Theor Appl Genet (2000) 101:733–741

P.K. Subudhi · D.T. Rosenow · H.T. Nguyen

Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments

Received: 12 January 2000 / Accepted: 12 February 2000

Abstract Stay green in sorghum (*Sorghum bicolor* L. Moench) is characterized by the plant's ability to tolerate post-flowering drought stress, thereby delaying the premature leaf and plant death. It contributes to normal grain filling and reduces the incidence of stalk lodging and charcoal rot disease during the late stages of grain development. Breeding for improving post-flowering drought tolerance in sorghum hybrids remains an important objective of sorghum breeders. Since evaluation of the stay green response is difficult and unreliable under field conditions, due to the timing and intensity of moisture stress and large environmental interaction, progress in improving drought tolerance by conventional breeding methods has been slow. The objective of the present study was to determine the consistency of quantitative trait loci (QTLs) controlling stay green in sorghum. We re-evaluated the Recombinant Inbred Line (RIL)-mapping population from the cross B35 x Tx7000 in two locations over 2 years and compared it with earlier reports. Analysis using the combined stay green-rating means of seven environments and the expanded molecular map reconfirmed all four stay green QTLs (*Stg1*, *Stg2*, *Stg3* and *Stg4*) that were identified earlier by Xu et al. (2000). Similarly, comparison of the stay green QTL locations with earlier reported results indicated that all four stay green QTLs showed consistency across different genetic backgrounds. Examination of the stay green QTL profiles of the best and poorest stay-green lines indicated that three stay green QTLs, *Stg1*, *Stg2* and *Stg3*, appear to be important for the expression of this trait when the percent phenotypic variation, and the consistency in different backgrounds and different environments, are considered. A significant epistatic interaction involving *Stg2* and a region on linkage group C was also identified for the stay green and chlorophyll content. We concluded that *Stg2* is the most important QTL controlling stay green, explaining the maximum amount of phenotypic variation. This report further strengthens our view to target the *Stg2* QTL region for gene discovery in order to improve the basic understanding of the stay green phenomenon, which might be helpful in manipulating this trait not only in sorghum but also in other cereal crop species.

Key words Drought tolerance · Genetic mapping · Gene interaction · Marker-assisted selection

Communicated by H.F. Linskens

P.K. Subudhi · H.T. Nguyen (✉)
Plant Molecular Genetics Laboratory,
Department of Plant and Soil Science, Texas Tech University,
Lubbock, TX 79409-2122, USA
e-mail: henry.nguyen@ttu.edu
Fax: +1-806-742-2888)

D.T. Rosenow
Texas A & M University Agricultural Research and Extension Center,
Route 3, Box 219, Lubbock, TX 79401, USA

Introduction

The excellent drought tolerance of sorghum makes it one of the most important food and feed crops in the arid and semi-arid regions of the world. Development and utilization of crop cultivars adapted to drought-prone conditions is a long-term solution for improving and stabilizing crop productivity. According to Bohnert et al. (1995), little progress has been documented on the use of specific physiological traits to enhance drought tolerance, partly because of the poor understanding of the physiological mechanisms associated with it. Drought response in sorghum has been characterized at both pre- and post-flowering stages. Drought stress during the post-flowering stage needs serious consideration because the negative impact of post-flowering drought on yield can be very drastic. Yield reduction can result from the loss of yield associated with premature plant death, stalk rot, lodging and the reduced seed size of post–flowering drought-susceptible cultivars.

