Theor Appl Genet (2008) 117:283–296
DOI 10.1007/s00122-008-0773-8

ORIGINAL PAPER

Development of genic-microsatellite markers for sorghum staygreen QTL using a comparative genomic approach with rice

G. Srinivas · K. Satish · S. Murali Mohan · R. Nagaraja Reddy · R. Madhusudhana · D. Balakrishna · B. Venkatesh Bhat · C. J. Howarth · N. Seetharama

Received: 24 August 2007 / Accepted: 8 April 2008 / Published online: 26 April 2008

Abstract The already available comprehensive genome sequence information of model crops along with the transcriptomic resource from other crops provides an excellent opportunity for comparative genome analysis. We studied the synteny between each of the four major sorghum staygreen quantitative trait loci (QTL) regions with that in the rice genome and attempted to increase marker density around the QTL with genic-microsatellites from the sorghum transcriptomic resource using the rice genome as template. For each of the sorghum QTL regions, the reported RFLP markers were compiled, used for sequence similarity searches against the rice genome which identified syntenous regions on rice chromosome 1 for *Stg1* and *Stg2* QTL, on chromosome 9 for *Stg3* QTL, and on chromosome 11 for *Stg4* QTL. Using the Gramene genome browsing tool, 869 non-redundant sorghum expressed sequence tags (ESTs) were selected and 50 genic-microsatellites (18, 12, 15, and 5, for *Stg1*, *Stg2*, *Stg3*, and *Stg4* QTL, respectively) could be developed. We could experimentally establish synteny of the *Stg1*, *Stg2*, *Stg3*, and *Stg4* QTL regions with that of the rice genome by mapping ten polymorphic genic-microsatellite markers (20%) to the positions of the staygreen QTL. The simple strategy demonstrated in the present study could readily be extrapolated to other cereals of the Poaceae family. The markers developed in this study provide a basis for the isolation of genes underling these QTL using an association study or map-based gene isolation approach, and create an additional option for MAS of the staygreen trait in sorghum.

Communicated by T. Sasaki.

G. Srinivas · K. Satish · S. Murali Mohan · R. Nagaraja Reddy · R. Madhusudhana (✉) · D. Balakrishna · B. Venkatesh Bhat · N. Seetharama
National Research Center for Sorghum,
Rajendranagar, Hyderabad 500030, India
e-mail: madhusudhana@nrcsorghum.res.in

C. J. Howarth
Institute of Grassland and Environmental Research,
Aberystwyth SY23 3EB, UK

Introduction

Different kinds of anonymous DNA-based markers have been developed and used in higher plants during the last two decades for a variety of purposes (Phillips and Vasil 2001). However, 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 agronomically important traits. The construction of functional maps consisting of genes of known function may allow verification if any of the mapped genes is a good candidate for any of the mapped quantitative trait loci (QTL) (Aubert et al. 2006).

The complete genome sequence information from model crops such as rice and *Arabidopsis*, coupled with the transcriptomic resources available from other crops provides an excellent opportunity for development of gene based markers for targeted regions through comparative genome analysis. The rice genome exhibits substantial colinearity with the genomes of other grasses, such as sorghum, maize, wheat, and barley (Ahn et al. 1993; VanDeynze et al. 1995; Chen et al. 1997; Gale and Devos 1998; Tarchini et al. 2000). In spite of the enormous differences in genome size, comparative genetic mapping using common DNA markers has revealed that the relative location of markers and mapped genes show remarkable conservation among the

cereals, although numerous segmental duplications and deletions have been observed (Helentjaris et al. 1988; Hulbert et al. 1990; Binelli et al. 1992; Chen et al. 1997; Ventelon et al. 2001). Thus, comparative mapping based on the regions of microsynteny between two organisms provides a powerful technique for enriching molecular markers in the target regions of interest. This synteny-based marker saturation approach of target regions using sequence information from model crops such as rice and *Arabidopsis* and the expressed sequence tag (EST) information available in many crops has been demonstrated previously (Liu and Anderson 2003; Perovic et al. 2004; Mammadov et al. 2005; Hwang et al. 2006).

The staygreen trait in sorghum, which is an important component of the post-flowering drought resistance mechanism (Rosenow and Clark 1981), is characterized by the plant's ability to maintain greater functional photosynthetic leaf area during the grain filling stage even under severe post-flowering drought stress. Sorghum genotypes with this trait continue to fill their grain normally under drought (Rosenow and Clark 1981) and exhibit increased resistance to charcoal rot (Rosenow 1984) and lodging (Henzell et al. 1984). This trait is also reported to be associated with resistance to diseases (Borrell and Hammer 2000), increased cytokinin concentration (McBee 1984) and stem sugars in basal nodes (Duncan 1984) in staygreen genotypes. Since the correlation of the trait with drought tolerance and stover quality were established, the genetic and physiological basis of the trait has been studied by many authors using different staygreen sources in sorghum (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; Harris et al. 2007) and in other crops such as rice (Cha et al. 2002; Jiang et al. 2004; Abdelkhalik et al. 2005), soybean (Guiamet et al. 1990; Luquez and Guiamet 2002) and *Festuca pratensis* (Moore et al. 2005).

In the present study, we adopted a comparative genomics approach for the development of genic-microsatellites in sorghum at the staygreen QTL regions, by integrating the rice genome sequence information and the available sorghum transcriptomic resource. QTL studies in sorghum have resulted in the identification of several genomic regions associated with the staygreen trait. Comparison of all the staygreen QTL from Xu et al. (2000) and Subudhi et al. (2000) along with results obtained by other workers (Tuinstra et al. 1997; Tao et al. 2000) using B35 inbred line as a staygreen source showed that four major QTL named *Stg1* (SBI-03), *Stg2* (SBI-03), *Stg3* (SBI-02), and *Stg4* (SBI-05) are consistent in different genetic and environment backgrounds and accounted for up to 53.5% phenotype variance (Subudhi et al. 2000). The *Stg1*, *Stg2*, and *Stg3* QTL were significantly correlated with the chlorophyll content at physiological maturity and were observed to overlap with the QTL for chlorophyll content (Subudhi et al. 2000). Significantly, the recent study by Harris et al. (2007), which reported on near-isogenic lines (NILs) developed for the same four staygreen QTL (*Stg1-Stg4*) using BTx642 (formerly B35) as the staygreen donor, has shown that these staygreen QTL individually reduce the post-flowering drought-induced leaf senescence in the recipient senescent genetic background of RTx7000.

These four staygreen QTL, which are expressed in different genetic studies, are all flanked primarily by RFLP markers. As RFLP markers are cumbersome, it is pertinent to explore the availability of microsatellite markers owing to their ease for deployment in marker assisted selection (MAS). A limited number of PCR-based microsatellite markers are already available at these QTL regions, following the compilation of the genomic SSRs from other mapping studies reported in sorghum. Therefore, with a long term goal of isolation of genes underlying these QTL through map-based cloning, candidate gene or an association study approach (Prioul et al. 1999; Thornsberry et al. 2001), our aim was to increase the marker density around the staygreen QTL using sorghum ESTs. This would enable high resolution mapping of the QTL for map-based gene cloning, and the development of functional markers (FM), besides providing additional microsatellite markers for these QTL for MAS of the staygreen trait in sorghum.

Materials and methods

Identification of sorghum-rice staygreen syntenous regions through in silico mapping

For each QTL, the reported flanking RFLP markers were listed, other RFLP markers located in and around the QTL were collected, the corresponding DNA sequences were obtained from the NCBI and other databases (www.cytomaize.org) and mapped on to the rice genome by "virtual Southern blot" based on similarity searches (Salse et al. 2004) using BLASTn tools with their standard servers' settings (http://www.gramene.org/Multi/blastview; http://www.ncbi.nlm.nih.gov/blast/).

An E value of $\leq 10^{-10}$ was adopted to claim a significant match between RFLP sequence and the rice genome. In cases, hits with $10^{-10} < E$ value $< 10^{-4}$ were also considered to be syntenous with the rice genome when consistent with the QTL synteny results. A marker giving a single BLAST hit was given priority for synteny identification and assigned to a chromosomal position on the rice genome. When multiple BLAST hits were noted, the marker hit showing the best colinearity with the staygreen QTL region was given priority. Rice chromosomes hosting at least two marker positions consistent with those of the sorghum map

were considered syntenous. Finally, syntenous regions were defined based on the number and relative order of markers supporting colinearity with the staygreen QTL regions.

