Genomic Approaches for Abiotic Stress Tolerance in Sorghum

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Santosh Deshpande, Sujay Rakshit, K.G. Manasa, Sarita Pandey and Rajeev Gupta

Abstract

Although sorghum is a crop grown under harsh environments, its productivity is adversely affected by various abiotic stresses including drought, temperature extremes, low fertility, and mineral toxicity among others. In recent years a large number of genetic and genomic resources have become available in sorghum, which provide researchers opportunities to relate sequence variations with phenotypic traits of interest and their utilization in sorghum improvement programs. The application of the molecular marker and genomic technologies has shown promise for efficient breeding. However, very few successful examples are available in the public domain of research in this direction. Some of these successes specifically related to application of molecular marker technologies for improving abiotic stresses are explained in this chapter. With recent advances in next-generation sequencing technologies and high-throughput phenotyping platforms/technologies, utilizing the new/advanced mapping populations such as nested-association mapping (NAM), backcross-derived NAM has shown great potential. These recent advancements will be the drivers for integration of genomics technologies in routine breeding programs in the immediate future.

1 Introduction

Sorghum [*Sorghum bicolor* L. (Moench)] is vital to the food security of many of the world's poorest people living in fragile agroecological zones. Sorghum is produced by about 100 countries in the world and mainly used as staple food in parts of Asia and Africa, whereas in the United States, Mexico, and Australia it is used as a major feed crop (Rakshit et al. 2014). The genome of sorghum is unique as there is only ~3 % differential sorghum–rice gene loss

S. Deshpande (✉) · K.G. Manasa · S. Pandey · R. Gupta
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Telangana, India
e-mail: s.deshpande@cgiar.org

S. Rakshit
ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India

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and comparatively less structural rearrangement for sorghum–rice in 50 million years than sorghum–maize rearrangement (with genome duplication events) in ~15 million years in evolutionary lineage (Bowers et al. 2003). The sorghum karyotype shows 70 million years of "abstinence" from genome duplication. Researchers have exploited this great opportunity to study functions of sorghum genes which may still resemble those of the common cereal ancestor. Accordingly, the sequenced ~740 Mb sorghum genome (Paterson et al. 2009) is a logical complement to that of *Oryza* (rice) for grass functional genomics.

In addition to its importance as a model crop, sorghum is the most drought-resistant among the world's top five cereal crops, and an important dual-purpose crop. Sorghum cultivation ranges from the equator (approximately 50° latitude) to elevations of 2500 m (Rao et al. 2015). Sorghum is exceptionally tolerant to low input levels (especially in West Africa), which is an important characteristic for the areas receiving little rainfall. Thus, sorghum plays a vital role in feeding the world's most vulnerable population under the context of increasing demand for limited fresh water supply, increasing use of marginal farmland, and climatic trends. These interesting facts about sorghum along with recent advances in genomics with the advent of the next-generation sequencing (NGS) platforms for genotyping, expression studies, and high-throughput phenotyping facilities make it a model plant species not only to study evolutionary relationships across other grass species but also to help understand the mechanisms and functions of the genome that will lead to better adaptation to climate change, especially for abiotic stresses such as drought, salinity, cold tolerance, and nutrient use efficiency. This is more relevant in the current scenario where global food production has to be increased by 70 % in the background of ever-shrinking cultivable land and water resources (MacIntyre et al. 2009), and increased incidences of environmental extremes such as floods, drought, and extreme temperatures (Mickelbart et al. 2015). Wang et al. (2014a, b) made a detailed review of the status of abiotic stress genomics in sorghum vis-à-vis other cereals, and Anami et al. (2015) reviewed biotic and abiotic stress resistance with specific reference to sweet sorghum. In this chapter, efforts have been made to update the status in this area, keeping the developments in perspective.

2 Stay-Green as a Post-flowering Drought Tolerance Trait

2.1 Importance of Stay-Green

Among the various abiotic stress factors affecting crop growth and productivity, water stress is the single greatest factor and this will be the most important factor under a changed climatic regime (Araus et al. 2002). The impact of drought may largely be addressed through genetic improvement for drought response (Mutava et al. 2011a, b). In sorghum, two distinct drought-stress responses have been identified based on the time of occurrence: a pre-flowering drought response occurring prior to anthesis and a post-flowering drought response during the grain-filling stage (Harris et al. 2007). Even though a number of commonly grown cultivars show tolerance at the pre-anthesis stage, they are not tolerant at post-anthesis stages (Sanchez et al. 2002), which is of more economic consequence. Post-flowering drought susceptibility symptoms are characterized by premature leaf and plant senescence, stalk lodging, charcoal rot, and reduction in seed sizes (Rosenow and Clark 1995). Retention of green leaf area during the grain-filling stage has been found to be associated with post-flowering drought tolerance in sorghum (Rakshit et al. 2016). This capacity of certain genotypes in sorghum is referred as the "stay-green" phenotype, and is one of the most well-characterized and utilized traits in sorghum improvement (Rosenow and Clark 1982; Borrell et al. 2000a, b; Borrell and Hammer 2000; Rakshit et al. 2016). The expression of stay-green has been reported to improve the quality of crop residues (van Oosterom et al. 1996), support the continuation of carbon fixation and supply of starch to the sink (McBee

et al. 1983), prevent premature death and lodging of crop (Rosenow and Clark 1982), sustain grain filling under water stress (Rosenow et al. 1983), and improve grain yield under moisture stress (Borrell and Douglas 1996). Even though some progress has been reported in identification of factors underpinning stay-green expression (Vadez et al. 2011; Kholová et al. 2014), understanding the genetic regulation of the mechanisms that lead to the expression of the stay-green phenotype in sorghum is incomplete. Early works considered the benefit of stay-green in terms of extending the period during which a leaf could actively fix carbon (McBee et al. 1983). Subsequently, it was related to the carbon economy of the plant, addressing the nitrogen status of the plant and, in particular, the balance between nitrogen demand and nitrogen capture (Borrell et al. 2001). Owing to its importance as a most widely characterized drought tolerance component trait in sorghum breeding, several quantitative trait locus (QTL) mapping studies (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Xu et al. 2000; Kebede et al. 2001; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007; Srinivas et al. 2009; Habyarimana et al. 2010; Sabadin et al. 2012) for stay-green are reported in sorghum and are well documented (Mace and Jordan 2010; Kiranmayee et al. 2015). There are several well-documented reviews about the progress of research on stay-green in sorghum (Vadez et al. 2013; Wang et al. 2014a, b).

