

# Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum

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**Abstract** The identification of quantitative trait loci (QTLs) affecting agronomically important traits enable to understand their underlying genetic mechanisms and genetic basis of their complex interactions. The aim of the present study was to detect QTLs for 12 agronomic traits related to staygreen, plant early development, grain yield and its components, and some growth characters by analyzing replicated phenotypic datasets from three crop seasons, using the population of 168 F<sub>7</sub> RILs of the cross 296B × IS18551. In addition, we report mapping of a subset of genic-microsatellite markers. A linkage map was constructed with 152 marker loci comprising 149 microsatellites (100 genomic- and 49 genic-microsatellites) and three morphological markers. QTL analysis was performed by using MQM approach. Forty-nine QTLs were detected, across environments or in individual environments, with 1–9 QTLs for each trait. Individual QTL accounted for 5.2–50.4% of phenotypic variance. Several genomic regions affected multiple traits, suggesting the phenomenon of pleiotropy or tight linkage. Stable QTLs were identified for studied traits across different environments, and genetic backgrounds by comparing the QTLs in the study with previously reported QTLs in sorghum. Of the 49 mapped genic-markers, 18 were detected associating either closely or exactly as the QTL positions of agronomic traits. EST marker Dsenhsbm19, coding for a key regulator (EIL-1) of

ethylene biosynthesis, was identified co-located with the QTLs for plant early development and staygreen trait, a probable candidate gene for these traits. Similarly, such exact co-locations between EST markers and QTLs were observed in four other instances. Collectively, the QTLs/markers identified in the study are likely candidates for improving the sorghum performance through MAS and map-based gene isolations.

## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops globally after wheat, maize, rice, barley and has a predominant role in the food and fodder security for millions of rural families in arid and semi-arid regions of the world. Progress in genetic manipulation of agronomically important traits is essential for the sustained improvement of sorghum to meet the future demand; nevertheless, progress has been hindered by the lack of understanding of inheritance of quantitative nature of the traits and their complex interrelations.

The advent of molecular marker technology in quantitative genetics greatly facilitates the study of complex traits and has made it possible to dissect the polygenes controlling such traits into individual Mendelian factors (Paterson et al. 1988). Using molecular linkage maps and quantitative trait loci (QTLs) mapping technology, it is possible to estimate the number of loci governing a particular trait of agronomic importance and to determine their map positions in the genome (Tanksley 1993). The identification of such genomic regions/QTLs governing traits of agronomic importance can create a base for rapid, detailed, and direct genetic manipulation of them through marker-assisted selection (MAS).

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Different kinds of genomic-DNA (non-genic) based markers such as RFLPs, RAPDs, AFLPs, and SSRs (microsatellites) have been developed in sorghum and used to construct linkage maps. QTLs have been identified using these genetic linkage maps predominantly containing anonymous molecular markers for many agronomically important traits including plant early development (anthesis and maturity), yield and its component traits, plant height and other growth characters (Pereira and Lee 1995; Tuinstra et al. 1998; Rami et al. 1998; Hart et al. 2001; Brown et al. 2006; Feltus et al. 2006), pre- and post-flowering drought stress (staygreen) tolerance (Tuinstra et al. 1996, 1997; Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000; Tao et al. 2000; Kebede et al. 2001; Haussmann et al. 2002) and for important biotic stresses.

During the last few years, emphasis has shifted towards the development of molecular markers from the transcribed region of the genome in order to associate the molecular polymorphisms of genes with phenotypic variability of the traits. Construction of genetic map by mapping functionally defined genes permits evaluation of co-location between genic-markers and QTLs of any trait (Aubert et al. 2006). It may also increase our understanding of the biochemical pathways and mechanisms affecting agronomically important traits (Matthews et al. 2001; Zhang et al. 2004). For instance, a functional map containing genes involved in carbohydrate metabolism and transport has been used to detect co-locations with QTLs for starch content of tubers in potato (Chen et al. 2001). Prioul et al. (1997) showed that the gene *Sh1* was the more likely candidate that controls the sucrose or hexose content of maize leaves. Similarly, studies have been performed with functionally defined genes for many agronomically important traits such as flowering time, grain quality, and disease resistance in many crop species (Lagercrantz et al. 1996; Faris et al. 1999; Pflieger et al. 2001; Huh et al. 2001; Francki et al. 2004; Li et al. 2004a). However, such applications in association of genic-markers with QTLs regulating the traits of agronomic importance have not been known in case of sorghum due to the presence of a few gene based markers on the constructed linkage maps used for the QTL studies.

Drought is an important abiotic stress in crop plants, which influences many agronomically important traits. Staygreen (persistent green leaf area) in sorghum is considered an important stress resistance mechanism, since it improves plant adaptation to post-flowering drought stress conditions by ensuring normal grain filling, particularly in environments where the crop depends on stored soil moisture (Rosenow et al. 1983). Due to the importance of the trait in sorghum, it has been characterized by many authors using different staygreen sources (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000; Tao et al. 2000; Kebede et al. 2001; Haussmann et al. 2002). In

general, under terminal drought stress conditions, many of the known high grain yielding sorghum cultivars and their derived hybrids are moderate staygreen types or senescent types, indicating the probable connection between the translocation of stem reserves into sink (seeds). Therefore, characterization of the staygreen trait under high yielding genetic background is more important to study its relative influence on other agronomic traits such as grain production than considering it alone (Borrell et al. 1999). However, many of the genotypes which had been used to characterize the trait were primarily selected based on their high staygreen expression. So it is important to compare the trait expression between high yield genetic backgrounds and the high staygreen genotypes used earlier for characterization of the trait before exploiting them for sorghum improvement under terminal drought conditions. Furthermore, the establishment of relation of the staygreen trait with plant early development is also the most critical point since these two traits are genetically inversely related. Crasta et al. (1999) highlighted the establishment of the genetic relationship between these two traits and identified QTLs that influence the staygreen trait but not maturity, in order to maximize crop improvement under drought stress environments. Therefore, it is essential to better understand the genetic mechanisms underlying these traits and to study the relationships among them before attempting the MAS of these important agronomic traits (staygreen, grain yield and its components, and plant early development).

The identification of QTLs controlling the agronomically important traits would improve our genetic understanding of these traits, enable to analyze association between these traits, clarify the relationships of QTLs to candidate genes and finally provide the basis for MAS of these traits. The objectives of the present study were to understand the genetic basis of agronomic traits related to staygreen trait, plant early development, yield and its component traits, and growth characters plant height and total number of leaves, and to determine the relationships among them under terminal drought stress conditions. In addition, we report the development and mapping of some gene derived microsatellite markers on to the previously constructed microsatellite linkage map.

## Materials and methods

### Plant material

The experimental material consisted of a set of 168 F<sub>7</sub> recombinant inbred lines (RILs) derived from the cross between 296B and IS18551. The 296B is a high yielding variety and the seed parent of several commercial sorghum hybrids in India. It is characteristically late maturing, short

in stature, having long compact panicle with bold seeds protruded from glumes; tan genotype with juicy leaf midrib, and shows moderate levels of staygreen. IS18551 is a landrace from Ethiopia: it is early maturing, long in stature, has short compact panicle with copious number of infertile branches; purple genotype with small seeds covered by glumes, dry leaf midrib, and shows much less staygreen than 296B.

