terms of responses to stress in relation to grain filling, nitrogen utilization, fixation, and transport, root architecture, and interactions, all physiological processes that are fundamentally different in legumes. Hence, the usefulness of developing a legume model has become increasingly relevant in recent years. Moreover, the induction of gene expression by environmental stress must be exploited to unravel mechanisms dealing with abiotic stress tolerance in the agriculturally important grain legumes. In legumes, the gene expression patterns following biotic stresses have been more extensively studied than those following abiotic stresses. With respect to abiotic stress, gene expression analyses have been mainly based on studies with cloned genes [32]. Significant progress is being made at the genetic and genomic levels using the model legume *M. truncatula* through macro- and microarray analysis, reverse genetics, genome sequencing, and other high-throughput techniques [33, 34]. The analysis of almost 200 000 ESTs of *M. truncatula*, isolated from many different libraries constructed from diverse stages and treatments, was facilitated by searchable databases such as MtDB2 [35] and the TIGR Gene Index (http://www.tigr.org).

The advent of next-generation sequencing platforms [36], most recently the "third generation" (also called "next–next generation" or NGS) sequencing systems will enable plant genome to be sequenced within hours. The NGS approaches allow deciphering the cell's transcripts on the sequence level, which will truly revolutionize the research of organisms that are not now in line for genomic sequencing. This approach could circumvent the problems posed by extremely large genomes such as legumes. The next-generation sequencing not only is a dramatic advance over capillary-based sequencing but also presents significant challenges in assembly and sequence accuracy due to short read lengths, method-specific sequencing errors, and the absence of physical clones. However, the promise of much lower sequencing cost

with the now proven concept of next-generation expressed sequence tag sequencing will allow assessment of plant genomes at least at the functional level [37]. At ICRISAT, these NGS approaches are being used to develop EST-based markers to map the QTL for stress response in grain legumes. Recent reports have also shown that transcriptomic tools are a good option for legume breeding to environmental stresses as discussed in the next sections.

39.3.1 Soybean

Among the grain legumes, soybean has been more intensively studied and according to the legume information system data, over 1.3 million ESTs were developed from different cDNA libraries, which is the largest in number among the individual grain legume ESTs. The availability of a large number of EST and BAC sequences facilitated the discovery of new SNP and SSR markers in soybean toward the construction of high-resolution genetic maps. Besides, using a modified cDNA-AFLP technique in soybean, 140 differentially expressed cDNA fragments were obtained by comparing control and isoosmotic treated plants where some of the responsive genes encoded for ion transporters, transcription factors (TFs), and redox enzymes [38].

39.3.2 Chickpea

Chickpea is the most important food legume of semiarid tropics (SAT) and taxonomically one of the closest crops to the model legume *Medicago*. Sensitive and tolerant varieties of chickpea have been characterized under abiotic stress conditions, although very little is known about the genes involved in these responses. However, the characterization of genes involved in the differential behavior of these cultivars may constitute a good basis to extrapolate these results to other grain legumes. Five differentially expressed cDNAs were identified using differential display reverse transcriptase PCR (DDRT-PCR) under drought conditions with drought-tolerant cv. ICCV2 and drought-susceptible cv. ILC3279 of chickpea [39]. Moreover, 319 unique ESTs available from different libraries have been analyzed for differences in transcript profiling during drought stress treatment in two chickpea varieties having contrasting levels of drought tolerance (*C. arietinum* cv. PUSABGD72 and ICCV2). These ESTs were clustered in four groups according to their expression patterns [40].

A transcriptional profiling study in chickpea under drought, cold, and high salinity was carried out using cDNA microarray approach to look at the gene expression in the leaf, root, and/or flower tissues in tolerant and susceptible genotypes [41]. The differentially expressed transcripts in response to the particular stress were analyzed and a transcriptional change of over twofold was observed for 109, 210, and 386 genes after drought, cold, and high-salinity treatments, respectively. Among these, 2, 15, and 30 genes were consensually differentially expressed between tolerant and

susceptible genotypes studied for drought, cold, and high salinity, respectively. The differentially expressed genes coded for various functional and regulatory proteins, highlighting the multiple gene control and complexity of abiotic stress response mechanism in chickpea.

Two nonnormalized cDNA libraries from the seedling leaves of a drought-tolerant chickpea cultivar under PEG-treated and nontreated conditions have been constructed where 92 differentially expressed genes were identified [42]. Most of the upregulated genes were related to drought tolerance, while the downregulated genes were mainly involved in the photosynthesis. A set of over 2800 chickpea ESTs have been generated from a library constructed after subtractive suppressive hybridization (SSH) of root tissue from two closely related chickpea genotypes possessing different sources of drought avoidance and tolerance, ICC4958 (tester) and Annigeri (driver), respectively [43]. A total of 106 EST-based markers were designed from 477 sequences with functional annotations that were tested on *C. arietinum*. Forty-four EST markers were polymorphic when screened across nine *Cicer* species (including the cultigen) [44]. The chickpea root EST database developed in these studies provide researchers with a major new resource for data mining associated with root traits and drought tolerance [43]. More recently, a total of 20 162 drought- and salinity-responsive ESTs were generated from 10 different root tissue cDNA libraries of chickpea and 177 new EST-based SSR markers were developed [45].

Besides, SuperSAGE analysis for gene expression in chickpea roots in response to drought was carried out resulting in sequencing of 80 238 of 26 bp tags [46]. Among these tags, 7532 (43%) UniTags were more than 2.7-fold differentially expressed and 880 (5.0%) were regulated more than 8-fold upon stress resulting in unambiguous annotation of 22% (3858) of these tags. Microarray analysis of these 3000 annotated UniTags confirmed 79% of the tag-based results, whereas RT-PCR confirmed the SuperSAGE data in all cases. This is the first study to prove the potential of SuperSAGE technology for molecular breeding in the nonmodel crops. However, lack of availability of a chickpea reference genome limits the value of SuperSAGE tags, as only a fraction of them could be annotated.

39.3.3 Peanut

In peanut, differential DDRT-PCR has been used to identify differentially expressed genes in peanut grown under drought stress versus irrigation conditions where some drought-responsive mRNA transcripts were identified based on expression pattern [47, 48]. Besides, DDRT-PCR studies have been carried out with transgenic peanut events overexpressing rd29A:DREB1A to detect the differentially expressed transcripts under abiotic stress [49]. Here, 51 differentially expressed transcripts were identified under stress treatments; among them 35 transcripts were newly expressed, 11 were upregulated, and 5 were downregulated. In the BLAST search of differentially expressed partial cDNAs, only 17 clones showed a significant similarity to the ESTs in the database, indicating that the majority of the cDNAs cloned in this study may be novel and needs further research to identify their role in stress response. These

results also suggested that the increased plant tolerance against drought stress in transgenic peanut may not be attributable only to the expression of DREB1A-targeted cold-responsive (COR) genes identified in *Arabidopsis* [49].