Development of EST-SSR markers

The Gramene genome browsing tool (http://www.gramene.org/genome_browser/index.html) is a comparative genomics tool which allows users to move along the rice genome back and forth using the navigation buttons provided in the tool. It aligns ESTs and clusters derived from ESTs from rice and other cereals (sorghum, maize, barley, sugarcane, wheat, and millets) on to the rice genome and also integrate additional information relating to the genome. Using the Gramene genome browsing tool, we surveyed the sorghum EST clusters aligned between the in silico defined flanking markers for each of the QTL region (described later) by navigating the rice genome. A single EST sequence representing each of the sorghum EST cluster were manually selected for marker development to avoid redundancy, and its sequence was downloaded from the NCBI database. The simple sequence repeat identification tool (SSRIT) (http://www.gramene.org/db/searches/ssrtool) was applied to identify simple sequence repeats (SSRs) from sorghum ESTs, with parameters set to identify repeats up to decamers, with a minimum of 5 repeats. The Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was utilized to design primer pairs flanking SSRs. The key parameters set for primer design were as follows: primer length 18–24 bp with 20 bp as the optimum; PCR product size 100–300 bp; optimum annealing temperature 54°C; GC content 35–60% with 50% as the optimum. The primers were synthesized by MWG Biotech Pvt. Ltd, Bangalore, India. The BLASTx tool (http://www.ncbi.nlm.nih.gov/BLAST) was employed to search the non-redundant peptide databases, using the default settings for the assigning of putative functions. The marker nomenclature proposed by De Vicente et al. (2004) was followed for the naming the markers developed in this study. The canonical name they proposed consists of [Function][Lab Designator][Species][Type of marker][serial # of clone]. Hence, the markers developed in this study were named Stgnhsbm1-Stgnhsbm50. The marker name denotes staygreen (Stg), developed by NRCS-Hyderabad (nh), in *Sorghum bicolor* (sb), a microsatellite marker (m) and followed by serial # of clone.

DNA extraction, PCR, and electrophoresis

The genomic DNA was extracted using the CTAB method (Saghai-Maroof et al. 1984). PCR reactions were set up in a 5 µL reaction in 96-well PCR plates (Axygen, PCR-96-HS-C). Each PCR reaction contained 2–4 pmol of primer, 1–4 mM $MgCl_2$, 0.1–0.2 mM dNTP, 0.2 U Taq DNA polymerase and 1× PCR buffer (Invitrogen S. Giuliano, Milanese, Italy). Temperature cycling was carried out for both genic and genomic microsatellite markers using the Bio-Rad iCycler version 3.3032 and touch-down PCR amplification: one 15-min denaturation cycle, followed first by ten cycles of 94°C for 10 s, 61°C for 20 s (reducing 1°C per cycle) and 72°C for 30 s, then by 31 cycles of 94°C for 10 s, 54°C for 20 s, and 72°C for 30 s. After completion of the 31 cycles, a final extension of 20 min at 72°C was included to minimize the +A overhang (Smith et al. 2000). PCR products were separated on a Bio-Rad Sequi-Gen^{TM} sequencing electrophoresis apparatus in 5% polyacrylamide gel containing 8 M urea and 1× TBE buffer at 80 W of constant power. The DNA fragments were visualized by silver staining (Fritz et al. 1999) and scored as either parental (A or B), heterozygous (H), or missing data (−).

Mapping of EST-SSRs on sorghum linkage map

In all, 236 genomic-microsatellites and 50 genic-microsatellite markers were considered for screening polymorphism between parents 296B and IS18551 of 168 F_7 recombinant inbred lines (RILs). The total of 118 polymorphic markers which consisted of 108 genomic-microsatellites and 10 genic-microsatellites were used for genotyping the RILs. Additionally, the genomic-microsatellites developed by Brown et al. (1996), Taramino et al. (1997), Kong et al. (2000), and Bhattramakki et al. (2000) were selected based on their positions on linkage maps and used for construction of the frame-work map for 296B × IS18551. The computer software JOINMAP 3.0 (Van Ooijen and Voorrips 2001) was utilized for map construction. The allocation of markers to linkage groups was mostly stable for a wide range of LOD grouping thresholds (from ≤4.0 to ≥7.0). More stringent parameter settings were not applied where the marker location were already known from the earlier published reports. The Kosambi mapping function was used to convert recombination into the genetic distance (Centimorgans, cM). A "ripple" was performed after three marker additions/insertions. The "jump" and "triplet" thresholds were set to 4 and 9, respectively. The goodness-of-fit of the constructed maps reflecting the discrepancy between final recombination frequencies in the map and those apparent from individual marker data pairs, was expressed as a chi-square value and computed according to Stam and van Ooijen (1995). We have used the nomenclature proposed by Kim et al. (2005) throughout this paper.

Alignment of the linkage groups and consensus map construction around the staygreen QTL

As our linkage map consists wholly of SSR markers, and the QTL reported by Xu et al. (2000) and Subudhi et al.

(2000) are RFLP based linkage maps, we initially aligned these linkage groups harboring the QTL on to the high density reference maps of Menz et al. (2002), and Bhattramakki et al. (2000) with common bridging RFLP markers mapped between them. Further using the common genomic SSRs that were mapped between our linkage map and that of Menz et al. (2002) and Bhattramakki et al. (2000), we aligned our frame work map, along with the newly mapped genic-microsatellite markers, with the corresponding linkage groups. Finally, for each staygreen QTL region, all RFLP and SSR marker that are reported and mapped were compiled and used for the construction of consensus map around the respective QTL (Fig. 1).

Results

Identification of rice genomic regions syntenous to sorghum staygreen QTL

Xu et al. (2000) and Subudhi et al. (2000) genetically mapped the *Stg1* QTL on SBI-03, which maps near RFLP markers BNL15.20 and BNL6.16 between the flanking markers NPI414-TXS1114 (Fig. 1A). Two RFLP markers (BNL 15.20 and TXS584) are common between the SBI-03 and the corresponding linkage group of reference maps. Nine genomic SSRs are common between the linkage group of the above reference maps (Bhattramakki et al. 2000; Menz et al. 2002) and the corresponding SBI-03 of our map (Fig. 2a). Finally, using the consensus map developed for the *Stg1* QTL region using Menz et al. (2002) and Bhattramakki et al. (2000) reference maps as a base, the RFLP markers HSP70 and UMC17 were selected as flanking markers for the QTL region since these markers flanks all the reported markers for the QTL at the proximal and distal end, respectively.

The *Stg2* QTL maps on the same LG, SBI-03, between RFLP markers WG889 and TXS584 (Fig. 1A). Since the sequence of TXS584 was not available, the next RFLP marker CDO920, whose sequence is known, was selected and the QTL was bracketed between these markers WG889-CDO920.

The *Stg3* QTL was reported on SBI-02 by Xu et al. (2000) and Subudhi et al. (2000) between the RFLP markers UMC5 and UMC116 (Fig. 1B). Three RFLP markers (UMC5, TXS1111, and UMC116) are common between this linkage group and the corresponding SBI-02 of Menz et al. (2002) and Bhattramakki et al. (2000) (Fig. 2b). The number of common genomic SSR markers between the linkage group SBI-02 of Menz et al. (2002) and Bhattramakki et al. (2000) and corresponding linkage group of our map are 15. Using the consensus map developed for the *Stg3* QTL region, the RFLP markers UMC139 and UMC22 were selected for bracketing the QTL region.

The *Stg4* QTL was identified on linkage group SBI-05 near the RFLP markers TXS387, TXS1628, TXS713 and a morphological marker rcb (Fig. 1C). This linkage group has three common RFLP markers (TXS722, TXS713, and TXS387) between the corresponding linkage group of Bhattramakki et al. (2000) (Fig. 2c). Eight genomic SSRs are common between the linkage group of our study and SBI-05 of Bhattramakki et al. (2000). The staygreen QTL was bracketed between the RFLP marker TXS387 and a morphological marker "rcb". However, the sequence was not available for the RFLP marker at the proximal end and the presence of morphological marker at the distal end, the next RFLP marker UMC52 was selected at the proximal end and the RFLP marker RZ900 reported by Crasta et al. (1999) as a flanking marker to the staygreen QTL in B35 × Tx430 population was selected at the distal end.

Thus, the RFLP markers bracketing the QTL, HSP70-UMC17 (for *Stg1* QTL region), WG889-CDO920 (for *Stg2* QTL region), UMC139-UMC22 (for *Stg3* QTL region), and UMC52-RZ900 (for *Stg4* QTL region), were selected and used as queries for BLASTn similarity searches against the rice genome to identify putative syntenic regions. In addition, other RFLP and SSR markers in and around the QTL region, whose sequences were available, were utilized for the final confirmation of syntenic regions. For *Stg1* QTL, out of 15 RFLPs compiled, seven markers whose sequence was available were tested in BLASTn hits and all markers were hit on rice chromosome 1 spanning a region from 28 to 37.1 Mb (Fig. 1A-a). The Xtxp38 genomic-microsatellite was also included for in silico analysis for the *Stg1* QTL by using the sequence of its maize homologue (AF036949) (Bhattramakki et al. 2000). Four of the eleven markers listed for the *Stg2* QTL whose sequence was known were employed in the in silico analysis. As four markers hit rice chromosome 1, we defined a region on rice chromosome 1 spanning 21.6−27.3 Mb to be syntenic to the *Stg2* QTL. Out of 13 RFLP markers compiled for the *Stg3* QTL only seven RFLP markers were sequenced, of which four markers were discarded in the analysis due to the lack of synteny with the rice genome, and hence only the remaining three RFLPs and an AFLP (Xtxa482) marker near UMC139 were considered for BLASTn in silico analysis. Although AFLP markers are not ideal for comparative genome analysis, the Xtxa482 marker bracketing the QTL at the proximal end was included in syntenic analysis as its position on the rice genome could be determined using its homologous rice EST (AU032483) that was reported by Childs et al. (2001) using a cDNA selection technology. As all four markers were hit on rice chromosome 9 covering a region from 14.8 to 20.4 Mb, this region was considered as syntenic to the *Stg3* QTL (Fig. 1B-b). Six sequenced RFLP markers were considered for syntenic region identification for the *Stg4* QTL; only two RFLPs were homologous to the rice chromosome