#### Field trials and evaluation of agronomic traits

The RIL population along with their parental lines were evaluated under three environments during 2003 (season I) and 2006 (season III) at agricultural farm of NRCS, Hyderabad, and during 2004 (season II) at agricultural farm of ICRISAT, Patancheru, Hyderabad. All the experiments were conducted in post-rainy or Rabi season of the each year (Oct–Feb). The RIL population was planted in a random block design (RBD) with three replications. The experimental units were two-row plots, with each row 4-m long, plant to plant spacing was 15 cm and 0.75 m space between rows. The crop was protected from leaf feeding insect pests as well as stem borers by following the recommended plant protection measures. The crop was well-irrigated during the early growth stages to promote good growth and high yield potential. Irrigation was withheld just prior to anthesis in order to allow moisture stress to develop during the grain-filling stage. These conditions are ideal for evaluating the expression of staygreen trait under terminal moisture-deficit conditions and to study its relation with other important agronomic traits. All phenotypic measurements of each of the agronomic traits were recorded from a total of six tagged individuals representing three uniform individuals in the center of each row. The 12 agronomic traits studied in the study included *plant height* (PHE, in centimeter), *days to anthesis* (DAN, no. of days from planting to 50% anthesis), *days to maturity* (DMA, no. of days from planting to form a black tip on seed at the junction between seed and plant at the base of the head), *total number of leaves* (TNL, no. of leaves counted from the base of the plant to flag leaf including dry leaves at the base at the anthesis stage); staygreen component traits such as *green leaf area at anthesis* (GLAA, in centimeters), *green leaf area at maturity* (GLAM, in centimeters), and *percent green leaf area preserved at maturity* (PGLAM, in percentage). As described by Haussmann et al. (2002), the upper six leaves of plant were considered for measuring the green leaf area. In general, the upper leaves are photosynthetically active at the stage of anthesis and direct assimilate mostly to the grain (Joshi et al. 2003). Briefly, green leaf area was derived from measuring the length and breadth of upper six leaves in centimeters from each of the six representative individuals and the leaf area was

estimated as: leaf length  $\times$  leaf width  $\times$  0.70. The average percentage green leaf area preserved at maturity (PGLAM) from each plot was calculated by dividing the total green leaf area of each plot at the maturity by the total green leaf area of that plot at anthesis; *grain yield* (GYL, in grams) and its component traits *panicle length* (PLE, in centimeters), *panicle weight* (PWE, in grams), *number of primary branches* [NPB, number, counted according to Brown et al. (2006)], and *seed weight* (SWE, weighing 100 seeds in grams) were recorded on the population.

In the present study, genes regulating three qualitative traits, viz., leaf midrib type (Mrco; juicy vs. dry), seed glume cover (GlumeT; covered vs. open) and plant coloration (Plcor; purple vs. tan) were scored and mapped as morphological markers in the population.

#### Phenotypic data analysis

The software Windostat package (<http://www.windostat.org/>) was used for all statistical analysis of phenotypic data on agronomic traits. Analysis of variance (ANOVA) was performed for studying the effect of genotype (RIL) as well as environment and genotype  $\times$  environment interactions for observed variance among the RILs for agronomic traits by residual maximum likelihood algorithm (REML) suggested by Patterson and Thompson (1971). The REML estimates the components of variances by maximizing the likelihood of all contrasts with zero expectation. Varietal means were estimated by generalized least squares with weights depending on the estimated variance components according to Patterson (1997). Pearson's correlation coefficients were calculated to determine relationships between various traits under study according to standard procedures using the varietal means obtained over three seasons. Broad-sense heritability ( $h^2$ ) of agronomic traits across seasons was calculated using trait mean values derived over seasons according to Falconer (1989).

#### Marker development and linkage mapping

As a preliminary analysis, primer pairs were designed flanking 50 unigene sequences containing microsatellite motifs from a total of 13,548 sorghum unigene sequences available at the NCBI database ([ftp://ftp.ncbi.nih.gov/repository/UniGene/Sorghum\\_bicolor/](ftp://ftp.ncbi.nih.gov/repository/UniGene/Sorghum_bicolor/)). In addition, a microsatellite marker was derived from a gene coding for caffeic acid *O*-methyltransferase (designated BMR) enzyme involved in lignin biosynthesis (Bout and Vermerris 2003). The parameters set for marker development and PCR amplification conditions were followed as described in our previous studies (Srinivas et al. 2008, 2009). In contrast to previous marker developments, in the study we used at least seven repeats as cut off for identification of all

microsatellite motifs for the development of unigene markers. The markers were named Ungnhsbm1-Ungnhsbm50 according to the marker nomenclature proposed by De Vicente et al. (2004). The genetic linkage map constructed previously on the same 296B × IS18551 RIL population (Srinivas et al. 2008, 2009) was further improved in the study by adding ten polymorphic unigene-microsatellites, the BMR gene and three morphological markers (Mrco, GlumeT, and Plcor). Collectively, the genetic map was produced with 152 loci consisting of 100 genomic-microsatellite markers, 49 genic-microsatellite markers (including the genic-markers mapped in the study) and the three morphological markers. The linkage map was constructed using JOINMAP 3.0 software (Van Ooijen and Voorrips 2001). The construction of the linkage map was done as described earlier (Srinivas et al. 2009).

### QTL mapping

The QTL analysis was performed with trait mean values in each season and averaged mean values of each trait across the three environments using the software MAPQTL version 5 (Van Ooijen 2005). First, the non-parametric Kruskal–Wallis (KW) test (Lehmann 1975) was performed to associate between markers and traits individually. Then, interval mapping (IM) analyses was performed (Lander and Botstein 1989) to locate preliminary QTL positions on the map. IM was used to select markers significantly associated with the trait to constitute an initial set of cofactors. A backward elimination procedure was applied to the initial set of cofactors. Only significant markers at  $P < 0.02$  were used as cofactors in the multiple QTL method (MQM) (Jansen 1993; Jansen and Stam 1994) analysis for QTL detection. After the selection of cofactors, MQM analyses

were performed. A thousand-permutation test was applied to each data set to decide the LOD (logarithm of odds) thresholds ( $P = 0.05$ ) for considering significant level of identified QTLs (Churchill and Doerge 1994). A 1-LOD support interval was calculated for each QTL to obtain a 95% confidence interval. Adjacent QTLs on the same chromosome were considered different when the curve had a minimum between peaks that were at least one-LOD unit below either peak or when the support intervals were non-overlapping. The phenotypic variance explained by a single QTL was calculated as the square of the partial correlation coefficient ( $R^2$ ) with the observed variable, adjusted for cofactors. The additive effect of a putative QTL was estimated by half the difference between two homozygous classes.

The identified QTLs were designated with italicized symbol composed of a *Q*, a trait name, a hyphen, the symbol for the chromosome in which the QTL is located, and, in cases where more than one QTL controlling a trait were detected in the same LG, they were numbered serially. For instance, the QTL name *QPhe-sbi06-1* refers to the plant height QTL detected on sorghum SBI-06.

## Results

### Phenotypic data analysis

Trait mean values of parents 296B and IS18551 and their RIL population for 12 agronomic traits over environments were shown in Table 1. The parental lines differed for most of the characters except for TNL, and staygreen component traits (GLAA, GLAM, and PGLAM). However, a wide range of variation for the investigated traits in the RIL population (Table 1), normal phenotypic distri-

**Table 1** Mean phenotypic values of parental lines, and min, max, mean phenotypic values and standard deviation in RILs for different agronomic traits

Trait	Parental lines		RIL population			
	296B	IS18551	Min	Max	Mean	SD
Plant height (PHE) (cm)	105.0	199.0	96.0	220.0	165.1	27.0
Days to anthesis (DAN) (days)	93.0	80.0	70.0	95.0	85.1	4.5
Days to maturity (DMA) (days)	125.0	115.0	110.0	130.0	118.7	3.5
Total number of leaves (TNL) (no./plant)	11.0	12.0	8.0	13.0	11.2	0.9
Green leaf area at anthesis (GLAA) (cm <sup>2</sup> /plant)	1,390.0	1,285.0	662.0	1,714.0	1,233.6	210.0
Green leaf area at maturity (GLAM) (cm <sup>2</sup> /plant)	830.0	750.0	276.0	1,301.0	713.1	228.5
% Green leaf area preserved at maturity (PGLAM) (%)	62.0	49.0	25.0	91.0	56.8	13.3
Grain yield (GYL) (g/panicle)	32.2	20.2	2.0	43.0	23.4	7.5
Panicle length (PLE) (cm/panicle)	23.0	17.0	11.0	35.0	19.0	4.0
Panicle weight (PWE) (g/panicle)	42.0	21.0	12.0	56.0	33.9	8.6
No. of primary branches (NPB) (no./panicle)	87.0	95.0	37.0	118.0	74.1	7.5
Seed weight (SWE) (g/100 seeds)	2.9	1.8	1.4	3.4	2.3	0.3

butions and transgressive segregations (data not shown) suggested a polygenic inheritance of the traits. The estimated broad-sense heritability ( $h^2$ ) values for agronomic traits were moderate to quite high and ranged from 53 to 88% depending on agronomic traits (Table 2). The calculated  $F$  values of agronomic traits using ANOVA analysis showed the presence of significant differences among the RILs and also highly significant environmental effects on traits and significant genotype  $\times$  environment interactions (Table 2).