In a recent study, six different cDNA libraries were constructed from developing peanut seeds at three reproduction stages (R5, R6, and R7) from a resistant and a susceptible cultivated peanut genotype, "Tifrunner" that is susceptible to *Aspergillus* infection with higher aflatoxin contamination and resistant to *tomato spotted wilt virus* (TSWV) and "GT-C20" that is resistant to *Aspergillus* with reduced aflatoxin contamination and susceptible to TSWV. The developing peanut seed tissues of these genotypes were challenged by *Aspergillus parasiticus* and drought stress in the field and 21 777 high-quality EST sequences were generated from cDNA clones of 6 libraries [50]. Similarly, EST libraries for cultivated peanut were developed from leaves of peanut line C34-24 (resistant to leaf spots and TSVW) and immature pods of peanut line A13 (tolerant to drought stress and preharvest aflatoxin contamination). A total of 1825 ESTs, 769 from the C34-24 and 1056 from the ESTs were identified and 44 EST-derived simple sequence repeat (SSR) markers have been characterized for cultivated peanut [51]. A total of 6264 high-quality ESTs were generated from leaves and roots of a wild peanut *Arachis stenosperma*, and 188 microsatellite markers have developed form these ESTs [52].

More recently, nearly 700 genes were identified in subtractive cDNA library from gradual process of drought stress adaptation in peanut. This study also showed the functional importance of HSP70 gene and key regulators such as Jumonji in drought stress response [53]. A high-density oligonucleotide microarray for peanut has also been developed using 49 205 publicly available ESTs and tested the utility of this array for expression profiling in a variety of peanut tissues [54]. Over 108 putatively pod-specific/abundant genes, as well as transcripts, whose expression was low or undetected in pod compared to peg, leaf, stem, or root were detected. Several transcripts that significantly overrepresented in the peanut pod included genes responsible for seed storage proteins and desiccation (e.g., late-embryogenesis abundant proteins, aquaporins, legumin B), oil production, and cellular defense [54].

39.4 Prote"omics"

Since the 1990s, genomics has been the most active research field in biological science generating a huge amount of information, while structural genomics has emerged at the methodological level to understand gene expression and function. A complete knowledge of the proteins expressed by the genome of a cell, tissue, or organism at a specific time point (proteome) is necessary to understand the biology of a cell or an organism. The proteome reflects the actual state of the cell or the organism and is an essential bridge between the transcriptome and the metabolome. Proteins act directly on biochemical processes, and thus must be closer to the phenotype, compared to DNA-based markers. Although research on plant responses to stress on the DNA or RNA level provided an important insight into stress tolerance, the

proteomics approach is very important in evaluating stress responses since the mRNA levels may not always correlate with protein accumulation [55]. In addition, many proteins are modified by posttranslational modifications such as phosphorylation, glucosylation, and ubiquitinylation, which significantly influence protein functions. Proteomics, understood as protein biochemistry on an unprecedented and high-throughput scale, is becoming a promising and active approach in this postgenomic period. However, its application to plants is rather limited compared to other biological systems [56].

Compared to analysis of the transcriptome, analysis of the plant proteome in response to abiotic and biotic stresses is still limited, although good technical progress has been achieved in the separation of proteins and their identification by mass spectrometry. Studies have evaluated changes in protein levels in plant tissues in response to stresses [57, 58]. However, these studies have mainly focused on nonlegume species such as *Arabidopsis* and rice [57] and some legumes recently [56]. As a result, only a handful of studies have been carried out in legumes, although in the next few years there should be a significant increase in the number of legume species and stresses analyzed. So far, pea has been more intensively studied, with the analysis of induced protein expression in roots in response to salt [59] and to cadmium stress [60]. Recently, *M. truncatila* has been the subject of several proteomic studies that represent the most extensive proteomic description of *M. truncatula* suspension cells to date and provide a reference map for future comparative proteomics and functional genomics studies of biotic and abiotic stress responses [61].

39.4.1 Soybean

Some reference maps of soybean that are available in the proteomics database provide a starting point for ongoing functional genomics studies associated with biotic/abiotic stress in soybean. The Soybean Proteome Database is aimed to be a data repository for functional analyses of soybean responses to flooding injury that is recognized as a major constraint for the establishment and production of this plant. The latest release contains 21 reference maps of soybean (*G. max* cv. Enrei) proteins electrophoresized on two-dimensional polyacrylamide gels of which the samples were collected from several organs, tissues, and organelles. These reference maps included 7311 detected proteins and 532 identified proteins, or proteins for which a sequence or peptide peak has been determined. The Soybean Proteome Database also integrates multiple "omes," where an "omics" table reveals relationships among 106 mRNAs, 51 proteins, and 89 metabolites that vary over time under flooding stress. The tabulated metabolites are anchored to a metabolome network. A unified temporal profile tag attached to the mRNAs, proteins, and metabolites facilitates retrieval of the data based on the temporal expression profiles. A graphical user interface based on dynamic HTML facilitates viewing of both the metabolome network and the profiles of multiple "omes" in a uniform manner. The entire database is available at http://proteome.dc.affrc.go.jp/soybean/ [62].

39.4.2 Chickpea

Most of the earlier understanding of dehydration-responsive cellular adaptation in chickpea has evolved from transcriptome analysis and the comparative analysis of dehydration-responsive proteins, particularly proteins in the subcellular fraction, is limited. Bhushan *et al*. [63] have initiated a proteomics approach to identify dehydration-responsive ECM proteins in JG-62, a drought-tolerant variety of chickpea where the dehydration-responsive temporal changes in ECM proteins revealed 186 proteins with variance at a 95% significance level. The comparative proteomics analysis led to the identification of 134 differentially expressed proteins that include predicted and novel dehydration-responsive proteins. This study, for the first time, demonstrated that over a 100 ECM proteins are presumably involved in a variety of cellular functions, namely, cell wall modification, signal transduction, metabolism, and cell defense and rescue, and impinge on the molecular mechanism of dehydration tolerance in plants. Since the nuclear proteins constitute a highly organized, complex network that plays diverse roles during cellular development and other physiological processes. Another study provided insights into the complex metabolic network operating in the nucleus during dehydration in chickpea [64]. Approximately, 205 protein spots were found to be differentially regulated under dehydration; mass spectrometry analysis allowed the identification of 147 differentially expressed proteins, presumably involved in a variety of functions including gene transcription and replication, molecular chaperones, cell signaling, and chromatin remodeling. The dehydration-responsive nuclear proteome of chickpea revealed a coordinated response, which involves both the regulatory and the functional proteins.

