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Genetic Determinants of Abiotic Stress Tolerance in Foxtail Millet

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Abstract

Foxtail millet is one of the most important C_4 Panicoid crops known for its small genome size ($\sim$490 Mb), short life cycle, inbreeding nature, and remarkable abiotic stress tolerance properties. It is a widely-grown food and fodder crop in the dry and semi-arid regions of Asia and Africa, including North China and India. *Setaria italica* (cultivated) and *Setaria viridis* (wild) are two widely known species of *Setaria* genus that serve as excellent model systems for evolutionary, architectural, and physiological studies in related potential bioenergy Panicoid grasses such as switch grass, napier grass, and pearl millet. Foxtail millet is rich in genetic diversity, with several core and mini core collections of its diverse germplasm. There are significant phenotypic variations that provide scope for association mapping and allele mining of new variants of abiotic stress tolerance that could be effectively utilized for crop improvement. Several of the foxtail millet accessions could also be abiotic stress tolerant particularly to drought and salinity, and exploiting their agronomic and stress tolerant traits could be particularly important for marker-assisted selection and genetic engineering. Furthermore, with the release and availability of the foxtail millet genome sequence, several of its distinctive attributes, including abiotic stress tolerance, have been discovered that may help in a better understanding of its evolution, stress physiology, and adaptation. The foxtail millet genome sequence thus not only helps toward identification and introgression of agronomically important traits but also helps in deciphering the abiotic stress tolerance mechanisms of this exceptionally stress tolerant crop and is also useful in developing climate resilient crops which are very crucial in this era of global climate change.

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8.1 Introduction

Millets that represent small-grained cereal crops are among the developing world's major crops grown for human consumption and livestock feed, including pearl millet, foxtail millet, finger millet, proso millet, little millet, kodo millet, Japanese and Indian barnyard millet, teff, and fonio. They have typically been originated, domesticated, and cultivated in semi-arid and tropical regions of Africa and Asia (Dvořáková et al. 2015). However, they are also grown in parts of America and Eurasia (Goron and Raizada 2015). Millets also play a crucial role in the economy of developing countries where a large acreage of marginal land is used for cultivation. Notably, India contributes significantly to global millet production with around 30% of total world millet production obtained from 25% of the total world area under millet production in 2013, indicating the importance of millets for the resource poor and marginal farmers of the Indian subcontinent (Tadele 2016). Millets distinctively are well-adapted to adverse agro-ecological conditions, are typically grown in marginal soils with the least amounts of input yet still rich in nutrients, and are a source of staple dietary supplements for millions of inhabitants of these regions. Interestingly, millets exhibit sufficient variability among themselves, which is not only exhibited in terms of morphology such as plant stature, seed color and size, panicle size, etc. but also demonstrated at the genetic level in terms of chromosome number and ploidy level that ranges from $2n = 2x = 14$ in pearl millet to $2n = 6x = 54$ in fonio (Tadele 2016). They have been broadly grouped into two major subfamilies, namely Panicoideae that comprises pearl millet, foxtail millet, proso millet, little millet, and Japanese barnyard millet and Chloridoideae that includes finger millet and teff, belonging to eight genera, namely *Pennisetum*, *Setaria, Echinocloa, Elusine, Panicum, Paspalum, Eragrostis,* and *Digitaria*. Thus, agronomically the Panicoideae subfamily is the most important grass family as it includes not only the most economically important C_4 cereal crop maize but also sorghum and most of the small-grained millets. Sugarcane, a major biofuel crop and *Miscanthus* and *Panicum virgatum* (switchgrass), the emerging bioenergy feedstocks, also belong to this subfamily (Li and Brutnell 2011). Both *Setaria italica* (foxtail millet) and *Setaria viridis* (green foxtail) are the closest relatives of the important bioenergy feedstock, switch grass, and thus are considered excellent models to study the evolution, architecture, and physiology of switchgrass and related C_4 Panicoid grasses (Lata et al. 2013).

Foxtail millet is a self-pollinating, diploid ($2n = 2x = 18$), C_4 Panicoid small-grained millet crop with a very small genome size (~515 Mb) and short life cycle (Lata et al. 2013). It is one of the world's oldest cultivated grain crops, domesticated ~8,000 years ago in Northern China (Li and Wu 1996; Lata et al. 2013). It ranks second in total global millet production and is presently one of the minor food crops of dry regions of Southern Europe and Asia, including India and China, whereas in North America it is primarily grown for silage, bird feed, and fodder or cover crop (Goron and Raizada 2015). Foxtail millet grains are nutritionally very rich, with comparatively higher seed protein, crude fat, iron, and mineral content as compared to staple cereal crops such as rice and wheat (Zhang et al. 2007a; Lata et al. 2013). Its grains are also high in fiber content (Amadou et al. 2011) and its bran is enriched with linoleic and oleic acids (Liang et al. 2010). Additionally, foxtail millet is widely known for its exceptional drought tolerance and high water use efficiency (WUE) (Li and Brutnell 2011; Lata et al. 2011a), and some of its cultivars are also found to be salt tolerant (Jayaraman et al. 2008). Considering both its agronomic and economic importance, several research groups across the world have recently focused their attention on the development of genetic and genomic resources for this crop as well as on understanding the physiological and molecular basis of its excellent abiotic stress tolerance characteristics. Much work has now been done on exploring existing germplasm resources for identification of newer alleles and stress resistance traits, as well as generation and utilization of genomic resources for introgression of agronomically important traits and marker-assisted selection (MAS) for crop improvement and cultivar development. This chapter thus focuses on the recent progress toward unravelling the genetic determinants of abiotic stress tolerance in foxtail millet and their exploitation in crop improvement programs for developing varieties.

8.2 Phylogeny and Genomic Relationship Among *Setaria* Species

Setaria is a diverse genus with approximately 125 species reported to date with different phenotypic traits, life cycles, and ploidy levels (Lata et al. 2013). *Setaria* species have been classified into three gene pools based on genome organization. For example, *S. italica* (domesticated and cultivated) and *S. viridis* (wild ancestor) which are considered very important from genetic studies perspective and represent the primary gene pool have AA genome (Benabdelmouna et al. 2001). *S. italica* was domesticated from *S. viridis* around 8,000 years ago in Northern China (Barton et al. 2009). *S. faberi* (giant foxtail or nodding millet) and *S. verticillata* (bristly foxtail) with AABB genome form the secondary gene pool and are thought to originate from a cross between *S. viridis* (AA) and *S. adhaerens* (bristly grass; BB) (Benabdelmouna et al. 2001). The remaining *Setaria* landraces such as *grisebachii, queenslandica, pumila,* etc. constitute the tertiary gene pool. Several phylogenetic and cytogenetic studies suggested that *S. viridis, S. adhaerens*, *S. faberi*, *S. verticillata*, and *S. pumila* are discrete taxa (Benabdelmouna et al. 2001; Benabdelmouna and Darmency 2003; Layton and Kellogg 2014). *S. pumila*, whose genome organization is not yet known, is confirmed to be unrelated despite its widespread habitat similarity with other *Setaria* species (Layton and Kellogg 2014). The *Setaria* genus complexity has been reviewed in detail by Lata et al. (2013). A detailed understanding of the complex and intricate phylogenetic and genomic relationships among *Setaria* species would be beneficial for identification and selection of germplasm with desired traits, as well as for competent parent selection and hybridization required for improved breeding strategies.

