A Meta-Analysis of Quantitative Trait Loci Associated with Stay-Green in Sorghum

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Abstract

A strong association between stay-green and post-flowering drought tolerance in sorghum has been established previously. In this study, a meta-analysis was conducted to find relevant genetic intervals that regulate stay-green in sorghum and thereby affect drought resilience. Meta-QTL analysis was performed to combine 115 QTLs mapped in previous studies and locate regions where recurrent QTLs occurred across experiments. This approach overcomes the constraints of a single QTL association experiment by increasing heterogeneity in populations and environments. The analysis included the construction of consensus maps, QTL projection, and QTL clustering. We report 32 Meta-QTL for stay-green and their position relative to genetic markers on the sorghum genome. The results from this study will facilitate future attempts aiming to improve and understand drought tolerance in sorghum by the use of fine mapping and marker-assisted selection.

Keywords: Meta-QTL, drought, genetic map, stress, QTL, clustering

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important staple and fodder crop, especially for the people living in semi-arid regions of Asia and Africa. Sorghum's resilience to osmotic stress has made it an exceptional model for studying genomics for drought tolerance. Despite its high tolerance, drought significantly hampers the productivity and nutritional quality in sorghum. The mechanisms by which sorghum responds to water deficit are complex and studies have identified a vast number of traits that govern this response (Amelework et al., 2015). Furthermore, significant variation also exists among genotypes for drought tolerance level (Abreha et al., 2022), and therefore underpinning the genetic loci for drought tolerance is vital to minimize the detrimental effect of drought. The stay-green trait, which confers the ability to resist premature senescence in response to post-flowering drought, has been recognized as one of the most crucial drought-related traits studied in sorghum (Abebe et al., 2021). Stay-green genotypes in sorghum can retain photosynthetically active green leaf area and normal grain filling under water-limited conditions (Borrell et al., 2000; Harris et al., 2007).

The underlying genetic factors affecting stay-green, a quantitative trait, have been extensively studied through QTL mapping experiments. Over 100 QTL for stay-green traits in sorghum have been mapped in different populations using a different set of markers. Differences in population, environment, and choice of markers limit the transferability of QTL across breeding programs. Also, the genetic effects of QTLs identified in any single study need to be tested for their presence in diverse genetic backgrounds and environments. QTL meta-analysis is an approach to identify consensus genomic regions by combining QTLs from diverse populations and environments (Goffinet and Gerber, 2000). These consensus QTL, also called Meta-QTL (MQTL), are stable and robust regions where QTLs were mapped recurrently in experiments. Additionally, the meta-QTL analysis leads to a significant reduction in confidence interval in the MQTL compared to the original QTLs. The reduced interval and reliability of MQTLs over QTLs enable Candidate Gene (CG) identification and Marker Assisted Selection (MAS) with improved precision.

In this study, we have performed the first meta-QTL analysis for stay-green traits in sorghum using results from 9 previous QTL mapping experiments (Table 1). The analysis involved the construction of consensus maps, QTL projection, and statistical clustering of QTLs.

Material and Methods

Collection of Stay-green QTLs in Sorghum

Through a rigorous literature search, a database of sorghum stay-green QTLs was created on a spreadsheet. The database consisted of 130 QTLs published across 9 studies from 1999 to 2014. The columns of the spreadsheet contained for each QTL the following information: (1) Name, (2) Chromosome, (3) Linkage group, (4) Phenotypic variance explained or R^2, (5) Position, (6) 95% Confidence Interval and (7) Source. For each QTL a 95 percent confidence interval was calculated using the formula $163/NR^2$ (Guo et al., 2006). 4 of the QTLs which had confidence intervals greater than 50cM were screened from further analysis. Additionally, data about each of the 9 studies were collected and saved separately as metadata. The metadata consisted of the name, population type, population size, mapping function, and cross performed in each study.

Consensus Map Building

To study relative positions of the range of QTLs mapped with different types of markers, a consensus map was built which contained markers from all the experiments. Apart from the maps described in the studies that reported QTLs, two sorghum consensus maps published on the Gramene database were also used for building the consensus map (Tello-Ruiz et al., 2021; Mace et al., 2011; Menz et al., 2004). The ConsMap tool from the MetaQTL package, available in Biomercator V4.2, was used to compile all the maps into a single consensus map (Veyrieras et al., 2007; de Oliveira et al., 2014).