39.4.3 Peanut

In peanut very few proteomic studies were conducted on stress response; in a recent study with selected tolerant and susceptible peanut genotypes from the US minicore collection were analyzed for changes in leaf proteins under water deficit stress [65]. A total of 102 protein bands/spots were analyzed by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) and by quadrupole time-of-flight tandem mass spectrometry (Q-TOF MS/MS) analysis. Forty-nine nonredundant proteins were identified that implicated a variety of stress response mechanisms in peanut. It was observed that lipoxygenase and 1L-myo-inositol-1-phosphate synthase, which aid in inter- and intracellular stress signaling, were more abundant in tolerant genotypes under water deficit stress. Here, the acetyl-CoA carboxylase, a key enzyme of lipid biosynthesis, increased in relative abundance along with a corresponding increase in epicuticular wax content in the tolerant genotypes suggesting an additional mechanism for water conservation and stress tolerance. In addition, there was a marked decrease in the abundance of several photosynthetic proteins in the tolerant genotype along with a concomitant decrease in net photosynthesis in response to water deficit stress.

39.5 Metabol"omics"

Undoubtedly, transcriptomic and proteomic data are important steps in deciphering a complex biological process, but they are still insufficient since most biological processes are ultimately mediated by cell metabolites. Metabolomics is considered to provide a direct "functional readout of the physiological state" of an organism. Besides, alternative mRNA splicing, protein turnover rates, and posttranslational modifications that modulate protein activity imply that changes in the transcriptome or proteome do not always correspond to alterations in the cell metabolome [66]. Target analysis, metabolite profiling, and metabolic fingerprinting are different conceptual approaches in metabolomics that can be used for a large range of applications, including phenotyping of genetically modified plants, substantial equivalence testing, determining gene function, and monitoring responses to biotic and abiotic stresses. Metabolomics can therefore be seen as bridging the gap between genotype and phenotype. Metabolic changes underpin plant development and responses to applied stresses, and that metabolic information reflects biological endpoints more accurately than transcript or protein analysis. Hence, the only way to the complete understanding of both gene function and molecular events controlling complex plant processes is to analyze the transcriptome, the proteome, and the metabolome in an integrative manner [67].

In legumes, the metabolomic approach has been used in *M. truncatula* suspension cells to determine the responses to various stimuli [68]. Although, large-scale comprehensive metabolomic studies are difficult, a number of targeted analyses have been performed to assess the involvement of subsets of metabolites in various stresses. Although the preliminary results from combining metabolic approaches with transgenics indicates the potential of increasing intrinsic stress resistance levels in legume crops and strengthens the potential role of biotechnology in crop improvement [69, 70], it must be emphasized that most metabolic pathways are interconnected in highly complex networks. Thus, modulating one metabolic pathway may have negative impacts on another, leading to concomitant deleterious traits in the modified crop. Large-scale metabolic analyses are therefore necessary to observe the metabolic networks important for plant growth and development under a range of environmental conditions.

39.6 Gen"omics"

Genomics involves the development of molecular markers for genetic diversity analysis and it provides novel opportunities to manipulate QTL through marker-assisted selection to develop improved cultivars. The use of genetic and genomic analysis to help identify DNA regions tightly linked to agronomic traits in crops, the so-called 'molecular markers, can facilitate breeding strategies for crop improvement. The use of molecular markers for the indirect selection of improved crops can

speed up the selection process by alleviating time-consuming approaches of direct screening under greenhouse and field conditions.

39.6.1 Soybean

The availability of the soybean genome sequence in combination with the integrated genetic and physical maps are valuable resources providing soybean researchers powerful and efficient genomic tools to identify and characterize genes or QTL for agronomic traits of soybean, facilitating marker-assisted breeding and soybean improvement. In soybean, *G. max* (L.) Merr., substantial genetic variation exists for salt response. In order to identify QTL associated with salt tolerance in soybean, lines from the cross of "S-100" (salt tolerant) × "Tokyo" (salt sensitive) were evaluated in saline fields where each line was characterized with RFLP markers and an initial QTL single-factor analysis was completed. These results were used to identify genomic regions associated with the trait and to saturate the selected genomic regions with SSR markers to improve mapping precision. Subsequently, a major QTL for salt tolerance was discovered near the Sat_091 SSR marker on linkage group (LG) N. The strong relationship between the SSR marker alleles and salt tolerance suggested that these markers could be used for marker-assisted selection in commercial breeding [71] (Table 39.1).

Table 39.1 List of major identified QTL associated with abiotic stress in important legume crops.

Legume	Abiotic stress	Marker type	References
L. culinaris	Cold	RAPD	[156]
	Winter hardiness	SSR	
	Winter hardiness	SSR, RAPD AFLP	[157]
G. max	Manganese toxicity	SSR, RAPD	[158]
	Salt stress	SSR	[71]
	Waterlogging	SSR	[159]
	Phosphorus deficiency	SSR, RFLP, EST	
	Phosphorus deficiency	SSR	[160]
Medicago sativa	Aluminum toxicity	RFLP	[161]
A. hypogaea	Transpiration	SSR	[74]
	Transpiration efficiency	SSR	
	Specific leaf area (SLA)	SSR	
	SPAD chlorophyll meter reading (SCMR)	SSR	
	SPAD at stage of harvest	SSR	

Q5 SPAD: ; RAPD: random amplified polymorphism DNA; RFLP: restriction fragment length polymorphism; AFLP: amplified fragment length polymorphism; SSR: simple sequence repeat; EST: expressed sequence tag.

39.6.2
Chickpea

MAS is being deployed in chickpea at ICRISAT to introgress QTL alleles associated with a large root size into elite germplasm [72]. Terminal drought can curtail chickpea yield from 20% to more than 50%. Hence, a deep root system capable of extracting additional soil moisture should positively impact yield in drought-prone areas [73].

39.6.3
Peanut

At ICRISAT, the first genetic map for cultivated peanut (*A. hypogaea*), an amphidiploid (4X) species, was developed that its utility demonstrated for molecular mapping of QTL controlling drought tolerance-related traits and establishing relationships with diploid AA genome of groundnut and model legume genome species. In order to develop a genetic linkage map for tetraploid cultivated groundnut, 1145 microsatellite or simple sequence repeat (SSR) markers available in public domain as well as unpublished markers from several sources were screened on two genotypes, TAG 24 and ICGV 86031, which are parents of a recombinant inbred line mapping population. As a result, 144 (12.6%) polymorphic markers were identified that amplified 150 loci. A total of 135 SSR loci could be mapped into 22 linkage groups (LGs) [74] (Table 39.1).

39.7
Functional Genomics

Large-scale analysis by using different "omics" technologies are providing extensive data sets that will help identify potential candidate genes for an increase in intrinsic resistance and/or tolerance levels in important legume crops. Identification of these candidate genes may allow their direct application in crop improvement through MAS or genetic engineering. However, in most cases, the roles of these candidate genes remain unknown and it will be important to carry out functional studies as a preliminary step toward their use in genetic improvement. To date, the *Arabidopsis*, rice, *M. truncatula*, and *L. japonicus* genomes have been sequenced and the genome sequencing projects of some other plants is underway. The traditional pursuit of a gene starting with a phenotype (forward genetics) has paved the way for the opposite situation where the gene sequences are known but not their functions. The challenge is to decipher the function of thousands of genes identified by genome projects where reverse genetics methodologies will be the key tools. The ability to knockout genes or suppress their expression are powerful tools to determine the function of a gene. This can be done by antisense RNA suppression, targeted gene replacement, insertional mutagenesis, gene silencing through RNAi, and targeted induced local lesion in genome (TILLING) approaches.