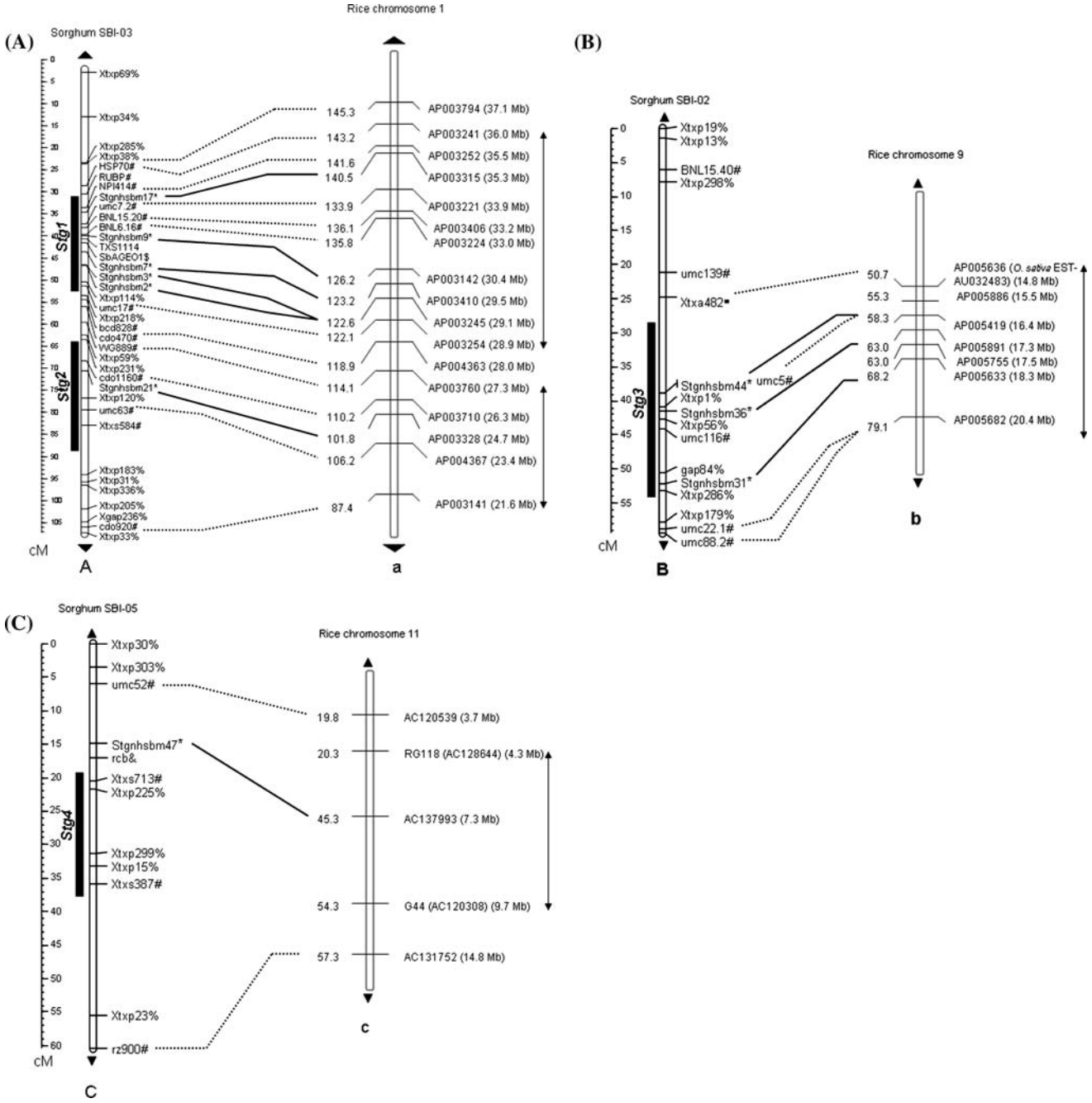
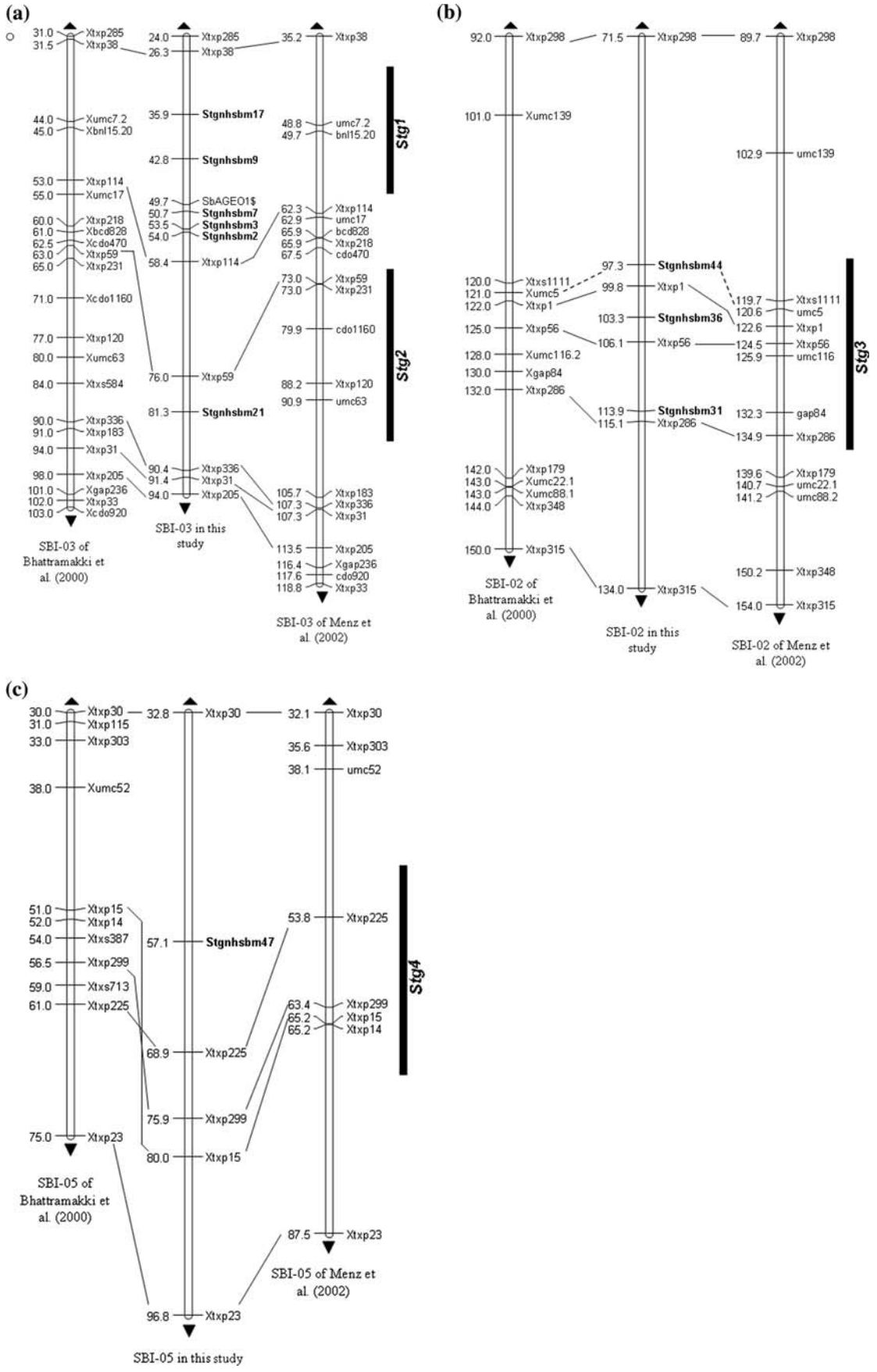