QTL Projection

Using the QTLproj tool in Biomercator V4.2, adapted from the MetaQTL package, 115 out of 126 QTLs were projected on the consensus map developed in this study (Veyrieras et al., 2007; de Oliveira et al., 2014). QTLproj uses flanking markers on the original map and the marker interval distances to find the optimal position and confidence interval for the QTL on the consensus map.

Meta-Analysis

The QTLClust tool from the MetaQTL package was used to probabilistically cluster QTLs. The projected QTLs on the consensus map was used for the meta-analysis. The clustering procedure for the algorithm is based on a gaussian distribution of the observed QTLs around their position with variance derived from the confidence interval. The best meta-model was chosen according to the Akaike Information Criterion (AIC). The meta-QTLs corresponding to only a single QTL was not considered.

Result and Discussion

The present work is motivated by the numerous attempts made to dissect genomic regions for stay-green in sorghum in the last two decades (Table 1). From the initial pool of 130 QTLs, 115 QTLs from 9 studies were aligned on a single consensus map consisting of 10 chromosomes of Sorghum. The remaining QTLs were not projected either due to high CI (>50 cM) or couldn't be projected due to a lack of common markers. The meta-analysis was able to identify 32 Meta-QTL regions from the projected QTLs. A summary of the MQTL regions identified in this study is shown in Table 2. Meta-QTL analysis overcomes the constraints of a single QTL association experiment by increasing heterogeneity in populations and environments. It also provides more reliable molecular markers and helps in gene identification. A summary of the chromosome-wise distribution of these QTLs and MQTLs is shown in (Figure 1).

Table 1. Summary of QTL association studies considered for the Meta-QTL analysis

Serial No.	Experiment Name	No. of Stay-green QTL(s)	Parents of population	Population size
1	Crasta et al. 1999	7	B35 x Tx430	96
2	Haussmann et al. 2002	19	IS9830 ×E36-1	226
3	Haussmann et al. 2002	21	N13 × E36-1	226
4	Xu et al. 2000	3	B35 x Tx7000	98
5	Kebede et al. 2000	14	SC56×Tx7000.	125
6	Sabadin et al. 2012	4	BR007 x SC283	100
7	Srinivas et al. 2008	9	296B x IS18551	168
8	Subudhi et al. 2000	4	B35 x Tx7000	98
9	Tau et al. 1999	5	QL39/QL41	152
10	Reddy et al. 2014	43	M35-1 X B35	245

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Table 2. Summary of all the Meta-QTLs generated

Meta QTL Name	Chromosome	Position on the consensus map (cM)	Confidence interval (cM)	N QTLs	N studies
MQTL_1	SBI-1	31.29	4.99	4	3
MQTL_2	SBI-1	46.89	3.12	3	1
MQTL_3	SBI-1	71.81	4.23	9	3
MQTL_4	SBI-1	82.65	7.69	4	2
MQTL_5	SBI-1	94.95	9.72	3	2
MQTL_6	SBI-1	129.78	8.9	4	2
MQTL_7	SBI-1	182.71	6.41	3	2
MQTL_8	SBI-2	20.55	8.95	2	2
MQTL_9	SBI-2	115.13	8.01	5	4
MQTL_10	SBI-2	123.26	6.09	6	4
MQTL_11	SBI-2	141.39	5.69	2	1
MQTL_12	SBI-2	155.23	3.79	6	1
MQTL_13	SBI-2	162.91	5.25	3	1
MQTL_14	SBI-3	35.36	5.21	2	2
MQTL_15	SBI-3	69.05	4.78	5	2
MQTL_16	SBI-3	83.64	3.96	6	2
MQTL_17	SBI-3	90.4	3.46	8	2
MQTL_18	SBI-3	106.97	7.51	5	4
MQTL_19	SBI-3	121.63	6.46	5	5
MQTL_20	SBI-3	132.01	2.91	5	3
MQTL_21	SBI-3	147.67	7.95	2	1
MQTL_22	SBI-4	43.25	9.27	4	3
MQTL_23	SBI-4	111.19	2.32	5	2
MQTL_24	SBI-5	48.59	2.29	5	3
MQTL_25	SBI-7	60.61	3.43	5	2
MQTL_26	SBI-7	147.62	2.05	4	2
MQTL_27	SBI-8	71.12	10.45	3	2
MQTL_28	SBI-8	131.66	7.02	3	2
MQTL_29	SBI-9	83.41	11.44	2	2
MQTL_30	SBI-9	126.31	0.62	7	1
MQTL_31	SBI-10	112.58	9.33	3	2
MQTL_32	SBI-10	127.06	12.53	2	2