8.3 Population Structure and Genetic Diversity Among Foxtail Millet Accessions

S. viridis has been projected as a Panicoid grass model because of its distinctive attributes such as small stature, diploidy, self-pollination, short generation time, minimal growth requirements, and excellent genetic transformation system that make it an exceptional model for genetic and evolutionary analyses. It also closely shares several of its cell wall characteristics with Panicoid grasses as compared to rice (Brutnell et al. 2015). Efforts have therefore recently been made to assess the genetic diversity of both wild and domesticated forms of *Setaria* in natural populations (Wang et al. 2010; Huang et al. 2014; Gupta et al. 2014). Various investigations on *Setaria* population structure showed the existence of subpopulations associated with distinct geographical locations (Brutnell et al. 2015). For example, an earlier population genetic study of 168 *S. viridis* and *S. italica* accessions from Eurasia and North America could not differentiate between the accessions from these two geographical locations but could distinctly identify southern and northern populations from central North America (Wang et al. 1995). Another genetic diversity study of a worldwide collection of 200 *S. viridis* accessions using genotyping-by-sequencing) and *S. italica* genome as reference revealed two distinct groups of *S. viridis* and a third group comprising the Chinese *S. viridis* accessions that resembled *S. italica*-like accessions, and all three groups also showed considerable admixture among themselves owing to rapid linkage disequilibrium (LD) decay in the overall sample (Huang et al. 2014). Intriguingly, the study reported a very strong correlation between genetic distance and climate, and genetic distance and geography in the North American *S. viridis* accessions, suggesting a balance between genetic drift and gene flow and multiple introductions and/or local adaptation to climate. A rapid decay in LD within 150 bp in wild green foxtail was also reported earlier (Wang et al. 2010). The authors reported that the level of LD in domesticated foxtail millet extends up to 1 kb. The genetic diversity study was carried out across 9 loci in 50 domesticated and 34 wild foxtail millet accessions that suggested a low level of genetic diversity in wild foxtail and also that domestication bottleneck of foxtail millet is more severe compared to maize and somewhat less prominent compared to rice (Wang et al. 2010). Another population structure and relative kinship study involving 184 foxtail millet accessions from diverse Indian geographical locations and 50 simple sequence repeat (SSR) markers showed a significant association ($R^2 = 18\%$) between 8 SSR markers and 9 agronomic traits, including flag leaf width and grain yield (Gupta et al. 2014).

8.4 Identification of Germplasm with Stress Resistance Traits

8.4.1 Biotic Stress Resistance

Millet crops are usually infected by fungal diseases and to a lesser extent by bacterial and viral diseases (Dwivedi et al. 2012). Blast, downy mildew, rust, and smut are the common fungal diseases that infect foxtail millet. Recently a new leaf and sheath brown spot fungus *Bipolaris australiensis* has also been reported to infect this crop (Mirzaee et al. 2010). Bacterial blight caused by *Xanthomonas* spp. is the most important bacterial disease affecting foxtail millet. This crop is also a carrier for both wheat curl mite *(Eriophyes tullipae* Keifer), which is a carrier of wheat streak mosaic virus and the virus itself (Baltensperger 2002). This viral disease, however, does not impact the already adapted foxtail millet cultivars, but they act as an over-summering host and severely affect nearby wheat fields. The effects of the above-mentioned diseases range from mild symptoms to severe infections, leading to devastations when large swathes of land are affected. However, not much work has been done regarding disease management in foxtail millet compared to pearl millet.

There has been a recent report where 17 foxtail millet germplasms were screened for leaf blast and rust at Regional Agricultural Research Station, Andhra Pradesh, India (Munirathnam et al. 2015). The study suggested DHFtMV 2-5 to be moderately resistant to both leaf blast and rust and it could be recommended for blast and rust prone areas. In an earlier study a core collection of 155 foxtail millet accessions were screened for new and diverse sources of blast resistance against Patancheru isolate (Fx 57) of *Magnaporthe grisea* (Sharma et al. 2014). The study reported two accessions, namely ISe 1181 and ISe 1547, to be free from head blast infection as well as resistance to neck, leaf, and sheath blast. On the other hand, ISe 1067 and ISe 1575 showed high levels of resistance to blast. However, in an earlier study, preliminary genetic analysis of four Japanese fungus isolates from foxtail millet suggested that blast resistance may be controlled by more than two dominant genes in this crop (Nakayama et al. 2005). Several foxtail millet germplasms have been identified and advanced lines for wheat streak mosaic resistance with enlarged head size and improved yield have also been developed (Siles et al. 2001).

8.4.2 Abiotic Stress Tolerance

Although foxtail millet is generally well-adapted to various abiotic stresses including drought, salinity, high temperature, and poor soil, even then this crop is not totally immune to abiotic stresses. Several of its varieties and accessions are also prone to various abiotic stresses. For example, foxtail millet being thinner stemmed is badly affected by lodging (Dwivedi et al. 2012). The worldwide foxtail millet accessions preserved in national and international genebanks can serve as an excellent resource for identification and exploitation of genetic variations for abiotic stress tolerance through precise phenotyping that can ultimately be utilized for crop improvement programs aimed at developing stress tolerant crops. In recent years foxtail millet has garnered substantial attention from the research community because of its projection as an excellent model for studying architecture, evolution, physiology, and stress tolerance attributes (Lata et al. 2013). Considerable work has been done toward germplasm identification and understanding the genetic basis of abiotic stress tolerance, particularly drought, salinity, lodging, and water logging in foxtail millet. Some progress has been made toward understanding the physiology of abiotic stress tolerance and the genomic regions associated with drought stress tolerance.