2.2 QTL Analysis for Stay-Green and Associated Root Traits

Overall, seven sources of the stay-green trait have thus far been used for identification of QTLs for this phenotype, and these are B35 (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000; Sanchez et al. 2002; Harris et al. 2007), E36-1 (Haussmann et al. 2002), QL41 (Tao et al. 2000), SC56 (Kebede et al. 2001), 296B (Srinivas et al. 2009), SC283 (Sabadin et al. 2012), and SDS 1948-3 (Habyarimana et al. 2010). Among these, the most commonly used source is B35 [BTx642, a BC1 derivative of IS12555, a *durra* sorghum from Ethiopia (Rosenow et al. 1983)]. All studies showed that stay-green is quantitatively inherited, and the QTLs varied across environments and years. However, six major stay-green QTLs, *stgC* (SBI-01), *stg3A* and *stg3B* (on SBI-02), *stg1* and *stg2* (on SBI-03), and *stg4* (on SBI-05) have been detected across several studies (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Xu et al. 2000; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007). Although the mapped QTLs are from different mapping populations, their physical positions are consistent across the maps (Wang et al. 2014a, b). A simple sequence repeat (SSR) framework map aligned to sorghum genome assembly and the consensus QTL intervals for stay-green traits from these studies (Mace and Jordan 2011a, b) are integrated (Fig. 1; http://cmap.icrisat.ac.in/cmap/) for selecting additional markers in the intervals, especially to support introgression work. Many studies have attempted to use the identified stay-green QTLs for developing drought-tolerant cultivars through marker-assisted backcrossing (Kassahun et al. 2010; Jordan et al. 2012; Vadez et al. 2013).

Root traits are assumed to play a major role towards improved drought tolerance. (For details refer to Chap. 11.) The nodal root angle in sorghum is hypothesized to influence both horizontal and vertical exploration of roots in the soil (Kato et al. 2006; Hammer et al. 2009; Singh et al. 2010; Mace et al. 2012). A putative association between narrow root angle and moderate to high levels of stay-green was made while studying genetic diversity for nodal root angle in a set of 44 diverse sorghum genotypes (Singh et al. 2011). As sorghum produces only one seminal root and the major root system forms nodal root axes, the root angle measured on nodal roots, the first flush of which appears approximately at the five-leaf stage, is suggested to be the most important parameter (Singh et al. 2010). Thus, small soil-filled root chambers can be used effectively to grow plants for a few