The term 'stay green' has been used to describe the post–flowering drought tolerance response in sorghum (Rosenow and Clark 1981). This is a mechanism of drought tolerance characterized by the maintenance of

green stems and upper leaves when water is limiting during grain filling. The expression of post-flowering symptoms becomes more prominent when crop growth is favorable prior to flowering, followed by severe moisture stress, particularly during the grain-filling stage. 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). Since grain yield and lodging are critically important to producers and are often related, the stay green trait should have a major direct benefit to sorghum producers by reducing moisture stress-type lodging associated with premature leaf and stalk death. Lodging often results in a complete grain loss from the lodged plants in mechanized agriculture, as in the USA. Grain yield is an important consideration when attempting to improve drought tolerance. The stay green trait does not appear to reduce the grain yield of hybrids, while at the same time it improves lodging resistance and resistance to diseases like charcoal rot (Borrell et al. 2000). Stay green genotypes also contain more cytokinins (McBee 1984) and basal stem sugars (Duncan 1984) than senescent genotypes. Increased accumulation of soluble sugars in stay green types appeared to be associated with a greater functional leaf area during grain filling, thereby reducing their dependence on stored assimilates from the stem to fill the grain (Duncan et al. 1981, McBee 1984). A higher concentration of stem sugars improves the digestible energy content of the stover, making stay green a valuable trait for both grain and fodder production in dual purpose sorghums (Oosterom et al. 1996). If photosynthesis is maintained for longer in stay green types, they may yield more in crops for which carbohydrate is a main harvest component (Thomas and Smart 1993). A direct relationship between senescing tissue and susceptibility to stalk rots was also found (Katsanos and Pappelis 1965). Dodd (1980) hypothesized that probably root and stalk rots begin with the senescence of root tissue due to a decline in the supply of carbohydrate. The rate of photosynthesis and the subsequent translocation of carbohydrates to the roots affect the predisposition to infection. The above information highlights the importance of manipulation in the stay green trait in sorghum not only to improve drought tolerance during the post-anthesis stage but also to improve the quality of the product in dual-purpose sorghum.

Stay green can be manipulated in sorghum and is quite independent of grain yield (Rosenow, personal communication). Specific drought responses have been observed to be heritable and can be transferred through conventional breeding methods. In some lines (e.g. B35) the stay green trait appears to be dominant in F_1 hybrids, while in others (e.g. R9188) it appears to be recessive (Rosenow 1984; Rosenow et al. 1988). Further experiments conducted using a cross combination B35 (stay-green inbred line) x Tx7000 (non-stay green inbred line) in our laboratory revealed the dominant action of major genes for this trait (Walulu et al. 1994). Broad-sense and narrow-sense heritability estimates were 0.80 and 0.60, respectively, indicating that the stay green trait is heritable and progress from selection can be attained.

The progress in improving drought tolerance in sorghum with traditional breeding methods has been slow. Stay green QTLs and the markers linked to them have been identified (Crasta et al. 1999; Xu et al. 2000) in order to accelerate breeding activities for the incorporation of drought tolerance into elite cultivars. However, there is an urgent need to understand the expression of this important trait at the molecular level. Sorghum is a diploid grass species (2n=20) with a relatively small genome (748–772 Mbp, Arumuganathan and Earle 1991) and is considered as a model species for drought-tolerance studies due to its inherent drought-tolerance characteristics. To achieve this, we moved a step ahead through this paper in order to determine the consistency and importance of stay green QTLs that were identified in two RIL mapping populations in different environments and a different genetic background, analyzing the individual recombinant inbred lines in detail.

In this paper we report the consistency of QTLs controlling stay green, an important post-flowering-drought tolerance trait across different environments and different genetic backgrounds in grain sorghum. The QTL regions or markers showing significant epistatic interaction in the expression of the stay green trait were also identified. It was concluded that the *Stg2* QTL that was identified consistently in two different genetic backgrounds and in all the environments studied is the most important QTL, and should be a focus for further investigation. This report sets the stage for targeting this *Stg2* QTL region for the discovery of genes controlling stay green.