Chr 1 had the highest number of QTLs reported (N=26), followed by Chr 3 (N=23) and Chr 2 (N=19). Chr 3 harbored the maximum number of MQTLs per chromosome whereas Chr 6 harbored no MQTL. The average phenotypic variance explained (PVE) in all the QTLs was 10% with 97% QTLs having PVE over 5%. The average CI for the MQTLs was 6.1 cM, which corresponds to a 46% reduction from the average CI of original QTLs. The number of individual QTLs per MQTL ranged from 2 to 9. The MQTLs with a higher number of underlying QTLs and also distinctness in experiments reporting the underlying

QTLs are thought of as more stable through genetic backgrounds and environments (Löffler et al., 2009). By this criteria MQTL_3, MQTL_9, MQTL_10, MQTL_18, MQTL_19 can be selected as the most stable MQTLs found in this study. Whereas MQTL_30 on Chr 9 is the most refined MQTL, i.e., it has the smallest confidence interval on the genetic map.

This study reports significant Meta-QTL regions based on 10 QTL experiments done on completely independent locations, time frame and population, but all related to stay-green in sorghum (Figure 2). The
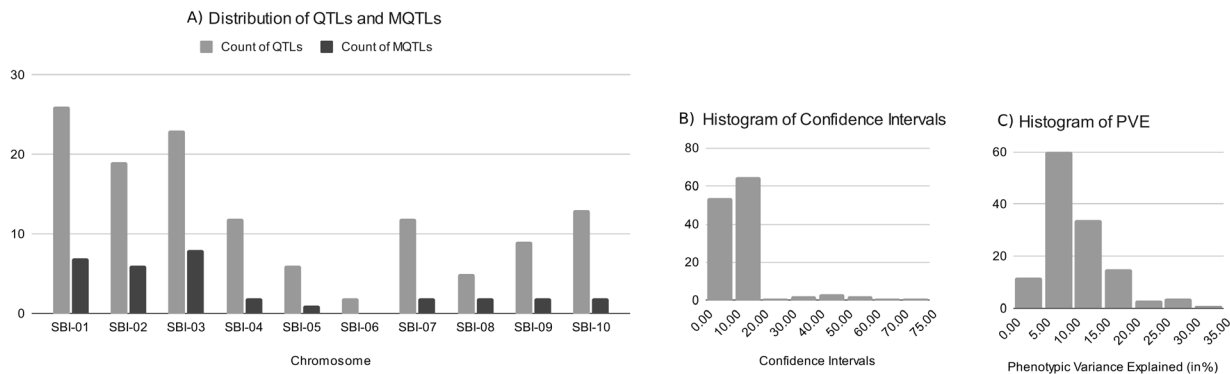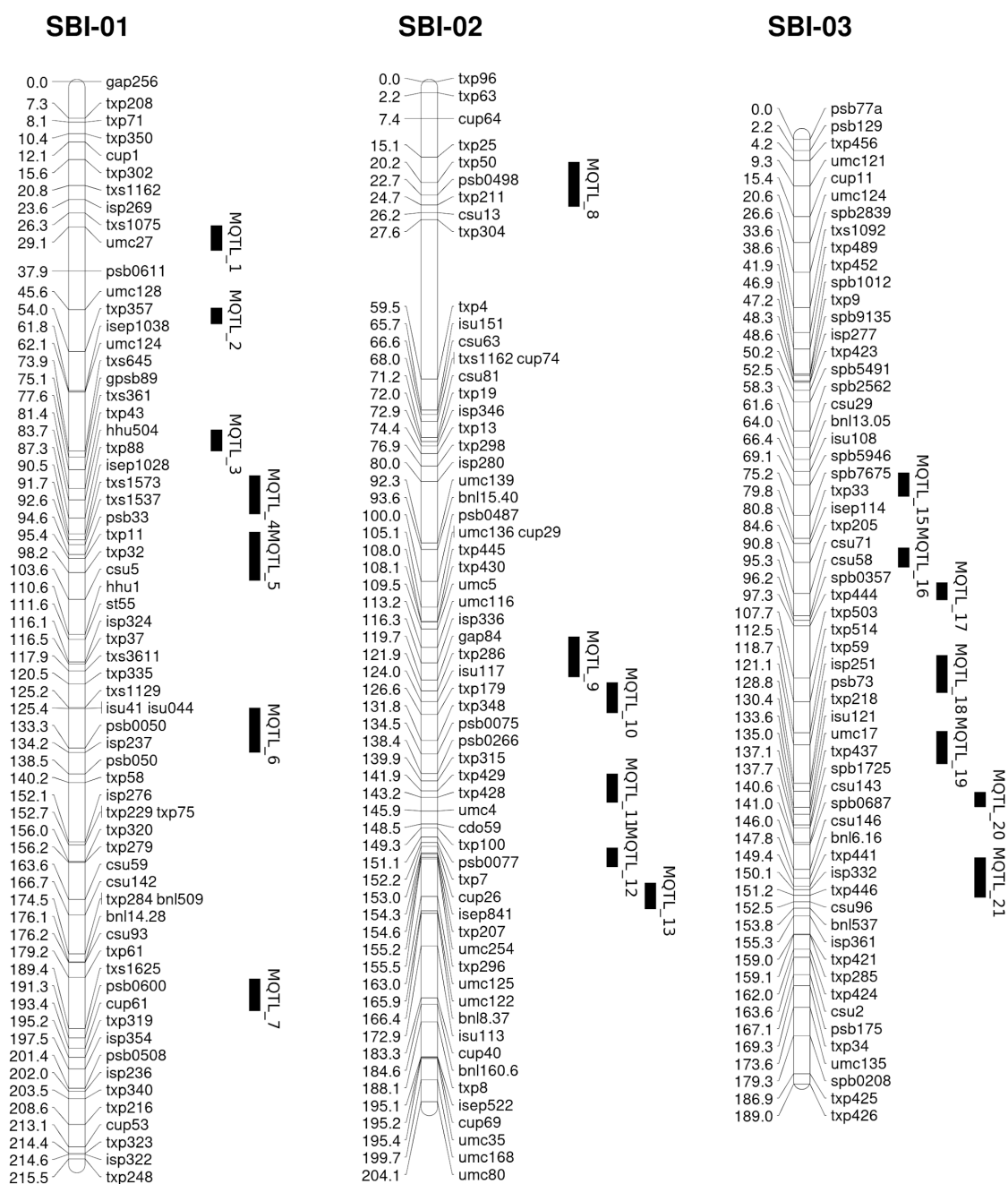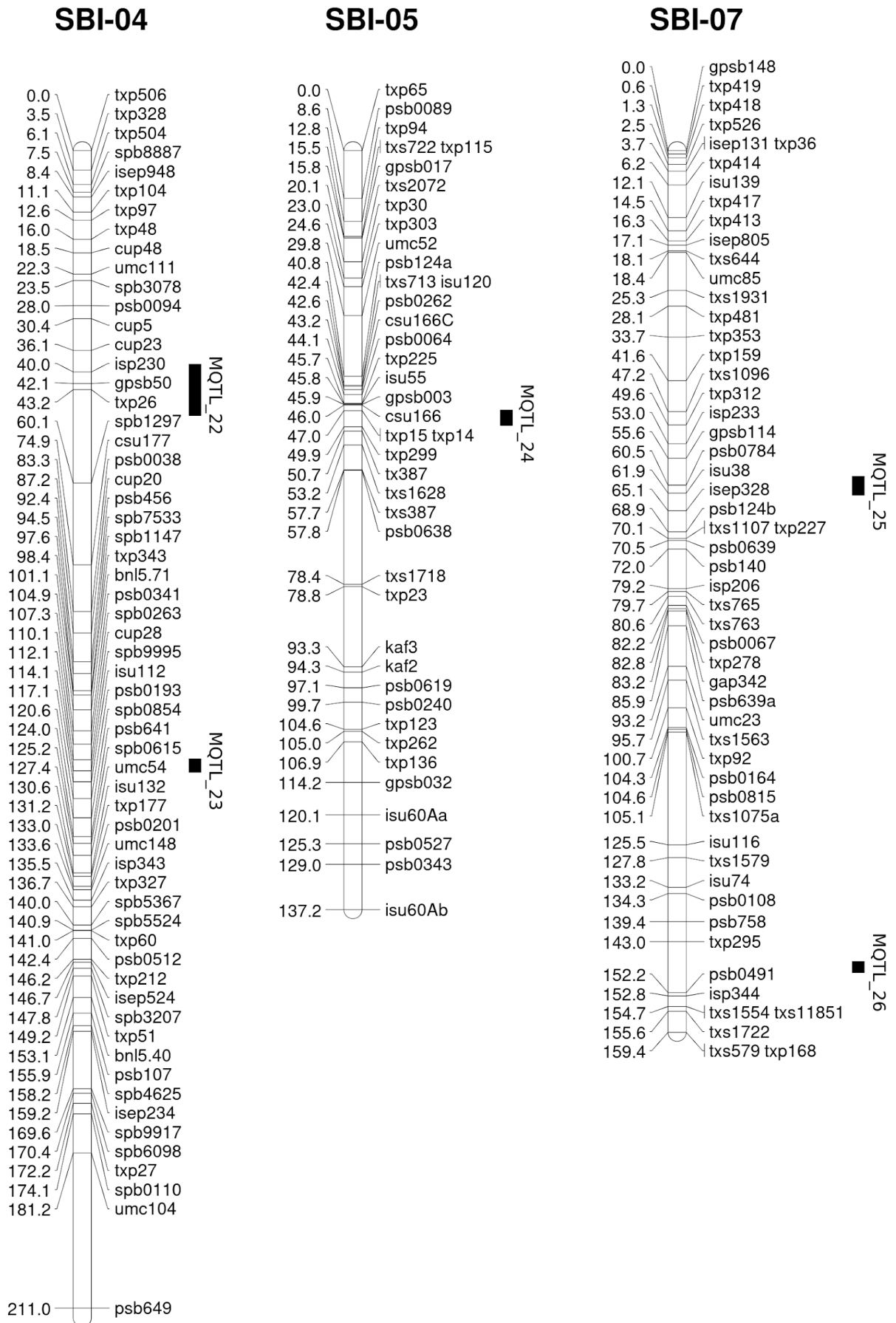
Figure 1. A) Chromosome wise distribution of the original QTL and MQTLs; B) A histogram of confidence intervals of the original QTLs; C) Histogram of the phenotypic variance explained (R2 of the original QTLs in percent)