8.4.2.1 Drought

Foxtail millet is known to be an abiotic stress tolerant crop, particularly to drought, and its WUE is reported to be higher than that of maize, wheat, and sorghum (Shantz and Piemeisel 1927; He and Bonjean 2010). Its high WUE and short life cycle make it an elite drought-tolerant crop (Zhang et al. 2007b; Lata et al. 2011a). However, it is most sensitive to drought at the inflorescence and spikelet development stage (about 35–50 days after sowing). About 17,799 drought-tolerant accessions (17,313 landraces and 486 elite cultivars) of foxtail millet have been divided into five major classes on the basis of their survival capacity in different intensities of drought stress (Li 1991, 1997). In China, researchers had developed screening methods for assessing drought tolerance capacity of foxtail millet germplasms using polyethylene glycol (PEG 6000) or mannitol and identified relative water content and germination rate parameters as indicators of drought stress tolerance (Wen et al. 2005). Foxtail millet genotypes have been screened at the seedling stage for drought tolerance using 20% PEG (Zhang et al. 2005; Zhu et al. 2008). Lata et al. (2011b) screened about 107 foxtail millet cultivars from different geographical locations for drought tolerance at the seedling stage using 20% PEG, and two highly tolerant (cv. IC-403579 and cv. Prasad) and two highly sensitive (cv. IC-480117 and cv. Lepakshi) foxtail millet cultivars were identified. The study also indicated lipid peroxidation as an important marker for drought stress tolerance at the seedling stage in foxtail millet. The identified dehydration-tolerant genotypes may be used for crop improvement purposes (Lata et al. 2011b). Many drought-inducible genes with various functions have been identified on the basis of genomic and molecular functions in foxtail millet (Li et al. 2014; Lata et al. 2010, 2011a, 2014; Muthamilarasan et al. 2014a, b).

8.4.2.2 Salinity

A limited number of reports have been available on soil salinity response in foxtail millet, unlike other cereals. The shoot Na^+ concentration could be considered as a potential nondestructive selection criterion for vegetative-stage screening (Krishnamurthy et al. 2007). Zhi et al. (2004) has screened 260 foxtail millet landraces and cultivars using relative germination rate at 1.0 and 1.5% NaCl concentration and the results showed a high range of variations from 0 to 90% in different cultivars and landraces (0–20% in 29 accessions, 21–50% in 45 accessions, 51–90% in 153 accessions, and over 90% in 33 accessions). On salinity stress, genes of glutamine synthetase (GS) and pyrroline-5-carboxylate (P5C) reductase were found to be up-regulated, resulting in higher transcript levels of these two enzymes, which play an important role in the biosynthesis of proline, a molecule for osmotic regulation in stressed plants (Huang et al. 2013). Sreenivasulu et al. (1999) checked the peroxidase activity on two cultivars of foxtail millet and categorized them as salt tolerant (cv. Prasad) and salt sensitive (cv. Lepakshi). Furthermore, Sreenivasulu et al. (2004) identified 620 differentially expressed-sequence-tags (ESTs) from cv. Prasad and cv. Lepakshi, representing unigenes of a barley EST collection using a 711-cDNA inserts macroarray filter. Among these transcripts, hydrogen peroxide-scavenging enzymes including phospholipid hydroperoxide glutathione peroxidase (PHGPX), ascorbate peroxidase (APX), and catalase 1 (CAT1) as well as different oxidoreductase enzymes such as glutamine synthetase and pyrroline-5-carboxylate (P5C) and some genes of cellular metabolism were found to be significantly up-regulated under high salinity stress in the tolerant cultivar compared to the sensitive. The result showed significantly higher proline content in seedlings of both the cultivars, although the concentration of proline was found to be more in the tolerant than in the sensitive cultivar and also showed a positive correlation with increased glutamine synthetase and P5C reductase activities (Veeranagamallaiah et al. 2007).

To get a better perspective of the salinity stress responses at the molecular level, the temporal changes in total protein profile of cv. Prasad seedlings were examined under different salt stress conditions (Veeranagamallaiah et al. 2008) which led to the identification of 29 differentially-expressed salt-responsive proteins (both up- and down-regulated). Puranik et al. (2011a) analyzed the biochemical responses of 21-day-old seedlings of foxtail millet at 250 mM NaCl stress for 1–48 h for a better understanding of salt stress response in these two cultivars. The two cultivars showed differential salt stress responses when analyzed for lipid peroxidation, and different reactive oxygen scavenging enzymes such as glutathione reductase and catalase under short-term salinity stress. To understand the molecular mechanisms in response to short-term salinity stress, researchers constructed two suppression subtractive hybridization cDNA libraries (forward and reverse) leading to the identification of a total of 249 non-redundant ESTs which were grouped into 11 different categories. cDNA-macroarray analysis of these clones revealed 159 to be differentially expressed under salinity treatment with 115 up- and 44 down-regulated. These transcripts were reported for the first time representing untapped gene sources allowing specific responses to short-term salt-stress in an orphan crop known to possess a natural adaptation capacity to abiotic stress

8.4.2.3 Other Abiotic Stresses

Foxtail millet can also be tolerant to other abiotic stresses such as low temperature, lodging, and water logging. The northern limit of foxtail millet cultivation in China was 50°N. However, researchers in China have developed an extremely-cold-tolerant foxtail millet cultivar Liggu No 26, which helped in extending its cultivation up to 385 km farther north to 54°N (Chen and Qi 1993; Dwivedi et al. 2012). Foxtail millet production is also reportedly being constrained by lodging, resulting in substantial yield losses as well as poor grain quality. According to Tian et al. (2010), a lodging coefficient based on stem and root traits of foxtail millet could be a fitting indicator for estimating lodging resistance in field conditions. The mechanical strength of the stem, heights and weights of the above and underground plant tissues were recommended as the major donors of lodging coefficient across foxtail millet germplasm (Tian et al. 2010; Dwivedi et al. 2012). In another study, path analyses pointed out that breaking strength of stem, associated with greater culm diameter and culm wall thickness, is the most crucial factor determining lodging coefficient (Tian et al. 2015). Longgu 28 and Nenxian 13 are two lodging resistant cultivars that have been developed in China (Dwivedi et al. 2012). Foxtail millet production is also affected by water logging. A waterlogging tolerant foxtail millet cv. Lugu No.7 has been reportedly developed to combat this constraint (Chen and Qi 1993). Considering the above facts, it is apparent that discovery and utilization of untapped novel variations and precise phenotyping for abiotic stress tolerance are key to improving adaptation to adverse environmental conditions in foxtail millet. Furthermore, an improved understanding of the physiological and molecular mechanism(s) of abiotic stress tolerance in foxtail millet can be effectively utilized for developing more stress-tolerant cultivars.

