Chapter 2
Towards Understanding the Transcriptional Control of Abiotic Stress Tolerance Mechanisms in Food Legumes

Rebecca Ford, Saleem Khan, and Nitin Mantri

Abstract A multitude of environmental and subsoil conditions cause abiotic constraints to the growth and productivity of legume food species. These stresses often occur simultaneously, leading to compounded effects of low and unreliable yields. Since legumes are a major food source, particularly in regions of major population growth, it is imperative that better tolerant and adapted varieties are developed. For this, transgenic approaches integrated within traditional breeding programs are proposed to offer substantial productivity gains through fast-tracking the development and deployment of well adapted and tolerant varieties to regions of greatest need. For this to occur, knowledge of the major tolerance genes and more importantly their regulators is required. Accordingly, recent functional genomics approaches have begun to shed light on the transcriptional, and hence regulatory and mechanistic controls governing tolerances to several of the major abiotic stresses, such as drought, temperature and salinity within temperate legume food species. Functional validation of these regulatory signals, their action on downstream genes and associated pathways is underway within several large international programs. This chapter will review these advances in knowledge to date within the model and crop grain legume species, to identify and characterize the molecular targets for the future selection and breeding of sustainably tolerant crops. Specifically, this chapter aims to summarize progress towards identifying and understanding the functions of the WRKY transcription factors involved in instigating and regulating abiotic stress tolerance mechanisms and their potential for improving abiotic stress tolerance within temperate legume food species.

R. Ford (✉)
School of Natural Sciences, Griffith University, QLD, Australia
e-mail: rebecca.ford@griffith.edu.au

S. Khan
Faculty of Veterinary and Agricultural Sciences, The University of Melbourne,
Melbourne, VIC, Australia

N. Mantri
School of Applied Sciences, Health Innovation Research Institute, RMIT University,
Melbourne, VIC, Australia

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Abbreviation

ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
AP2/ERF	APETALA2/Ethylene-responsive factor
DREB	Dehydration-responsive element binding
IAA	Indole Acetic Acid
LAP	Legume anthocyanin production
MYB	Myeloblastosis
NCED	9-cis-epoxycarotenoid dioxygenase
PEG	Polyethylene glycol
TF	Transcription factor
TMV	Tobacco mosaic virus
WRKY	Tryptophan Arginine Lysine

2.1 Regulation of Abiotic Stress and Tolerance

The primary causes of crop loss globally are the extremes of water availability (mainly drought), temperature (heat and cold) and soil toxicities and deficiencies (mainly salinity). These limit the growth and development and agro-geographical distribution of crops worldwide, which causes significant reduction in productivity. Plants respond to adverse environmental conditions through many physiological, biochemical and molecular changes, which enable plants to survive and reproduce (Mantri et al. 2010a). These are governed by an array of genes encoding proteins with diverse functions. The ability of a plant to grow and survive under such conditions is dependent on its ability to adapt growth and metabolic processes, governed through complex networks of molecular switches and regulators. This includes the ability to perceive the stress, respond to the stress through instigation of nearby and/or systemic signals (signal transduction) and the expression of relevant tolerance genes and metabolites. Hence, abiotic stress tolerance traits are multigenic and quantitative in nature and difficult to manipulate or select for within the constraints of traditional breeding approaches. It would be unlikely that selection of a single or few functional genes would result in sustained tolerance since the impacts of abiotic stresses are varied and complex. In the case of salinity tolerance, effects caused by altered ionic strengths and osmotic pressures around membranes lead to potential ion compartmentation and/or exclusion and even changing hormone levels that stimulate cell division for avoidance through altered root physiology (Lv et al. 2012; Mantri et al. 2012). Therefore, selection or manipulation of the stress perceivers and gene pathway regulators is proposed to be a far more efficient approach. In particular, the group of genes that encode regulatory proteins may be useful for the management of crops under stress conditions (Cabello et al. 2014). This group includes protein kinases, enzymes involved in phospholipid metabolism, transcription factors and other signalling molecules. Understanding their function is critical for determining the possibilities to manipulate or select for improved stress tolerance.