A total of 39 significant correlation coefficients ( $P < 0.05$  and  $P < 0.01$ ) were observed among the traits (Table 3). The highest correlation coefficient was observed

between GYL and PWE ( $r = 0.92$ ), suggesting that the crop productivity in the RIL population was largely due to number of seeds produced per head rather than seed weight since there was no correlation between SWE and GYL. Significant positive correlations were observed among DAN, DMA, TNL, GLAA, GLAM, and PGLAM. NPB significantly positively correlated with DAN ( $r = 0.68$ ), DMA ( $r = 0.54$ ), TNL ( $r = 0.54$ ) and staygreen traits GLAA ( $r = 0.49$ ), GLAM ( $r = 0.56$ ), and PGLAM ( $r = 0.38$ ) suggesting that plant development and high photosynthetic capacity is directly related to number of primary branches (NPB). However, NPB was not correlated positively with GYL and its related traits.

**Table 2** The  $F$  value of ANOVA for genotype, environment, genotype  $\times$  environment interactions and heritability of 12 agronomic traits in the RIL population derived from the cross 296B  $\times$  IS18551

Trait	Genotype			Environment			Genotype $\times$ environment			
	<i>df</i>	<i>F</i> value	<i>P</i> value	<i>df</i>	<i>F</i> value	<i>P</i> value	<i>df</i>	<i>F</i> value	<i>P</i> value	Heritability $h^2$ (%)
Plant height (PHE) (cm)	167	17.95	0.00	2	365.81	0.00	334	1.87	0.00	82.0
Days to anthesis (DAN) (days)	167	16.53	0.00	2	5,218.61	0.00	334	3.72	0.00	88.0
Days to maturity (DMA) (days)	167	11.22	0.00	2	234.97	0.00	334	1.79	0.00	75.8
Total number of leaves (TNL) (no/plant)	167	13.83	0.00	2	266.90	0.00	334	1.68	0.00	80.4
Green leaf area at anthesis (GLAA) (cm <sup>2</sup> /plant)	167	9.49	0.00	2	10.78	0.00	334	1.79	0.00	57.6
Green leaf area at maturity (GLAM) (cm <sup>2</sup> /plant)	167	9.75	0.00	2	9.42	0.00	334	1.75	0.00	55.8
% Green leaf area preserved at maturity (PGLAM) (%)	167	5.98	0.00	2	8.50	0.00	334	1.67	0.00	53.0
Grain yield (GYL) (g/panicle)	167	7.90	0.00	2	12.50	0.00	334	1.50	0.00	56.6
Panicle length (PLE) (cm/panicle)	167	45.88	0.00	2	511.97	0.00	334	3.62	0.00	86.0
Panicle weight (PWE) (g/panicle)	167	7.89	0.00	2	10.50	0.00	334	1.45	0.00	55.0
No. of primary branches (NPB) (no/panicle)	167	30.18	0.00	2	24.20	0.00	334	2.56	0.00	76.4
Seed weight (SWE) (g/100 seeds)	167	45.92	0.00	2	701.30	0.00	334	3.16	0.00	86.5

**Table 3** Correlation among the agronomic traits in the 296B  $\times$  IS18551 RIL population

Trait	Plant height (PHE)	DAN	DMA	TNL	GLAA	GLAM	PGLAM	PLE	PWE	NPB	GYL
Days to anthesis (DAN)	0.18*										
Days to maturity (DMA)	0.02	0.69**									
Total no. of leaves (TNL)	0.06	0.67**	0.62**								
Green leaf area at anthesis (GLAA)	0.04	0.53**	0.48**	0.48**							
Green leaf area at maturity (GLAM)	0.15*	0.64**	0.54**	0.56**	0.62**						
% Green leaf area preserved at maturity (PGLAM)	0.20**	0.47**	0.37**	0.41**	0.16*	0.84**					
Panicle length (PLE)	0.12	-0.03	-0.18*	-0.13	0.08	0.10	0.06				
Panicle weight (PWE)	-0.28**	-0.04	0.06	0.03	0.15*	-0.09	-0.24**	-0.17*			
No. of primary branches (NPB)	0.13	0.68**	0.54**	0.54**	0.49**	0.56**	0.38**	0.04	-0.00		
Grain yield (GYL)	-0.25**	-0.08	0.01	0.00	0.17**	-0.15*	-0.25**	-0.25**	0.92**	-0.03	
Seed weight (SWE)	0.02	-0.19*	-0.00	-0.23**	-0.01	-0.11	-0.13	-0.27**	0.08	-0.20**	0.06

\*\* Significant at  $P < 0.01$ , \* Significant at  $P < 0.05$



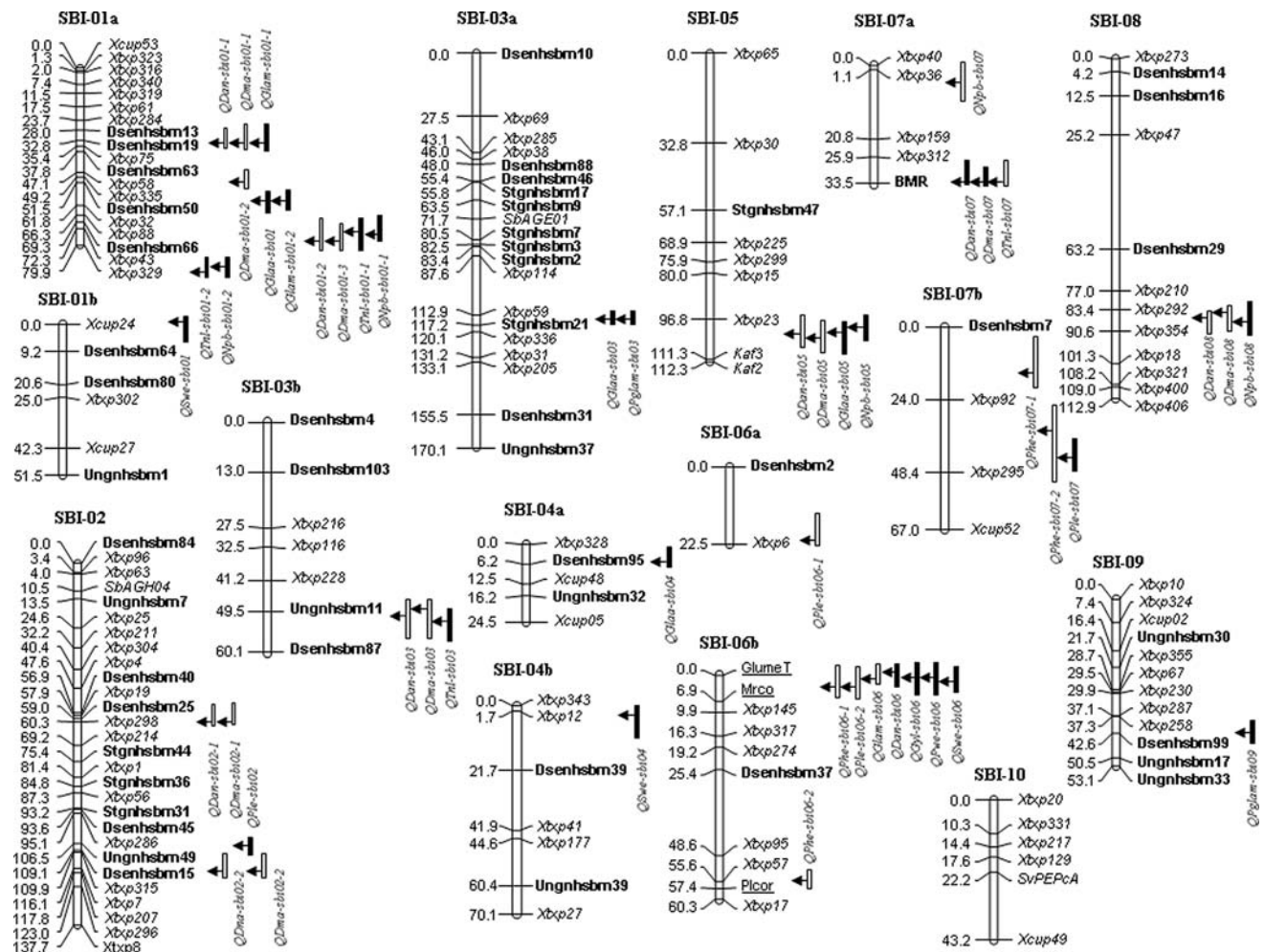
Marker development and the genetic linkage map

Out of 13,548 unigene sequences mined for microsatellites, a total of 336 sequences (2.48%) were identified with microsatellite motifs. Among them, a total of 50 unigene-SSR markers were developed. The ten unigene-SSR markers were mapped on five sorghum chromosomes including one (Ungnhsbm1), two (Ungnhsbm7, Ungnhsbm49), two (Ungnhsbm11, Ungnhsbm37), two (Ungnhsbm32, Ungnhsbm39), and three (Ungnhsbm17, Ungnhsbm30, Ungnhsbm33) on linkage groups SBI-01, SBI-02, SBI-03, SBI-04 and SBI-09, respectively. The BMR gene was mapped on linkage group SBI-07. All three morphological markers scored in the population co-segregated and subsequently were mapped on linkage group SBI-06 (Fig. 1). Collectively, the genetic linkage map included 152 marker loci, consisting of 100 genomic-microsatellites, ten genic-

microsatellite markers mapped at the four staygreen QTL regions (Srinivas et al. 2008), 28 mapped subtracted drought stress EST-microsatellites (Srinivas et al. 2009) and the markers mapped in this study. However, mapping of new markers in the study did not reduce the number of linkage groups; the markers Ungnhsbm1 and Ungnhsbm17, Ungnhsbm33 extended linkage groups SBI-01 and SBI-09, respectively, and by overall the map length was enhanced from 1,074.5 cM in previous study (Srinivas et al. 2009) to 1,098.7 cM in this study.