8.5 Factors Responsible for Abiotic Stress Tolerance in Foxtail Millet

8.5.1 Transcription Factors

Plant responses under abiotic stress conditions are a complex phenomenon that has various dynamic responses manifested at physiological, biochemical and molecular levels. Plants subjected to stress environments perceive stress signals through specific receptors and communicate via sophisticated signal transduction pathways, resulting in activation of stress-inducible transcription factors (TFs) and downstream stress-responsive gene expression. TFs associated with abiotic stress responses in foxtail millet are listed in Table 8.1. To open the insights of dehydration stress regulatory pathway in foxtail millet, a comparative transcriptome analysis has been carried in a drought-tolerant cultivar cv. Prasad using a suppression subtractive hybridization (SSH) technique. This study resulted in identification of a novel stress- responsive TF *SiDREB2* belonging to DREB (dehydration-responsive element-binding proteins) sub-family (Lata et al. 2010). The transcript level of *SiDREB2* was found to be significantly up-regulated in tolerant cultivar, suggesting this gene might play an important role in stress-responsive mechanisms in foxtail millet. DREB is an important subfamily of AP2/ERF (APETALA2/ethylene-responsive element-binding factor) TFs and participates in the regulation of stress-responsive gene expression through abscisic acid (ABA)-independent pathways (Lata and Prasad 2011).

A novel *SiDREB2* has been cloned and characterized from foxtail millet and used to develop an allele-specific marker (ASM) for dehydration tolerance (Lata et al. 2011a; Lata and Prasad 2013b, 2014). Furthermore, to understand the role of AP2/ERF TFs in foxtail millet, a genome-wide analysis has been carried out using in silico approaches (Lata et al. 2014). A total of 171 AP2/ERF encoding genes in the *S. italica* genome have been identified, of which 48 were DREB TFs evaluated with the help of phylogenetic and domain architecture analysis. NAC TFs are also well-known for their regulatory role in biotic as well as abiotic stress in many crop plants, and a subtractive hybridization study in *S. italica* showed significant up-regulation of *SiNAC* in salinity stress libraries (Puranik et al. 2011a). Puranik et al. (2011b) reported the cloning of *SiNAC*, a novel membrane-associated NAC gene that functions as an activator of transcription in stress responses and also regulates plant developmental pathways. Puranik et al. (2013) also carried out genome-wide identification and characterization of NAC TFs in *S. italica*. Among the

Table 8.1 Details of important transcription factors linked with abiotic stress responses in foxtail millet

Transcription factor	Numbers identified	Stress response	Functional characterization	References
SiDREB2	1	Dehydration, drought and salt response	Yes	Lata et al. (2011a)
SiNAC2	1	Dehydration, drought and salt response	Yes	Puranik et al. (2011b)
SiNAC	147	Drought and salt stress	–	Puranik et al. (2013)
SiAP2/ERF	171	Drought and salinity response	–	Lata et al. (2014)
SiARDP	1	Drought	Yes	Li et al. (2014)
SiMYB	209	Abiotic stress and hormone response	–	Muthamilarasan et al. (2014b)
SiC2H2	124	Abiotic and hormone response		Muthamilarasan et al. (2014a)
SiWRKY	105	Dehydration, salt and hormone stress	–	Muthamilarasan et al. (2015)
SvWRKY	44	Dehydration, salt and hormone stress	–	Muthamilarasan et al. (2015)
SiNF-YA1 and *SiNF-YB8*	01	Drought and salt stress	Yes	Feng et al. (2015)
SiASR1	01	Drought and oxidative stress	Yes	Feng et al. (2016)
SiDof	35	Drought stress	–	Zhang et al. (2017)

identified 147 *SiNAC* genes, 50 candidate genes were selected for quantitative expression analysis under various abiotic stress treatments. Results of the above study suggested *SiNAC128* as a potential candidate gene for further in-depth characterization (Puranik et al. 2013).

Zhang et al. (2012) and Bennetzen et al. (2012) released a draft genome sequence of *S. italica* that revealed various novel aspects of foxtail genome and facilitated the identification and characterization of a few important stress-responsive TFs, including MYB and C2H2, and miRNAs (Lata and Prasad 2013a). These two (MYB and C2H2 proteins) TFs constitute the largest TF families in plants, playing crucial roles in various developmental and stress-responsive processes (Ambawat et al. 2013). To analyze their role in abiotic stress response, a comprehensive genome-wide study has been performed on foxtail millet (Muthamilarasan et al. 2014a, b). A total of 209 and 124 gene family members of MYB and C2H2, respectively, were identified. Furthermore, on the basis of phylogenetic analysis, *SiMYB* proteins grouped into ten groups (I–X) and *SiC2H2* proteins into five groups (I–V). It has also be found that *SiMYB* and *SiC2H2* protein sequences show significant similarity with their orthologs in sorghum, maize, and rice that show conservation in the overall protein structure of these TFs (Muthamilarasan et al. 2014a, b). Expression patterns of *SiMYB* and *SiC2H2* candidate genes in response to abiotic stresses and hormone treatments using qRT-PCR revealed specific and/or overlapping expression patterns of these genes. Out of analyzed expression profiles of 11 candidate *SiMYB* genes, 3 (*SiMYB124, SiMYB126*, and *SiMYB150*) showed significant up-regulation during drought stress.

In the case of *SiC2H2*, nine candidate genes were selected for expression analysis under abiotic stress response that suggested late expression of *SiC2H2 031*, whereas *SiC2H2 78, SiC2H2 85*, and *SiC2H2 94* showed higher expression during the early phase of drought stress. WRKY proteins also play a significant role in signaling pathways associated with different stress responses. A genome-wide analysis of the WRKY TF family in *S. italica* (*SiWRKY*) and *S. viridis* (*SvWRKY*) was conducted, leading to the identification of 105 *SiWRKY* and 44 *SvWRKY* proteins, respectively (Muthamilarasan et al. 2015). Sequence alignment of these WRKY proteins classified them into three major groups, namely groups I, II, and III. Most of the WRKY proteins fall into group II (53 *SiWRKY* and 23 *SvWRKY*), followed by group III (39 *SiWRKY* and 11 *SvWRKY*) and group I (10 *SiWRKY* and 6 *SvWRKY*). Phylogeny analysis further divided group II into five sub-groups (IIa–e). Comparative mapping of *SiWRKY* and *SvWRKY* genes among related C_4 Panicoid genomes demonstrated the orthologous relationships between these genomes. Expression profiling of candidate *SiWRKY* genes in response to stress (dehydration and salinity) and hormone treatments (ABA, salicylic acid, and methyl jasmonate) suggested the putative involvement of *SiWRKY 066* and *SiWRKY 082* in stress and hormone signaling (Muthamilarasan et al. 2015).