Materials and methods

Plant materials and extension of molecular map

An F_7 RIL (recombinant inbred line) population from the cross B35 x Tx7000 was used for this study. The development of this RIL mapping population was earlier described by Xu et al. (2000). B35, a BC1 derivative of IS12555, durra sorghum from Ethiopia, responds distinctly differently to the drought at pre-flowering and post-flowering stages compared to Tx7000. It is susceptible to pre-flowering drought and highly resistant to post-flowering drought (stay green trait) with a relatively low yield potential. Tx7000 is an elite high yielding public line commonly used for developing sorghum hybrids in the United States and is resistant to pre-flowering drought but very susceptible to post-flowering drought.

The map of B35 x Tx7000, which was developed earlier by Xu et al. (2000) was expanded further by the addition of 91 RFLP, SSR, and RAPD markers. Additional RFLP clones were obtained from Dr. Gary Hart (TXS clones) and Dr. Andrew Paterson (pSB clones) at Texas A & M University, Maize RFLP Laboratory, University of Missouri-Columbia, and Dr. S.R. McCouch, Cornell University.

Post-flowering stress evaluation and environments

The parental lines and 98 F_7 RILs of B35 x Tx7000 were evaluated under post-flowering drought stress conditions at Lubbock dryland and Halfway limited-irrigation in 1997 and 1998, respectively. The

field trials used a randomized complete block design with three replications and single row plots of 4.9-m long and 1.0-m apart. All limited-irrigation trials were irrigated adequately up to the flowering stage; however, irrigation was withdrawn just before anthesis to allow moisture stress to develop during the grain-development stage. No irrigation was provided in the dryland trial. The use of RILs allowed us to accurately evaluate the stay-green and drought-stress responses at multiple locations in different years. The stay-green expression of individual RILs, along with parental lines, were estimated visually on a scale of 1 to 5 based on the degree of premature leaf and plant death at physiological maturity on a plot basis in the field under post-flowering drought stress. A rating of 1 indicates essentially no leaf death, while the rating of 5 corresponds to complete plant (leaves and stem) death. Visual ratings of leaf and plant senescence have been demonstrated to be a reliable indicator of the stay green response (Wanous et al. 1991).

Data analysis

The additional markers were placed on to the existing map of B35 x Tx7000 by using computer program MAPMAKER Macintosh Version 2.0 (Lander et al. 1987) and the Haldane mapping function (Haldane 1919). Standard statistical procedures, such as analysis of variance, frequency distribution and correlation, were done according to Steel and Torrie (1980). The trait-value in most cases was log-transformed to more closely fit a normal distribution. Simple interval mapping with MAPMAKER/QTL (Lander and Botstein 1989) was performed to identify QTLs for the two traits of interest, stay green and chlorophyll content. For the chlorophyll content, the data employed by Xu et al. (2000) was used, which included the data from Halfway 1993, 1994, and Lubbock 1993, 1994. Initially RIL mean-values of Lubbock dryland 1997 and Halfway 1998 were used for identifying the QTLs. Finally, the pooled means over years 1993, 1994, 1997 and 1998 (data over five environments during 1993 and 1994 from Xu et al. 2000) were also used for drawing conclusive evidence for the segregation of the stay green QTLs. A LOD threshold of 2.4 was employed to declare the presence of a putative QTL in a given genomic region for both traits. This threshold level corresponds to a test of the presence of a QTL at the 0.05 level of significance according to the ”sparse-map” case (Lander and Botstein 1989). However, the consistent QTLs with a lower LOD score were also reported. For comparing the QTL location for stay green, the information already published by Crasta et al. (1999) and Tuinstra et al. (1997) was used. The best and poorest stay green RIL lines from the B35 x Tx7000 population were identified, taking into consideration the average stay green rating over all the years, such as 1993, 1994, 1997 and 1998. The QTL profiles of those lines were determined from the raw marker data to identify the major and consistent QTLs for expression of the stay green trait. The location of the QTLs for this population was compared with the QTLs for stay green identified by Crasta et al. (1999) and Tuinstra et al. (1997). All four stay green QTLs were fitted into the multiple QTL model in all possible combinations to determine the percent phenotypic variation explained, as well as the interaction among them. In order to detect the significant 2-locus interaction among the markers for stay green and chlorophyll content, the computer program Q-gene (Nelson 1997) was employed and a threshold *F*-value of 10.0 was used to declare significant interactions. This program was also used to perform single-marker analysis in order to detect association of the marker with the trait of interest.