SBI-04

Position	Marker
0.0	txp506
3.5	txp328
6.1	txp504
7.5	spb8887
8.4	isep948
11.1	txp104
12.6	txp97
16.0	txp48
18.5	cup48
22.3	umc111
23.5	spb3078
28.0	psb0094
30.4	cup5
36.1	cup23
40.0	isp230
42.1	gpsb50
43.2	txp26
60.1	spb1297
74.9	csu177
83.3	psb0038
87.2	cup20
92.4	psb456
94.5	spb7533
97.6	spb1147
98.4	txp343
101.1	bnl5.71
104.9	psb0341
107.3	spb0263
110.1	cup28
112.1	spb9995
114.1	isu112
117.1	psb0193
120.6	spb0854
124.0	psb641
125.2	spb0615
127.4	umc54
130.6	isu132
131.2	txp177
133.0	psb0201
133.6	umc148
135.5	isp343
136.7	txp327
140.0	spb5367
140.9	spb5524
141.0	txp60
142.4	psb0512
146.2	txp212
146.7	isep524
147.8	spb3207
149.2	txp51
153.1	bnl5.40
155.9	psb107
158.2	spb4625
159.2	isep234
169.6	spb9917
170.4	spb6098
172.2	txp27
174.1	spb0110
181.2	umc104
211.0	psb649