There has been a recent flurry of publications demonstrating the isolation and characterization of abiotic stress-responsive genes and their potential transcriptional controllers from temperate legumes using several methods. This has included partial elucidation of the transcriptional responses and controls to drought, salinity and cold in chickpea using microarrays (Mantri et al. 2007, 2010b) and most recently the more in-depth identification of cold stress tolerance genes and transcription factors in chickpea using cDNA-AFLP (Dinari et al. 2013). There exists tremendous opportunity to employ the high throughput and next-generation sequencing technologies to start to characterize the key functional roles of many genes and gene regulators that have been identified.

2.1.1 Transcription Factors

Among the regulatory proteins, transcription factors (TFs) play a major role in defensive gene expression involved in tolerance mechanisms (Puranik et al. 2012). TFs contain key proteins that interact with *cis*-acting elements within the stress-responsive gene promoters and enhancer sequences to regulate through activation or repression of downstream gene networks. Hence, TFs are the subject of many studies aimed at determining how they may best be manipulated to change the regulation of the whole suit of genes under their control through up- or downregulation (Liu et al. 2013).

TFs are grouped into large gene families related to their characteristic DNA-binding domains (DBDs), such as AP2/ERF, B3, NAC, SBP and WRKY. They are also characterized based on their role in responding to a particular stimulus/stimuli and controlling physiological and metabolic responses involved in adaptive plant growth and development. Members of a family may respond uniquely to different stress stimuli (Yamasaki et al. 2013). TFs that control the complex signals for flowering in pea that are associated with the FT locus have recently been well characterized (Hecht et al. 2011).

TF expression has been characterized in several legume and non-legume species using large-scale quantitative reverse transcription-PCR (Czechowski et al. 2004; Caldana et al. 2007; Kakar et al. 2008; Libault et al. 2009). Using this information, several attempts have been made to quantify TF gene expression in different plant parts to assess for tissue specificity related to function (Gruber et al. 2009; Libault et al. 2009). Additionally, the availability of whole genome sequences has paved the way for elucidating the complex network of gene expressions and regulations in response to abiotic stresses. Sequences are available for *Medicago truncatula* (http://www.plantgdb.org/MtGDB/), *Lotus japonicus* (http://www.plantgdb.org/LjGDB/), *Glycine max* (http://www.plantgdb.org/GmGDB/) and *Cicer arietinum* (Varshney et al. 2013; Jain et al. 2013).

The model legume *M. trucatula* was originally used to undercover abiotic stress-specific TFs and their functions. For example, *WXP1*, an *AP2/EREBP* TF was found to enhance drought tolerance in transgenic plants by increasing cuticular wax and thereby improving the water retaining capacity (Zhang et al. 2005). The same gene and a closely related paralog (*WXP2*) also enhanced drought tolerance in transgenic *Arabidopsis* plants, as well as enhanced freezing tolerance in the *WXP1* overexpressing lines (Zhang et al. 2007). Further, *MtHAP2-1* was shown to be a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 (Combier et al. 2006).

A *Legume Anthocyanin Production 1* (*LAP1*) gene that serves as an *MYB* TF was identified and constitutively expressed in transgenic alfalfa. *LAP1* induced massive accumulation of anthocyanin pigments comprising multiple glycosidic conjugates of cyanidin. Constitutive expression of *LAP1* induced many genes involved in anthocyanin biosynthesis including glucosyltransferase *UGT78G1*, that when expressed transgenically resulted in increased anthocyanin accumulation when plants were exposed to abiotic stress (Peel et al. 2009).

In another study, a *M. truncatula* 16,000+ gene microarray was used to identify 84 TF sequences differentially expressed in the root apex in response to salt stress. Analysis of salt-stress regulation in root apexes versus whole roots showed that several TF genes have more than 30-fold expression differences including specific members of *AP2/EREBP*, *HD-ZIP* and *MYB* TF families. Several salt-induced TF genes also respond to other abiotic stresses such as osmotic stress, cold and heat, suggesting that they participate in general stress response pathways (Gruber et al. 2009).