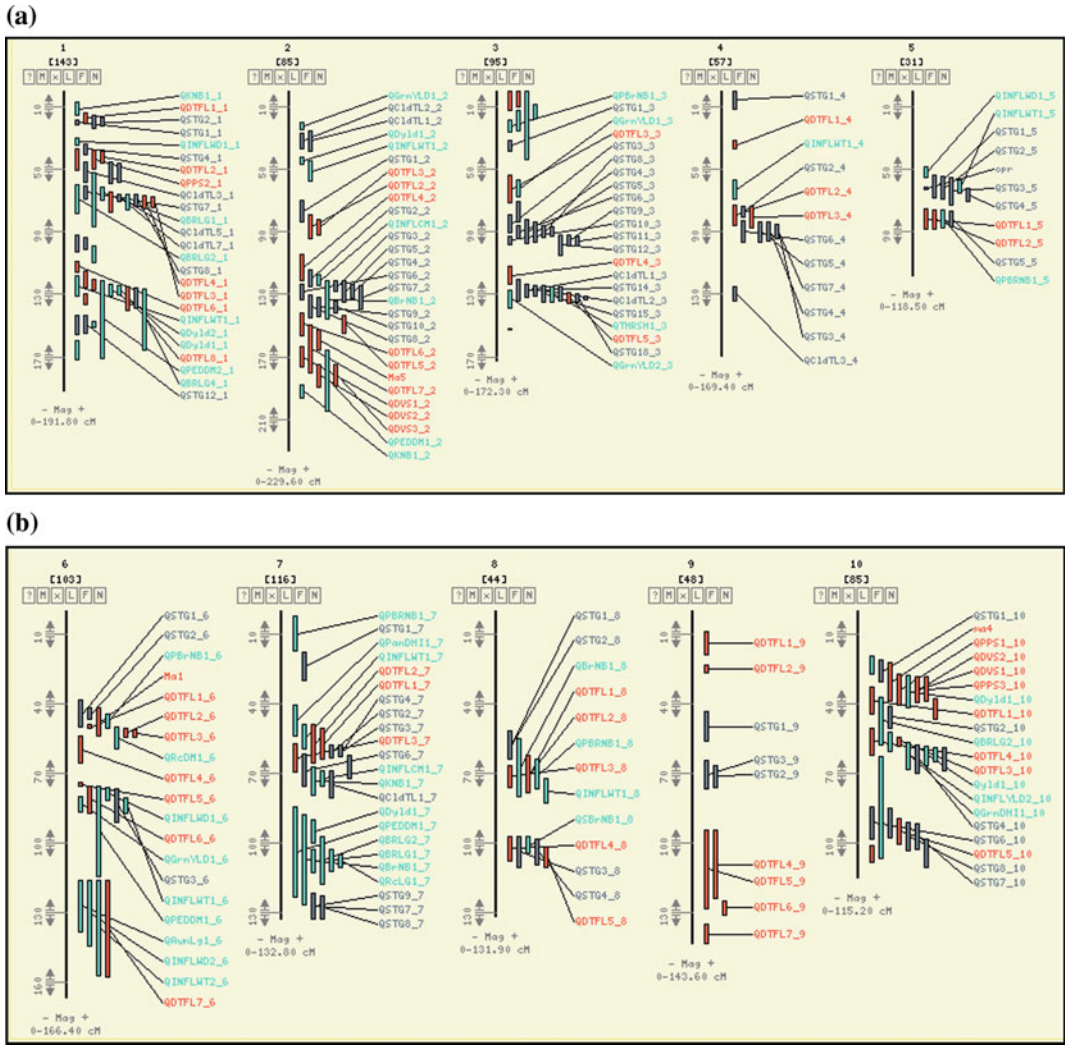

Fig. 1 Consensus QTL intervals for stay-green QTLs from Mace and Jordan (2011a, b) aligned to physically aligned SSRs (vertical bars indicate QTL interval tracks; red tracks for maturity genes/QTLs; aqua for panicle traits, and grey tracks for stay-green). **a** QTL tracks for sorghum chromosome SBI-01 to SBI-05. **b** QTL tracks for sorghum chromosome SBI-06 to SBI-10

weeks to study the nodal root angles (Singh et al. 2010, 2012). A seedling root angle tends to have medium to high heritability (Mace et al. 2012; Singh et al. 2011). Using the rapid root angle screening strategy, Mace et al. (2012) identified four QTLs for nodal root angle in sorghum, explaining 29.78, 11.65, 10.01, and 6.72 % of total phenotypic variation, respectively. These were further confirmed in a backcross-derived nested association mapping population. Three of the four identified QTLs showed homology to previously identified root angle QTL in rice and maize, whereas all four QTLs colocalized with previously identified QTL for stay-green in sorghum. This has opened up the scope to use these reported QTLs towards developing sorghum genotypes with better rooting systems, making them water- and nutrient-efficient genotypes. The

Table 1 SNPs associated with drought-related QTLs (Sujay Rakshit personal communication unpublished data)

Chromosome no.	Trait	QTL	Physical position		No. of SNP
			Starting point	Ending point	
SB01	Shoot dry weight	qSDW1_1[#]	59236915	64917463	75
SB02	Stay-green	Stg3[@]	26010527	28923791	42
	Root dry weight	qRDW1_2[#]	71213786	77627131	124
SB03	Stay-green	Stg2[@]	19344798	22773106	35
	Stay-green	Stg1[@]	25307987	25969626	5
SB05	Stay-green	Stg4[@]	5166739	12984849	111
	Nodal root angle	qRA1_5[#]	13464752	45972507	278
	Root dry weight, Shoot dry weight	qRDW1_5[#]	50053880	51943001	15
	Nodal root angle	qRA2_5[#]	54167643	55807231	28
SB08	Nodal root angle	qRA1_8[#]	8026484	41653341	358
	Root dry weight, Total leaf area	qRDW1_8, qTLA1_8, qTLA2_8[#]	47089939	50926404	66
	Total leaf area	qTLA3_8[#]	54033969	54997392	20
SB10	Bloom	BLMC[$]	56693	11980745	257
	Nodal root angle	qRA1_10[#]	57009865	58763524	25

[$]Burow et al. (2009); [#]Mace et al. (2012); [@]Srinivas et al. (2008)

projection of the six additional significant marker–trait associations for nodal root angle identified in the association mapping study identified that five out of six (83 %) of these new potential QTLs were also found to colocate with regions of the genome containing previously mapped stay-green QTLs (Mace et al. 2012).

In a recent study at the ICAR-Indian Institute of Millets Research, Hyderabad, India, a reference set for drought study comprising 96 genotypes has been developed through a combination of detailed multilocation drought response phenotyping of 258 diverse sorghum genotypes and their molecular diversity analysis using 39 polymorphic SSR markers (Rakshit et al. 2014). Through double digest restriction-site associated DNA ddRAD sequencing of this reference set, 235,009 genomewide single nucleotide polymorphisms (SNPs) have been identified, 1439 of which fall within the coordinates of reported QTLs associated with drought tolerance in sorghum (Table 1; Sujay Rakshit unpublished data).

2.3 Stay-Green QTL Introgression: Successes and Lessons Learned

The initial introgression work carried out at ICRISAT, Patancheru, India, has further dissected the genetic function of the stay-green QTLs. These studies suggest that *stg3A* and *stg3B* QTLs are responsible for transpiration efficiency and vapor pressure deficit response, and are also found to be most stable across several genetic backgrounds and across years (personal communication: Santosh Deshpande). The marker-assisted backcross (MABC) research at ICRISAT till the late 2000s were based on SSRs alone (Vadez et al. 2013), and a very large QTL interval that almost covered the complete chromosome arm was used. These datasets are publically available (www.icrisat/cmap/ac.in). With progress in the understanding of the genetic regulation of the stay-green phenotype in sorghum, the current introgression of genomic regions involved in expression of the stay-green

phenotype will further lead a well-targeted manipulation of individual components. For instance, it is reported that B35 donor parent alleles at stay-green QTL *stg1* contribute to increased water extraction by the moderately senescent *caudatum* line, S35 (Vadez et al. 2011); however, *stg1* failed to show the same phenomenon in the genetic background of the highly senescent *durra* line, R16 (Vadez et al. 2011). Therefore, it is necessary to identify better donors for each of the component traits of the stay-green phenotype, which may vary with the genetic backgrounds, specific soil, water, and temperature regimes in which improved drought tolerance is desired. Among the investigated six QTLs, *stg3A* and *stg3B* QTLs are more stable across genetic backgrounds and environments. The *stg3A* and *stg3B* QTLs are located next to each other on SBI-02, and the mesocarp gene, *Z*, which governs the grain seed coat color, is located between the two QTL intervals (Mace and Jordan 2010). This poses a problem in selection for grain color coupled with stay-green traits. Similarly, location of the maturity gene Ma_5, just after the *stg3B* interval, also adversely affects fixation of the flowering time (Kim 2003). QTL analysis (Srinivas et al. 2008, 2009) along with in silico studies (Ramu et al. 2010) helped to add several SSRs in the region represented by the mentioned QTLs (Table 2). By exploiting existing backcross generations in ongoing MABC projects, the QTL interval for *stg3A* and