Fig. 1 **A**, **B**, and **C** are sorghum consensus maps SBI-03, SBI-02, and SBI-05 developed for the *Stg1&Stg2*, *Stg3*, and *Stg4* staygreen QTL regions, respectively, using compiled RFLP, genomic SSRs from the high-density reference map of Menz et al. (2002), staygreen QTL reported by Xu et al. (2000) and Subudhi et al. (2000), and genic and genomic SSRs of this study. The black bar indicates the QTL region on the linkage group. The symbols (#) indicate the RFLP and (%) genomic SSRs, that served as backbone markers for the construction of the consensus map. *Asterisks* (*) indicate the genic-microsatellite markers mapped in this study. The *filled square* denotes an AFLP marker. The genomic SSR marker mapped in this study, whose position previously was not reported, is indicated with a symbol ($). The symbol (&) indicate the morphological marker "rcb". *Dotted* and *bold lines* connecting sorghum RFLP and EST derived SSR markers, respectively, with the rice physical map indicate the syntenic rice BAC/PAC clones harboring the homologous genes or ESTs and their corresponding positions on the rice chromosome **a**, **b**, and **c**, are partial physical maps of homologous rice chromosomes syntenous to the sorghum *Stg1&2*, *Stg3*, and *Stg4* staygreen QTL, respectively. The *double headed arrow* shows the selected rice genomic region for the in silico selection of ESTs for marker development. The *scales* to the *left* of the sorghum and rice maps indicate map distances in centiMorgans (Kosambi function). *Arrowheads* at the *top* and *bottom* of the maps direct towards the flanking genomic regions


(a)
31.0 Xtxp285
31.5 Xtxp38
44.0 Xumc7.2
45.0 Xbnl15.20
53.0 Xtxp114
55.0 Xumc17
60.0 Xtxp218
61.0 Xbcd828
62.5 Xcdo470
63.0 Xtxp59
65.0 Xtxp231
71.0 Xcdo1160
77.0 Xtxp120
80.0 Xumc63
84.0 Xtxs584
90.0 Xtxp336
91.0 Xtxp183
94.0 Xtxp31
98.0 Xtxp205
101.0 Xgap236
102.0 Xtxp33
103.0 Xcdo920
SBI-03 of Bhattramakki et al. (2000)
24.0 Xtxp285
26.3 Xtxp38
35.9 Stgnhsbm17
42.8 Stgnhsbm9
49.7 SbAGEO1$
50.7 Stgnhsbm7
53.5 Stgnhsbm3
54.0 Stgnhsbm2
58.4 Xtxp114
76.0 Xtxp59
81.3 Stgnhsbm21
90.4 Xtxp336
91.4 Xtxp31
94.0 Xtxp205
SBI-03 in this study
35.2 Xtxp38
48.8 umc7.2
49.7 bnl15.20
62.3 Xtxp114
62.9 umc17
65.9 bcd828
65.9 Xtxp218
67.5 cdo470
73.0 Xtxp59
73.0 Xtxp231
79.9 cdo1160
88.2 Xtxp120
90.9 umc63
105.7 Xtxp183
107.3 Xtxp336
107.3 Xtxp31
113.5 Xtxp205
116.4 Xgap236
117.6 cdo920
118.8 Xtxp33
SBI-03 of Menz et al. (2002)
Stg1
Stg2
(b)
92.0 Xtxp298
101.0 Xumc139
120.0 Xtxs1111
121.0 Xumc5
122.0 Xtxp1
125.0 Xtxp56
128.0 Xumc116.2
130.0 Xgap84
132.0 Xtxp286
142.0 Xtxp179
143.0 Xumc22.1
143.0 Xumc88.1
144.0 Xtxp348
150.0 Xtxp315
SBI-02 of Bhattramakki et al. (2000)
71.5 Xtxp298
97.3 Stgnhsbm44
99.8 Xtxp1
103.3 Stgnhsbm36
106.1 Xtxp56
113.9 Stgnhsbm31
115.1 Xtxp286
134.0 Xtxp315
SBI-02 in this study
89.7 Xtxp298
102.9 umc139
119.7 Xtxs1111
120.6 umc5
122.6 Xtxp1
124.5 Xtxp56
125.9 umc116
132.3 gap84
134.9 Xtxp286
139.6 Xtxp179
140.7 umc22.1
141.2 umc88.2
150.2 Xtxp348
154.0 Xtxp315
SBI-02 of Menz et al. (2002)
Stg3
(c)
30.0 Xtxp30
31.0 Xtxp115
33.0 Xtxp303
38.0 Xumc52
51.0 Xtxp15
52.0 Xtxp14
54.0 Xtxs387
56.5 Xtxp299
59.0 Xtxs713
61.0 Xtxp225
75.0 Xtxp23
SBI-05 of Bhattramakki et al. (2000)
32.8 Xtxp30
57.1 Stgnhsbm47
68.9 Xtxp225
75.9 Xtxp299
80.0 Xtxp15
96.8 Xtxp23
SBI-05 in this study
32.1 Xtxp30
35.6 Xtxp303
38.1 umc52
53.8 Xtxp225
63.4 Xtxp299
65.2 Xtxp15
65.2 Xtxp14
87.5 Xtxp23
SBI-05 of Menz et al. (2002)
Stg4

◀ **Fig. 2** **a**, **b**, and **c** show alignment of linkage groups SBI-03, SBI-02, and SBI-05 of two sorghum reference linkage groups (Bhattramakki et al. 2000; Menz et al. 2002) with the corresponding linkage groups developed in this study using the RIL mapping population of the cross 296B × IS18551 for sorghum *Stg1&Stg2*, *Stg3*, and *Stg4* staygreen QTL regions, respectively. The marker positions on the linkage groups of Bhattramakki et al. (2000) and Menz et al. (2002) are drawn at an approximate scale. Selected markers were shown on the linkage groups of Bhattramakki et al. (2000) and Menz et al. (2002) for the staygreen QTL regions that were used in consensus map constructions for clarity. The markers in bold are new genic-microsatellite markers mapped to the positions of staygreen QTL regions. The new genomic SSR marker mapped for the *Stg1* QTL, whose position was not reported earlier, is indicated by a symbol ($). *Solid lines* unite anchor genomic SSR markers that mapped between this study and maps of Bhattramakki et al. (2000), Menz et al. (2002). The *dotted line* unites the RFLP marker UMC5 with its overlapping genic-microsatellite marker mapped in this study. The *black bar* indicates the position of the staygreen QTL region with respect to the linkage groups. The *scales* indicate map distances in centiMorgans (Kosambi function). *Arrowheads* at the *top* and *bottom* of the maps direct towards the flanking genomic regions of individual linkage groups

11 covering a region from 3.7 to 14.8 Mb whereas the other four markers did not yield homologous sequences in rice. Due to the low marker resolution for the *Stg4* QTL, combined with the flanking markers distance from the target region, we roughly confined the target QTL region between rice markers RG118-G44, which spanned a region from bp 4.3 to 9.7 Mb (Fig.1C–c). The summary of markers used for the identification of syntenous regions and their corresponding positions on the rice genome is provided in Table 1.

Based on this in silico analysis with rice we defined the *Stg1* QTL to a 21.1 cM (122.1–143.2 cM) interval on rice chromosome 1; *Stg2* QTL to 26.7 cM (87.4–114.1 cM) on chromosome 1; *Stg3* QTL to 28.4 cM (50.7–79.1 cM) on rice chromosome 9; and *Stg4* QTL to 34 cM (20.3–54.3 cM) on rice chromosome 11. The QTL, bordered by the proximal and distal BAC clones AP003241-AP003254 for *Stg1*; AP003760-AP003141 for *Stg2*; AP005636-AP005682 *Stg3*; and AC128644-AC120308 for *Stg4* were then utilised for SSR marker development. The BAC/PAC clones (Release 5 of the TIGR Rice Pseudomolecules) and their corresponding positions on rice chromosomes syntenous to the four sorghum staygreen QTL can be seen in the website (http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml).

Genic-microsatellite marker development

Of the 869 ESTs selected in the intervals of targeted syntenous regions of rice for microsatellite development, 93 ESTs were detected with microsatellite motifs, and with only 50 EST sequences suitable for primer design for the regions flanking the microsatellite motifs. Consequently, a total of 50 genic-microsatellite primer pairs (designated Stgnhsbm1–50), could be developed, that is, 18, 12, 15, and 5, for *Stg1*, *Stg2*, *Stg3*, and *Stg4* QTL regions, respectively. Among the 50 EST-SSR markers the most common repeat type was di-nucleotides (46%) followed by tri-nucleotides (38%) and tetra-nucleotides (4%). Six (12%) ESTs contained two adjacent SSR repeats (Compound SSRs). BLASTx analysis for assigning putative functions of these 50 EST revealed that 39 EST (78%) showed significant homology to previously characterized genes, most belonging to transcription factor families, signaling cascade, photosynthesis and drought related metabolism. A total of 11 EST (22%) did not show homology to known characterized genes. The primer sequence information and summary of the putative functions of the 50 EST used in this study is provided in Table 2.