Despite the historical lag in availability of genomic information for the food legumes, the recent development of online transcriptomic databases for model legumes and reference species holds great promise to aid with TF identification and functional assignment in these orphan species. In particular, "LegumeIP" is a freely available tool (plantgrn.noble. org/LegumeIP) that contains large-scale gene expression data from *L. japonicas* and *M. truncatula* microarrays, as well as RNA-Seq-based gene expression data from *G. max* and time-course expression data from nodule, flower, root and leaf tissues (Li et al. 2012). The ability to perform systematic synteny analyses and construct gene family phylogenies across these species and *A. thaliana* will better enable the accurate classification of TF family members from the legumes once the sequences become available.

The following summarizes the current status of knowledge regarding TF characterization in some important food legumes.

2.1.2 Soybean

The availability of the soybean genome sequence has allowed large-scale identification and annotation of regulatory TFs for functional studies. This has enabled capturing of stress-responsive TFs and their regulatory networks and identification of the full complement of TF encoding genes (Mochida et al. 2009). A total of 5,035 TF models have been found within the soybean genome and grouped into 61 families. The relevant annotations of soybean TF genes can be accessed via the soybean TF database (soybeantfdb.psc.riken.jp).

In functional studies, overexpression of the *GmHSFA1* TF gene conferred tolerance to heat stress (Zhu et al. 2006). Further, 131 *bZIP* genes were identified from soybean and their response to abscisic acid (ABA), drought, salt and cold was analysed. From these, Soybean *GmbZIP44*, *GmbZIP62* and *GmbZIP78* functioned as negative regulators of ABA signalling and conferred salt and freezing tolerance in transgenic *Arabidopsis* (Liao et al. 2008). Recently, a novel bZIP transcription factor gene, *GmbZIP1* was shown to enhance multiple abiotic stress tolerances (Gao et al. 2011).

Members of the ethylene response factor (*ERF*) TF family regulate gene expression in response to biotic and abiotic stresses. In soybean, 98 unigenes that contained a complete *AP2/ERF* domain were identified and their phylogeny, gene structures, and putatively conserved motifs in soybean *ERF* proteins were analysed and compared with those of *Arabidopsis* and rice. Expression analysis 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 cross-talk between biotic and abiotic stress-responsive signalling pathways. Overexpression of two full-length genes from two different subgroups enhanced the tolerances to drought, salt stresses, and/or pathogen infection (Zhang et al. 2008).

Several TF genes from the *DREB* (dehydration-responsive element binding) gene subfamily of the *AP2/EREBP* family have also been identified and characterized in soybean. For example, the overexpression of *GmDREB2* activated expression of downstream genes in transgenic *Arabidopsis*. This resulted in enhanced tolerance to drought and high-salt stresses but did not cause growth retardation (Chen et al. 2007). In addition, constitutive expression of *GmDREB3* in transgenic *Arabidopsis* caused growth retardation, whereas its expression under control of the stress-inducible *Rd29A* promoter minimized negative effects on plant growth under normal growth conditions. This indicated that a combination of the *Rd29A* promoter and *GmDREB3* might be useful for improving tolerance to environmental stresses (Chen et al. 2009).

A new member of the soybean *AP2/ERF* TF family, *GmERF3*, was also analysed for biotic and abiotic stress tolerance. Ectopic expression of the *GmERF3* gene in transgenic tobacco plants induced the expression of some *PR* genes and enhanced resistance against infection by *Ralstonia solanacearum*, *Alternaria alternata* and tobacco mosaic virus (TMV), and gave tolerance to high salinity and dehydration stresses. This suggested that *GmERF3* might play dual roles in the responses to biotic and abiotic stresses (Zhang et al. 2009a).

NAC TFs play important roles in plant growth, development and stress responses. In soybean, *GmNAC11* acts as a transcriptional activator, whereas *GmNAC20* functions as a mild repressor. Overexpression of *GmNAC20* enhanced salt and freezing tolerance in transgenic *Arabidopsis* plants whilst *GmNAC11* overexpression only improved salt tolerance (Hao et al. 2011). In another recent study, *GmNAC5* was induced by mechanical wounding, high salinity, and cold treatments but was not induced by ABA (Jin et al. 2013).