Results

Mapping stay green in additional environments

We have re-evaluated the B35 x Tx7000 RIL population during 1997 and 1998 in two different locations. Analysis of the 1997 data on stay green rating identified *Stg2* and *Stg3* in the same linkage groups A and D, respectively, by interval mapping using the MAPMAKER-QTL program. However, another new QTL on linkage group E (interval UMC85–TXS644) was identified with a LOD score of 2.22 and with 13.6% phenotypic variance explained (PVE). The single-marker analysis also detected a significant correlation between the stay green rating and the markers in the *Stg1* region. Data on the stay green-scoring for 1998 (Halfway location) indicated the consistency of *Stg1* and *Stg2*. In both years the LOD score for *Stg3* was lower that 2.0. However, these markers were significantly correlated in single-marker analysis at the P=0.05 level and was consistent. Therefore, we retained these QTLs in our analysis. We also carried out a QTL analysis using the mean values of stay green scores over 1993, 1994, 1997 and 1998, which included seven environments. This analysis detected all four stay green QTLs, *Stg1*, *Stg2*, *Stg3* and *Stg4*. All the stay green QTLs except *Stg4* were identified at a LOD score higher than 3.5. Irrespective of environment, *Stg2* explained the highest % of phenotypic variation (29.2%) with the highest LOD (LOD score 5.52) and a higher additive effect (Table 1). The mean over several environments was found to increase the precision of QTL mapping through increasing the LOD scores as well as the of percentage phenotypic variation explained. The chromosomal regions bracketing the NPI414 and TXS1114, and the WG889 and TXS584, markers were concluded to harbor the stay green QTLs *Stg1* and *Stg2*, respectively.

Consistency of QTLS in different environments and genetic backgrounds

We have summarized the QTL analysis done in the two RIL mapping populations and observed that two QTLs are completely consistent between both populations (Fig. 1). These are *Stg2* and *Stg4* of the B35 x Tx7000 RIL population (Xu et al. 2000), which correspond to *StgA* and *StgJ* of the B35 x Tx430 population (Crasta et al. 1999), respectively. *Stg2* in both populations explained the highest percentage of phenotypic variation. Even *Stg3* of the B35 x Tx7000 population was close to the *StgD2* of Crasta et al. (1999). Although QTL analysis using interval mapping did not detect *Stg1* in the B35 x Tx430 population, single-marker analysis revealed that markers bracketing this QTL in the B35 x Tx7000 population were highly correlated with the stay green ratings in the B35 x Tx430 population. Tx430 contributed favorable alleles for the stay green trait at two QTL regions, *StgB* and *StgI.1*. The two QTLs *StgG* and *StgI.2* were not identified in the B35 x Tx7000 population. Overall comparative analysis reflects the consistency of all the major QTLs of the B35 x Tx7000 population with that of B35 x Tx430. The discrepancy may be due to the different threshold LOD values and QTL mapping programs used in identifying QTLs. Our

Table 1 Quantitative trait loci for stay-green rating and chlorophyll content under post-flowering drought stress in sorghum RILs of the cross B35 x Tx7000