Genetic mapping of EST-SSRs for Staygreen QTL

With the exception one primer pair (Stgnhsbm29) all genic-microsatellite primer pairs developed in this study amplified PCR products of the expected sizes. The unexpected product size of the marker Stgnhsbm29 may be attributed to the possible presence of intron sequences in the genomic DNA between the primer sites. Most of the primers developed amplified informative products when used to screen our mapping population. In cases where the markers produced multiple bands we considered only the band of the expected size to avoid ambiguity. In all, of the 50 genic-microsatellites tested, ten genic-microsatellites (20%) were found to be polymorphic when used to screen the parental lines 296B and IS 18551. Polymorphic markers were tested for linkage analysis using the framework linkage map of 108 genomic-microsatellites produced by JOINMAP version 3.0 (Van Ooijen and Voorrips 2001). The positions of genomic-microsatellite markers in the final linkage map produced in this study are in close agreement with that of Menz et al. (2002) and Bhattramakki et al. (2000) (Fig. 2a−c). In one instance for the *Stg4* QTL we could observe change in marker order for three consecutive genomic SSRs (Xtxp225, Xtxp299, and Xtxp15) between SBI-05 of our study and SBI-05 of Bhattramakki et al. (2000). However, the order of these markers is in agreement with SBI-05 of Menz et al. (2002) (Fig. 2c). All ten genic-microsatellite markers were mapped to their expected target locations, based on the in silico mapping results.

For the identification of the locations of newly mapped genic-microsatellites with respect to the staygreen QTL, consensus maps were constructed for the four staygreen QTL regions using the marker data generated in this study and common RFLP and genomic-microsatellites compiled from different individual linkage groups (Crasta et al. 1999; Xu et al. 2000; Subudhi et al. 2000; Menz et al. 2002; Bhattramakki et al. 2000). The mapping of the genic-microsatellites between the genomic-microsatellites known

Table 1 Sorghum RFLP and genic-microsatellite markers and syntenous rice BAC/PAC clones and their corresponding positions on rice chromosomes

Staygreen QTL	Marker	Gene bank accession	Marker type	Sorghum LG	Rice BAC/ PAC clone	Rice chromosome	Start	End	*E* value
Stg1	Xtxp38[a]	AF036949 (Maize homologue)	SSR	3	AP003794	1	37177456	37177728	2.00E-102
	HSP70	U41653	RFLP	3	AP003241	1	36034487	36036152	0.00
	NPI414	AY772453	RFLP	3	AP003252	1	35505782	35506058	1.00E-42
	UMC7[b]	umc7.x3.b	RFLP	3	AP003221	1	33948177	33948660	0.00
	BNL15.20[b]	bnl15.20.x4.b	RFLP	3	AP003406	1	33221743	33222034	6.00E-37
	BNL6.16	G10769	RFLP	3	AP003224	1	33059732	33060048	2.00E-129
	UMC17	AY771218	RFLP	3	AP003254	1	28949401	28949599	2.00E-63
	CDO470	AA231703	RFLP	3	AP004363	1	28076243	28076384	7.00E-30
	Stgnhsbm2	CX616697	SSR	3	AP003245	1	29125764	29126080	1.00E-39
	Stgnhsbm3	BG464180	SSR	3	AP003245	1	29126746	29126854	3.00E-36
	Stgnhsbm7	CB925941	SSR	3	AP003410	1	29599165	29599586	1.00E-106
	Stgnhsbm9	CD223691	SSR	3	AP003142	1	30408775	30408515	7.00E-50
	Stgnhsbm17	CF760991	SSR	3	AP003315	1	35307976	35308105	5.00E-20
Stg2	CDO920	BE439196	RFLP	3	AP003141	1	21698637	21698871	1.00E-24
	UMC63	DQ123897	RFLP	3	AP004367	1	23452278	23459980	5.00E-39
	CDO1160	AA231698	RFLP	3	AP003710	1	26388059	26388314	4.00E-12
	WG889	BH854380	RFLP	3	AP003760	1	27369347	27369666	2.00E-49
	Stgnhsbm21	CN127248	SSR	3	AP003328	1	24702438	24702517	1.00E-20
Stg3	UMC5	AY771216	RFLP	2	AP005419	9	16403432	16403619	5.00E-33
	UMC22[b]	umc22.x3.b	RFLP	2	AP005682	9	20473447	20473541	1.00E-04
	UMC88[b]	umc88.x3.b	RFLP	2	AP005682	9	20474538	20474088	6.00E-68
	Xta482[a]	AU032483 (Rice EST homologue)	AFLP	2	AP005636	9	14867285	14869977	4.00E-08
	Stgnhsbm31	CF761081	SSR	2	AP005633	9	18393851	18394067	4.00E-16
	Stgnhsbm36	CN137941	SSR	2	AP005891	9	17345381	17346134	1.00E-30
	Stgnhsbm44	CD221844	SSR	2	AP005419	9	16403430	16403646	2.00E-43
Stg4	UMC52	DQ123899	RFLP	5	AC128644	11	3740314	3739669	9.00E-13
	RZ900	AA231825	RFLP	5	AC131752	11	14844254	14844358	6.00E-46
	Stgnhsbm47	AW745398	SSR	5	AC137993	11	7381621	7381762	2.00E-53

[a] Indicates the markers their positions on rice chromosomes deduced by using their homologue sequences (Bhattramakki et al. 2000; Childs et al. 2001)

[b] Indicates RFLP markers whose sequences were taken from www.cytomaize.org

to flank the QTL confirms the linkage with the QTL. The map locations of newly developed EST markers for QTL were further confirmed by using BLASTn similarity searches of EST markers along with RFLP markers in the QTL region against the rice genome.

For the *Stg1* QTL, which is reported to be tightly linked to the RFLP markers BNL15.20, BNL6.16 on SBI-03 (Subudhi et al. 2000; Xu et al. 2000), five EST markers (Stgnhsbm2, Stgnhsbm3, Stgnhsbm7, Stgnhsbm9, and Stgnhsbm17) were placed covering the QTL region on the corresponding linkage group SBI-03 of our map. One genomic-microsatellite marker (SBAGE01) developed by Taramino et al. (1997), whose position was previously unknown was also mapped in the QTL target region. The BLASTn searches of the genic markers Stgnhsbm2, Stgnhsbm3, Stgnhsbm7, Stgnhsbm9, and Stgnhsbm17, along with sequenced RFLP markers against the rice genome showed that they are colinear and there was no DNA rearrangement observed between the corresponding regions of the rice genome (Fig. 1A-a).

Only one genic-microsatellite (Stgnhsbm21) out of twelve developed for the *Stg2* QTL was found to be polymorphic between the parental lines. As a result only this marker was subsequently mapped near the genomic-micro-

Table 2 Primer sequences of genic-microsatellite markers designed for four staygreen QTL