39.7.1
Gene Silencing Approaches

Antisense RNA suppression requires considerable effort for any given target gene before even knowing whether it will be successful [75]. In *Arabidopsis*, collections of random T-DNA (over 225 000 independent *Agrobacterium* T-DNA insertions) or transposable element insertion mutants are available [76]; such a collection does not exist yet for the legumes. Targeted gene replacement via homologous recombination has not yet been reproducibly achieved for higher plants. Although collections of T-DNA mutants may be very useful, they produce a limited range of allele types and do not always produce null alleles [77, 78]. Recently, the use of the tobacco retrotransposon Tnt1 has been successfully applied for large-scale insertional mutagenesis in *M. truncatula* that promises to be a useful tool for functional genomics [79].

The term RNA silencing broadly has been adopted to describe phenomena such as posttranscriptional gene silencing (PTGS) in plants, quelling in fungi, and RNA interference in animals [80]. Researchers have developed different RNA silencing strategies as tools for selective knockout of targeted genes. Virus-induced gene silencing (VIGS) has been developed to suppress plant gene expression through infection with virus vectors that harbor a target region of the host gene [80, 81]. There are vectors available that have the ability to support VIGS in plants [82, 83]; these have not yet been used extensively in legumes.

Since VIGS in peanut is not yet feasible, 25 peanut water deficit stress-induced cDNAs were characterized in a heterologous species *Nicotiana benthamiana* [84]. Increased membrane damage was seen under water deficit stress in most of the silenced plants signifying that many of these stress-induced genes were important to confer drought tolerance. Under water stress, silencing of homologue of flavonol 3-*O*-glucosyltransferase (F3OGT), a homologue of alcohol dehydrogenase, a homologue of salt-inducible protein, and a homologue of heat shock protein 70 showed more visible wilting symptoms compared to the controls. Interestingly, downregulation of two genes, homologous to aspartic proteinase 2, and Jumonji class of transcription factor showed relative drought-tolerant phenotypes. Moreover, F3OGT-silenced plants showed more wilting symptoms, membrane damage, and chlorophyll degradation than any other type during water deficit. These results demonstrated that VIGS approach can be used to characterize and assess the functional relevance of water-deficit-stress-induced cDNAs in a heterologous species.

39.7.2
TILLING

The limitations of RNA silencing or insertational mutagenesis can be overcome by TILLING that combines chemical mutagenesis with a powerful screening method for potential mutations [75, 85, 86]. The generation of phenotypic variants without introducing foreign DNA in the plant makes TILLING very suitable not only for functional analysis but also for agricultural applications. The TILLING facility for collection of mutants is available for *L. japonicus* [87] and *M. truncatula* (U.C. Davis,

USA; CNRS, Gif-Sur-Yvette, France). TILLING facilities are also being extended to a wider variety of legumes including soybean and peanuts.

39.8 Transgenomics

The use of transgenic technology or "transgenomics" potentially offers a more targeted gene-based approach for gaining valuable information to understand the mechanisms governing stress tolerance, providing a complementary means for the genetic enhancement of field crops, thereby alleviating some of the major constraints to crop productivity in developing countries [88]. Tissue culture has been repeatedly described as difficult in grain legumes. Regeneration from both organogenesis and embryogenesis has been reported to be recalcitrant in this plant group [89, 90] and has been attributed as a major constraint in transgenic development for many legumes. Since advances in molecular genetics, for example, gene overexpression, gene suppression, promoter analysis, and T-DNA tagging require efficient transformation systems [91]. Implementation of robust protocols for regeneration in legumes is therefore a necessary condition for genetic transformation.

In plants, upon exposure to abiotic stress, a number of genes are turned on resulting in increased levels of several osmolytes and proteins that may be responsible for conferring a certain degree of protection from these stresses. Therefore, it may be necessary to transfer several potentially useful genes into the same plant in order to obtain a high degree of tolerance to drought or salt stress. Novel genes accessed from exotic sources of plants, animals, bacteria, and even viruses can be introduced into the crop through various genetic transformation methods [9] with the possibility of controlling the timing, tissue specificity, and expression level of transferred genes for their optimal function.

The feasibility of using *Agrobacterium tumefaciens*-mediated gene transfer has been an important breakthrough in legume transgenic research although the rate of recovery of transgenic lines is still low in many cases [90, 91]. To date, genetic transformation has been reported in all the major legume crops such as *Vigna* species, *C. arietinum*, *C. cajan*, *Phaseolus* spp., *Lupinus* spp., *Vicia* spp., *P. sativum*, and soybean. Despite being crucial to tropical agriculture, transgenic grain legumes with an exception of soybean have not moved out from laboratories to large farm lands compared to their counterparts, "cereals" [92]. For example, the increase in tolerance to aluminum toxicity in transgenic alfalfa [93] and cyanamide toxicity in transgenic soybean [94] demonstrates the potential of this approach in legumes (Table 39.2). At ICRISAT, efficient transformation protocols have been developed for legume crops including groundnut, pigeonpea, and chickpea. A more exhaustive review of the application of transgenesis to overcome abiotic stresses in plants is provided in Ref. [9].

Various transgenic technologies for improved stress tolerance have been developed involving the expression of functional genes including those encoding for enzymes required for the biosynthesis of osmoprotectants [95–97] or modifying membrane lipids [98, 99], late embryogenesis proteins [100], and detoxification

Table 39.2 Selective reports on production of abiotic stress-tolerant transgenic legumes.

Gene	Protein	Source(s)	Cellular role	Transhost	Promoter used	Performance of transgenics	References
NTR1 (nectarin1)	Jasmonic acid carboxyl methyltransferase	*B. campestris*	Methyl jasmonate	*G. max*	CaMV35S		[119, 120]
Cah	Cyanamide hydratase	*Myrothecium verrucaria*	Cyanamide hydratase	*G. max*	*A. thaliana* actin-2 promoter	Tolerance to cyanamide toxicity	[94]
DREB1A	DRE-binding protein	*A. thaliana*	Transcription factor	*A. hypogaea*	*A. thaliana* RD29A promoter		[112]
DREB1A	DRE-binding protein	*A. thaliana*	Transcription factor	*C. arietinum*	*A. thaliana* RD29A promoter		[Development of transgenic chickpea for drought tolerance (ICRISAT unpublished data).]
p5cs	O1-pyrroline 5-carboxylate synthase	*V. aconitifolia*	Proline biosynthesis	*C. arietinum*	CaMV35S		[122]
codA	Choline oxidase A	*Arthrobacter globiformis*	Glycine-betaine biosynthesis	*C. arietinum*	CaMV35S with a chloroplastic transit peptide		[121]
GmDREB1	DRE-binding protein	*G. max*	Transcription factor	*M. sativa*	*A. thaliana* RD29A promoter	Salt tolerance	[137]
WXP1	AP2 domain	*M. truncatula*	Wax biosynthesis	*M. sativa*	CaMV35S	Tolerance to drought	[162]
MDH	Malate dehydrogenase	*M. sativa*	Malate dehydrogenase	*M. sativa*	CaMV35S	Tolerance to aluminum toxicity	[93]