Trihelix TFs condition light-regulated responses and other developmental processes involved in abiotic stress signalling. Two trihelix TF genes, *GmGT-2A* and *GmGT-2B*, from soybean conferred stress tolerance through regulation of common and specific sets of genes (Xie et al. 2009). Lastly, the role of soybean *MYB* TFs in response to abiotic stresses has also been evaluated. Three *GmMYB* genes (*GmMYB76*, *GmMYB92* and *GmMYB177*) whose expression changed in response to ABA, salt, drought and/or cold stress were chosen for functional analysis from about 156 soybean *GmMYB* genes. The transgenic *Arabidopsis* plants overexpressing *GmMYB76* or *GmMYB177* showed better performance than the *GmMYB92-transgenic* plants in response to salt and freezing tolerance (Liao et al. 2008).

2.1.3 Chickpea

A chickpea NAC gene *CarNAC5* was found to be expressed in many chickpea tissues including seedling leaves, stems, roots, flowers, seeds and pods, but mostly accumulated in flowers. The *CarNAC5* was strongly expressed during seed maturation, in germinating seeds, and strongly induced by drought, heat, wounding, salicylic acid (SA) and indole-3-acetic acid (IAA) treatments (Peng et al. 2009a). Subsequently, among other NAC TFs, *CarNAC3*, was also shown to be significantly induced by drought stress, ABA, ethephon and IAA (Peng et al. 2009b) and the expression of *CarNAC1* was strongly induced by drought, salt, cold, wounding, H_2O_2, ethephon, salicylic acid, indole-3-acetic acid and gibberellin (Peng et al. 2010). From the members of *ERF*/*AP2* proteins that play a crucial role in growth and stress response, expression of *CAP2* from chickpea enhanced growth and tolerance to dehydration and salt stress in transgenic tobacco (Shukla et al. 2006).

2.1.4 Peanut

In peanut, six *ERF* TF genes designated as *AhERF1–6* were cloned and their expression patterns were analysed under cold, salt and drought stress. Of these, the expression of *AhERF4* and *AhERF6* was rapidly and substantially enhanced under abiotic stress. Whereas *AhERF3* was downregulated in leaves under salt stress and *AhERF2* was downregulated in leaves under drought stress. Interestingly, the expression of *AhERF3* and *AhERF5* exhibited contrary expression patterns in peanut leaves and roots upon PEG treatment. These results suggested that different *ERF* TFs might have different functions in abiotic stress acclimation in peanut (Chen et al. 2012a).

Previously, a drought-induced NAC gene, *AhNAC2*, was isolated from peanut and transgenic *Arabidopsis* overexpressing *AhNAC2* was hypersensitive to ABA in root growth, seed germination and stomatal closure compared to the wild-type plants. The transgenic lines exhibited enhanced tolerance to drought and salinity stress, and the expression levels of 12 stress-related genes in the *AhNAC2* transformed plants were higher than the wild type (Liu et al. 2011). Finally, the oxidative cleavage of *cis*-epoxycarotenoids catalysed by *9-cis-epoxycarotenoid dioxygenase* (*NCED*) is considered to be the rate-limiting step in ABA biosynthesis. The constitutive expression of peanut *AhNCED1* gene in wild-type *Arabidopsis* resulted in increased ABA accumulation in transgenic plants in response to drought stress (Wan and Li 2006).