Trait and location[a]	Flanking markers	Linkage groups	Interval length[c]	QTL position	Additive effects[d]	Peak LOD score	% Variance explained
SG97DLL[b]	RZ323-A12RFLP	A	2.2	2.0	0.0838	2.65	14.0
	UMC5-UMC116	D	5.5	2.0	0.0728	1.90	10.7
	UMC85-TXS644	E	7.2	4.0	0.0821	2.22	13.6
	Total					6.48	32.6
SG98HW	NPI414-BNL15.20	A	4.4	2.0	0.2333	3.18	15.4
	WG889-TXS584	A	12.8	6.0	0.2677	3.66	19.9
	BNL15.40-PSB605	D	7.2	0.0	0.1850	1.91	9.1
	Total					6.86	30.1
SG3478[b]	NPI414-BNL15.20	A	4.4	3.2	0.0205	3.61	18.1
	WG889-TXS584	A	12.8	5.5	0.0703	5.52	29.2
	TXS1307-UMC5	D	0.7	0.0	0.0573	3.49	17.5
	Txs387-CSU166C	J	14.8	0.3	0.0305	1.81	9.4
	Total					12.70	53.5
CHL34	CSU143-dhn6	A	7.1	5.9	–2.0755	3.67	16.9
	WG889-TXS584	A	12.8	2.3	–5.2845	5.44	22.6
	TXS1307-UMC5	D	0.7	0.7	–4.4913	2.80	12.4
	Total					8.56	34.6

[a] SG97DLL: stay green rating at Lubbock dryland 1997, SG98HW: stay green rating at Halfway 1998, SG3478: mean stay green rating over seven stress environments such as Lubbock 1993, Halfway 1993, Lubbock 1994, Lubbock dryland 1994, Halfway 1994, Lubbock dryland 1997, and Halfway 1998. CHL34: mean chlorophyll rating of Lubbock 1993, Halfway 1993, Lubbock 1994, and Halfway 1994