Table 2 A list of SSRs available for stg3A and stg3B QTL region including the original SSRs listed in Vadez et al. (2013)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
1	Xtxp013	Stg3A	SBI-02	55.95	119
2	Xiabtp193	Stg3A	SBI-02	56.08	218
3	Dsenhsbm055	Stg3A	SBI-02	56.79	–
4	Dsenhsbm025	Stg3A	SBI-02	56.86	–
5	m13_Xtxp304	Stg3A	SBI-02	57.04	–
6	Xtxp298	Stg3A	SBI-02	57.08	199
7	Stg3a_1	Stg3A	SBI-02	57.17	189
8	Stg3a_2	Stg3A	SBI-02	57.28	442
9	Stg3a_3	Stg3A	SBI-02	57.60	114
10	Stg3a_4	Stg3A	SBI-02	57.70	294
11	Stg3a_5	Stg3A	SBI-02	57.76	197
12	Xisp 280	Stg3A	SBI-02	57.76	–
13	Xisp10278	Stg3A	SBI-02	57.77	–
14	Stg3a_6	Stg3A	SBI-02	57.87	267
15	Stg3a_7	Stg3A	SBI-02	58.13	154
16	XSbAGB03	Stg3A	SBI-02	58.13	144
17	Stg3a_8	Stg3A	SBI-02	58.20	216
18	Stg3a_9	Stg3A	SBI-02	58.33	188
19	Dsenhsbm108	Stg3A	SBI-02	58.43	–
20	Stg3a_10	Stg3A	SBI-02	58.56	212
21	Stg3a_11	Stg3A	SBI-02	58.61	134
22	Xiabtp391	Stg3A	SBI-02	58.87	403

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
23	Xiabtp265	Stg3A	SBI-02	58.91	246
24	Stg3a_12	Stg3A	SBI-02	58.95	166
25	Stg3a_13	Stg3A	SBI-02	59.07	166
26	Xcup63	Stg3A	SBI-02	59.10	145
27	Xtxp464	Stg3A	SBI-02	59.20	140
28	Xiabtp80	Stg3A	SBI-02	59.20	176
29	Stg3a_14	Stg3A	SBI-02	59.75	153
30	Stg3a_15	Stg3A	SBI-02	59.82	201
31	Stg3a_16	Stg3A	SBI-02	59.88	156
32	Stg3a_17	Stg3A	SBI-02	59.95	253
33	Stg3a_18	Stg3A	SBI-02	59.97	204
34	msbcir339	Stg3A	SBI-02	60.19	176
35	Stg3a_19	Stg3A	SBI-02	60.19	131
36	Stg3a_20	Stg3A	SBI-02	60.21	202
37	Stg3a_21	Stg3A	SBI-02	60.23	227
38	Stg3a_22	Stg3A	SBI-02	60.28	208
39	Stg3a_23	Stg3A	SBI-02	60.28	176
40	Stgnhsbm34	Stg3A	SBI-02	60.44	–
41	Xtxp214	Stg3A	SBI-02	60.44	220
42	Xtxp445	Stg3A	SBI-02	60.45	238
43	Xcup29	Stg3A	SBI-02	60.45	191
44	Stg3a_24	Stg3A	SBI-02	60.46	125
45	Stg3a_25	Stg3A	SBI-02	60.48	224
46	Stg3a_26	Stg3A	SBI-02	60.51	162
47	Xtxp466	Stg3A	SBI-02	60.65	159
48	Xtxp465	Stg3A	SBI-02	60.67	177
49	Xiabtp509	Stg3A	SBI-02	60.82	240
50	Stg3a_27	Stg3A	SBI-02	60.84	103
51	Xtxp430	Stg3A	SBI-02	61.09	158
52	Xtxp430	Stg3A	SBI-02	61.09	158
53	Xsbarslbk2.61	Stg3A	SBI-02	61.09	–
54	Stg3a_28	Stg3A	SBI-02	61.13	221
55	Stg3a_29	Stg3A	SBI-02	61.13	113
56	Xisep0934	Stg3A	SBI-02	61.22	196
57	Stg3a_30	Stg3A	SBI-02	61.26	148
58	Stg3a_31	Stg3A	SBI-02	61.33	169
59	Xtxp001	Stg3A	SBI-02	61.37	211
60	Stg3a_32	Stg3A	SBI-02	61.37	184