MQTL_22

MQTL_23

SBI-05

Position	Marker
0.0	txp65
8.6	psb0089
12.8	txp94
15.5	txs722 txp115
15.8	gpsb017
20.1	txs2072
23.0	txp30
24.6	txp303
29.8	umc52
40.8	psb124a
42.4	txs713 isu120
42.6	psb0262
43.2	csu166C
44.1	psb0064
45.7	txp225
45.8	isu55
45.9	gpsb003
46.0	csu166
47.0	txp15 txp14
49.9	txp299
50.7	tx387
53.2	txs1628
57.7	txs387
57.8	psb0638
78.4	txs1718
78.8	txp23
93.3	kaf3
94.3	kaf2
97.1	psb0619
99.7	psb0240
104.6	txp123
105.0	txp262
106.9	txp136
114.2	gpsb032
120.1	isu60Aa
125.3	psb0527
129.0	psb0343
137.2	isu60Ab

MQTL_24

SBI-07

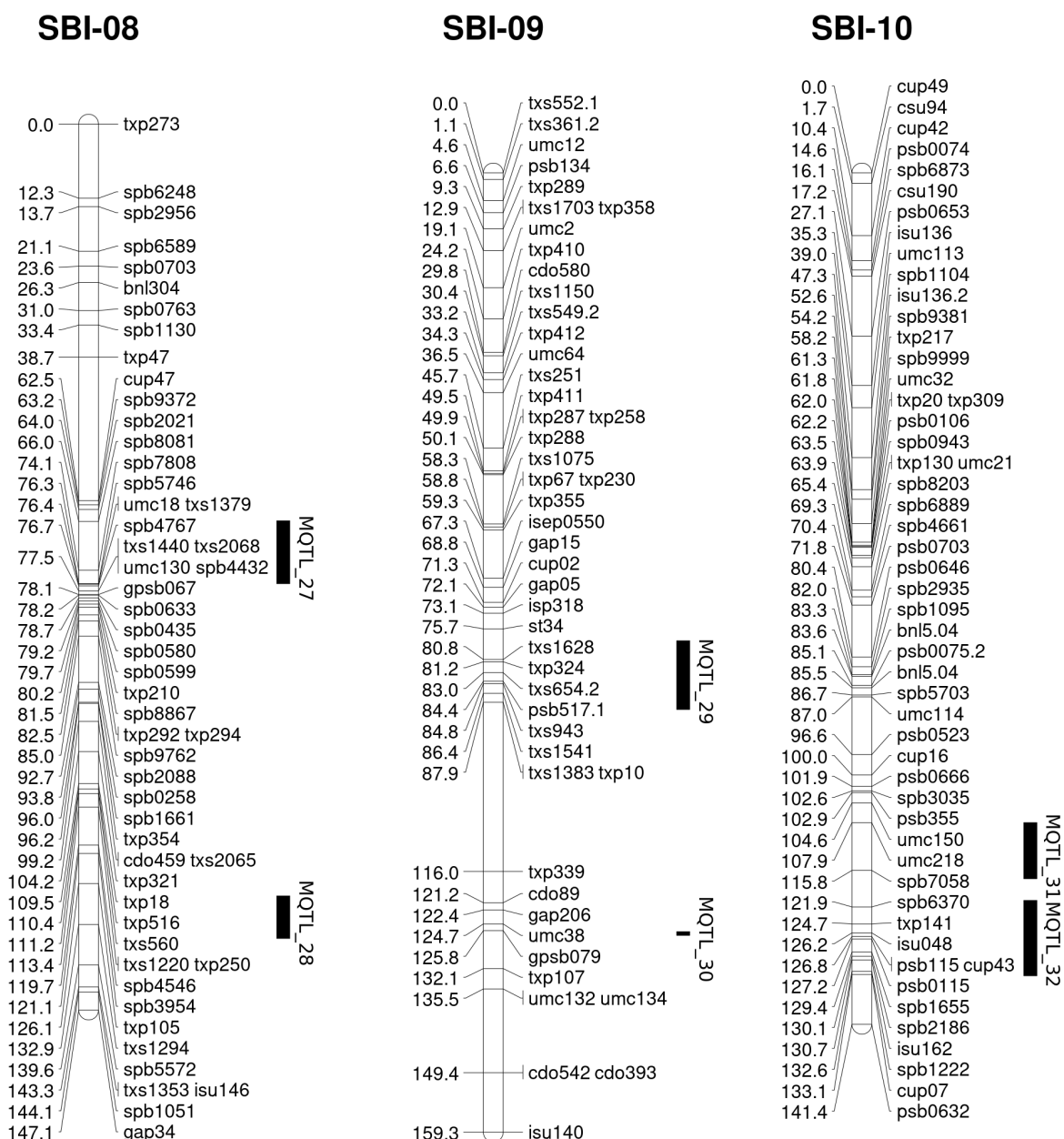| Position	Marker
0.0	gpsb148
0.6	txp419
1.3	txp418
2.5	txp526
3.7	isep131 txp36
6.2	txp414
12.1	isu139
14.5	txp417
16.3	txp413
17.1	isep805
18.1	txs644
18.4	umc85
25.3	txs1931
28.1	txp481
33.7	txp353
41.6	txp159
47.2	txs1096
49.6	txp312
53.0	isp233
55.6	gpsb114
60.5	psb0784
61.9	isu38
65.1	isep328
68.9	psb124b
70.1	txs1107 txp227
70.5	psb0639
72.0	psb140
79.2	isp206
79.7	txs765
80.6	txs763
82.2	psb0067
82.8	txp278
83.2	gap342
85.9	psb639a
93.2	umc23
95.7	txs1563
100.7	txp92
104.3	psb0164
104.6	psb0815
105.1	txs1075a
125.5	isu116
127.8	txs1579
133.2	isu74
134.3	psb0108
139.4	psb758
143.0	txp295
152.2	psb0491
152.8	isp344
154.7	txs1554 txs11851
155.6	txs1722
159.4	txs579 txp168