[b] The data was log transformed to ensure normality

[c] Interval estimated at a LOD fall of –1.0

[d] Additive effect of every allele of Tx7000

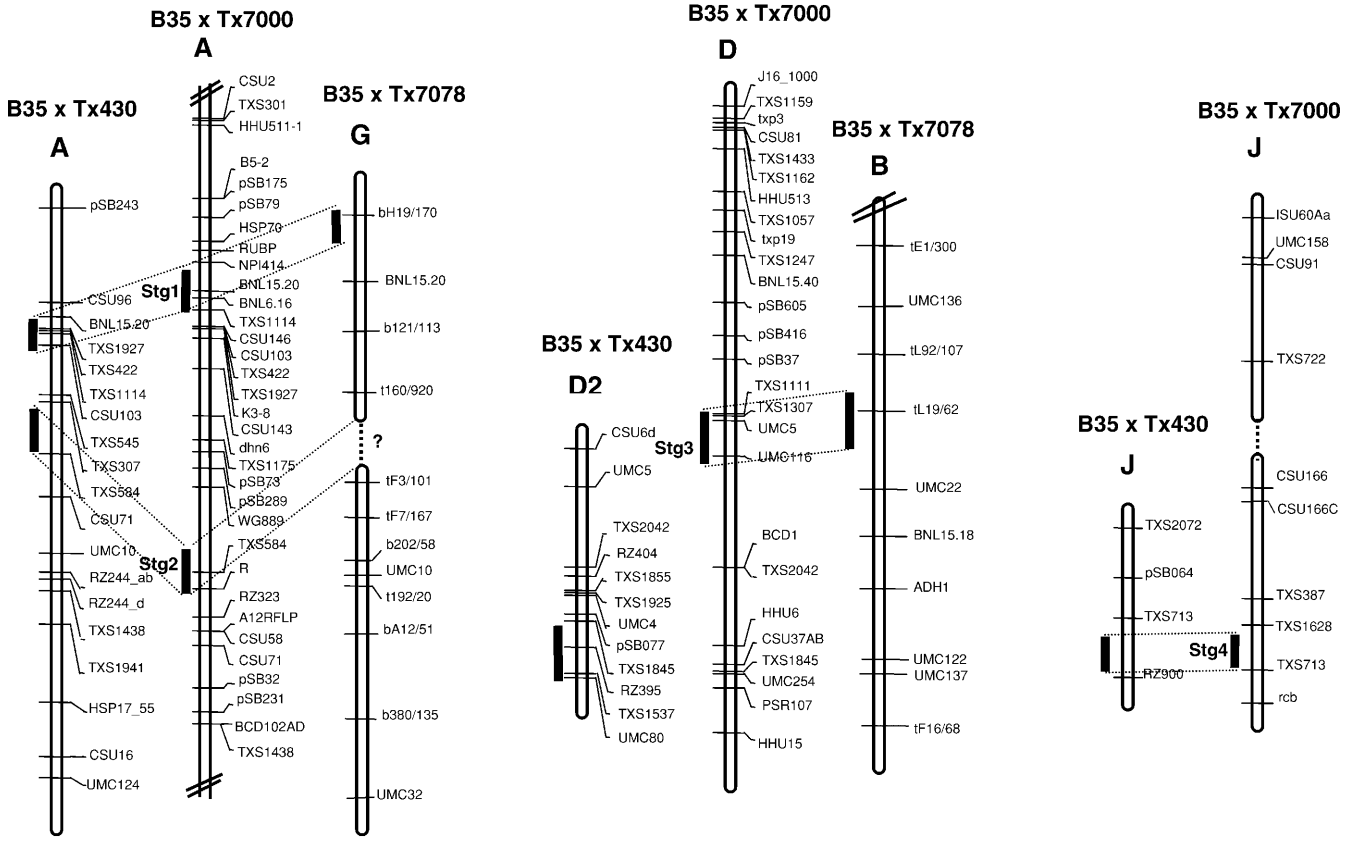

Fig. 1 Linkage groups of sorghum showing the consistency of stay green QTLs in the B35 x Tx7000, B35 x Tx430 and B35 x Tx7078 RIL populations. The map of the B35 x Tx7000 population (Xu et al. 2000) was expanded by the addition of 91 more markers and the QTL analysis was undertaken using pooled mean data on stay green rating over seven environments, whereas the information on stay green QTLs in the B35 x Tx430 population was from Crasta et al. (1999) and in the B35 x Tx7078 population from Tuinstra et al. (1997). A partial linkage map was shown in the case of linkage groups A and B of the B35 x Tx7000 and B35 x Tx7078 populations respectively. The *dotted lines* indicate the corresponding QTL location in other populations. The nomenclature of stay green QTLs such as *Stg1*, *Stg2*, *Stg3*, and *Stg4* was adopted from Xu et al. (2000)

Table 2 Significant epistatic (2-locus) interaction for stay-green and chlorophyll content revealed by Q-gene analysis

Trait: SG3478[a]					
Marker pair	*F*-value[b]	aabb[c]	AAbb	aaBB	AABB
TXS584 x BNL5.71 (linkage group C)	16.74	3.30	**2.52**	2.88	2.79
UMC116 x TXS1695 (linkage group B)	11.87	2.86	2.74	3.19	**2.41**
TRAIT: CHL34[d]					
TXS584 x TXS1090 (linkage group C	19.60	30.84	**41.69**	35.76	35.56
WG889 x TXS1090 (linkage group C)	20.60	30.24	**42.61**	34.76	36.50
dhn6 x TXS1224 (linkage group C)	10.90	31.42	**40.97**	36.95	38.28
UMC5 x PSB416 (linkage group D)	11.19	33.54	**45.71**	39.58	38.10

[a] Average stay green rating over seven stress environments during 1993,1994,1997 and 1998
[b] A threshold *F*-value of 10.0 was used to declare the interactions significant
[c] A-a: alleles of first marker, B-b: alleles of the second marker, lower-case letters denote the Tx7000 allele and upper case letters denote the B35 allele. Values in bold indicate favorable interaction most likely to favour drought tolerance for both stay green and chlorophyll content
[d] Average chlorophyll rating over four stress environments during 1993 and 1994

experience in this investigation reveals that it is sometimes useful to report the consistent QTLs even with a lower LOD score that might have gone undetected otherwise.