fe-sod	Fe-superoxide dismutase	*N. plumbaginifolia*	Dismutation of reactive oxygen intermediates in chloroplasts	*M. sativa*	CaMV35S with a chloroplastic transit peptide	Showed increased Fe-SOD activity, which was associated with increased winter survival	[163]
mn-sod	Mn-superoxide dismutase	*N. plumbaginifolia*	Dismutation of reactive oxygen intermediates in mitochondria	*M. sativa*	CaMV35S with a chloroplastic and mitochondrial transit peptide	Showed significantly greater survival in field under water stress and in winter	[100, 164]
sod	Superoxide dismutase	*N. plumbaginifolia, P. sativum*	Dismutation of toxic reactive oxygen intermediates	*M. sativa*	CaMV35S	Showed increased regrowth after freezing stress	[165]

enzymes [101]. A widely adopted strategy has been to engineer certain osmolytes for their overexpression in plants to develop stress-tolerant crops [102–107]. However, the approaches involving the transfer of a single functional gene have not proven very effective in improving plant tolerance beyond the short-term effects that have been reported [108, 109]. Hence, multiple mechanisms to engineer water stress tolerance have been utilized and studies involving regulatory genes have been more efficient [108–112].

39.8.1 Soybean

The first report of soybean (*G. max*) transformation was published in 1988 where both *Agrobacterium*-mediated transformation [113] and particle bombardment method were used [114]. At present, soybean is the only transgenic legume crop that is under commercial cultivation. Roundup ready soybean was the first transgenic soybean resistant to herbicide, commercially released in the United States in 1996 by Monsanto company (http://www.monsanto.com/history.asp), which was grown commercially in seven countries, the United States of America, Argentina, Canada, Mexico, Romania, Uruguay, and South Africa in 2001 [115]. Globally, herbicide-tolerant soybean occupied 33.3 million hectares, representing 63% of the global transgenic crop area of 52.6 million hectares for all crops by 2001 [115]. There have been numerous excellent reviews on gene technology applications in soybean [91, 116–118]. Recent reports on transgenic soybean for abiotic stress tolerance include transformation with coding sequence for cyanamide hydratase (Cah), an enzyme that converts toxic cyanamide to urea, from the soil fungus *Myrothecium*. Cah expression detoxified cyanamide in leaf callus and embryogenic cultures of soybean as well as in whole plants as shown by cyanamide resistance [94]. Another study on the constitutive expression of *nectarin1* (*ntr1*) gene from *Brassica campestris* in transgenic soybean resulted in enhanced accumulation of methyl jasmonate (MeJA). *NTR1* gene encodes jasmonic acid carboxyl methyl transferase, which is an important plant regulator involved in plant development that regulates the expression of plant defense genes in response to various stresses such as wounding, drought, and pathogens. The higher levels of MeJA in the transgenic soybean plants conferred tolerance to dehydration during seed germination and seedling growth as reflected by the percentage of the fresh weight of seedlings. In addition, the transgenic soybean plants also conferred better capacity to retain water than wild-type plants when drought tolerance was tested using detached leaves [119, 120].

39.8.2 Chickpea

Since it is believed that osmoregulation is one of the best strategies for abiotic stress tolerance, especially if osmoregulatory genes could be triggered in response to drought, salinity, and high temperature. A prokaryotic osmoregulatory choline oxidase gene (*codA*) has been targeted at the chloroplasts to enhance the potential

of photosynthetic machinery of chickpea to withstand oxidative damage. Chloroplasts from plants of transgenic lines were evaluated for their efficacy to withstand photoinhibitory damage where the loss in PS II activity in chloroplasts of wild-type plants exposed to high light intensity was significantly higher than that in chloroplasts of transgenic chickpea. The results indicated that H_2O_2 produced by *codA* as a by-product during synthesis of glycine-betaine is responsible for building stronger antioxidant system in chloroplasts of transgenic chickpea plants [121]. Similarly at ICRISAT, the P5CSF129A gene encoding the mutagenized *D1-pyrroline-5-carboxylate synthetase* (P5CS) for the overproduction of proline was introduced in chickpea. The accumulation of proline in several of these transgenic events was more pronounced and increased significantly in the leaves when exposed to water stress along with a decrease in free radicals as measured by a decrease in the malonaldehyde (MDA) levels, a lipid peroxidation product [122]. However, the overexpression of proline appeared to have no beneficial effect on biomass accumulation since only a few events showed a significant increase in the biomass production toward the end of the progressive drying period. In any case, the overexpression of P5CSF129A gene resulted only in a modest increase in the transpiration efficiency (TE), thereby indicating that the enhanced proline had little bearing on the components of yield architecture that are significant in overcoming the negative effects of drought stress in chickpea. These results agree with the previous reports in other crops [123–125], and, in our own assessment, the gene affecting single protein might be less efficient in coping with water-limiting conditions [122].

To address the multigenicity of the plant response to stress, a strategy to target transcription factors that regulate the expression of several genes related to abiotic stress was considered. Regulatory genes or transcription factors, more specifically those belonging to the AP2/ERF family, have previously been shown to improve stress tolerance under lab conditions by regulating the coordinated expression of several stress-related genes in heterologous transgenic plants [111, 112, 126]. Hence, a large number of transgenic plants of chickpea carrying the *DREB1A* transcription factor from *Arabidopsis thalianna*, driven by a stress-inducible promoter from *rd29A* gene from *A. thaliana*, have been developed [Development of transgenic chickpea for drought tolerance (ICRISAT unpublished data).].

39.8.3 Peanut

The transfer of individual genes to plants, for acquiring higher stress tolerance, has so far had only a limited impact. However, the simultaneous transcriptional activation of a subset of those genes, by transferring transcription factors, has been revealed as a promising strategy [127, 128]. Using transgenic plants carrying regulatory genes, specifically those belonging to the AP2/EREBP family (DREB1A), proved an efficient method to improve the abiotic stress tolerance of crop plants [111, 112, 126]. The overexpression of *DREB1A* under the control of a constitutive promoter was detrimental when stress was not applied, although it had a positive effect on plants under stress. The use of the stress-inducible promoter from rd29A, instead of the

CaMV35S promoter, to overexpress *DREB1A* minimized the negative effects on plant growth [111]. Since improving the water use efficiency (WUE) of a plant is a complex issue, efforts to breed groundnut genotypes for high TE and stomatal conductance have obtained limited success. At ICRISAT, the transgenic groundnut plants carrying *DREB1A* transcription factor from *A. thaliana* driven by a stress-inducible promoter from *rd29A* gene also from *A. thaliana* have been shown to improve drought tolerance under greenhouse conditions [112]. A few transgenic events with contrasting responses have been selected for further detailed studies on the gas exchange characteristics of leaves. Besides, the biochemical responses of plants under identical conditions of water stress have been examined critically to further understand the mechanisms underlying environmental stress resistance in these transgenic events [109].