MQTL_25

MQTL_26

Figure 2. Meta-quantitative trait loci (MQTL) associated with stay-green on sorghum genetic map. Genetic markers(right) and the corresponding position(left) on the consensus genetic map are shown for each chromosome. Meta-QTLs are represented as 95% confidence intervals (CIs) as determined by Biomercator v4.2. NOTE: Chromosome 6 (SBI-06) is not shown as no Meta-QTL was detected on it.

meta-analysis has verified that several QTLs related to stay-green detected in the different experimental studies are co-incident and can be represented by a common confidence interval. We report a comprehensive set of genetic markers linked to stay-green. In addition, we report the number of underlying QTLs and number of studies for each Meta-QTL. These parameters give insight about the stability and robustness of the given MQTLs. Thus, results from the Meta-QTL analysis would potentially benefit breeders aiming to improve breeding for stay-green to indirectly improve drought resilience in sorghum.

Conclusion

This study reports relevant genetic intervals that regulate stay-green in sorghum and thereby affect drought resilience. Results from this study could aid in future attempts aiming to improve drought tolerance in sorghum. The study was completed using in-silico methods only. Further investigations can focus on fine mapping and validation of the MQTL regions and markers reported in this study.

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References

Abebe, T., Belay, G., Tadesse, T., and Keneni, G. (2021). Stay-green genes contributed for drought adaptation and performance under post-flowering moisture stress on sorghum (*Sorghum bicolor* L. Moench). *Journal of Plant Breeding and Crop Science* **13**,190-202. https://doi.org/10.5897/JPBCS2020.0894

Abreha, K. B., Enyew, M., Carlsson, A. S., Vetukuri, R. R., Feyissa, T., Motlhaodi, T., Ng'uni, D., and Geleta, M. (2022). Sorghum in dryland: morphological, physiological, and molecular responses of sorghum under drought stress. *Planta* **255**,1-23. https://doi.org/10.1007/s00425-021-03799-7

Amelework, B., Shimelis, H., Tongoona, P., and Laing, M. (2015). Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: a review. *African Journal of Agricultural Research* **10**, 3029-3040. https://doi.org/10.5897/AJAR2015.9595

Crasta, O. R., Xu, W. W., Rosenow, D. T., Mullet, J., and Nguyen, H. T. (1999). Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. *Molecular and General Genetics* **262**, 579-588. https://doi.org/10.1007/s004380051120

De Oliveira, Y., Sosnowski, O., Charcosset, A., and Joets, J. (2014). BioMercator 4: a complete framework to integrate QTL, meta-QTL, and genome annotation *In* European Conference on Computational Biology 2014.