Genic-microsatellite markers and their functions

Out of ten unigene-microsatellite markers mapped in the study, eight markers showed significant homology to previously characterized proteins belonging to genes involved in diverse biologically important functions, while two markers



**Fig. 1** Genetic linkage map of sorghum showing 49 quantitative trait loci (QTLs) identified for the 12 agronomic traits studied in the 296B × IS18551 RIL population. The useful alleles contributed for the traits by 296B and IS18551 are presented by *black bars* and *open bars*, respectively, on the right hand side of each chromosome. Supported

intervals for each QTL are indicated by the length of *vertical bars*. LOD maximum is pointed by an *arrow* which shows possible QTL position on chromosomes. The *markers in bold* are gene derived microsatellite markers. Genomic-SSR markers are *italicized*. Genes regulating the morphological traits are *underlined*

did not show homology to any of the characterized proteins. A microsatellite marker was developed from the gene coding for caffeic acid *O*-methyltransferase involved in lignin biosynthesis that is involved in brown midrib production in sorghum (Bout and Vermerris 2003). The primer sequence information and putative functions of ten unigene-microsatellites and the BMR gene is provided in Table 4. The information of the genic-microsatellites mapped on the map other than this study can be found in our earlier reports (Srinivas et al. 2008, 2009).

#### QTL identification

A total of 12 agronomic traits were evaluated on the RIL population derived from the parental lines, 296B and IS18551. The putative QTLs for each trait identified by MQM in the population were listed in Table 5, and chromosomal location of QTLs is depicted in Fig. 1. The QTL results were based on average trait values of agronomic traits over three seasons. In case QTLs were not identified from average data of a trait but from other season of the three environments studied, those QTLs were also presented using the data of the corresponding environment.

#### QTLs for plant height (PHE)

Four QTLs were identified to control PHE in the population. The QTLs were distributed on two linkage groups with two each on SBI-06, and SBI-07. At all QTLs, increasing the plant height was contributed by the tall parent IS18551. These QTLs were consistently identified in both combined analyses and individual analysis of three environments investigated. Three QTLs viz., *QPhe-sbi06-1* (21.6% PV), *QPhe-sbi07-1* (14.8% PV), and *QPhe-sbi07-2* (24.2% PV) explaining larger phenotypic variation were identified in the population.

#### QTLs for days to anthesis (DAN)

Nine QTLs were detected for DAN in the population and were distributed on seven linkage groups with two on SBI-01, two on SBI-02, one each on SBI-03, SBI-05, SBI-06, SBI-07, and SBI-08. Among these, only four QTLs were identified in combined analysis while other QTLs were specifically detected in different individual environments. Majority of the QTLs detected for decreasing the trait (early flowering) were from the early flowering parent IS18551. But on SBI-06 and SBI-07, the QTLs detected for earliness were contributed by late flowering parent 296B. The phenotypic variation explained by each QTL ranged from 6.1 to 13.5%. A major QTL, *QDan-sbi01-2* was detected on SBI-01 near the EST marker Dsenhsbm66, which explained 13.5% of phenotypic variance.

#### QTLs for days to maturity (DMA)

Similarly, nine QTLs were identified for DMA on six chromosomes. Since DAN and DMA are related traits, eight out of the nine QTLs identified for DMA coincided exactly with their genomic positions with the corresponding DAN QTL. Five QTLs were consistently identified in combined analysis, while other four QTLs were specifically identified for individual environments. The effect of QTL alleles influencing the trait (increasing or decreasing the trait) from parents were in agreement with the corresponding DAN QTL. The phenotypic variation explained by each QTL ranged from 5.9 to 15.3%.

#### QTLs for total number of leaves (TNL)

Four QTLs located on three linkage groups including two on SBI-01, one each on SBI-03, and SBI-07 were detected for TNL. The 296B allele increased TNL at three QTLs regions, whereas the IS18551 allele increased the trait at the QTL on SBI-07. The phenotypic variation explained by each QTL ranged from 5.3 to 14.1%. All QTLs were significantly identified in individual environments as well as in combined analysis.

#### QTLs for green leaf area at anthesis (GLAA)

Four QTLs on chromosomes SBI-01, SBI-03, SBI-04, and SBI-05 were detected for GLAA. At all the QTL regions, alleles from 296B parent increased the green leaf area. Out of four QTLs identified, three QTLs *QGlaa-sbi01* (on SBI-01), *QGlaa-sbi03* (on SBI-03), and *QGlaa-sbi05* (on SBI-05) share similar genomic regions as the earlier identified staygreen QTL regions *StgG*, *Stg2*, and *Stg4*, respectively, from the popular staygreen donor B35 (Crasta et al. 1999; Xu et al. 2000, Subudhi et al. 2000). All the QTLs were consistently identified both in combined analysis and in individual environments. Individually each QTL explained the phenotypic variation in the range of 6.1–11.8%.

#### QTLs for green leaf area at maturity (GLAM)

Three QTLs were identified on two chromosomes including two QTLs on SBI-01 and one on SBI-06 for GLAM. The alleles from 296B associated with higher green leaf area were at the QTL regions *QGlam-sbi01-1* and *QGlam-sbi01-2* on SBI-01, while the allele from this parent associated with less green leaf area at the QTL, *QGlam-sbi06* on SBI-06. The phenotypic variation explained by each QTL ranged from 6.6 to 8.7%. In spite of less phenotypic variation, these QTLs were detected both in combined analysis and analysis from three individual environments.

**Table 4** Primer sequence information and putative functions of genic-microsatellite markers mapped in the study

Marker name	NCBI Unigene/ GeneBank ACC no	Repeat motif	Forward primer (5' → 3')	Reverse primer (5' → 3')	Ann. temp (°C)	Expected size (bp)	Putative function	E value	Species
Ungnhsbm1	Sbi.63	(CGA) <sup>7</sup>	GCCCCACCAAAATACAAGAACC	CTTTGGGGCACTAAAACACA	54	236	Cytochrome P450 CYP71E1 (CYP71E1)	0.00	<i>Sorghum bicolor</i>
Ungnhsbm7	Sbi.249	(TA) <sup>9</sup>	GAGGACGAGTGCATGATGAG	GAGAAAGAACACGACGAGCA	53	165	Phytosulfokine peptide precursor	4.00E-10	<i>Zea mays</i>
Ungnhsbm11	Sbi.360	(CTT) <sup>11</sup>	CGTCCTCGTCTCCTTCGTC	TGCATGTTCAAGGAGAGCATC	54	216	Hypothetical protein	0.009	<i>Oryza sativa</i>
Ungnhsbm17	Sbi.543	(TCTG) <sup>10</sup>	TGTTTCATCTCCAACGTCCTCG	GCAGCATCTCGTCAGAAATCA	54	213	Metallothionein	1.10	<i>Oryza rufipogon</i>
Ungnhsbm30	Sbi.1096	(CT) <sup>8</sup>	CATCTCCACCCCTTCTCTCC	GCGTGTCCGATGGCCATATC	56	154	Lisencephaly type-1-like homology motif; CTLH	2.00E-44	<i>Medicago truncatula</i>
Ungnhsbm32	Sbi.1164	(AG) <sup>8</sup>	TTCCAAATCCAAATCCATCC	CGAGGAGGTCGAGTAGAACG	54	169	Bax inhibitor-1	7.00E-54	<i>Oryza sativa</i>
Ungnhsbm33	Sbi.1175	(GT) <sup>10</sup>	GCGGACTTAGTTGGACCTGA	TTCACAGCATAAAGCGCAAC	54	197	Hypothetical protein	2.00E-07	<i>Oryza sativa</i>
Ungnhsbm37	Sbi.1311	(GCA) <sup>7</sup>	ATAGACGATGGGGGTGCAT	CTGCCCCCTATTACTGTGCTGCT	54	174	Putative pectinacetyltransferase precursor	4.00E-70	<i>Oryza sativa</i>
Ungnhsbm39	Sbi.1364	(GT) <sup>7</sup>	ATTCAGCTTCATCGCCATT	TGGATTCTCTGCACTTGACG	54	207	Putative family II lipase EXL4	3.00E-47	<i>Oryza sativa</i>
Ungnhsbm49	Sbi.1731	(GTG) <sup>7</sup>	CCCAAGGCGTACCTGAGAT	TGGCAAAAACCTCCGAGTACAA	54	227	Phytoene synthase radicle isoform	6.00E-60	<i>Oryza sativa</i>
BMR	AY217766	(CTAT) <sup>6</sup>	CATGTCGATCGATCAGTGCT	TCGAGGAGCCTTCTTGCTGAT	54	232	Caffeic acid O-methyltransferase	2.00E-130	<i>Sorghum bicolor</i>