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
61	Stg3a_33	Stg3A	SBI-02	61.47	99
62	Dsenhsbm032	Stg3A	SBI-02	61.47	–
63	Xisep0926	Stg3A	SBI-02	61.54	191
64	Xtxp056	Stg3A	SBI-02	61.57	347
65	Stgnhsbm35	Stg3A	SBI-02	61.66	242
66	Stg3a_34	Stg3A	SBI-02	61.83	268
67	Stg3a_35	Stg3A	SBI-02	61.84	146
68	Stgnhsbm36	Stg3A	SBI-02	61.95	180
69	Stg3a_36	overlap region	SBI-02	62.13	212
70	Stg3a_37	overlap region	SBI-02	62.29	186
71	Xisep0913	overlap region	SBI-02	62.40	208
72	Xisp10336	overlap region	SBI-02	62.69	–
73	Xisp 336	overlap region	SBI-02	62.69	–
74	Xisep0941	overlap region	SBI-02	63.12	188
75	Dsenhsbm045	overlap region	SBI-02	63.13	–
76	Stgnhsbm31	overlap region	SBI-02	63.13	–
77	Xiabtp231	overlap region	SBI-02	63.13	159
78	Stgnhsbm39	overlap region	SBI-02	63.20	–
79	Xtxp286	overlap region	SBI-02	63.39	–
80	Xgap84	overlap region	SBI-02	63.39	–
81	Stgnhsbm40	overlap region	SBI-02	63.44	–
82	Xisep0938	overlap region	SBI-02	63.44	205
83	Xisep0938	overlap region	SBI-02	63.44	–
84	Xiabtp226	overlap region	SBI-02	63.49	283
85	Xcup41	overlap region	SBI-02	63.62	231
86	Stgnhsbm42	overlap region	SBI-02	64.87	–
87	Xisep0849	overlap region	SBI-02	64.91	–
88	Xisp10334	overlap region	SBI-02	65.28	–
89	Xisep0944	overlap region	SBI-02	65.30	–
90	Xisep0939	overlap region	SBI-02	65.48	–
91	Xisep1022	overlap region	SBI-02	65.48	–
92	Xisep0942	overlap region	SBI-02	65.83	–
93	Xisep0910	overlap region	SBI-02	65.95	194
94	Xgpsb128	overlap region	SBI-02	66.17	285
95	Ungnhsbm49	overlap region	SBI-02	67.12	–
96	XmSbCIR187	overlap region	SBI-02	67.19	–
97	Xtxp348	overlap region	SBI-02	67.32	–
98	Xisep0935	overlap region	SBI-02	67.41	–

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
99	Xisp10200	Stg3B	SBI-02	67.80	–
100	Xiabtp323	Stg3B	SBI-02	68.22	191
101	Dsenhsbm015	Stg3B	SBI-02	68.22	–
102	Xtxp428	Stg3B	SBI-02	68.39	213
103	Xisp10259	Stg3B	SBI-02	68.39	–
104	Xisp 259	Stg3B	SBI-02	68.39	–
105	Xiabtp397	Stg3B	SBI-02	68.69	188
106	Xtxp429	Stg3B	SBI-02	68.85	214
107	Stg3b_1	Stg3B	SBI-02	69.31	152
108	Stg3b_2	Stg3B	SBI-02	69.61	209
109	Xtxp100	Stg3B	SBI-02	69.64	116
110	Stg3b_3	Stg3B	SBI-02	70.09	188
111	Stg3b_4	Stg3B	SBI-02	70.20	164
112	Xtxp207	Stg3B	SBI-02	70.26	184
113	Xtxp007	Stg3B	SBI-02	70.26	230
114	Xcup26	Stg3B	SBI-02	70.26	220
115	Stg3b_5	Stg3B	SBI-02	70.42	188
116	Stg3b_6	Stg3B	SBI-02	70.60	99
117	Stg3b_7	Stg3B	SBI-02	70.66	175
118	Stg3b_8	Stg3B	SBI-02	70.67	151
119	Xisep0733	Stg3B	SBI-02	70.75	330
120	Stg3b_9	Stg3B	SBI-02	70.85	225
121	Xiabtp190	Stg3B	SBI-02	70.89	215
122	Xisep0841	Stg3B	SBI-02	70.89	215
123	Xiabtp388	Stg3B	SBI-02	71.03	129
124	Xtxp296	Stg3B	SBI-02	71.11	168
125	Stg3b_10	Stg3B	SBI-02	71.20	277
126	Stg3b_11	Stg3B	SBI-02	71.43	219
127	Xiabtp484	Stg3B	SBI-02	71.60	117
128	Xiabtp205	Stg3B	SBI-02	71.79	165
129	Xiabtp076	Stg3B	SBI-02	72.17	292
130	Xiabtp317	Stg3B	SBI-02	72.33	236
131	Xcup40	Stg3B	SBI-02	75.36	193
132	Stg3b_1	Stg3B	SBI-02	77.29	140
133	Xtxp019	Stg3B	SBI-02	–	–
134	Xtxp008	Stg3B	SBI-02	–	–

stg3B QTL has been reduced. At ICRISAT additional efforts are currently being made to identify SNPs representing this region of the genome and currently a set of ~70 SNPs from genic regions have already been identified (Santosh Deshpande, personal communication).

These additional SSRs facilitate not only the selection for the stay-green QTLs independently but also for stringent mesocarp color in segregating populations. Similarly, as elaborated earlier, a major nodal root angle QTL distal to *stg3B* (Mace et al. 2012) has provided further opportunities for improvement of drought tolerance by selecting these QTLs simultaneously or in combination. The QTL intervals reported by Vadez et al. (2013) also overlap this root angle QTL within the *stg3B* region. This in turn is an opportunity to evaluate the available introgression lines (Vadez et al. 2011) for the combined effort of stay-green QTL(s) and root angle QTL in different combinations. Recent advances in the area of accurate and precise phenotyping platforms are adding values to dissect complex traits such as stay-green. Vadez et al. (2011) utilized the lysimeter-based estimations for the mechanism responsible for *stg3A* and *stg3B* as water-use efficiency under water-limited conditions and vapor pressure deficit for better transpiration efficiency. These traits help direct trait value measurement rather than stay-green expression. But they are cumbersome to measure for a large breeding or segregating population. At ICRISAT, a high-throughput phenotyping platform called the "LeasyScan" facility has been established to measure leaf area in a quicker way to access the dynamics of leaf development and leaf conductance (Vadez et al. 2015). This further helps to develop an early stage assay for screening the large breeding populations. The technique is based on a novel 3D scanning technique to capture leaf area development continuously, increasing imaging throughput and analytical scales by combining gravimetric transpiration measurements. Utilization of these new phenotyping advances has a great scope for high-throughput trait screening and breeding selections in large breeding populations.

