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 $163/N R^2$ (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                       | SC56xTx7000.          | 125             |
| 6          | Sabadin et al. 2012 | 4                        | BR007 x SC283         | 100             |
| 7          | Srinivas et al. 2008 | 9                        | 296B x IS18551        | 168             |
| 8          | Subduhi 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             |
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

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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         |
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 (R^2 of the original QTLs in percent)
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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.

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.

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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