Staygreen QTL	Primer name	EST gene bank accession No.	Type(s) of SSR and number of repeats	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Ann. temp (°C)	Expected size (bp)	BLAST X Putative function	Species	*E* value
Stg1	Stgnhsbm1	AW286961	(AGC)5	CGAATCGAAATCCCTCTCC	GAGGCGTTGTTGTTGTCATC	54	156	Un known protein	*Oryza sativa*	7.00E-08
	Stgnhsbm2	CX616697	(AT)7 + (TA)5	CATTTCGACTTCGACATCAGC	CTCCCAATTGTTCCGTGTG	55	163	Putative myb-related protein	*Oryza sativa*	1.00E-35
	Stgnhsbm3	BG464180	(CT)6	CAACTGCAACTGCAAGCTCT	CTAGTCCGTCCGTCGTCACT	54	367	Putative myb-related protein	*Oryza sativa*	1.00E-14
	Stgnhsbm4	CF432983	(TG)5	TTTTGCAGCAGGTCTCACTG	GGTAAAATCAGTGCCAGCAT	53	225	N-myristoyl transferase	*Triticum aestivum*	2.00E-29
	Stgnhsbm5	CN126720	(CT)7	CCTTTCTTCCTTCGTCCGTA	GACTGGAGGTTGGAGACGAG	54	204	Acid phosphatase	*Arabidopsis thaliana*	1.00E-42
	Stgnhsbm6	CN142368	(ACC)5	CCACCCAACCAGATGGAG	ACGTGTCGCTCGGCTGTAG	55	247	Glycine dehydrogenase P protein	*Oryza sativa*	2.00E-64
	Stgnhsbm7	CB925941	(CTCC)5	CTCTCTCTCCCTCCCTCCCTC	CCATTGCTCACTCAGATCCA	56	182	Cytidine/deoxycytidine deaminase-like	*Oryza sativa*	6.00E-56
	Stgnhsbm8	CD231604	(CGC)5	AACAAGCTCTGCTCCTCCAC	CGAGGAAGCCCTTGTAGTTG	54	178	Satase isoform I	*Zea mays*	6.00E-60
	Stgnhsbm9	CD223691	(CAG)7	TCGATCGGATTAGGTTACCG	CAGAACGAGACCATCGAACA	54	180	Putative aminotransferase	*Oryza sativa*	3.00E-64
	Stgnhsbm10	CD222411	(CCG)5	TCGATTCGAGCGGTCAGTA	GATGGACTCCGAGGAGGAG	54	223	Unknown protein	*Oryza sativa*	6.00E-61
	Stgnhsbm11	CF433669	(TC)6	GTTCCTTCCACCCACACATC	GAGCTGGACCTGCACCTTC	55	162	Putative GTP-binding protein	*Oryza sativa*	9.00E-94
	Stgnhsbm12	CD228063	(TC)6	CCCACACATCCACACTCCTC	CACGATGTTGGAGCCCTTA	55	248	Putative GTP-binding protein	*Oryza sativa*	7.00E-68
	Stgnhsbm13	AW672515	(CA)6 + (ACG)6	GCACGAGGCACACACACAC	CTCATGCAGCTGGGAATGT	55	211	Unknown protein	*Oryza sativa*	2.00E-46
	Stgnhsbm14	CF427777	(TCG)5	CAATGGAGGTTTCCACCAAG	CTTGAACGAGGTGGTGTCG	54	233	VQ Motif-containing protein-like	*Oryza sativa*	8.00E-40
	Stgnhsbm15	CN146684	(GC)6 + (GCT)7	CCCTTTTGGCACTGGTACAT	ATCACATCGCAAACGGTACA	54	171	WRKY transcription factor 13	*Oryza sativa*	2.00E-35
	Stgnhsbm16	BG946801	(ATCC)5	TCTACACCTCCGACCTCGAC	GCTGCTAACATCTCGCAAAAG	54	183	Putative photosystem II reaction center W protein	*Oryza sativa*	1.00E-10
	Stgnhsbm17	CF760991	(TA)8	GGCAGCATCTACCAGACCAT	GAAGGCATCTAGCGATCACC	54	210	Putative VHS2 protein	*Oryza sativa*	1.00E-22
	Stgnhsbm18	CN147567	(GCT)5	GGGTTCTAGGAGGCGTAGGT	ATATACAGCGCCGCAAATG	54	199	Unknown protein	*Oryza sativa*	5.00E-75
Stg2	Stgnhsbm19	CN144249	(GC)6	GGTGAAGGAGCTCAAGAACG	ATGGTACAAGCCGAAACCAG	54	224	Chlorophyll a-b binding protein 2, LHCII type I CAB-2) (LHCP)	*Oryza sativa*	4.00E-126
	Stgnhsbm20	CN144349	(GA)5 + (CCG)5	CCCATCCATCTCCATTTCC	ATGACGTCCTCCACCTTCG	55	250	Cytochrome c oxidase subunit Vb precursor	*Oryza sativa*	9.00E-11
	Stgnhsbm21	CN127248	(CGC)13	GGACCCGGAACGCCGTAAATC	GGGAGTCATAGACGGAGACG	54	245	SEC13 protein homolog YGL100w-like	*Oryza sativa*	1.00E-106
	Stgnhsbm22	CB925228	(GAC)6	TCGATCGAGCTCCAAAGAAC	AGCAGCACCAGCTTCCAC	54	197	Cytochrome P450-like	*Oryza sativa*	4.00E-18
	Stgnhsbm23	CX617365	(TA)5	GGCCCAACTCCTACATCATC	ACCACAAGCCGGTCAATTAT	53	235	Carbonic anhydrase	*Zea mays*	2.00E-42
	Stgnhsbm24	BG739315	(AT)5	CTACGGCAACCTGAAGGTTG	GCCAGTTGAAACTGACAGGAG	54	161	Aspartic protease	*Oryza sativa*	8.00E-72
	Stgnhsbm25	BE919148	(CTC)5	GGTGGTTGCTTTTGCTCAG	GCTCGTCATGGAATTGGAG	54	181	Aspartic proteinase oryzasin 1 precursor	*Oryza sativa*	2.00E-36
	Stgnhsbm26	CD221566	(CG)5	TGGGTCCTACCTACCTCGTG	CCTTTCGGATCAATTCCAGA	54	246	Ribokinase-like	*Oryza sativa*	5.00E-73
	Stgnhsbm27	CF434308	(GA)8	GGTACCCGGTAGTTCGATCA	CTGGTCTCGAGGCTGAACTC	54	235	Putative GTP-binding protein Rab11b	*Oryza sativa*	4.00E-45
	Stgnhsbm28	BE357014	(GA)6 + (GTC)5	CACGAGGCACATCTATCCAC	CTCGCTCCAGCAATCCTC	54	209	6b-interacting protein 1-like	*Oryza sativa*	2.00E-48

Table 2 continued

Staygreen QTL	Primer name	EST gene bank accession No.	Type(s) of SSR and number of repeats	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Ann. temp (°C)	Expected size (bp)	BLAST X Putative function	Species	*E* value
	Stgnhsbm29	BF704597	(GGC)5	CTGCTCGAACTAACCAGAAAGA	TCCCATCAAGCCTGTAAACC	54	183	Unknown protein	*Arabidopsis thaliana*	1.00E-30
	Stgnhsbm30	CN133175	(AG)6	CTGCAGCTGAGAGCTGACC	GACCACGACGGTGTAGGAC	54	152	Cgi67 serine protease-like	*Oryza sativa*	2.00E-55
Stg 3	Stgnhsbm31	CF761081	(AG)10	ATAGGGACACGGCAGCACTA	ACCCAGGTGAAGATGATCCA	54	159	Os09g0480600	*Oryza sativa*	3.00E-08
	Stgnhsbm32	CF430486	(GA)12	ACTCATCAACAGCCAACAGC	AGGTTGGACAGCCACAGC	54	162	Putative hydroxycinnamoyl transferase	*Oryza sativa*	8.00E-60
	Stgnhsbm33	CD209273	(AT)6	AGTGCTCTGCTCCCTGTCTC	GGTAAATAAAGGCATCCA	50	231	Senescence-associated protein DH	*Zea mays*	3.00E-29
	Stgnhsbm34	CF482416	(CCA)5	CCCCAAAACTCTCGAAATCA	CTCCTCGAAGTGCTGCTTCT	54	190	Putative ABI3-interacting protein 2, AIP2	*Oryza sativa*	2.00E-70
	Stgnhsbm35	BG559930	(GC)8 + (TG)7	AGAACGCACGCATCACTCT	AACCACGTCGTATGCCGTAT	54	242	Basic helix-loop-helix (bHLH)-like protein	*Oryza sativa*	6.00E-13
	Stgnhsbm36	CN137941	(AGG)9	CTTTCGCCTGGTCGTACACT	AGAAGAACGCCTCGCTCTC	54	180	Unknown protein	*Oryza sativa*	2.00E-55
	Stgnhsbm37	CX620916	(CCT)5	CCCATGGAGAGCAGCATC	AGAGGCTGCAGGAGGTCAT	54	197	Glutamate/malate translocator	*Sorghum bicolor*	2.00E-88
	Stgnhsbm38	BE366552	(GT)5	CCACATCGTCGCCTACTACA	AATCTTTGAGCTCAGATCATGC	54	151	Putative Photosystem I reaction center subunit V	*Oryza sativa*	9.00E-22
	Stgnhsbm39	CN137647	(CAG)6	CCATTCTTCGCCACTTCG	CTCGGATTTGGCTTCGTCTA	54	180	Unknown protein	*Oryza sativa*	4.00E-43
	Stgnhsbm40	CN130386	(TGG)5	CGGAGGAAGAGATTGCAGAG	ACCCAACCCATTTGTTCATC	54	250	Putative multiple stress-responsive zinc-finger protein	*Oryza sativa*	1.00E-11
	Stgnhsbm41	AW923884	(CGG)6	TCGTCTCCCACTAGGGTTTG	CTCCAGTTCGGGGACGAGT	55	246	Putative plastid protein	*Oryza sativa*	2.00E-38
	Stgnhsbm42	CD462612	(TC)5	ATCGACCTCGTCTCTCTCTCG	GGAAACTGCGCAGAAGGA	54	147	Unknown protein	*Oryza sativa*	2.00E-59
	Stgnhsbm43	CD204746	(GCG)7	GGATCTCGGCGGTTCTTC	GTCCTTTGAGCATTCCTTGC	54	194	Putative tbc1 domain family protein	*Oryza sativa*	2.00E-52
	Stgnhsbm44	CD221844	(AT)6	GAGATCCAGCGCATGTATGA	GTTCAGGACAGGGGTACAGC	53	232	Putative plastid (p)ppGpp synthase	*Oryza sativa*	2.00E-30
	Stgnhsbm45	BG355722	(GCG)5	AGGATCAGACGGCAGCAG	AACACAACGCCGAGAGTCTT	54	246	Putative potasium transporter	*Oryza sativa*	1.00E-49
Stg 4	Stgnhsbm46	CX611411	(GA)16	GTTCTTGGAGTGGTCCATCG	CGGTGTAGACGATCTTGACG	54	214	Alcohol dehydrogenase 2	*Oryza sativa*	6.00E-41
	Stgnhsbm47	AW745398	(GA)9	GCACGAGGACAAAACAAAACA	CTGCTTCTCCCATGGTGGT	55	150	PrMC3	*Oryza sativa*	2.00E-34
	Stgnhsbm48	BG947503	(GT)6	GCATCTTGAGTTGGCTCTCC	AGATGGCCAGTCCATGATC	54	245	Expressed protein	*Oryza sativa*	9.00E-09
	Stgnhsbm49	BG159333	(GC)6	AGGTGTACGAGACGGAGTGG	CGAAACTTTTCGCAGAGGAA	55	208	Uncharacterized plant-specific domain TIGR01627 family protein	*Oryza sativa*	4.00E-44
	Stgnhsbm50	CN126147	(GC)5	CTGCTCGGTACAGGCATCTT	GAGCAAGTCCTGCATGTCAA	54	198	Cytochrome P450 family protein	*Oryza sativa*	7.00E-78

satellite Xtxp59. In silico analysis of the marker with rice genome showed it was located on chromosome 1 between the RFLP markers CDO1160 and UMC63 selected for the *Stg2* QTL.