In another study, six ABA stress ripening (ASR) genes were identified from foxtail millet (Feng et al. 2016). The proteins contain ABA/WDS domain and are a class of plant-specific TFs. Overexpression of *SiASR1* in tobacco led to enhanced drought and oxidative stress tolerance as well as altered expression levels of *NtSOD*, *NtAPX*, *NtCAT*, *NtRbohA*, and *NtRbohB* genes, suggesting its important role in stress-related signaling. Furthermore, the expression level of *SiASR4*, a target gene of *SiARDP*, increased under drought and salt stress in transgenic *Arabidopsis* and foxtail millet plants (Li et al. 2017). The *SiASR4* transgenic plants showed enhanced transcription of stress-responsive and reactive oxygen species (ROS) scavenger-associated genes. Together, these findings suggested that *SiASR4* functions in drought and salt stress environments and is regulated by *SiARDP* via an ABA-dependent pathway (Li et al. 2017). Similarly, *SiARDP* an ABA-responsive DRE-binding protein-coding gene enhances drought and salt stress tolerance in Arabidopsis and improves drought stress tolerance in transgenic foxtail millet (Li et al. 2014). The gene was also found to be regulated by two ABA-responsive element binding (AREB)-type TFs, namely *SiAREB1* and *SiAREB2*, which were able to bind SiARDP both in vitro and in vivo physically. Furthermore, in view of the role of TFs in modulating stress-responsive gene regulatory networks, an in silico study was undertaken to identify and characterize total TF-encoding genes in *S. italica* genome (Bonthala et al. 2014). The study identified 2,297 putative TFs and categorized them in 55 families. This information is available in the Foxtail millet Transcription Factor Database (http://59.163.192.91/FmTFDb/) in which complete details of the TFs are compiled, including their sequences, physical positions, tissue-specific gene expression data, gene ontologies, and phylogeny (Bonthala et al. 2014). This database is useful in pinpointing candidate TFs for stress-related studies and performing large-scale investigations.

8.5.2 Other Stress Responsive Genes

Other than TFs, several stress-responsive genes have also been analyzed for their expression profiles and activities under various abiotic stresses in foxtail millet (Table 8.2). Isolation and functional characterization of osmotic stress-responsive genes from foxtail millet could be an important measure for deciphering abiotic stress tolerance mechanisms. Sreenivasulu et al. (2004) examined the expression profile of a phospholipid hydroperoxide

Table 8.2 Functional validation of important genes linked with abiotic stress response in foxtail millet

Gene	Gene function	Source	Plant/organism tested	Type of tolerance	References
SiOPR1	12-Oxophytodienoic acid reductase 1	Foxtail millet	Foxtail millet	Drought tolerance	Zhang et al. (2007b)
Aldose reductase	Detoxify free toxic aldehydes	Foxtail millet	Foxtail millet	Salt tolerance	Veeranagamallaiah et al. (2009)
DNAj	Chaperon protein	Foxtail millet	Wheat	Drought and heat tolerance	Wang et al. (2009)
SiWD40	WD protein	Foxtail millet	Foxtail millet	Salt and Drought tolerance	Mishra et al. (2012)
SiPLDa1	Phospholipase D	Foxtail millet	*Arabidopsis Thaliana*	Drought stress	Peng et al. (2010)
SiARDP	ABA-responsive DRE-binding protein gene	Foxtail millet	*Arabidopsis Thaliana*, Foxtail millet	Salt and Drought tolerance	Li et al. (2014)
SiLEA14	LEA protein	Foxtail millet	*E. coli*, *Arabidopsis Thaliana*, Foxtail millet	Salt and Drought tolerance	Wang et al. (2014a)
SiREM6	Encode remorin protein	Foxtail millet	*Arabidopsis Thaliana*	Salt tolerance	Yue et al. (2014)
SiALDH	Aldehyde dehydrogenase	Foxtail millet	*Escherichia coli*	Salt tolerance	Chen et al. (2014)
SiLTP	Lipid transfer protein	Foxtail millet	Tobacco, Foxtail millet	ABA, salinity and drought stress tolerance	Pan et al. (2016)
SisHSP-27	Heat shock proteins	Foxtail millet	Yeast	Drought, heat, salt and cold	
SiASR4	Encode abscisic acid, stress-and ripening induced proteins	Foxtail millet	*Arabidopsis Thaliana*, Foxtail millet	Salinity and drought tolerance	Li et al. (2017)

glutathione peroxidase gene *PHGPX* in the tolerant foxtail millet cultivar Prasad and proposed its crucial role in conferring salt stress tolerance and in oxidative stress-induced defense reactions. A 12-oxophytodienoic acid reductase 1 gene *SiOPR1* was reported to be highly expressed in foxtail millet roots under drought stress. However, the gene was not found to be influenced by NaCl, ABA, and methyl jasmonate treatments, indicating its important role in drought stress tolerance (Zhang et al. 2007c). The comparative expression profiles of glutamine synthetase (*GS*) and pyrroline-5-carboxylate (*P5C*) reductase under salinity stress in a salt tolerant (cv. Prasad) and a salt sensitive (cv. Lepakshi) foxtail millet cultivar indicated a positive correlation with higher proline accumulation (Veeranagamallaiah et al. 2007). An improved aldose reductase activity with increasing salt stress was also reported in these two foxtail millet cultivars (Veeranagamallaiah et al. 2009). Interestingly, the increase in the enzyme activity was positively correlated with accumulation of sorbitol, essential for osmotic balance, and 4-hydroxynon-2-enal, a major product of lipid peroxidation.

Zhao et al. (2009) reported improved aluminum stress tolerance in transgenic Arabidopsis plants overexpressing *Si69*, a Wali7 homologue. In another study, foxtail millet *DNAj* gene, associated with drought and heat tolerance, was introgressed into four wheat cultivars via the pollen-tube pathway, paving the way for developing drought-tolerant wheat lines (Wang et al. 2009). Overexpression of a phospholipase D gene *SiPLDα1* in Arabidopsis remarkably enhanced drought tolerance of transgenic plants (Peng et al. 2010). The Arabidopsis transgenic plants had higher biomass, increased relative water content, reduced electrolytic leakage, and higher survival percentages, with no undesirable effect on their growth and development compared to control plants. Augmented expression levels of several stress-related genes were also observed in the *35S::SiPLDα1* transgenic Arabidopsis plants, suggesting *SiPLDα1* as a useful target gene for improving drought stress tolerance (Peng et al. 2010). Mishra et al. (2012) reported a nuclear-localized WD-repeat-containing protein gene *SiWD40* to be induced under various abiotic stresses such as dehydration, salt, cold, and ABA. It was also suggested that an AP2-domain-containing protein SiAP2 might regulate the environmental stress-responsive *SiWD40* expression. Recently, 11 plant-specific remorin protein family genes were identified from foxtail millet (Yue et al. 2014). The remorin proteins have the ability to attach to the plasma membrane (Jacinto et al. 1993). One of the remorin family genes, *SiREM6*, was found to improve high salt stress in transgenic Arabidopsis at germination and early seedling growth stages. Interestingly, the *SiREM6* promoter contained two DRE elements and one AREB element. However, only a DRE-binding TF *SiARDP* could physically bind to the DRE elements of the *SiREM6* promoter whereas *SiAREB1* could not bind to the AREB-element, indicating *SiREM6* to be a target gene of *SiARDP* (Yue et al. 2014).