39.8.4 Candidate Genes from Legumes

There are several reports on candidate genes being cloned from legumes and tested in model plants for abiotic stress tolerance (Table 39.3). These advances suggest good prospects for developing transgenic legumes with enhanced tolerance to abiotic stress in the near future. There have been reports on manipulating the expression of pea DNA *helicase45* or the glyoxalate pathways conferring high salinity tolerance in tobacco [129, 130]. Similarly, ectopic expression of the *AhNCED1* gene (which results in oxidative cleavage of *cis*-epoxycarotenoids) in *Arabidopsis* improved the water stress tolerance levels by causing accumulation of endogenous ABA [131]. Besides, a *CarNAC1* gene (for NAM, ATAF1,2, and CUC2) was isolated from a cDNA library constructed from chickpea (*C. arietinum* L.) seedling leaves treated by polyethylene glycol and has been found to play important roles in plant development and stress responses [132]. Another cDNA clone encoding a dehydrin gene, *cpdhn1*, was isolated from a cDNA bank prepared from ripening seeds of *C. pinnatifidum* [133]. Since the gene expression was induced not only during seed development but also in leaves in response to drought, chilling, and salinity and to treatment with ABA or methyl jasmonate, the CpDHN1 protein may have a role in tolerance to a variety of environmental stresses, both abiotic and biotic. In another effort, a *CAP2* gene from chickpea encoding a novel AP2 family transcription factor that increased under dehydration has been characterized [134]. The CaMV35S promoter-driven expression of CAP2 in tobacco resulted in increased tolerance to dehydration and salt stress than the wild-type plants. Besides, transgenic plants expressed higher steady-state transcript levels of abiotic stress response genes NtERD10B and NtERD10C and auxin response genes IAA4.2 and IAA2.5, indicating a mutual interrelation between plant growth and development and abiotic stress response pathways and a probable involvement of CAP2 in both the signaling pathways.

Several transcription factors of AP2 family including DREB homologue and ERF transcription factors have been isolated from soybean and were characterized by their expression in transgenic plants. GmDREB2 [135] GmDREB3 [136] from soybean was expressed in *Arabidopsis* and has shown tolerance to drought and salt stress, whereas

Table 39.3 Abiotic stress-responsive genes characterized from legumes.

Gene	Protein	Source(s)	Cellular role	Transhost	Promoter used	Tolerance	Reference
CAP2	AP2/EREBP	*C. arietinum*	Transcription factor	*N. tabacum*	CaMV35S	Drought and salt tolerance	[134]
bip	Binding protein	*G. max*	Molecular chaperone involved in unfolded protein response	*N. tabacum*	CaMV35S	Water stress	[166]
GmbZIP44, GmbZIP62, and GmbZIP78	bZIP	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Salt and freezing stresses	[141]
GmCHI	Chilling inducible	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Cold, drought, and salt tolerance	[143]
GmDREB2	AP2/EREBP	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Drought and salt tolerance	[135]
GmDREB3	AP2/EREBP	*G. max*	Transcription factor	*Arabidopsis* and *N. tabacum*	CaMV35S *A. thaliana* RD29A promoter		[136]
GmDREBa, GmDREBb, and GmDREBc	AP2/EREBP	*G. max*	Transcription factor	Yeast one hybrid	—	Response to abiotic stresses	[138]
GmERF057 and GmERF089	AP2/EREBP	*G. max*	Transcription factor	*N. tabacum*	CaMV35S promoter	Drought and salt	[139]
GmGT-2A and GmGT-2B	Trihelix	*G. max*	Transcription factor	*Arabidopsis*	—	Abiotic stresses	[144]
GmMYB76, GmMYB177, and GmMYB92	MYB	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Salt and freezing	[140]
GmPHD2	Alfin1-type PHD finger protein	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Salt tolerance	[145]
GmTP55, antiquitin homologue	ALDH7 family	*G. max*	Abiotic stress responsive	*N. tabacum* and *Arabidopsis*	CaMV35S	Drought and salt	[146]

(*Continued*)

Table 39.3 (*Continued*)

Gene	Protein	Source(s)	Cellular role	Transhost	Promoter used	Tolerance	Reference
GmWRKY13	WRKY	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Increased sensitivity to salt and mannitol stress	[142]
GmWRKY21	WRKY	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Cold stress	[142]
GmWRKY54	WRKY	*G. max*	Transcription factor	*Arabidopsis*	CaMV35S	Salt and drought	[142]
SCOF-1 soybean cold-inducible factor-1	C2H2 (Zn)	*G. max*	Transcription factor	*Arabidopsis* and *N. tabacum*	CaMV35S	Cold	[167]
alfin1	Zn finger family of proteins	*M. sativa*	Transcription factor	*M. sativa*	CaMV35S	Salt	[168]
msalr	NADPH-dependent aldose/aldehyde	*M. Sativa*	Detoxification	*N. tabacum*	CaMV35S	Improved recovery after rehydration	[169]
Mszpt2-1	Kruppel like	*M. truncatula*	Transcription factor	*M. truncatula*	CaMV35S	Gene-silenced transgenics became more sensitive to recover from salt stress	[170]
WXP1, WXP2	AP2 domain	*M. truncatula*	Wax biosynthesis	*Arabidopsis*	CaMV35S	Drought	[171]
Ph_acut_ AY026054	bZIP	*Phaseolus acutifolius*	Transcription factor	—	—	Water deficit stress	[172]
Ph_vulg_AF350505	bZIP	*P. vulgaris*	Transcription factor	—	—	Water deficit stress	[172]
PvNAP	NAC	*P. vulgaris*	Transcription factor	atnap null mutant, *Arabidopsis*	AtNAP	Leaf senescence	[173]