Goffinet, B., and Gerber, S. (2000). Quantitative trait loci: a meta-analysis. *Genetics* **55**, 463-473. https://doi.org/10.1093/genetics/155.1.463

Guo, B., Sleper, D. A., Lu, P., Shannon, J. G., Nguyen, H. T., and Arelli, P. R. (2006). QTLs associated with resistance to soybean cyst nematode in soybean: meta-analysis of QTL locations. *Genomics, Molecular Genetics and Biotechnology* **46**, 595-602. https://doi.org/10.2135/cropsci2005.04-0036-2

Harris, K., Subudhi, P.K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R. and Mullet, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany* **58**, 327-338. https://doi.org/10.1093/jxb/erl225

Haussmann, B., Mahalakshmi, V., Reddy, B., Seetharama, N., Hash, C., and Geiger, H. (2002). QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theoretical and Applied Genetics* **106**, 133-142. https://doi.org/10.1007/s00122-002-1012-3

Kebede, H., Subudhi, P. K., Rosenow, D. T., and Nguyen, H. T. (2001). Quantitative trait loci influencing drought tolerance in grain sorghum *(Sorghum bicolor* L. Moench*). Theoretical and Applied Genetics* **103**, 266-276. https://doi.org/10.1007/s001220100541

Löffler, M., Schön, C. C., and Miedaner, T. (2009). Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis. *Molecular Breeding*, **23**, 473-488. https://doi.org/10.1007/s11032-008-9250-y

Mace, E. S., and Jordan, D. R. (2011). Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. *Theoretical and Applied Genetics* **123**, 169-191. https://doi.org/10.1007/s00122-011-1575-y

Menz, M. A., Klein, R. R., Unruh, N. C., Rooney, W. L., Klein, P. E., and Mullet, J. E. (2004). Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. https://doi.org/10.2135/cropsci2004.1236

Reddy, N. R. R., Ragimasalawada, M., Sabbavarapu, M. M., Nadoor, S., and Patil, J. V. (2014). Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. *BMC Genomics* **15**, 1-16. https://doi.org/10.1186/1471-2164-15-909

Sabadin, P.K., Malosetti, M., Boer, M.P., Tardin, F.D., Santos, F.G., Guimaraes, C.T., Gomide, R.L., Andrade, C.L.T., Albuquerque, P.E.P., Caniato, F.F. and Mollinari, M. (2012). Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. *Theoretical and Applied Genetics* **124**, 1389-1402. https://doi.org/10.1007/s00122-012-1795-9

Srinivas, G., Satish, K., Madhusudhana, R., Reddy, R. N., Mohan, S. M., and Seetharama, N. (2009). Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. *Theoretical and Applied Genetics* **118**, 1439-1454. https://doi.org/10.1007/s00122-009-0993-6

Subudhi, P. K., Rosenow, D. T., and Nguyen, H. T. (2000). Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor L. Moench*): consistency across genetic backgrounds and environments. *Theoretical and Applied Genetics* **101**, 733-741. https://doi.org/10.1007/s001220051538

Tao, Y. Z., Henzell, R. G., Jordan, D. R., Butler, D. G., Kelly, A. M., and McIntyre, C. L. (2000). Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. *Theoretical and Applied Genetics* **100**, 1225-1232. https://doi.org/10.1007/s001220051428

Tello-Ruiz, M.K., Naithani, S., Gupta, P., Olson, A., Wei, S., Preece, J., Jiao, Y., Wang, B., Chougule, K., Garg, P. and Elser, J.(2021). Gramene 2021: harnessing the power of comparative genomics and pathways for plant research. *Nucleic Acids Research* **49**, D1452-D1463. https://doi.org/10.1093/nar/gkaa979

Veyrieras, J. B., Goffinet, B., and Charcosset, A. (2007). MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. *BMC Bioinformatics* **8**, 1-16. https://doi.org/10.1186/1471-2105-8-49

Xu, W., Subudhi, P. K., Crasta, O. R., Rosenow, D. T., Mullet, J. E., and Nguyen, H. T. (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor L. Moench*). *Genome*, **43**, 461-469. https://doi.org/10.1139/g00-003

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