In a similar kind of study, researchers have characterized a late embryogenesis abundant (LEA) gene *SiLEA14* from foxtail millet (Wang et al. 2014a). The LEA proteins are known to play important roles in protecting plants from various abiotic and biotic stresses. *SiLEA14* gene was expressed in different developmental stages including roots, stems, leaves, inflorescence, and seeds. The gene was found to be highly induced by salt stress and exogenous ABA application. Overexpression of *SiLEA14* improved *Escherichia coli* growth performance under salt stress as compared to control. The Arabidopsis seedlings overexpressing *SiLEA14* exhibited enhanced salt and osmotic stress tolerance in comparison to the wild-type (WT) plants. The transgenic foxtail millet seedlings also displayed better growth under salt and drought stresses than the WT. The results thus indicated *SiLEA14* to be a novel atypical LEA protein that might play significant roles in abiotic stress resistance of crop plants. One of the studies highlighted the role of aldehyde dehydrogenases (ALDH) in various abiotic stress responses such as osmotic stress, cold, H_2O_2, and ABA in foxtail millet. ALDH genes are known to detoxify ROS indirectly and help in reducing lipid peroxidation mediated cellular toxicity under various environmental stresses (Chen et al. 2014). The genome-wide survey led to the identification of 20 *SiALDH* genes. Expression analysis suggested organ- and stress-specific expression of these genes in foxtail millet, and the transformation of *SiALDH2B2*, *SiALDH10A2*, *SiALDH5F1*, *SiALDH22A1*, and *SiALDH3E2* genes in *E. coli* led to improved tolerance to salt stress (Chen et al. 2014). A similar investigation for identification and characterization of cytokinin oxidase/dehydrogenase (CKX) from foxtail millet was performed (Wang et al. 2014b). The results indicated high up-regulation of *SiCKX* genes under various abiotic stresses, hinting at their possible role in stress regulation.

Pan et al. (2016) isolated a non-specific lipid transfer protein (LTP) coding gene *SiLTP* from foxtail millet. The LTPs are low molecular weight cysteine-rich soluble proteins with diverse roles in different developmental stages as well as in biotic and abiotic stress responses. The *SiLTP*-overexpressing foxtail millet lines showed improved tolerance to salt and drought stresses whereas the *SiLTP* RNA interference (RNAi)-based transgenic lines of foxtail millet were found to be more sensitive to salt and drought stress compared to control. Recently, genome-wide investigation for various heat shock proteins (HSPs) have also been performed in foxtail millet, leading to the identification of 20, 9, 27, 20, and 37 genes belonging to *SiHSP100*, *SiHSP90*, *SiHSP70*, *SiHSP60*, and *SisHSP* families, respectively (Singh et al. 2016). Expression profiling indicated up-regulation of several HSPs in the tolerant cultivar compared to the sensitive one under dehydration, heat, salinity, and cold stresses. Furthermore, overexpression of a small HSP gene *SisHSP-27* in yeast conferred tolerance to various abiotic stresses.

Altogether, these studies have identified potential candidate genes that could be effectively utilized in crop improvement programs for improving abiotic stress tolerance. However, the lack of an efficient transformation system for expressing the candidate genes in foxtail millet remains a bottleneck in *Setaria* genomics, even though these genes are reported to be effective in enhancing stress tolerance of transgenic plants by regulating the expression of broad-spectrum stress-related genes. As a consequence, the detailed molecular, cellular, and physiological mechanisms responsible for variation in tolerance to various abiotic stresses, including drought tolerance among foxtail millet lines, have not yet been fully elucidated.

8.5.3 Small RNAs

Small RNAs (sRNAs) are noncoding RNAs, including miRNA, siRNA, snRNA, snoRNA, etc. (Sunkar and Zhu 2004). Among them, microRNAs (miRNAs) and endogenous small interfering RNAs (siRNAs) represent two major classes of small RNAs involved in gene regulation. Both miRNAs and siRNAs act as modulators of gene expression at the post-transcriptional level and are key players in stress response (Sunkar 2010). miRNAs regulate the expression of the target transcripts/genes by binding to reverse complementary sequences, causing cleavage of the target RNA, whereas siRNAs bind to the target sequence in a similar manner and direct DNA methylation (Khraiwesh et al. 2011). Several studies have been conducted to date that elucidate the involvement of miRNAs in various abiotic and biotic stresses including drought (Zhao et al. 2007; Liu et al. 2008; Zhou et al. 2010), cold (Zhou et al. 2008), salinity (Liu et al. 2008; Sunkar et al. 2008), UV-B radiation (Zhou et al. 2007), mechanical stress (Lu et al. 2005), and bacterial infection (Navarro et al. 2006) in various model plants. With the advancement in high-throughput sequencing and small RNA profiling techniques, along with the availability of advanced data analysis tools and software, the sequencing of a large number of small RNA libraries simultaneously and identification of stress-responsive miRNAs with precision have become faster and cheaper (Ding et al. 2013; Rajwanshi et al. 2014). However, only recently there have been a few reports on the identification of stress-responsive miRNAs from foxtail millet. A genome-wide transcriptome study in *S. italica* under drought stress was performed by Qi et al. (2013).

Two RNA and sRNA libraries were constructed from two treatments, namely drought stressed, and unstressed whole seedlings *S. italica* Yugu1 and sequenced. sRNAs of varied nucleotide sequence lengths were identified—24-nt (nucleotide) sRNAs were found to be predominant followed by 21-, 22-, and 23-nt sRNAs. The study also revealed that a number of 24-nt siRNAs were low across the genic regions, indicating their negative role in influencing gene expression in response to drought stress. Differential expression analysis led to the identification of 19 long maximally expressed noncoding RNAs during drought stress and, among these, two natural antisense transcripts (NATs of Si003758m and Si038715m) showed drought-regulated expression patterns (Qi et al. 2013). Similarly, Yi et al. (2013) constructed two small RNA libraries from shoot tissue of *S. italica* inbred line Yugu1 followed by sequencing using Illumina HighSeq 2000 platform leading to the identification of a total 43 known miRNAs, 172 novel miRNAs, and 2 miRNA precursor candidates. The targets of the selected miRNAs were identified, annotated, and functionally validated by stem-loop RT-PCR in four tissues (Yi et al. 2013). The raw reads generated from the studies of Yi et al. (2013) and Qi et al. (2013) are available in the NCBI SRA database under accession numbers SRA062640 and SRA062827, respectively.