**Table 5** Quantitative trait loci (QTLs) detected for 12 agronomic traits in the 296B × IS18551 RIL population

Trait	QTL name	Environment	Chromosome	Flanking markers	QTL position	LOD	Increased effect	Additive effect	R <sup>2</sup> (%)	
Plant height (PHE)	<i>QPhe-sbi06-1</i>	AV, I, II, III	SBI-06	<u>GlumeT-Xtxp145</u>	3	7.49	IS18551	-13.79	21.6	
	<i>QPhe-sbi06-2</i>	AV, I, II, III	SBI-06	<u>Xtxp57-Plcor</u>	1	3.12	IS18551	-7.96	8.2	
	<i>QPhe-sbi07-1</i>	AV, I, II, III	SBI-07	<u>Dsenhsbm7-Xtxp92</u>	10	4.33	IS18551	-11.25	14.8	
	<i>QPhe-sbi07-2</i>	AV, I, II, III	SBI-07	<u>Xtxp92-Xtxp295</u>	9	6.98	IS18551	-17.93	24.2	
Day to anthesis (DAN)	<i>QDan-sbi01-1</i>	AV, II, III	SBI-01	<u>Dsenhsbm13-Dsenhsbm19</u>	0	2.57	296B	1.24	6.3	
	<i>QDan-sbi01-2</i>	AV, I, II, III	SBI-01	<u>Xtxp32-Dsenhsbm66</u>	1	4.77	296B	1.96	13.5	
	<i>QDan-sbi02-1</i>	II	SBI-02	<u>Xtxp298-Dsenhsbm25</u>	0	2.71	296B	1.21	6.5	
	<i>QDan-sbi02-2</i>	II	SBI-02	<u>Ungnhsbm49-Dsenhsbm15</u>	0	3.13	296B	1.41	8.5	
	<i>QDan-sbi03</i>	AV, I, II	SBI-03	<u>Dsenhsbm87-Ungnhsbm11</u>	1	3.40	296B	1.61	9.8	
	<i>QDan-sbi05</i>	II	SBI-05	<u>Xtxp23-Kaf3</u>	6	2.53	296B	1.41	7.7	
	<i>QDan-sbi06</i>	III	SBI-06	<u>GlumeT-Mrco</u>	0	2.70	IS18551	-1.98	8.1	
	<i>QDan-sbi07</i>	AV, II	SBI-07	<u>Xtxp312-BMR</u>	0	2.68	IS18551	-1.32	7.3	
Days to maturity (DMA)	<i>QDma-sbi01-1</i>	AV, II, III	SBI-01	<u>Dsenhsbm13-Dsenhsbm19</u>	0	5.40	296B	1.18	13.9	
	<i>QDma-sbi01-2</i>	AV, II	SBI-01	<u>Xtxp58-Dsenhsbm63</u>	0	5.96	296B	1.24	15.3	
	<i>QDma-sbi01-3</i>	AV, I, II, III	SBI-01	<u>Xtxp32-Dsenhsbm66</u>	1	4.26	296B	1.11	11.3	
	<i>QDma-sbi02-1</i>	II	SBI-02	<u>Xtxp298-Dsenhsbm25</u>	0	3.69	296B	0.97	9.7	
	<i>QDma-sbi02-2</i>	II	SBI-02	<u>Ungnhsbm49-Dsenhsbm15</u>	0	2.44	296B	0.82	6.6	
	<i>QDma-sbi03</i>	III	SBI-03	<u>Dsenhsbm87-Ungnhsbm11</u>	0	2.57	296B	1.36	6.5	
	<i>QDma-sbi05</i>	AV, II	SBI-05	<u>Xtxp23-Kaf3</u>	7	2.58	296B	0.94	7.8	
	<i>QDma-sbi07</i>	I	SBI-07	<u>Xtxp312-BMR</u>	0	2.70	IS18551	-1.12	5.9	
Total no. of leaves (TNL)	<i>QTnl-sbi01-1</i>	AV, I, II, III	SBI-01	<u>Xtxp32-Dsenhsbm66</u>	3	2.55	296B	0.21	5.3	
	<i>QTnl-sbi01-2</i>	AV, II, III	SBI-01	<u>Xtxp43-Xtxp329</u>	0	3.74	296B	0.31	9.9	
	<i>QTnl-sbi03</i>	AV, I, II, III	SBI-03	<u>Dsenhsbm87-Ungnhsbm11</u>	3	2.59	296B	0.27	7.8	
	<i>QTnl-sbi07</i>	AV, I, II, III	SBI-07	<u>Xtxp312-BMR</u>	0	5.54	IS18551	-0.34	14.1	
	Green leaf area at anthesis (GLAA)	<i>QGlaa-sbi01</i>	AV, I, II, III	SBI-01	<u>Dsenhsbm50-Xtxp335</u>	1	3.97	296B	82.07	11.8
		<i>QGlaa-sbi03</i>	AV, II, III	SBI-03	<u>Xtxp59-Stgnhsbm21</u>	1	2.65	296B	60.29	6.1
		<i>QGlaa-sbi04</i>	AV, II, III	SBI-04	<u>Dsenhsbm95-Xtxp328</u>	0	2.52	296B	63.18	6.9
		<i>QGlaa-sbi05</i>	AV, I, II, III	SBI-05	<u>Xtxp23-Kaf3</u>	6	3.42	296B	83.65	10.2
Green leaf area at maturity (GLAM)	<i>QGlam-sbi01-1</i>	AV, I, II, III	SBI-01	<u>Dsenhsbm13-Dsenhsbm19</u>	0	2.69	296B	67.40	7.4	
	<i>QGlam-sbi01-2</i>	AV, I, II, III	SBI-01	<u>Dsenhsbm50-Xtxp335</u>	1	3.31	296B	76.74	8.7	
	<i>QGlam-sbi06</i>	AV, II, III	SBI-06	<u>GlumeT-Mrco</u>	1	2.85	IS18551	-68.35	6.6	
% Green leaf area preserved at maturity (PGLAM)	<i>QPglam-sbi03</i>	AV, I, II, III	SBI-03	<u>Xtxp59-Stgnhsbm21</u>	1	2.60	296B	3.54	5.2	
	<i>QPglam-sbi09</i>	II	SBI-09	<u>Dsenhsbm99-Xtxp258</u>	3	2.90	296B	4.15	7.5	
Grain yield (GYL)	<i>QGyl-sbi06</i>	AV, I, II, III	SBI-06	<u>GlumeT-Xtxp145</u>	1	5.10	296B	2.85	14.6	
Panicle length (PLE)	<i>QPle-sbi02</i>	AV, II	SBI-02	<u>Ungnhsbm49-Xtxp286</u>	3	4.87	296B	1.35	14.7	
	<i>QPle-sbi06-1</i>	AV, I, II, III	SBI-06	<u>Dsenhsbm2-Xtxp6</u>	0	2.54	IS18551	-0.90	6.5	
	<i>QPle-sbi06-2</i>	AV, I, II, III	SBI-06	<u>GlumeT-Xtxp145</u>	3	20.28	IS18551	-3.28	50.4	
	<i>QPle-sbi07</i>	AV, I, II, III	SBI-07	<u>Xtxp92-Xtxp295</u>	1	2.97	296B	1.33	9.0	
Panicle weight (PWE)	<i>QPwe-sbi06</i>	AV, I, II, III	SBI-06	<u>GlumeT-Xtxp145</u>	1	4.90	296B	3.65	14.7	
No. of primary branches (NPB)	<i>QNpb-sbi01-1</i>	AV, I, II, III	SBI-01	<u>Xtxp32-Dsenhsbm66</u>	2	3.03	296B	5.67	9.8	
	<i>QNpb-sbi01-2</i>	AV, I	SBI-01	<u>Xtxp43-Xtxp329</u>	1	2.70	296B	4.36	6.1	
	<i>QNpb-sbi05</i>	AV, I, II, III	SBI-05	<u>Xtxp23-Kaf3</u>	5	2.75	296B	4.99	8.1	
	<i>QNpb-sbi07</i>	AV, I, II, III	SBI-07	<u>Xtxp40-Xtxp159</u>	3	2.64	IS18551	-4.47	6.7	
	<i>QNpb-sbi08</i>	AV, I, II, III	SBI-08	<u>Xtxp292-Xtxp354</u>	4	2.66	296B	4.72	7.7	