3 Aluminum (Al) Tolerance

3.1 Importance and Mechanism of Al-Tolerance

Soil acidity imposes one of the most severe constraints on crop productivity in the tropics and subtropics (Wang et al. 2014a, b). Many tropical soils are acidic because percolating rainwater leaches the cations such as calcium, magnesium, and potassium, which are replaced by aluminum (Al), manganese, and hydrogen, leading to toxic levels of these elements (Rao et al. 1993). In acidic soils, particularly at pH below 5, Al is solubilized into ionic forms (Al^{3+}) and this leads to phosphorus (P) deficiency as P is fixed as aluminum phosphate (a highly insoluble and unavailable form to plants; Rao et al. 1993). Plants growing in Al toxicity display stunted growth and become susceptible to drought (Marschner 1991; Kochian et al. 2004; Wang et al. 2014a, b). This happens mainly due to inhibition of cell division, cell elongation, or both under low availability of P leading to poor root growth (Delhaize et al. 2004, 2009; Magalhaes et al. 2004). Al toxicity is the single most important factor affecting crop production on 2/3 of the acid soil affected area (Eswaran et al. 1997), and it is a major constraint for sorghum production in tropical and subtropical regions (Doumbia et al. 1993, 1998). Plant adaptation to acid soils is mainly through tolerance and avoidance, of which avoidance occurs more commonly. Avoidance is achieved either through changes in the rhizosphere ecology such as increase in pH through root exudates or release of chelators for Al (such as citrate or malate), or increase in root surface area via mycorrhizae (Marschner 1991).

3.2 Genes for Al-Tolerance in Sorghum

Al tolerance in sorghum is controlled by a single gene (*AltSB/SbMATE*) in a cross between tolerant, SC283, and sensitive, BR007, which has been mapped to sorghum chromosome 3

(Magalhaes et al. 2004). Positional cloning identified a gene coding for an aluminum-activated citrate transporter, which is a member of the multidrug and toxic compound extrusion (MATE) family, the causal component leading Al tolerance (Magalhaes et al. 2007). Markers from this region have been deployed by breeders to introgress favorable *SbMATE* alleles in susceptible sorghum genotypes (Anami et al. 2015). *SbMATE* expression is reported to be induced with time of exposure to Al and the expression is higher in the root apex compared to the rest of the root (Magalhaes et al. 2007). The region 1–3 mm behind the root tip where transition from cell division to cell elongation occurs was reported to be the most sensitive area (Sivaguru et al. 2013). After exposure to Al, sensitive genotypes accumulate several-fold more Al in their root apex compared to Al-tolerant genotypes (Delhaize et al. 1993). The coding region of *SbMATE* is identical between Al-tolerant and Al-sensitive genotypes. However, the second intron of *SbMATE* shows polymorphism between the two types. In the promoter region of *SbMATE*, a tourist-like miniature inverted repeat transposable element (MITE) transposon has been reported, whose repeat numbers are observed to be positively correlated with Al tolerance (Magalhaes et al. 2007). With this it is assumed that the causative mutations underlying aluminum tolerance may have a regulatory nature (Anami et al. 2015).

4 QTL/Marker Analysis for Other Abiotic Stresses

4.1 Early-Season Cold Tolerance

Sorghum, in general, cannot grow well under soil temperature below 15 °C. However, if early-season cold tolerance can be induced in sorghum cultivars, the area under it can be extended to more northern latitudes, and may allow early planting, particularly in the United States (Yu and Tuinstra 2001). Under this situation, improved emergence and early-season vigor are needed, which will ensure establishment of better crop stand, and prevent loss of seedlings due to cold. The "Kaoliangs" sorghum germplasm from temperate regions of China shows better cold tolerance than tropical germplasm (Cisse and Ejeta 2003). Thus, this particular germplasm is a promising source for improvement of cold tolerance (Yu and Tuistra 2001; Franks et al. 2006). However, these germplasms are in general not agronomically promising, and thus require extensive backcrossing to transfer cold tolerance in the breeding program. Gunaratna (2002) found that genetic control of seedling vigor traits under cold stress and under optimal temperatures is similar. In order to identify QTLs for seedling vigor traits under cold stress, Knoll et al. (2008) developed a recombinant inbred line (RIL) population of 153 RILs from a cross between cold-tolerant Chinese Kaoliang "Shan Qui Red" (SQR) and a cold stress-susceptible African *caudatum* SRN39. They could identify two QTLs for germination. One among them on linkage group SBI-03a was significant under cold and optimal temperature. The other, on SBI-07b, was contributed by SQR, and showed higher significance under cold temperatures. They also identified a region on SBI-01a from SQR, favoring seedling emergence and seedling vigor under early and late field plantings. Knoll and Ejeta (2008) further demonstrated favorable effects of SQR alleles towards seedling vigor and/or emergence in two new populations. Subsequently, Burow et al. (2011) reported an additional 16 QTLs for cold germinability, field emergence, and early seedling vigor in a mapping population consisting of 171 RILs derived from the cross between RTx430 (cold-sensitive) and PI610727 (cold-tolerant) using 141 SSR markers. The most promising region for improving field emergence identified in this study is located on SBI-01. Other QTL-rich regions were located on SBI-03, SBI-04, SBI-06, SBI-08, and SBI-09. Utilizing a genetic map based on an F_8 RIL population reported by Shiringani et al. (2010) and by phenotyping a subset of the same population for cold tolerance, Bekele et al. (2013) identified highly interactive epistatic QTL hotspots having a significant effect on prolonged chilling survival. The

major QTL regions on chromosome SBI06 harbor candidate genes that govern tolerance to abiotic stress. They identified several genes conferring maintenance of cell division and growth under early chilling stress within QTL hotspot regions, which can be a potential candidate for breeding cold tolerance.

In sorghum, a higher respiration rate has been correlated with higher germination under cold stress (Balota et al. 2010). Washburn et al. (2013) correlated a rhizome formation trait to overwintering ability of sorghum. The phenomenon of overwintering and rhizomatousness are reported to be controlled by seven QTLs (Paterson et al. 1995; Washburn et al. 2013) that were identified in a mapping population of BTx623/*S. propeinquum*. The ability of sorghum to overwinter and form rhizome has been suggested to be useful for biofuel sorghum production (Anami et al. 2015).