2.1.5 Cowpea

In cowpea, a stress-inducible gene for NCED, *VuNCED1*, involved in ABA biosynthesis under water stress was identified from drought-tolerant cowpea (Luchi et al. 2000). In a bid to generate genomic resources in cowpea, sequencing and analysis of the gene-rich, hypomethylated portion of the cowpea genome identified over

250,000 gene-specific sequence reads (GSRs) with an average length of 610 bp (Timko et al. 2008). A total of 62 out of the 64 well-characterized plant TF gene families were represented in the cowpea GSRs, which may provide a resource for functional markers linked to abiotic stress tolerance traits in cowpea. Recently, a comparative genomics approach was used to identify 18 conserved *V. unguiculata* miRNAs belonging to 16 distinct miRNA families. Using these potential miRNA sequences 15 potential target genes were predicted and all of them were identified as transcription factors (Paul et al. 2011).

2.2 Phaseolus

A root-specific *bZIP* TF was shown to be responsive to water deficit stress in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*) (Rodriguez-Uribe and O'Connell 2006). Meanwhile, a *NAC* family TF member *AtNAP* important for leaf senescence was characterized in *Arabidopsis* (Guo and Gan 2006). In this study, an orthologous *NAC* TF from *P. vulgaris*, PvNAP, was identified that shared the same leaf senescence-specific expression pattern as *AtNAP. P. vulgaris* leaves at five distinct developmental stages, were analysed and the *PvNAP* transcript was detected in senescing leaves only. In addition, AtNAP homologues in *P. vulgaris* were able to restore the *Arabidopsis AtNAP* null mutant to wild type (Guo and Gan 2006). Recently, analysis of genes in response to dehydration stress in *P. vulgaris* identified *CA1* with sequence similarity to the *ERF* family *AP2/EREBP* and was tenfold induced by drought stress (Kavar et al. 2008).

2.3 WRKY TFs and Their Roles in Abiotic Stress Tolerance

The WRKY (W=Tryptophan, R=Arginine, K=Lysine, Y=Tyrosine) family are one of the largest TF families and regulate many plant processes (Ulker and Somssich 2004; Zhang and Wang 2005). Members can either activate or repress multiple downstream gene networks along with MAP kinases, MAP kinase kinases, 14-3-3 proteins, calmodulin, histone deacetylases and other protein partners. WRKY TFs possess a DNA-binding domain of 60 amino acids with an evolutionary conserved WRKYGQK signature at the N terminal and a C terminal that contains a zinc finger motif (Eulgem et al. 2000). WRKY proteins have DNA-binding activity at a specific W BOX motif (C/T)TGAC(T/C) in their promoter region (Eulgem et al. 2000). Other sequences adjacent to the WRKY domain also assist in DNA-binding activity (Luise et al. 2013; Ciolkowski et al. 2008).

Structural classification of the WRKY family is based on configuration of the zinc finger motif, the number of WRKY domains and the size of the intron (Zhang and Wang 2005). The family is classified into three main groups 1, 2 and 3, which are further divided into subgroups 2a+2b, 2c, 2d and 2e (Eulgem et al. 2000; Zhang and Wang 2005) (Table 2.1). The family originated two billion years ago

Table 2.1 WRKY transcriptional factor family groups and functions

WKRY gene	Group/subgroup	Organism	Source of induction	Role in abiotic stress	References
At WRKY33	1	*Arabidopsis thaliana*	Drought, salt	Activate drought tolerance gene CesA8	Wang et al. (2013)
AtWRKY25	1	*Arabidopsis thaliana*	NaCl, heat	Increased salinity and heat tolerance	Jiang and Deyholos (2009)
AtWRKY34	1	*Arabidopsis thaliana*	Cold	Increased pollens viability during cold stress	Zou et al. (2010)
PtrWRKY2	1	*Poncirus trifoliata*	Low temperature	Positive regulation of cold stress	
AtWRKY26	1	*Arabidopsis thaliana*	High temperature	Upregulation of heat stress	Li et al. (2011)
GmWRKY1, 5, 15, 21, 30	1 and 2	*Glycine max*	Cold	Overexpression during cold stress	Zhou et al. (2008)
PtrWRKY1	2	*Poncirus trifoliata*	Drought, cold	Increased drought and cold tolerance	Şahin-Çevik and Moore (2013)
CgWRKY1	2	*Citrus grandis*	Drought, cold	Increased drought and cold tolerance	Şahin-Çevik and Moore (2013)
GmWRKY6, 41	2	*Glycine max*	NaCl, dehydration	Overexpression under salinity stress	Zhou et al. (2008)
Hv-WRKY38	2	*Hordeum vulgare*	Cold and dehydration	Positive regulation of cold and drought stress	
BhWRKY1	2	*Boea hygrometrica*	Dehydration, ABA	Increased expression of *BhGolS1*/drought tolerance	
OsWRKY11	2	*Oryza sativa*	Drought, heat	Binding with HSP101 and increased drought tolerance	
GmWRKY13, 17, 27	2	*Glycine max*	Drought, salt	Early positive regulation of drought stress	Zhou et al. (2008)
OsWRKY08	2	*Oryza sativa*	NaCl, PEG, ABA	Upregulation of osmotic stress in transgenic *Arabidopsis*	