Han et al. (2014) analyzed expression patterns of 43 miRNAs in different tissues of *S. italica*, including leaves, roots, stems, and spikes, and also experimentally validated five predicted targets of four miRNAs using 5′-RLM-RACE. Furthermore, a total of 355 mature miRNAs (Sit-miR) have been identified from the genomic and CDS sequences of *S. italica* and classified into 53 families (Khan et al. 2014). Expression profiling of candidate Sit-miRs was analyzed using Northern blot analysis and stem-loop RT-qPCR under different abiotic stresses in two *S. italica* cultivars (IC-403579, stress tolerant; IC-480117, stress susceptible). Three Sit-miRs, namely Sit-miR162a, Sit-miR167b, and Sit-miR171b, were found to be up-regulated in tolerant cultivar compared to sensitive and Sit-miR156c, Sit-miR397a, Sit-miR393, Sit-miR160d, and Sit-miR6248a were found to be down-regulated in the tolerant cultivar under drought stress (Khan et al. 2014). The complete information about chromosomal location, length, sequences of pre-miRNA and mature miRNA, secondary structure, and target gene information of identified sit-miRs has been made available to the global research community through an open-access web resource, Foxtail millet miRNA Database (http://59.163.192.91/FmMiRNADb/ index.html; Khan et al. 2014).

In another study, four small RNA libraries from control and drought stressed seedlings of IC-403579 (tolerant) and IC-480117 (susceptible) were constructed and sequenced using Illumina HiSeq 2000 platform (Yadav et al. 2016). Transcriptome results of this study led to the identification of a total of 55 known miRNAs, which were classified into 23 miRNA families and 136 novel miRNAs which could be classified into 47 miRNA families, respectively. Stem-loop quantitative real-time PCR further validated some of these candidate novel dehydration-responsive Sit-miRs. In a similar kind of study, drought-responsive miRNAs were identified from *S. italica* inbred An04-4783 seedlings using Illumina sequencing (Wang et al. 2016). A total of 81 known and 76 novel miRNAs were identified. Furthermore, degradome sequencing was carried out to confirm the target genes of the identified drought-responsive miRNAs. The above studies give an insight into the role of miRNAome of *S. italica* in response to abiotic stress. There is also a dire need for identification and characterization of target genes of sRNAs to delineate their roles in abiotic stress regulatory pathways in foxtail millet.

8.6 DNA Methylation

Alteration of gene expression during plant development and various environmental stresses may also occur because of epigenetic modifications such as DNA methylation, chromatin remodeling, and histone modification (Boyko and Kovalchuk 2008). Many cytosines in the genome, particularly those located at CG dinucleotide, may potentially act as transcriptional switches (Shibuya et al. 2009). Hypomethylation or hypermethylation of DNA may function as a major switch to control the expression of effector genes during stress response in plants (Chinuusamy and Zhu 2009). Very little is known about the global patterns of epigenetic modification such as DNA methylation in the case of abiotic stress, and whether DNA methylation plays a role in various abiotic stress responses in plants is still unclear. Considering this, a genome-wide salinity-induced differential methylation analysis was carried out on 3-weeks-old contrasting foxtail millet cultivars, namely salinity tolerant cv. IC403579 (IC04) and sensitive cv. IC480117 (IC41) using a methylation-sensitive amplified polymorphism (MSAP) technique. The study indicated a reduction in DNA methylation level in the tolerant cultivar compared to the sensitive. It also led to the identification of a total of 86 MSAP fragments, which were functionally annotated and classified into various categories, namely transporters, TFs, phosphatases, oxidoreductases, transposable elements (TEs), etc. A validation of methylome data was also carried out through expression analysis of four selected genes. Overall, the study inferred the role of salinity stress in inducing genome-wide DNA methylation as well as gene expression modulation in foxtail millet (Pandey et al. 2017).

8.7 Advances in Foxtail Millet Genomic Resources and Their Utilization

Although foxtail millet is an important grain crop and a potential model for several bioenergy crops, the availability of genomic tools is limited and needs to be developed. ESTs and molecular markers can be excellent genomic resources for foxtail millet. Zhang et al. (2007b) analyzed the dehydration-induced transcriptome of foxtail millet cv. Mar51 using subtracted cDNA library and microarray. A total of 1,947 uniESTs were obtained of which 95 and 57 ESTs were up-regulated in roots and shoots, respectively. Jayaraman et al. (2008) used cDNA-AFLP markers for comparing the gene expression profiles of a salt tolerant and a salt sensitive cultivar of foxtail millet (*S. italica*) in response to salt stress. A total of 90 differentially expressed transcript-derived fragments (TDFs) were identified, out of which 86 TDFs were classified on the basis of their either complete presence or absence (qualitative variants) and 4 on differential expression pattern levels (quantitative variants) in the two varieties. Other than these, the genes identified in transcript profiling studies on drought (Lata et al. 2010) and salinity (Puranik et al. 2011a) are important genomic resources, which could be exploited for enhancing drought tolerance traits and can be functionally validated to understand the molecular genetics of foxtail millet in stress response and adaptation. Recently, Qi et al. (2013) analyzed the whole transcriptome of foxtail millet using the Illumina platform and identified a total of 2,484 drought responsive genes, out of which ~48% were up-regulated and ~52% were down-regulated. Sequence-based molecular markers, genetic linkage maps, and trait-genetics are important molecular tools in marker-aided selection and breeding for desired agronomic traits such as yield, quality, and improved abiotic stress tolerance. The availability of the foxtail millet genome sequence in the public domain has been an immense help in the development of large-scale genomic resources, comparative mapping, markers/quantitative trait loci (QTLs) identification and development, and molecular breeding of foxtail millet and related cereals crops (Muthamilarasan and Prasad 2015). The first assembled reference genome of foxtail millet and green foxtail was released independently by the United States Department of Energy-Joint Genome Initiative (USDOE-JGI) and Beijing Genome Initiative (BGI), China in 2012 (Bennetzen et al. 2012; Zhang et al. 2012; reviewed in Lata and Prasad 2013). Both studies predicted the genome size of foxtail millet to be ~490 Mb coding for ~30,000 genes. The studies also led to the identification of large number of stress-responsive genes and miRNA families in foxtail millet. In addition, thousands of SNPs, insertion-deletion polymorphisms, and structural variations were also identified in the sequenced genomes when compared with other lines.