Through a differentially expressed gene (DEG) analysis between contrasting genotypes, HongkeiZi (cold tolerant) and BTx623 (cold sensitive), Chopra et al. (2015) identified 41,603 SNPs. They could validate 89 % of the 114 selected SNPs using endpoint genotyping technology. By combining expression profile data and gene-based SNP information they generated a searchable database, which turned out to be an important resource for sorghum cold stress genomics research (http://www.csrl.ars.usda.gov/psgd/index-sorghum.aspx.html). All these markers are important resources to introgress cold tolerance in sorghum. Recently Upadhyaya et al. (2016) conducted association mapping in a sorghum mini-core collection (Upadhyaya et al. 2009) using 162,177 SNPs and identified only one marker locus (*Locus 7-2*) to be significantly associated with low-temperature germination and none with vigor. The *Locus 7-2* was found to represent field early-emergence QTL flanked by Xtxp278 and Xtxp295 (Burow et al. 2011). The locus was found next to three overlapping emergence QTLs close to sPb-5796 (Fiedler et al. 2012). Furthermore, the syntenic region of this locus colocalizes with two cold-tolerance rice QTLs, and it is found that when its wheat homologue is overexpressed in tobacco, cold-tolerance and germination rate are increased.

4.2 Salinity Tolerance

Salinity is also an emerging problem in sorghum cultivation in different parts of the world. It has been reported that upon Na^+ stress a high-affinity potassium transporter gene family in sorghum, *SbHKT1;4*, is strongly upregulated in salt-tolerant sorghum genotypes, leading to better Na^+/K^+ ratio and optimum plant growth (Wang et al. 2014b). A total of 38 QTLs influencing salt tolerance has been reported from an RIL population comprising 181 lines derived from a cross Shihong137/L-Tian (Wang et al. 2014a). Out of these, six are reported to be major QTLs explaining above 10 % phenotypic variation. Studies suggested that the mechanism of salt tolerance at the germination and seedling stages is different. Further research in this direction is essential before deploying the identified QTLs in MAS.

4.3 Nitrogen Use Efficiency

The demand for nitrogen (N) fertilizer in agriculture across the globe currently stands at ~110 million metric tons per year and is projected to reach up to ~250 million metric tons by the year 2050 (www.fao.org). Because of the high mobility of nitrate in the soil, up to 50 % of applied N is lost by the processes of leaching, runoff, and denitrification. This not only increases the cost of crop production but also adds to the pollution of the groundwater and adversely affects the soil structure. The process has detrimental effects on the environment by increasing greenhouse gases such as nitric oxide in the atmosphere. Hence, developing crop varieties with improved N absorption and utilization can mitigate these problems of modern agriculture. Sorghum is predominantly cultivated under low

fertility conditions, particularly in Africa and Asia. However, in developed countries it is cultivated under high fertility conditions. Significant genotypic differences in terms of nitrogen-use efficiency (NUE) have been reported in sorghum (Maranville et al. 1980; Youngquist et al. 1992). Expression analysis between four low-N tolerant sorghum genotypes (San Chi San, China17, KS78, and high-NUE bulk) and three sensitive genotypes (CK60, BTx623, and low-NUE bulk) under of low-N (LN, 0 kg ha^{-1}) and normal N (NN, 100 kg ha^{-1}) regimes revealed that in sensitive genotypes, N-stress increased the abundance of DEG-transcripts associated with stress responses including oxidative stress, whereas the tolerant genotypes produced greater root mass for efficient uptake of nutrients (Gelli et al. 2014). Higher abundance of transcripts related to high-affinity nitrate transporters (NRT2.2, NRT2.3, NRT2.5, and NRT2.6) and lysine histidine transporter 1 (LHT1) were detected in tolerant genotypes, which indicates possible improved uptake of inorganic and organic forms of nitrogen by these genotypes. Higher abundance of *SEC14* cytosolic factor family protein transcript in tolerant genotypes could lead to increased membrane stability and tolerance to N-stress.

Gelli et al. (2016) sequenced a population of 131 RILs derived from a cross between CK60 (inefficient N user) and China17 (efficient N user) using GbS. Following the composite interval mapping (CIM) technique using 642 polymorphic SNPs they could identify 38 QTLs for 11 agronomic traits under normal (100 kg ha^{-1}) and no N application condition on chromosomes 1, 5, 6, 7, and 9. Phenotypic variation explained by each QTL ranged from 6.2–50.8 %. Using Illumina RNA sequencing on seedling root tissues they could identify 726 differentially expressed transcripts between the parents. Among these 108 were mapped close to the QTL regions. Differentially expressed transcripts were related to nitrogen metabolism (Ferredoxin-nitrate reductase), glycolysis (Phosphofructo-2-kinase), seed storage proteins, plant hormone metabolism, and membrane transport. The study indicated that the differentially expressed transcripts underlying the pleiotropic QTL regions could be potential targets for improving sorghum performance under limited N fertilizer through marker-assisted selection.

5 Molecular Insight into Abiotic Stress Response

5.1 Noncoding RNAs

MicroRNAs (miRNAs) have been associated with regulations of different classes of genes across plant species. Pasini et al. (2014) reported upregulation of miRNA associated with the regulation of transcription, signal transduction, carbon metabolism, detoxification, osmoprotection mechanisms, and stability of protein membranes upon imposition of low moisture stress in four-leaf-old sorghum genotype, IS1945. The study suggested the possible utility of these drought-related genes in identifying drought-tolerant sorghum lines. In fact, miRNA 169 g, as reported to be upregulated under drought stress in rice, has five sorghum homologues (Zhao et al. 2007). Qi et al. (2013) also reported long noncoding RNAs (lncRNAs) from foxtail millet in response to drought stress, having sequence conservation and collinearity with sorghum. Through an in silico study, Ram and Sharma (2013) further indicated a probable role of miRNAs in water stress response in sorghum.