CaWRKY40	2-a	*Capsicum annum*	Heat	Increased resistance to hot environment	
AtWRKY8	2-c	*Arabidopsis thaliana*	NaCl	Positive regulation of salt stress	Hu et al. (2013)
DgWRKY1	2-c	*Dendranthema grandiflorum*	Salt, drought	Upregulation of salinity and drought stress	Liu et al. (2013)
AtWRKY39	2-e	*Arabidopsis thaliana*	Heat	Enhanced PR1 expression/heat tolerance	Li et al. (2010)
TcWRKY53	3	*Thlaspi caerulescens*	PEG, NaCl	Negative regulation of osmotic stress	
OsWRKY45	3	*Oryza sativa*	ABA, PEG, Dehydration	Overexpressed during dehydration	Qiu and Yu (2009)
AtWRKY63	3	*Arabidopsis thaliana*	ABA, drought	Increased drought tolerance	
GsWRKY20	3	*Glycine soja*	Drought, salt, ABA	Positive regulation of drought and salt stress	Luo et al. (2013)
BcWRKY46	3	*Brassica campestris*	Salt, cold, ABA	Increased salt and cold tolerance in transgenic tobacco	

and has subsequently increased in frequency and distribution throughout the plant kingdom (Ulker and Somssich 2004), playing roles in stress signalling and defence (Eulgem and Somssich 2007; Lai et al. 2008; Chavan and Kamble 2013; Chen et al. 2012a; Madrid et al. 2010). Involvement in plant biotic signalling has been extensively studied, however, much less is known about their involvement in abiotic stress tolerances. A summary of this knowledge, with a specific focus on each stress and application of knowledge to improving legume food species follows.

2.3.1 Drought

In *A. thaliana*, the AtWRKY33 TF interacts with the W BOX of the drought resistance gene *CesA8* to regulate its transcription (Wang et al. 2013). This was similar to expression of the rice OsWRKY45 TF in response to dehydration (Qiu and Yu 2009) and induction of PtrWRKY1 from *Poncirus trifoliata* and CgWRKY1 from *Citrus grandis* in response to drought (Şahin-Çevik and Moore 2013).

Of the soybean WRKY TFs, expression of GmWRKYs 13, 17 and 27 occurred in the early response to drought and those numbered 21, 41, 54 and 62 were induced under prolonged drought conditions (Zhou et al. 2008). Subsequently, in a wild soybean (*Glycine soja*), GsWRKY20 was highly induced under drought, salt and cold treatments in leaf and root tissues of transgenic *Arabidopsis* and showed increased drought tolerance by subsequent reduction in number of stomata and water loss (Luo et al. 2013). In *Medicago truncatula*, the genome-wide analyses of MtWRKY showed that 19 MtWRKYs belong to group 1 and 49 MtWRKYs were recognized in a bigger WRKY group 2. Only 12 MtWRKYs were identified in-group 3 (Song and Zhibiao 2014). The functional validation and possible roles in defence activation of MtWRKYs has not yet been achieved.