**Table 5** continued

Trait	QTL name	Environment	Chromosome	Flanking markers	QTL position	LOD	Increased effect	Additive effect	$R^2$ (%)
Seed weight (SWE)	<i>QSwe-sbi01</i>	AV, I, II, III	SBI-01	Dsenhsbm64- <u><i>Xcup24</i></u>	0	5.45	296B	0.12	14.8
	<i>QSwe-sbi04</i>	AV, I, II, III	SBI-04	<u><i>Xtxp12</i></u> -Dsenhsbm39	0	3.50	296B	0.10	9.5
	<i>QSwe-sbi06</i>	AV, I, II	SBI-06	<u>GlumeT</u> - <i>Xtxp145</i>	2	2.80	296B	0.09	7.0

Underlined markers are QTLs flanking markers which are closer to the QTL position, QTL position 0 indicate QTL position is exactly the same as the corresponding flanking marker. Environments I, II, III, and AV (average) indicate QTLs detected in the seasons 2003, 2004, 2006, and across all seasons, respectively

$R^2$  (%) is % phenotypic variation explained by individual QTL, QTL name indicates Q for QTL followed by trait name to which the QTL was detected and by the chromosome number on which it was detected. If more than one QTL were detected on the same chromosome for a trait, QTLs identified were serially numbered

#### QTLs for % green leaf area preserved at maturity (PGLAM)

Two QTLs were detected on SBI-03 and SBI-09 for PGLAM. At both the QTL positions, positive alleles were derived from 296B. The phenotypic variation explained by QTLs *QPglam-sbi03* and *QPglam-sbi09* was 5.2 and 7.5%, respectively. Only the QTL *QPglam-sbi03* (corresponding to the *Stg2* QTL) was identified consistently across the environments whereas the QTL *QPglam-sbi09* was detected in single environment (II-ICRISAT). Although identified in single environment, the QTL was located in the same genomic region as the staygreen QTL regions *Stg1.1* and *QLsn.txs-F* reported by Crasta et al. (1999), and Feltus et al. (2006), respectively.

#### QTLs for grain yield (GYL)

In three individual environments and combined analysis, only one QTL, *QGyl-sbi06*, was detected consistently on SBI-06 associated with GYL. This QTL was derived from the high yielding parent 296B, which increased the grain yield around 14.6% of phenotypic variation at LOD 5.1.

#### QTLs for panicle length (PLE)

Four QTLs were detected which significantly affected PLE in the population with two QTLs on SBI-06, one each on SBI-02 and SBI-07. Interestingly, the major QTL *QPle-sbi06-2* was detected on SBI-06 from IS18551 and explained alone 50.4% of the phenotypic variance at LOD 20.1 despite possessing small panicle length. At the QTL positions on SBI-02 and SBI-07, the positive alleles for the trait were contributed by 296B. The other major QTL, *QPle-sbi02* identified on SBI-02 for PLE explained a phenotypic variation of 14.7%. All QTLs were consistently detected in individual analysis as well as combined analysis.

#### QTLs for panicle weight (PWE)

A single significant QTL was detected for PWE in all environments as well as in combined analysis on SBI-06, exactly at the same genomic region to that of yield QTL. At the QTL region, *QPwe-sbi06*, 296B was associated with increasing PWE with 14.7% of phenotypic variation.

#### QTLs for number of primary branches (NPB)

Five QTLs were found for NPB on chromosomes consisting of two on SBI-01, and one each on SBI-05, SBI-07, and SBI-08. These QTLs explained 6.1–9.8% of phenotypic variance. At the QTL regions on SBI-01, SBI-05, and SBI-08, allele from 296B was associated with increasing NPB while the QTL *QNpb-sbi07* on SBI-07 was associated with decreasing the trait. All QTLs were consistently detected in individual environments and in combined analysis except the QTL *QNpb-sbi01-2* on SBI-01 was detected in combined analysis and in 2003 (I-NRCS).

#### QTLs for seed weight (SWE)

In the population, SWE was influenced by three QTLs residing on SBI-01, SBI-04 and SBI-06, respectively. The phenotypic variation explained by each QTL ranged from 7 to 14.8%. A major QTL was detected on SBI-01 which explained phenotypic variation of 14.8% with LOD value 5.1. At all the loci influencing the SWE, the alleles were from bold seed parent 296B that was associated with increasing the trait. All QTLs for SWE were consistently detected in individual environments and in combined analysis.

#### Association of genic-microsatellites and morphological markers with QTLs of agronomic traits

Out of the 49 mapped genic-markers, 18 were closely associated with 28 QTLs of ten agronomic traits. These QTLs

linked markers were mapped on seven chromosomes except on SBI-05, SBI-08, and SBI-10. Exact correspondence of gene positions with QTL likelihood positions of agronomic traits were observed in five instances. The drought EST marker *Dsenhsbm19* coding for ETHYLENE-INSENSITIVE3-1 (EIL-1), a key transcription regulator of ethylene biosynthesis, was mapped exactly at the same locus on SBI-01 where QTL influencing the plant early development traits (DAN, 6.3% PV; DMA, 13.9% PV) and GLAM (7.4%, PV) were mapped. Similarly, drought EST marker *Dsenhsbm15* (coding for a permease protein) and unigene marker *Ungnhsbm11* (hypothetical protein) mapped on SBI-02 and SBI-03, respectively, corresponded exactly with their positions with the QTL influencing plant early development. However, the EST markers explained phenotypic variance of less than 10% for the traits. The drought EST marker *Dsenhsbm95*, coding an unknown protein, was exactly located in the region where a QTL was detected for GLAA on SBI-04 explaining a phenotypic variance up to 6.9%. The BMR gene was located on SBI-07 and was found to be associated with the traits TNL, DAN and DMA, explaining phenotypic variance of 14.1, 7.3 and 5.9%, respectively. The staygreen marker *Stgnhsbm21* mapped at the *Stg2* QTL region in our previous study (Srinivas et al. 2008) was closely associated (about 1 cM distant) with the QTLs *QGlaa-sbi03* and *QPglam-sbi03* detected for the staygreen components GLAA and PGLAM, respectively, on SBI-03 in the study. Among the three genes mapped as morphological markers, *GlumeT* and *Mrco* genes were closely associated with the major genomic region identified on SBI-06 influencing seven agronomic traits (PHE, PLE, DAN, GLAM, GYL, PWE, and SWE). The plant color gene, *Plcor*, was closely identified as a QTL flanking marker for PHE on the same linkage group, which explained 8.2% of phenotypic variance.

## Discussion

In the present study, we detected 49 QTLs for 12 agronomic traits using the RIL population of the cross 296B × IS18551. The map constructed majorly based on SSR markers in the study was aligned with the high density reference maps of Menz et al. (2002) and Bhatramakki et al. (2000) containing both RFLP and common genomic-microsatellites mapped in the study in order to compare the positions of QTLs detected in the study with that reported earlier for the corresponding agronomic traits in sorghum.