5.2 Role of Genes Governing Auxin and Transcription Factors

Over 50 differentially expressed drought-responsive gene orthologues with enriched ABREs and CGTCA-motifs or motifs responsive to ABA specific to sorghum have been identified (Dugas et al. 2011). A large number of auxin-related gene families including *SbGH3*, *SbLBD*, *SbIAA1*, *SbGH3-13*, *SbLBD32*, and others have been reported that are highly induced by salt or drought stress (Wang et al. 2010). Moisture stress has been observed to trigger upregulation of transcription factor genes of MADS-box, Auxin Responsive Factors, Heme

Activator Protein 2, and so on, specifically in root tissues (Aglawe et al. 2012). Sorghum auxin transporters have been observed to be up- or downregulated in response to abiotic stresses, depending on the class of transporters (Shen et al. 2010).

Over 100 ethylene response factor (ERF) genes have been reported in sorghum (*SbERF*), which have been grouped into 12 classes (A-1 to A-6 and B-1 to B-6) on the basis of sequence homology (Yan et al. 2013). The ERF superfamily plays an important role in both abiotic and biotic stress response in plant systems. In addition to ERF, chloroplast glutathione reductase (*cpGRs*), G-protein complexes, drought response element-binding (*DREB*) proteins, and *SbEST* are reported to play very important roles in abiotic stress responses not only in sorghum but in other plant species as well. In a recent study, Chopra et al. (2015) identified a total of 1910 DEGs under cold and control temperature from cold-tolerant genotype HongkeZi and cold-sensitive genotype BTx623. They could identify TFs including *DREB*, *C-repeat binding factors*, and ERF TFs to be upregulated under cold stress in tolerant genotype HongkeZi. They also identified specific genes such as plant cytochromes, glutathione s-transferases, and heat shock proteins to be differentially regulated under cold stress between cold-tolerant and susceptible genotypes.

5.3 Role of Compatible Solutes

Betaise aldehyde dehydrogenase (*BADH1* and *BADH15*) is reported to be induced in response to moisture deficit leading to accumulation of glycine betaine (Wood et al. 1996). *SbGRRNP* (glycine-rich RNA-binding protein) expression is regulated by salinity and ABA, as well as blue and red light (Aneeta et al. 2002). This suggests that in sorghum a possible crosstalk between abiotic stress and light signal may exist. By engineering higher mannitol synthesis, Maheswari et al. (2010) could induce salinity tolerance in transgenic sorghum cv. SPV462. Studies further suggest that *SbP5CS1* and *SbP5CS2* are upregulated in response to drought, salt, and jasmonic acid treatment (Su et al. 2011). These two are important regulatory genes controlling proline synthesis. Thus, the study suggested a role of proline biosynthesis in imparting abiotic stress tolerance. All the above reports suggest the possibility to develop abiotic stress-tolerant genotypes by modulating the role of these genes, transcription factors, or miRNAs either through the transgenic route or through genome editing.

6 Next-Generation Sequencing (NGS) Tools and Next-Generation Populations

When combined with high-throughput and precise phenotyping platforms developed at ICRISAT (Vadez et al. 2015), NGS technologies provide a powerful and rapid tool for identifying the genetic basis of agriculturally important traits and for predicting the breeding value of individuals in a plant breeding population (Varshney et al. 2014). Next-generation populations such as nested-association mapping (NAM) populations (Yu et al. 2008; Buckler et al. 2009) and backcross-derived NAM (BCNAM) populations (Jordan et al. 2012) are two most potent next-generation populations to dissect genomics of complex traits. All these new tools play a major role in advancing breeding efficiencies by simultaneously aiding genetic studies and also providing access to new variability in comparatively elite backgrounds. These new-generation populations are required for linking phenotypic variations with sequence variations at high resolution. The BCNAM scheme was already well established by Jordan et al. (2011) to benefit both trait mapping and infusing diversity that was not accessible previously in the traditional plant breeding populations. In combination with genomewide sequencing approaches, these populations provide access to insights into the genetic architecture of important traits (Yang et al. 2014a, b). The major limitation in implementing these new advances routinely in the breeding programs is lack of data management skills and

database resources. In terms of applications of these tools in molecular breeding, the NGS-based genotyping platforms such as genotyping-by-sequencing (GbS) have the potential for background selection in traditional introgression breeding and genomic selection (GS) which is an emerging methodology in modern breeding programs. The main advantage and driving force for its implementation are availability of low-cost high-throughput genotyping platforms and access to high-throughput phenotyping facilities. These in turn feed the improved capacities of performance prediction of individual genotypes for quantitative traits. The genomic selection, to realize its full potential, will need to address specific issues related to the theory of GS such as design of training populations, predicting efficacy under an altered marker and population variable scenario, and an approach for integration of GS in ongoing breeding schemes (both in scale and dimension). Similarly, considering the different level of advancement of constituent technologies involved in GS, an appropriate resource investment strategy for every single breeding program needs to be developed for maximizing the returns in terms of genetic gain.

7 Future Prospects

Sorghum is an important failsafe crop providing food–feed–fodder–fuel to most of the resource-poor farming communities in drylands where it is a staple crop. The wide repertoire of germplasm, genetic, genomic, and breeding information/resources positions sorghum as one of the C_4-model crop species. Advancement in NGS, high-throughput phenotyping, and recent developments in advanced breeding populations will drive the new cycle of genetic gain in sorghum across agroecological zones. The sorghum research community has access to large genetic (germplasm with unique traits) and genomic (SSRs, SNPs, high-density genetic maps, genome sequence) resources, and many QTLs/candidate genes associated with agronomic traits are known in sorghum. These marker–trait associations need to be validated independently in breeding populations and suitable marker assays such as the KASPar SNP assay need to be generated. The integration of these new tools along with new-generation breeding populations will further help accumulate the required information to develop the framework for implementing genomic selection for sorghum improvement and thus historical division between breeding and genomics will become increasingly blurred.

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