GmDREB1 enhanced salt tolerance in transgenic *medicago* [137]. Besides, three more DREB homologues were identified in soybean, namely, GmDREBa, GmDREBb, and GmDREBc. While *GmDREBa* and *GmDREBb* genes were induced by salt, drought, and cold stresses in the leaves of soybean seedlings, the expression of *GmDREBc* was apparently induced in roots by salt, drought, and abscisic acid treatments [138]. In another study, expression analysis of ERF transcription factors in soybean showed that nine unigenes belonging to six ERF family subgroups were induced by both biotic/abiotic stresses and hormone treatment, suggesting that they were involved in crosstalk between biotic and abiotic stress-responsive signaling pathways. Overexpression of two full-length soybean genes GmERF057 and GmERF089 from two different subgroups enhanced the tolerances to drought, salt stresses, and/or pathogen infection of the tobacco plants [139]. Moreover, transcription factors of MYB.family GmMYB76, GmMYB177, and GmMYB92 [140] and of bZIP family GmbZIP44, GmbZIP62, and GmbZIP78 were isolated from soybean and tested in transgenic *Arabidopsis* for their role in stress tolerance [141]. Over 64 *GmWRKY*genes from soybean were identified that expressed differentially under various abiotic stresses. For example, GmWRKY21 responded to cold stress, while GmWRKY54 conferred salt and drought tolerance, possibly through the regulation of DREB2A and STZ/Zat10 [142]. Also, six GmPHD genes encoding Alfin1-type PHD finger proteins were identified in soybean and their expressions responded differentially to drought, salt, cold, and ABA treatments. Another gene GmCHI (chilling inducible) has been assumed to be regulated by ABA-dependent signal transduction pathway during cold acclimation in soybean. Overexpression of *GMCHI* in *Arabidopsis* under the control of CaMV35S promoter enhanced the tolerance to cold, drought, and NaCl stresses [143]. In another report, GmGT-2A and GmGT-2B, "GT" element binding transcription factors belonging to the trihelix family genes, were cloned from soybean and their overexpression improved plant tolerance to salt, freezing, and drought stress in transgenic *Arabidopsis* plants [144]. Transgenic *Arabidopsis* plants overexpressing the GmPHD2 showed salt tolerance compared to the wild-type plants by diminishing the oxidative stress through regulation of downstream genes [145]. Similarly, an ectopic expression of a soybean antiquitin homologue gene GmTP55 (closely related to the stress-induced plant antiquitin-like proteins belonging to the ALDH7 family) in both *Arabidopsis* and tobacco has been shown to confer tolerance to salinity during germination and to water deficit during plant growth [146].

39.9 Phen"omics"

Although occupying the last position in a long and wide array of gene-based "omics" approaches, phenomics, which can be viewed as a "modern phenotyping counterpart," is critical to the gene-"omics" approach. Indeed, it is often and wisely considered that unless the phenotypic expression of plants displaying different genomic/metabolomic/proteomic/transcriptomic/transgenomic content is properly understood and characterized, and then accurately and precisely measured, there is

little chance that any of the approaches above can be successful. There is unfortunately an increasing gap between the knowledge on the genotype and that on the phenotype [147] that urgently needs to be tackled. What is often viewed in the phenomics is the possibility to harness new technology to increase the throughput of "traditional" phenotypic assessments. While this has indeed a tremendous potential, it also bears the risk of making phenotyping a technology-driven activity generating (many) numbers, rather than a question/hypothesis-driven approach to the understanding of plant response to stress. In what follows, we attempt to lay out the basic principles that should be considered when attempting "phenomics" characterization for focusing on the type of abiotic stress (e.g., drought, salinity, etc.).

39.9.1 Relevant Protocols to Assess Plant Response to Stress: Drought as a Case

There have been a number of studies that explain the importance of using relevant protocols to assess drought stress response [108] or in the approach to look at specific traits that are likely to be beneficial under water limitation, like root systems [148]. In short, the principle of exposing plants to stress is about ensuring that the kinetics of stress impositions are relevant to those that plants would face in natural environments. The use of rapid stress imposition (uprooting, exposure to PEG, growth in very small pots, etc.) is not suitable to properly characterize plant response to stress, and especially to acquire knowledge on the genes involved in the plant response, as these are likely to be different from the genes that would be expressed under natural conditions. Therefore, while applying water stress, it is essential to have a rigorous control and record of the stress intensity and the kinetics of stress imposition. One school of thought proposes to look at stress intensity from the angle of the soil moisture available for transpiration [149], as it has the great and powerful advantage of allowing comparison across all plant species, across environments. Unfortunately, rarely care is taken for this index in many gene-based studies. The other school of thought is to measure leaf water potential as an indicator for stress intensity. It has the drawback of being more labor intensive and less sensible than simple gravimetrics of soil moisture measurement [150, 151], but has the value of providing information on leaf water status that can be useful for understanding the other "omics" responses. In any case, any of these two "stress indicators" is a key requirement to make any sense of "omics" responses to water deficit. Equally important is the need to measure the environmental conditions under which plants are assessed. Much of the gene-based "omic" work takes place in glasshouse or growth chamber environment, where it is essential to assess air temperature, humidity, and light intensity to understand the physical drivers of plant water use.

39.9.2 Relevant Protocols Used to Extract "Omics" Products in Grain Legumes

In recent past, a large number of studies have attempted to identify genes responsible for stress response. Besides the fact that there are often thousands of genes that are

expressed, making the choice of key ones, if any, the conditions under which the plants are challenged to stress are often questionable. One such example is a recent study [40] that reports 319 unique ESTs from two contrasting lines of chickpea, with 70% of these being more than twofold abundant in the tolerant cultivar. The protocol used to challenge the plant was withdrawal of irrigation at 12 days after sowing, for a period of 3, 6, and 12 days. Here, the plants were grown in pots (3L) containing a composite soil, without any indication of the soil water capacity. Besides, no measurement of soil moisture was done and only relative water content was measured, putatively as a control for moisture stress. In another study on chickpea [45], attempts were made to expose plants to stress conditions that were similar to those of the natural conditions. Here, a dry-down technique was used to expose the plants to a progressive water stress, similar to the one in the field conditions, by partially compensating the daily water loss and ensuring that water stress symptoms (apparent from a decrease in plant transpiration) do not occur until at least 10 days after stress imposition, that is, similar to the field conditions. In such experiments, the soil moisture, which indicates the level of stress, is kept rigorously constant across genotypes tested. It allows replication of the experiment across environments or plant materials. Moreover, the contrasting materials were also challenged for salinity tolerance. The protocol used here was the very same protocol as used to screen genotypes for seed yield under salt stress. Since the physiological analysis also indicates that reproduction is likely the most sensitive process under salt stress, the flower tissue samples collected during the study for genotyping.

Similarly, work has been carried out in peanut to identify ESTs involved in the contrasting drought response in two genotypes (TAG24 and ICGV86031) (unpublished – EST sequence posted in Genbank). While TAG24 appears to have a high threshold of soil moisture where its transpiration declines, ICGV86031 clearly declines transpiration at lower soil moisture (dryer soil). Such differences are expected to play a causal role in the transpiration efficiency differences between these two lines. To identify possible genes responsible for that response, a standard dry-down protocol was used [153, 154], where tissue sampling was performed precisely when genotypes displayed phenotypic differences (differences in the transpiration relative to the control) during the stress. These two examples illustrate that relevant protocols are needed to mimic as closely as possible the natural conditions, to extract genes that are most likely to be involved in the response under natural conditions.