### QTLs for growth characters and plant early development

In sorghum, four genes regulating plant height (*Dw1-Dw4*) have been identified (Quinby and Karper 1954). Pereira and

Lee (1995) mapped four QTLs for plant height and based on their co-localization, pleiotropy with other QTLs for morphology and yield components, they related their QTLs with dwarfing (*Dw*) genes. They inferred *Dw2*, *Dw3*, and *Dw4* genes with QTLs on their linkage groups LG H, LG A and LG E, respectively. A major QTL, *QPhe-sbi07-2*, explaining 24.2% of phenotypic variation identified in this study on SBI-07 corresponds to the genomic region where QTL conditioning *Dw3* gene was mapped on the linkage group by Brown et al. (2006). This QTL corresponding to the *Dw3* gene was also reported for plant height earlier at the similar positions on their corresponding LGs, LG A by Rami et al. (1998) and on LG E by Klein et al. (2001) and Hart et al. (2001), respectively. The QTL, *QPhe-sbi06-1*, explaining 21.6% of phenotypic variance identified on SBI-06 in the present population corresponds to the position of QTLs reported for plant height on LG 6 by Brown et al. (2006), and LG I by Hart et al. (2001). This QTL corresponds to the *Dw2* gene conditioning plant height QTL on LG D by Lin et al. (1995). The detection of all the major plant height QTL on SBI-06 and SBI-07 observed in this study also support the results by Hart et al. (2001) and Feltus et al. (2006) suggesting that these regions play an important role in the control of plant height variation in sorghum.

Number of leaves produced per plant is an important component of forage yield. No study in sorghum was earlier attempted for mapping this trait. The four QTLs identified for this trait in the population were consistent across all seasons hence they can be considered as ideal targets for MAS of the forage yield in sorghum.

Nine QTLs each were identified for DAN and DMA. The QTL, *QDan-sbi06*, on SBI-06 and the QTL, *QDma-sbi01-2*, on SBI-01 were mapped specifically for DAN and DMA, respectively. The eight QTLs positions that are common to DAN and DMA matched exactly with their highest LOD values, suggesting the same genes may be involved in regulating these related traits. Although these two traits are highly correlated and measures the earliness, QTL *QDan-sbi06* (*Ma1* gene described later) identified specifically for DAN probably involved in plant development during the vegetative growth phase, whereas the QTL *QDma-sbi01-2* (*Ma3* gene described later) identified specifically for DMA may be involved in plant development during the grain-filling stage.

A series of six maturity genes have been recognized in sorghum to alter flowering time: *Ma1-Ma6*, (Quinby 1967). Of these genes only *Ma1*, and *Ma3*, were mapped in sorghum on LG D (SBI-06) by Lin et al. (1995) and on LG A (SBI-01) by Childs et al. (1997), respectively. In this study, the position of the QTL *QDma-sbi01-2*, identified on SBI-01 for DMA near *Xtxp58* marker coincided with *Ma3* gene coding for phytochrome B (PHYB) (Childs et al. 1997).

Similarly, the QTL, *QDan-sbi06*, on SBI-06 identified for DAN matches its position with *Mal* gene (Quiby and Karper 1954) as well as the maturity QTL on LG D by Lin et al. (1995) and the maturity QTL on chromosome 6 by Feltus et al. (2006). The two QTLs on SBI-02 near *Xtxp298* and *Dsenhsbm15* share similar positions with the maturity QTL reported on LG B by Lin et al. (1995) and on LG G by Kebede et al. (2001), respectively. Other QTLs does not correspond to any of the QTLs reported earlier for flowering or maturity in sorghum. Therefore, they may be considered as new loci regulating plant development and they probably correspond to the maturity genes that were not mapped in sorghum.

#### QTLs for staygreen trait

For GLAA, which measures photosynthetic capacity at anthesis stage, four QTLs were detected. Among these, the QTL on SBI-01 (near the EST *Dsenhsbm50*), SBI-03, and SBI-05, corresponds to the major staygreen QTL identified from staygreen donor B35 viz., *StgG*, *Stg2*, and *Stg4*, respectively, (Crasta et al. 1999; Xu et al. 2000, Subudhi et al. 2000). A new QTL, *QGlaa-sbi04*, on SBI-04 was identified in the study. Out of three QTLs identified for GLAM, only one QTL on SBI-01 was common with the QTL identified for GLAA, indicating the involvement of different genes responsible for the trait at these stages. Similarly, the two QTLs were mapped for PGLAM in the study at similar positions as the staygreen QTL *Stg2* by Xu et al. (2000); and the staygreen QTL *Stg1.1* and *QLsn.txs-F* reported by Crasta et al. (1999), and Feltus et al. (2006), respectively. These results demonstrate that the staygreen QTLs *StgG*, *Stg2*, *Stg4*, *Stg1.1* and *QLsn.txs-F* are common in this and earlier studies on the staygreen trait in sorghum. The QTL results for the staygreen components (GLAA, GLAM and PGLAM) suggests that though they are inherited independently, there exist some common genetic mechanism regulating these traits (such as at the *Stg2* QTL). An independent inheritance of the staygreen trait components GLAA and GLAM had also been suggested by Van Oosterom et al. (1996) and Haussmann et al. (2002). The results also suggest that probability of presence of different alleles of the genes regulating the trait at the identified QTL regions in the 296B since they explained a minor portion of phenotypic variance. This is in contrast to the QTL alleles identified using high staygreen sources such as B35 which explained a major phenotypic variance (Crasta et al. 1999; Xu et al. 2000; Subudhi et al. 2000). Transferring these QTL alleles from the high staygreen donor to high yielding genetic backgrounds through MAS may improve the level of trait expression thereby increasing their agronomic performance under terminal drought stress conditions.

#### QTLs for yield and its component traits

A single QTL was identified for both yield and panicle weight on the chromosome SBI-06 and they explained about 14% of phenotypic variation. Due to the population size, only the QTL having large phenotypic effect might have been detected in the present study. Most other QTLs affecting grain yield probably had smaller effects and was undetected at the significant QTL identification threshold. The QTL detected for the trait was not reported earlier in sorghum indicating that presence of additional genetic loci regulating the trait in germplasm pool which has not been explored previously.

A significant QTL, *QPle-sbi06-2*, for panicle length explaining 50.4% of phenotypic variation was mapped on SBI-06 from IS18551 (tall parent with short panicle) exactly at the region of plant height QTL, *QPhe-sbi06-1*, which could be conditioned by *Dw2* gene identified in this study. At the corresponding location on the linkage group LG B, a QTL for panicle length was also identified by Rami et al. (1998) which explained only 10–15% of phenotypic variation. Further, the QTLs on SBI-02 and SBI-07 for the trait corresponds to the QTLs on LG F and LG A by Rami et al. (1998), respectively. Similarly, the QTL on SBI-07 also shares similar position with the QTL on LG E by Hart et al. (2001). The QTL, *QPle-sbi06-1* detected on SBI-06 in this study is not reported earlier for the trait. The *QPle-sbi07* QTL mapped for PLE on SBI-07 is near to the genomic region of *Dw3* gene. However, this QTL may not be conditioned by the *Dw3* gene since the genomic region from IS18551 is positively associated with plant height while it negatively associated with panicle length and their QTL positions also did not match. Similar phenomenon was also observed at this genomic region for QTL between panicle length and plant height by Hart et al. (2001) and Brown et al. (2006).

Sorghum has been less studied for inflorescence architecture than other members of the Poaceae family. Brown et al. (2006) have mapped two QTLs for panicle primary branch number and a single QTL for secondary branching number while characterizing the inflorescence architecture in sorghum. Five QTLs detected for NPB in the study did not relate to any of the QTL for panicle branching pattern identified by Brown et al. (2006). Therefore, it is likely that these QTLs may be new loci regulating the primary branching pattern of the panicle in sorghum resulting in their involvement in inflorescence architecture.

A major QTL explaining 14.8% of phenotypic variation for seed weight was detected near the genomic-microsatellite marker *Xcup24* on SBI-01. It was earlier detected in sorghum at similar position near the RFLP marker *umc84* by Tuinstra et al. (1998). Significantly, this QTL also corresponds to a seed weight QTL mapped in homologous

regions on rice chromosome 3 and on maize chromosome 1 (Li et al. 2004b). Li et al. (2004b) suggested that this QTL may be associated with the domestication in cereals. The seed weight QTL identified at homologous region in this study also supports this theory. Two new QTLs were also identified for this trait on chromosomes SBI-04 and SBI-06, respectively.

#### Mapping of genes regulating the morphological traits

In this study, the genes *GlumeT*, *Mrco*, and *Pcor* regulating three morphological traits glume type (open vs. closed), midrib color (juicy vs. dry) and plant color (tan vs. purple), respectively, were mapped on SBI-06. Based on the map position, the *GlumeT* gene probably is same to the QTL reported for the glume cover on SBI-06 by Feltus et al. (2006). Similarly, *Mrco* gene controlling the midrib color shared the similar location as the gene “D” for the trait by Xu et al. (2000). Likewise, the *Pcor* gene regulating plant color also matched its position with plant color gene reported by Klein et al. (2001).