In light of the availability of the genome sequence, comparative genomics studies have been geared up for the identification of genes underlying QTL(s) for determining key traits for genetic improvement of foxtail millet (Paterson et al. 1995; Doust and Kellogg 2006; Doust et al. 2009, 2010; Lata et al. 2013; Muthamilarasan and Prasad 2015). Foxtail millet genome sequence was also helpful in aligning the switchgrass genome (http://kdbioinfo.cropsoil.uga.edu/devoslab/prjctfxtlmllt.html), leading to translating the sequence and QTL information from this diploid crop to other candidate biofuel grasses, which are otherwise polyploid. A direct practical proposition of using foxtail millet genome sequence would therefore be an easy access for crop breeders searching for exploitable variations for crop improvement strategies. The genome sequence information has also served as a reference in whole-genome resequencing (WGR) of 916 *S. italica* accessions collected from different eco-geographical zones of the world and the construction of a high-density haplotype map using 85 million single nucleotide polymorphisms, which revealed genomic variations among these accessions (Jia et al. 2013). Similarly, the *S. italica* genome sequence data facilitate WGR of cultivated and wild varieties of *Setaria* with contrasting phenotypes to identify novel genes/alleles/QTLs underlying drought response and to execute NGS-based genomics-assisted breeding for drought tolerance. Although the *S. viridis* genome has also been sequenced (Bennetzen et al. 2012), the lack of publicly available sequence information has for a long time significantly impeded the development of genetic and genomic resources in this important model species. However, recent developments have made this data available in the web portal “*Setariabase*” (http://www.sviridis. org; Brutnell et al. 2015).

Sequence-based molecular markers, QTLs, and genetic linkage maps can also be effectively utilized for marker-aided breeding of economically important traits. Lata et al. (2011a) have identified an SNP in the *SiDREB2* gene of foxtail millet associated with dehydration tolerance. An ASM has also been developed for the same, which can amplify the specific products from the dehydration-tolerant and sensitive accessions (Lata et al. 2011a). It has also been shown that this ASM contributes to approximately one-fourth of the total variation in lipid peroxidation and relative water content at the seedling stage (Lata and Prasad 2013b, 2014). Furthermore, considering the importance of intron length polymorphic (ILP) markers in germplasm characterization, diversity studies, and molecular breeding, a set of 98 ILP markers was developed from foxtail millet (Gupta et al. 2011). In continuation of this, 5,123 ILP markers were also recently developed, and their utility in cross-species transferability, germplasm characterization, and comparative mapping among millets and non-millets was also demonstrated (Muthamilarasan et al. 2013). Foxtail millet has very limited resources of co-dominant microsatellite markers and saturated genetic linkage maps. In light of this, Pandey et al. (2013) performed a genome-wide analysis and identified 28,342 microsatellite repeat motifs spanning 405.3 Mb of foxtail millet genome. Among these microsatellites, trinucleotide repeats (~48%) were predominant followed by dinucleotide repeats (~75%). About 159 markers were validated successfully in 8 accessions of *Setaria sp.* with ~67% polymorphic potential for different traits. Similarly, Kumari et al. (2013) identified 495 EST-SSRs from 66,027 ESTs available at the NCBI database and developed 447 SSR markers successfully validated in green foxtail and other millets. In another study, Zhang et al. (2014) isolated 5,020 highly repetitive microsatellite motifs from Yugu1 genome and designed 788 SSR primer pairs based on the sequence comparison between *S. italica* and *S. viridis*. Out of these, 733 could produce reproducible amplicons and were found to be polymorphic for 28 *Setaria* genotypes. Furthermore, Yadav et al. (2014) developed 176 miRNA-based molecular markers. In a similar line, the highly polymorphic nature of 20,278 TE-based markers were developed which could be categorized into five different polymorphism types, namely retrotransposon-based insertion polymorphisms, inter-retrotransposon amplified polymorphisms, repeat junction markers, repeat junction–junction markers, insertion-site-based polymorphisms and retrotransposon-microsatellite amplified polymorphisms (Yadav et al. 2015). Open access databases (Foxtail millet Transposable Elements-based Marker Database; FmTEMDb; http://59.163.192.83/ltrdb/index.html; and Foxtail Millet microRNA Database; FmMiRNADb; http://59.163.192.91/FmMiRNADb/) for these resources were also constructed for open use by the researchers. The details of molecular markers and genetic linkage maps reported to date in foxtail millet are listed in Table 8.3. Furthermore, the ASM, microsatellites, and other molecular markers would assist and play a significant role in allele mining of germplasm resources, diversity and transferability studies, phylogenetics, and comparative mapping, thus paving the way for discovery and exploitation of novel alleles in crop improvement, and accelerating the foxtail millet breeding process for stress tolerance.

8.8 Conclusion and Future Perspectives

Considering the potential of both foxtail millet and green foxtail as an excellent model system for evolutionary, architectural and physiological studies of related bioenergy grasses and C_4 crops, much attention has been given to these crops in the last decade by the scientific community. In fact, foxtail millet has gained considerable attention in terms of both structural and functional genomics. Furthermore, the release of the *Setaria* genome sequence has paved the way for large-scale development of genomic resources for this important model crop. Notably, foxtail millet research pertaining to various abiotic stresses has led to the identification of several potential candidate genes that could be effectively utilized in crop improvement programs including transgenics and molecular breeding. Furthermore, the development of various databases would act as a valuable resource for large-scale genotyping applications in foxtail millet. Despite genome-wide investigation of several gene families, there is still much scope for further studies that could help to provide a better understanding of the complex abiotic stress

Table 8.3 Summary of DNA-based markers and QTLs available in foxtail millet related to abiotic stress tolerance

DNA markers/QTLs	References
100 polymorphic SSRs developed from the two genomic DNA libraries	Jia et al. (2009)
~1000 SNPs by sequencing pools of RILs (*S. italica* acc. B100 x *S. virdis* acc. A10)	http://www.plantbio.uga.edu/media/2010_grad_symposium(1).pdf
98 ILP markers	Gupta et al. (2011)
One Allele-specific marker for *SiDREB2* gene	Lata et al. (2011a)
147 genomic microsatellite markers	Gupta et al. (2011)
28 342 microsatellites	Pandey et al. (2013)
447 EST-SSRs	Kumari et al. (2013)
788 SSRs	Zhang et al. (2014)
5,123 ILP markers	Muthamilarasan et al. (2014a, b)
176 miRNA-based markers	Yadav et al. (2014)
20,278 TE-based markers	Yadav et al. (2015)
8 QTLs and 128 SSR markers spans 1293.9 cm with an average of 14 markers per linkage group of the 9 linkage groups	Qie et al. (2014)

regulatory mechanisms operating in foxtail millet. The role of small RNAs and epigenetic modifications in abiotic stress response remains elusive, and therefore concerted efforts should be directed toward understanding their roles in abiotic stress response and adaptation. Further whole genome resequencing of diverse foxtail millet accessions would help not only in discovering novel variants for various abiotic stresses but also in exploiting them for the development of molecular markers and identification of QTLs to be utilized in foxtail millet breeding programs. Taken together, the potential abiotic stress tolerance features and the development of large-scale genetic resources in foxtail millet would certainly help in accelerating the crop improvement programs in this climate resilient crop.

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