2.4 Salinity

In *A. thaliana*, AtWRKY25 and AtWRKY33 were upregulated quickly in leaf and root tissues (Jiang and Deyholos 2009) and AtWRKY8 was highly expressed in root tissues under salinity (Hu et al. 2013). Similarly, the transient high expression of soybean GmWRKY6, 13, 17, 27 and 41 was reported under salinity stress (Zhou et al. 2008). It was recorded that soybean WRKYs increased salt tolerance through regulating the salinity stress-related transcriptional factors DREB2A and STZ/Zat10 genes (Zhou et al. 2008).

2.4.1 Temperature

In *A. thaliana*, the viability of mature pollen increased with the induction of AtWRKY34 after cold treatment and pollen sensitivity towards cold stress was reduced after overexpression of AtWRKY34 in wild-type lines (Zou et al. 2010).

Meanwhile, AtWRKY25 was highly expressed under continuous high temperature stress (Li et al. 2009), and using mutant studies Li et al. (2010) showed that AtWRKY39 expression under thermal stress enhanced expression of heat responsive and the salicylic acid-inducible pathogenic-related protein PR1 gene for improved heat tolerance. Additionally, highlighting the very unique functional nature of individual WRKY family members, expression of AtWRKY26 increased and AtWRKY33 decreased under thermal stress (Li et al. 2011). Meanwhile, in the food legumes, the soybean GmWRKY1, 5, 15, 21, 30, GmWRKY43, GmWRKY48 and GmWRKY62 were upregulated under low temperatures (Zhou et al. 2008).

The functional biology and temperature activation of *Arabidopsis* WRKY TF (AtWRKY25, AtWRKY26 and AtWRKY33) indicated that in temperature stress, heat stress-related genes like Hsps, MBF1c and Zat10 are activated which further increased heat tolerance of the plant (Li et al. 2011). The AtWRKY25, 26 and 33 all belong to group 1 of the WRKY family (Eulgem et al. 2000; Zhang and Wang 2005).

2.5 Progress Towards Establishing Tolerance-Related Function of Candidate Transcription Factors

A diversity of functional genomics approaches have been employed to better characterize and validate the actions of TFs involved in particular abiotic stress tolerance responses. In *Arabidopsis*, the WRKY25 and 33 genes were found via microarray analysis to aid in NaCl tolerance, the function of these genes and an upstream-inducible region were validated through transgenic overexpression and found to be stimulated with ABA (Jiang and Deyholos 2009). In the legume model species *Medicago truncatula*, the functional response levels of over 1,000 TF transcripts were established using a 384-well plate qRT-PCR pipeline to produce a useful resource that has subsequently been used to study the function of TFs under several abiotic stress stimuli (Kakar et al. 2008). More recently, DeepSuperSAGE followed by RT-qPCR was used to identify and validate the functional responses of several transcription signals involved in the early perception of water deprivation in dehydrated soybean roots, including members of the WRKY and NAC families (Ribamar et al. 2013). In chickpea, TFs that lie within microsatellite loci have been used to genome map with, to determine those that may be functionally important (Kujur et al. 2013). This is a smart approach to hone in on a subset of TFs directly related to the functional control of a particular trait of interest, including abiotic stress tolerance in crop plants.

2.6 Concluding Remarks

Apart from a limited number of studies and mostly in the model legume species, to date there is very little knowledge of the functional role of TFs in abiotic stress responses and regulation in food legumes. Much investigation, using forward and reverse genetics techniques is required to address specific research questions to uncover the key transcriptional drivers and their regulators. Comprehensive program aimed at the elucidation of the genetic controls governing abiotic stress tolerances and the subsequent incorporation of these genes and mechanisms through transgenics into elite breeding lines and subsequently cultivars are beginning to emerge, for example, the chickpea program at ICRISAT. This includes research towards developing cultivars tolerant to drought, salinity and low temperatures. Genes already under investigation for transfer include those encoding for enzymes required for the biosynthesis of osmoprotectants, modifying membrane lipids, LEA proteins and detoxification enzymes, microRNAs (Mantri et al. 2013). However, more programs are predicted for the near future to address other mandate legume crops and to uncover stress-inducible transcription factors that may be used for tolerance induction and selection following functional validation.

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