#### Clustering of QTLs affecting the agronomic traits

It is more likely that clustering of QTLs for different characters may result from tight linkage of several genes controlling the traits since genes are often located in the gene-rich regions with hot spots of recombination (Gill et al. 1996; Faris et al. 2000; Sandhu et al. 2001), otherwise their inheritance is functionally linked through common mechanistic basis, in other words, a single gene affect multiple characters (pleiotropy) (Veldboom et al. 1994; Xiao et al. 1996).

The same phenomenon of clustering of QTLs affecting different traits was observed in the present study. A major QTL cluster of about 7-cM region was located on the chromosome SBI-06, near the *Glume* type gene (*GlumeT*), is significantly associated with seven of the 12 traits measured. At this major genomic region, alleles from IS18551 increased PHE, DAN, GLAM, PLE, but caused negative effects for GYL and its related traits such as PWE, and SWE. This genomic region probably conditioned by *Dw2* gene for plant height in this study since at this genomic region a plant height QTL was mapped and inferred it as *Dw2* gene by Lin et al. (1995). Graham and Lessman (1966) have studied the effect of plant height on yield and yield components in two isogenic lines differing at *Dw2* loci and reported that the *Dw2* gene may have pleiotropic effects on panicle length, yield, seed weight and leaf area in classical genetics. The result observed in the study is also in agreement with their result of involvement of the genomic region in affecting the traits. However, the mapping resolution achieved in this study is not sufficient to determine whether pleiotropy or tight linkage is the genetic cause of

the association of the multiple QTLs at this region. Similarly, in another classical study, Casady (1965) reported that the *Dw3* gene had a pleiotropic effect on the number of kernels per panicle, kernel weight, as well as on tiller number and panicle size. Rami et al. (1998) have also found major QTLs for yield components and morphological traits such as germination rate, number of kernel per panicle, kernel weight per panicle (grain yield), thousand-kernel weight (seed weight), panicle compactness, plant height and panicle length at the *Dw3* chromosomal region on their linkage group A (SBI-07). However, in the present study, at the corresponding region on SBI-07, QTLs were identified only for plant height and panicle length.

To maximize crop performance under drought stress, it is important to establish the genetic relationship between staygreen and plant development (maturity) and to identify QTLs influencing the staygreen but not plant development. With this concept, Crasta et al. (1999) reported seven staygreen QTLs and two QTLs for maturity. They further reported that the QTL on LG G influenced both the traits in the population B35 × Tx430, while the remaining six staygreen QTLs are beneficial for the staygreen trait in sorghum for drought tolerance. In the present study, out of the nine QTLs identified for DAN and DMA, three were matching with the three staygreen QTLs identified in the study. Among these, one region each was identified on SBI-01 (at the EST *Dsenhsbm19*), and SBI-05 (*Stg4* QTL). The third QTL was identified on SBI-06 where the *Dw2* gene is located. Hence, the QTLs exclusively located for the staygreen trait on SBI-03 (*Stg2* QTL), SBI-04, and SBI-09 (*Stg1.1* and *QLsn.txs-F* QTL) could be employed in MAS for improving the sorghum performance under terminal drought stress.

Tight linkage of genes or pleiotropy of a single gene could be the possible explanation for the clustering of QTLs for the agronomic traits in the current study. However, in order to rule out either one of these possibilities and to elucidate the genetic basis of the association between these traits, more detailed studies such as increasing the population size and saturating the target genomic regions by adding more molecular markers with specifically constructed near-isogenic lines (NILs) for the QTLs would be needed and this may lead to map-based cloning of the gene(s) underlying the QTLs.

#### Consistency of QTLs among the environments

Of the 49 QTLs identified for the traits, 33 QTLs (67.3%) were significantly identified in combined analysis and at more than one location while 6 QTLs (12.2%) were detected in combined analysis and in a single environments. These results indicate that a large portion of the QTLs detected for the traits are stable across environments.



However, DAN and DMA gave inconsistent QTLs among locations. Five of the nine QTLs identified each for DAN and DMA were identified in single environment especially at the location ICRISAT (II-2004). These QTLs may be environmentally dependent since plant development is highly influenced by photoperiod and temperature. Nevertheless, some of the inconsistent QTLs identified for DAN were common with QTLs for DMA and were detected in more than one environment, suggesting that these QTLs can also be considered consistent since these traits were highly related (Fig. 1).

#### Association of ESTs with QTLs of agronomic traits

The association of genic-markers with quantitative traits may increase our knowledge of genes influencing the agronomic traits (Matthews et al. 2001; Zhang et al. 2004). Since ESTs represent a portion of the gene, the full length gene can be cloned with the availability of complete genome sequence of sorghum and analyzed for gene expression in order to associate to a particular trait. In all, in the study, 18 EST markers were found either as QTL locus or closely linked with the QTLs of 10 agronomic traits out of 12 traits studied, thus providing a simple PCR based microsatellite markers for MAS of these traits (Table 5; Fig. 1). The drought EST microsatellite Dsenhsbm19 coding for ETHYLENE-INSENSITIVE3-1 (EIL-1), a key transcription regulator of ethylene biosynthesis (Chao et al. 1997; Solano et al. 1998), was mapped exactly at the same locus where the QTL *QGlam-sbi01-1* regulating the green leaf area at maturity (GLAM), the QTL *QDan-sbi01-1* for days to anthesis (DAN), and for the QTL *QDma-sbi01-1* for days to maturity (DMA) were mapped (Fig. 1). This gene accounted for DMA, GLAM and DAN with a phenotypic variation of 13.9, 7.4 and 6.3%, respectively. It is well known that ethylene is involved in regulation of flowering (Trusov and Botella 2006; Achard et al. 2007) and leaf senescence (Gepstein and Thimann 1981; Jing et al. 2005; Yang et al. 2008). Hence, the Dsenhsbm19 marker may be the candidate gene for the traits which probably are influenced through the regulating of ethylene synthesis pathway under stress condition. Further detailed genetic analysis of Dsenhsbm19 should reveal its molecular mechanisms of these important agronomic traits in sorghum.

The staygreen marker Stgnhsbm21 mapped at the *Stg2* QTL region on SBI-03 in our previous study (Srinivas et al. 2008) was closely associated (about 1-cM distant) with the staygreen QTLs *QGlaa-sbi03* and *QPglam-sbi03* on SBI-03 identified in the present study. Since this EST was known to be up-regulated during senescence retardation by benzyl adenine in *Arabidopsis* (Guterman et al. 2003) and its close linkage with staygreen QTL, this marker can be considered as the candidate gene for the staygreen trait.

However, this assumption has to be confirmed through detailed association studies. Caffeic acid *O*-methyltransferase, a key enzyme involved in lignin biosynthesis in sorghum (Bout and Vermerris 2003) is known to be involved in improving forage quality by down-regulation of the gene (Guo et al. 2001). A microsatellite (designated BMR) from this gene coding for the enzyme was mapped on SBI-07 in the study, which was associated with the same locus influencing the QTLs of traits total number of leaves (TNL), days to anthesis (DAN) and days to maturity (DMA). However, involvement of the gene in the regulation of the traits has to be confirmed by further studies in order to understand the effect of the gene on the traits. Other EST markers that could be considered as candidate genes included the drought EST marker Dsenhsbm15 (coding for a permease protein), Ungnhsbm11 marker (hypothetical protein) and the drought EST marker Dsenhsbm95 (unknown protein) for DAN/DMA and GLAM, respectively.

#### Conclusions

Majority of the QTLs influencing the traits in the study were consistent among the environments studied indicating that they are highly reliable. Furthermore, stable QTLs were identified for the traits across diverse genetical and environmental backgrounds by comparing the QTLs of the study with previous QTL studies in sorghum. Therefore, the genic-microsatellite markers coupled with genomic-SSRs linked to the agronomically important QTLs can be used for improving the sorghum performance through marker-assisted selection (MAS) strategies under terminal drought stress conditions. Importantly, the EST markers found associating with some major QTLs detected in the present study will create a base for studying the molecular mechanisms of these genes for determining the traits in future. The new set of genic-microsatellite markers reported in this study will have applications in QTL mapping studies, diversity studies, and association mapping studies in sorghum, and also for comparative genomic studies between sorghum and other members of the Poaceae family.

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