Three markers (Stgnhsbm31, Stgnhsbm36, and Stgnhsbm44) were mapped to the *Stg3* QTL on SBI-02 and they showed colinearity with the corresponding region on rice chromosome 9. The BLASTn similarity searches of UMC5 and the marker Stgnhsbm44 against the rice genome showed that they were positioned at the same point bp 1643432 on rice chromosome 9 with a highly significant *E* value (2.00e−43) indicating that they are overlapping markers and that we have successfully converted the RFLP marker UMC5 into a PCR based SSR marker. The marker Stgnhsbm36 was positioned on rice chromosome 9 at 17.3 Mb, around 1 Mb away from UMC5, which could be very tightly linked to the *Stg3* QTL as it is mapped between the RFLP markers UMC5-UMC116 which were reported as flanking markers to the QTL by Xu et al. (2000).

The marker Stgnhsbm47 was mapped at the proximal end to the *Stg4* QTL, which was reported around the RFLP marker TXS713 (Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000), on SBI-05 flanked by the nearby genomic microsatellites Xtxp303, Xtxp30, and Xtxp225 at the proximal and distal ends, respectively (Fig. 1C-c). This was confirmed as Stgnhsbm47 marker mapped between the two genomic-microsatellite markers (Xtxp30 and Xtxp225) on to the corresponding linkage group SBI-05 of our map (Fig. 2c). The BLASTn search of Stgnhsbm47 with rice showed that this marker was positioned at 7.3 Mb on rice chromosome 11.

Discussion

In this study, we targeted development of microsatellite markers for the staygreen QTL using ESTs to provide additional options for MAS, development of FM (Andersen and Lübberstedt 2003), and to create a base for high resolution mapping of the QTL for map-based gene isolation.

We used the rice genome sequence information as the template for the development of gene derived markers for the four staygreen QTL in sorghum, which were identified using RFLPs in a population derived from the cross B35 × TX7000 (Subudhi et al. 2000; Xu et al. 2000). A total of 50 genic-microsatellite markers were developed, among them ten polymorphic markers and a previously unmapped genomic microsatellite (SBAGE01) mapped to the regions of staygreen QTL using our recombinant inbred line mapping population (296B × IS18551). Since the genic markers developed were not mapped in the same population (B35 × TX7000) used by Subudhi et al. (2000) and Xu et al. (2000), we confirmed the co-location of the mapped markers with the QTL by their linkage with genomic-microsatellite markers which are known to be underlying the QTL region from high density reference maps of Menz et al. (2002) and Bhattramakki et al. (2000). Further, the identification of syntenous regions on the rice genome for the mapped of genic markers, along with the RFLP markers reported to flank the QTL, confirmed the linkage of genic-microsatellite markers with the QTL.

Genes encoding MYB related transcription factor (markers Stgnhsbm2 and Stgnhsbm3), putative cytidine deaminase (marker Stgnhsbm7), putative amino transferase (marker Stgnhsbm9), and putative VHS2 protein (Marker Stgnhsbm17) were mapped at regular intervals covering the entire *Stg1* QTL region between the genomic-microsatellite markers Xtxp38 and Xtxp114, previously reported flanking SSRs for the QTL region. A gene coding for SEC 13 (Stgnhsbm21) was mapped to the position of *Stg2* QTL. For the *Stg3* QTL, genes coding for plastid (p)ppGpp synthase (marker Stgnhsbm44), and two unknown proteins (Stgnhsbm31 and Stgnhsbm36) were localized along with genomic SSRs Xtxp1 and Xtxp56 positioned near the QTL linked RFLPs UMC5-UMC116. The BLASTn similarity searches of the Stgnhsbm44 marker and reported UMC5 RFLP marker for the *Stg3* QTL showed that they are overlapping markers indicating conversion of the RFLP marker into an SSR marker. The marker Stgnhsbm36 appears to be very tightly linked to the QTL as it mapped between the RFLP markers UMC5-UMC116, reported as flanking markers to the QTL by Xu et al. (2000). A gene coding for PrMC3 protein (marker Stgnhsbm47) was mapped near the *Stg4* QTL at the proximal end near genomic-microsatellite Xtxp225. For the *Stg1* QTL, genes encoding the MYB related transcription factor (Stgnhsbm2 and Stgnhsbm3), cytedine deaminase (Stgnhsbm7), and putative amino transferse (Stgnhsbm9) are known to be associated with leaf senescence (Lee et al. 2001; Guo et al. 2004). A gene coding for SEC 13 protein (Stgnhsbm21) mapped at the *Stg2* QTL is involved in protein trafficking and was known to be up-regulated during senescence retardation by benzyladenine in Arabidopsis (Guterman et al. 2003). Detailed genetic studies, such as association studies, are further required to prove that the developed markers are the candidate genes underlying the QTL. The present study establishes the synteny of the sorghum *Stg1&Stg2*, *Stg3*, and *Stg4* staygreen QTL with that of genomic regions on rice chromosomes 1, 9, and 11, respectively.

For development of the genic-microsatellite markers at the QTL targets we utilized the Gramene genome browsing tool for the selection of ESTs at the staygreen QTL regions, unlike the in silico selection procedure utilized for the development of markers at barley *Rph*16 locus (Perovic et al. 2004), which involves blasting of all available ESTs on to the rice genome and subsequent clusterization of

selected ESTs, in order to reduce the redundancy at the syntenous region. The simple selection procedure used in this study avoided blasting the 260634 sorghum EST sequences that were available at the beginning of this work against the rice genome. As we selected only single EST sequences from each EST cluster in the target intervals, this selection procedure further simplified the computational work of assembling the sorghum ESTs into Tentative consensus sequences (TCs) for the reduction of redundant sequences. The reliability of this selection method was experimentally proven by genetic mapping of all the polymorphic makers to the target QTL intervals.

Genomic rearrangements in syntenic regions appear to be a common attribute at the DNA level among species (Tarchini et al. 2000; Dubcovsky et al. 2001; Song et al. 2002). The in silico mapping of RFLP and genic markers for staygreen QTL on to the rice genome showed that the QTL regions appear to be colinear with the corresponding genomic regions of rice especially at the *Stg1*, *Stg2*, and *Stg3* QTL (Fig. 1). It is to be noted that as this colinearity was observed with the deployment of only a few markers, minor rearrangements at the micro synteny level may have gone unnoticed, as evidenced by the absence of homologous sequences in rice to some RFLP markers used in in silico analysis.

The strategy described in the present study can be extrapolated for the establishment of synteny between QTL regions of targeted species with the rice genome and for rapidly filling gaps in linkage maps of less-studied crops. Moreover, mapping the genic-microsatellite markers at target regions, such as QTL, provide a basis for saturating these regions with additional markers designed from aligned EST sequences such as (1) development of cleaved amplified polymorphic markers by digesting the PCR products, (2) designing of new primers at the conserved regions of ESTs which flank the less conserved regions, such as introns, (3) expressed sequence tag polymorphisms (ESTPs) (4) single-stranded conformation polymorphism, and (5) by the screening of single nucleotide polymorphisms (SNPs) through direct sequencing of PCR products; all of which could serve the starting point for map-based cloning of genes underpinning the QTL. Furthermore, this strategy may have broad applications in the development of FM for the important traits in less-studied cereals at the targeted QTL regions by utilizing the functional information at the syntenous rice genomic region.

As MAS for any trait requires the tight linkage of the QTL with the markers, and as RFLPs are cumbersome for this purpose, the new gene-derived SSR markers developed in this study provide an additional option for the MAS of the staygreen trait in sorghum. These markers also help in anchoring sequences with sorghum BAC contigs of the QTL regions and thereby create a base for the isolation of underlying genes through a map-based gene isolation approach. Additionally, the set of 869 ESTs selected should be a useful resource for developing new molecular markers for the staygreen QTL regions and provide a base for the high resolution mapping.

Acknowledgments The authors are thankful to unanimous reviewers for their critical suggestions for improving the manuscript. The First and Second authors would like to thank The Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, for providing assistance in the form of Senior Research Fellowship for their doctoral programme. The authors wish to thank Dr. B.V.S Reddy, Principal Scientist, ICRISAT, India for providing the mapping population.