39.9.3 Adaptive versus Constitutive Genes

In most of the cases, the gene-based "omic" approaches tend to be influenced a lot by the idea that stress tolerance "results" from different stress-responsive genes intervening in the case of tolerant entries and being absent/unexpressed in sensitive lines. However, as far as water limitation is concerned, plants exposed to water deficit usually behave like fully irrigated plants until about 60% or more of the soil moisture has been depleted [152]. So, understanding how plants control plant water use before

stress symptoms appear is even more important than understanding how plants respond when they are left with only 40% or less of the soil water. A recent study on pearl millet shows this is critical for the terminal drought tolerance [153, 154]. Here, differences in leaf conductance under fully irrigated conditions were identified and related to the yield-based differences under stress. So, this means that genotypes have an array of development and functioning characteristics displayed under nonstressed conditions that can determine how well they would be adapted to a situation of stress. In the example of pearl millet cited above, a lower leaf conductance under fully irrigated conditions would simply limit water use when water is available and make it available for the grain filling period, a time when soil moisture has receded and plants are under stress. Therefore, constitutive traits become critical to consider in the "omic" approaches, including phenomics, to first identify their mechanisms (e.g., a slower leaf expansion rate or smaller leaf size) and then the related genes involved in development or functioning processes (e.g., a limited leaf conductance) that predispose particular genotypes to be better equipped to face a forthcoming water limitation.

39.9.4 Physiology Integration in a Novel Context of Environment-Specific Breeding

The growing genotype–phenotype gap is in part explained by a generational change in plant biologists, who have turned away from disciplines of physiology–biochemistry to molecular genetics, and by the belief that a single gene approach of "tolerance gene" identification would solve all problems. Rather, there is a clear need to have the phenotypic information guiding the gene-based "omics" work. Hence, phenomics should in part include a reductionist approach to break down integrated measurement of traits such as yield or biomass into smaller, more heritable components or traits, closer to the identification of cell- or organ-based mechanisms responsible for the integrated response differences. Again, molecular "omics" offer the potential for easier and more reliable way of predicting phenotypes with the condition that robust phenotype–genotype relationships have first been demonstrated. In any case, a reductionist approach to understand the mechanisms of tolerance to abiotic stress is needed to progress toward the identification of genes involved. It also fits the likely evolution of breeding approach from a one-variety-to-fit-all-situation to environment-specific breeding where it will be critical to understand/identify particular characteristics making a genotype adapted to particular environments.

Physiology as a discipline is an integral component of such a breeding perspective. The approaches and protocols that are developed by "phenomists" need to be adapted, or adaptable, to the requirement of a breeding program: these need to be large scale, simple, and applicable to a large number of entries, which is a prerequisite for QTL mapping, either through RIL population or through association panels. At the same time, these need to be capable of assessing cell- or organ-based mechanisms having potential importance. For instance, recent work in pearl millet indicates that lower leaf conductance leads to having water left in the soil profile to support seed filling, and this is attributed to differences in root hydraulics [154], for which precise

protocols are needed. This is a prerequisite to identify the genes involved in a cell- or organ-based mechanism.

39.9.5 Addressing Complexity of Plant Response to Abiotic Stress

Phenomics is also about addressing the complexity of plant response to stress. For instance, crop success under terminal drought could be explained by genotype's capacity to extract water deeper from the soil profile and make this water available for critical periods. In parallel, having water available for critical periods could be explained by differences in the pattern of water use (less water use) before reaching such critical development stages. The later could lead to less water use, while the former could lead to earlier/more water use. So, while this small example illustrates the need to target specific mechanisms, it also stresses on the need to look at different traits in a comprehensive manner. The difficulty lies in having an experimental approach that is enough reductionist to accurately phenotype cell- or organ-based actions, while being sufficiently integrated to have such reductionist measurements coupled to "integrated" measurements that have a meaning for the breeding community. At ICRISAT, work is ongoing where the initial target is to unravel the functionality of rooting traits in a way that their actual combination with terminal water deficit can be understood [148, 155]. As the work progresses, the initial focus on roots, root functionality, and water capture is getting complemented by a component of understanding of the regulation of water use by the crop canopy. Hence, modeling is surely a critical component of the breeding program, to reintegrate the pieces of the phenomics puzzle in a comprehensive and relevant framework. With the present phenomics development, allowing for measuring more and more, modeling remains a sort of safeguard that helps target what phenotype matters more than those that matter less. At the same time, the combination of phenomics and modeling offers a great potential of rapidly assessing the value of certain phenotypes on plant performance.

39.10 Conclusions

Over the years, biotechnology has emerged as a promising tool to overcome stresses in plants; but to date progress has been limited in legumes. Biotechnological applications, including all "omics," were direct and potential approaches for improving abiotic stress tolerance in grain legumes where the existing germplasm lacks the required traits for conventional breeding. However, successful application of "omics" to abiotic constraints requires knowledge of stress response at molecular level, which includes gene expression to protein or metabolite and its phenotypic effects. Availability of genome sequence of model legumes and soybean has a potential to facilitate positional cloning and other approaches and their applications for abiotic stress research on legumes. A genome-wide expression profiling with next-gener-

ation sequencing approaches could circumvent the problems posed by extremely large genomes like legumes.

Compared to analysis of the transcriptome, analysis of the plant proteome and metabolome in response to abiotic stresses is still limited to *M. truncatula* and protein reference maps of soybean to stress responses are now available. More recently, there are few proteomics studies on peanut and chickpea available, and they have to be extensively carried out in all grain legumes for abiotic stress tolerance. Moreover, the recent progress in the mass-scale profiling of the genome, transcriptome, proteome, and metabolome (i.e., "omics") offers the possibility of investigating the concerted response of thousands of genes to drought and other abiotic stresses. Hence, the research dealing with other strategies such as MAS or even classical breeding will be able to take advantage of the results being gathered from these "omics" technologies.

The mapping of abiotic stress QTL in legume is still at an early stage and gene pyramiding has not been applied yet. Nevertheless, with the establishment of the model legumes, *M. truncatula* and *L. japonicus*, there is now applicable information on legumes. Among the grain legumes, soybean has been more intensively studied, and the availability of more numbers of ESTs and genome sequences will facilitate mapping of major QTL in other legumes. The use of transgenic technology potentially offers a more targeted gene-based approach not only for gaining valuable information but also improving stress tolerance in legumes. However, the genetic engineering options addressing plant resistance to abiotic stress, mainly in relation to drought, have been confined to experimental laboratory work and to single gene approaches, lead to marginal stress improvement in grain legumes. Hence, there is a need for identification of candidate genes for abiotic stress tolerance in legumes that will allow their direct application in genetic engineering. Hence, multiple mechanisms to engineer abiotic stress tolerance and studies involving regulatory genes under the control of stress-inducible promoters have a potential to improve stress tolerance in grain legumes. Also, since only transgenic soybean has been commercialized in developed countries, there is a need to address the regulatory issues for transgenics' deployment in developing countries. Needless to point out that the current advances in tissue-derived techniques, genetic transformation and MAS, together with the advances in powerful new "omics" technologies offer a great potential to improve this situation. Besides, a thorough and meaningful assessment of phenotypic expression to understand the mechanisms of adaptation to stress is needed before genes responsible for these mechanisms can be identified and tagged. Indeed, it is now possible to target almost all legume crops with a variety of biotechnological approaches for genetic improvement.

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