References

Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-He′ naut I, Huguet T, Burstin J (2006) Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. Theor Appl Genet 112:1024–1041

Abdelkhalik AF, Shishido R, Nomura K, Ikehashi H (2005) QTL-based analysis of leaf senescence in an indica/japonica hybrid in rice (*Oryza sativa* L.). Theor Appl Genet 110:1226–1235

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice wheat and maize chromosomes. Mol Gen Genet 241:483–490

Andersen JR, Lübberstedt T (2003) Functional markers in plants. Trends Plant Sci 8:554–559

Bhattramakki D, Dong J, Chhabra AK, Hart GE (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Binelli G, Gianfranceschi L, Po ME, Taramino G, Busso C, Stenhouse J, Ottaviano E (1992) Similarity of maize and sorghum genomes as revealed by maize RFLP probes. Theor Appl Genet 84:10–16

Borrell AK, Hammer GL (2000) Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop Sci 40:1295–1307

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez-Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 93:190–198

Cha KW, Lee YJ, Koh HJ, Lee BM, Nam YW, Paek NC (2002) Isolation, characterization, and mapping of the stay green mutant in rice. Theor Appl Genet 104:526–532

Chen M, SanMiguel P, DeOliveira AC, Woo SS, Zhang H, Wing RA, Bennetzen JL (1997) Microcolinearity in *sh2*-homologous regions of the maize, rice, and sorghum genomes. Proc Natl Acad Sci 94:3431–3435

Childs KL, Klein RR, Klein PE, Morishige DT, Mullet JE (2001) Mapping genes on an integrated sorghum genetic and physical map using cDNA selection technology. Plant J 27:243–255

Crasta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT (1999) Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Mol Gen Genet 262:579–588

De Vicente C, Metz T, Alercia A (2004) Descriptors for genetic markers technologies. IPGRI

Dubcovsky J, Ramakrishna W, SanMiguel PJ, Busso CS, Yan L, Shiloff BA, Bennetzen JL (2001) Comparative sequence analysis of colinear barley and rice bacterial artificial chromosomes. Plant Physiol 125:1342–1353

Duncan RR (1984) The association of plant senescence with root and stalk diseased in sorghum. In: Mughogho LK (ed) Sorghum root and stalk diseases: a critical review. Proceedings of the consultative discussion of research needs and strategies for control of sorghum rot and stalk diseases (Bellagio, Italy, 27 November−2 December 1983). International Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 99–100

Fritz AK, Caldwell S, Worral WD (1999) Molecular mapping of Russian wheat aphid resistance from triticale accession, PI 386156. Crop Sci 39:1707–1710

Gale MD, Devos KM (1998) Comparative genetics in the grasses. Proc Natl Acad Sci 95:1971–1974

Guiamet JJ, Teeri JA, Nooden LD (1990) Effects of nuclear and cytoplasmic genes altering chlorophyll loss on gas exchange during monocarpic senescence in soybean. Plant Cell Physiol 31:1123–1130

Guo Y, Cai Z, Gan S (2004) Transcriptome of Arabidopsis leaf senescence. Plant Cell Environ 27:521–549

Guterman A, Hajouj T, Gepstein S (2003) Senescence-associated mRNAs that may participate in signal transduction and protein trafficking. Physiologia Plantarum 118:439–446

Harris K, Subudhi PK, Borrell A, Jordan DR, Rosenow DT, Nguyen HT, Klein PE, Klein RR, Mullet J (2007) Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J Exp Bot 58:327–338

Haussmann BIG, Mahalakshmi V, Reddy BVS, Seetharama N, Hash CT, Geiger HH (2002) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:143–148

Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Henzell RG, Dodman RL, Brengman AA, Mayers RL (1984) Lodging, stalk rot, and root rot in sorghum in Australia. In: Mughogho LK (ed) Sorghum root and stalk diseases: a critical review. Proceedings of the consultative discussion of research needs and strategies for control of sorghum rot and stalk diseases (Bellagio, Italy, 27 November−2 December 1983). International Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 225–235

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci 87:4251–4255

Hwang TY, Moon JK, Yu S, Yang K, Mohankumar S, Yu YH, Lee YH, Kim HS, Kim HM, Maroof MA, Jeong SC (2006) Application of comparative genomics in developing molecular markers tightly linked to the virus resistance gene Rsv4 in soybean. Genome 49:380–388

Jiang GH, He YQ, Xu CG, Li XH, Zhang Q (2004) The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross. Theor Appl Genet 108:688–698

Kebede H, Subudhi PK, Rosenow DT, Nguyen HT (2001) Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 103:266–276

Kim JS, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM (2005) Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics 169:1169–1173

Kong L, Dong J, Hart GE (2000) Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). Theor Appl Genet 101:438–448

Lee RH, Wang CH, Huang LT, Chen SC (2001) Leaf senescence in rice plants: cloning and characterization of senescence up-regulated genes. J Exp Bot 52:1117–1121

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Luquez VM, Guiamet JJ (2002) The stay green mutations d1 and d2 increase water stress susceptibility in soybeans. J Exp Bot 53:1421–1428

Mammadov JA, Steffenson BJ, Maroof MA (2005) High-resolution mapping of the barley leaf rust resistance gene Rph5 using barley expressed sequence tags (ESTs) and synteny with rice. Theo Appl Genet 111:1651–1660

McBee GG (1984) Relation of senescence, nonsenescence, kernel maturity to carbohydrate metabolism in sorghum. In: Mughogho LK (ed) Sorghum root and stalk diseases: a critical review. Proceedings of the consultative discussion of research needs and strategies for control of sorghum rot and stalk diseases (Bellagio, Italy, 27 November−2 December 1983). International Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 119–129

Menz MA, Klein RR, Mullet JE, Obert JA, Unruh NC, Klein PE (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and, SSR markers. Plant Mol Biol 48:483–499

Moore BJ, Donnison IS, Harper JA, Armstead IP, King J, Thomas H, Jones RN, Jones TH, Thomas HM, Morgan WG, Thomas A, Ougham HJ, Huang L, Fentem T, Roberts LA, King IP (2005) Molecular tagging of a senescence gene by introgression mapping of a stay-green mutation from *Festuca pratensis*. New Phytol 165:801–806

Perovic D, Stein N, Zhang H, Drescher A, Prasad M, Kota R, Kopahnke D, Graner A (2004) An integrated approach for comparative mapping in rice and barley with special reference to the Rph16 resistance locus. Funct Integr Genomics 4:74–83

Phillips RL, Vasil IK (2001) DNA-based markers in plants, advances in cellular and molecular biology of plants, 2nd edn, vol 6. Kluwer, Dordrecht

Prioul JL, Pelleschi S, Sene M, Thevenot C, Causse M, de Vienne D, Leonardi A (1999) From QTL for enzyme activity to candidate genes in maize. J Exp Bot 50:1281–1288

Rosenow DT, Clark LE (1981) Drought tolerance in sorghum. In: Loden HD and Wilkinson D (eds) Proceedings of the 18th Biennial Grain Sorghum Research (Lubbock, TX, 28 February−2 March 1993), pp 122–126

Rosenow DT (1984) Breeding for resistance to root and stalk rot in Texas. In: Mughogho LK (ed) Sorghum root and stalk diseases: a critical review. Proceedings of the consultative discussion of research needs and strategies for control of sorghum rot and stalk diseases (Bellagio, Italy, 27 November−2 December 1983). International Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 209–217

Saghai-Maroof MA, Soliman KM, Jordensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci 81:8014–8018

Salse J, Piegu B, Cooke R, Delseny M (2004) New in silico insight into the synteny between rice (*Oryza sativa* L.) and maize (*Zea mays* L.) highlights reshuffling and identifies new duplications in the rice genome. Plant J 38:396–409

Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000) Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Sci 40:226–232

Song R, Llaca V, Messing J (2002) Mosaic organization of orthologous sequences in grass genomes. Genome Res 12:1549–1555

Stam P, Van Ooijen JW (1995) JOINMAP. Software for the calculation of genetic linkage maps. CPRO-DLO: Wageningen, The Netherlands

Subudhi PK, Rosenow DT, Nguyen HT (2000) Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theor Appl Genet 101:733–741

Tao YZ, Henzell RG, Jordan DR, Butler DG, Kelly AM, Mcintyre CL (2000) Identification of genomic regions associated with stay-green in sorghum by testing RILs in multiple environments. Theor Appl Genet 100:1225–1232

Taramino G, Tarchini R, Ferrario S, Lee M, Pe ME (1997) Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. Theor Appl Genet 95:66–72

Tarchini R, Biddle P, Wineland R, Tingey S, Rafalski A (2000) The complete sequence of 340 kb of DNA around the rice *Adh1-Adh2* region reveals interrupted colinearity with maize chromosome 4. The Plant Cell 12:381–391

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, IV BucklerES (2001) Dwarf8 polymorphisms associate with variation in flowering time. Nat Genet 28:286–289

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1997) Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol Breed 3:439–448

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands

VanDeynze AE, Nelson JC, O'Donoughue JS, Ahn SN, Siripoonwiwat W, Harrington SE, Ylesias ES, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses Oat relationships. Mol Gen Genet 249:349–356

Ventelon M, Deu M, Garsmeur O, Doligez A, Ghesquiere A, Lorieux M, Rami JF, Glaszmann JC, Grivet L (2001) A direct comparison between the genetic maps of sorghum and rice. Theor Appl Genet 102:379–386

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen NT (2000) Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461–469