Six stay green QTLs were identified in a random amplified polymorphic DNA (RAPD)-based map by Tuinstra et al. (1997). Since they used only 20 RFLP markers, we could not work out the correspondence of their exact stay green QTL locations with those of ours. However, our linkage groups A, B, C, D, E, G and I may correspond to their linkage groups G, E, N, B, D, F and K, respectively. Our *Stg1* may be equivalent to their QTL on linkage group G (Fig. 1). Similarly, *Stg3* might correspond to one of the two stay green QTLs identified by them on linkage group B.

Interaction among the QTLs in B35 x Tx7000

We used two different approaches to study the interactions among the stay green QTLs. In one approach different combinations of QTLs were fit into a multiple regression model to determine the percentage of explained phenotypic variation (PV) (Table 3). In another approach, we used the Q-gene program (Nelson 1997) to study the significant two-locus interactions for stay green rating. We used the mean of the stay green score over seven stress environments (Lubbock 1993, Halfway 1993, Lubbock 1994, Halfway 1994, Lubbock dryland 1994, Lubbock dryland 1997 and Halfway 1998) for this analysis. All four stay green QTLs, *Stg1*, *Stg2*, *Stg3*, and *Stg4*, together explained 53.5% of the phenotypic variation. A multiple regression fit of all possible three QTL combinations revealed that *Stg2+Stg3+Stg4* explained 53.1% of the phenotypic variation, followed by *Stg1+Stg2+Stg3* (49.8% of PV), *Stg1+Stg2+Stg4* (38.6% of PV) and *Stg1+Stg3+Stg4* (36.9% of PV). Similarly, among all possible two-QTL combinations analyzed, the best QTL combination was *Stg2+Stg3* explaining 49.6% of the phenotypic variation, followed by *Stg2+Stg4* (34.8%), *Stg1+Stg2* (33.7%), *Stg1+Stg3* (31.8%), *Stg3+Stg4* (25.2%) and *Stg1+Stg4* (25.1%). It may be noted that the *Stg2+Stg3* combination explained even more than the sum of the phenotypic variation for individual QTLs. This shows clear evidence of an interaction between the stay-green QTLs. Further, among these stay-green QTLs, *Stg2* is the most important of which singly explains 30% of the phenotypic variation with a LOD score of 5.52.

Similarly, we also analyzed the chlorophyll rating using the mean data of four environments (Lubbock 1993, Halfway 1993, Lubbock 1994, and Halfway 1994) and the expanded molecular map. The MAPMAKER-QTL analysis result was almost the same with identification of all the *QTLs Chl1*, *Chl2*, and *Chl3* (Xu et al. 2000). The latter two QTLs were almost in identical locations whereas *Chl1* was located on the molecular map (CSU143-dhn6) very close to the earlier identified location (NPI414-BNL15.20). All three QTLs for chlorophyll content together explained 34.6% of the phenotypic variation whereas a two-QTL combination analysis revealed that *Chl2* and *Chl3* interacted additively, explaining 33.3% of phenotypic variation. This is close to the total phenotypic variation explained by all three *Chl* QTLs, followed by *Chl1+Chl3* (27.2% PV) and *Chl1+Chl2* (24.7% PV). The additive effect of each of the alleles is also highest in the case of *Chl2*, followed by *Chl3* and *Chl1*. This again provided strong evidence that *Chl2*, which is located in the same region as *Stg2*, is the most important QTL.

Using the Q-gene program, and the mean data, several significant 2-locus interactions were detected (Table 2). A threshold F-value of 10.0 was used for this analysis. The F-value and the phenotypic value of all possible four two-locus genotypes are given in Table 2. Most of the markers in the *Stg2* region showed epistatic interaction with the marker BNL5.71 or with TXS1090 on linkage group C for the traits stay green and chlorophyll rating. The most significant interaction was that of TXS584 with BNL5.71 (F-value of 16.74) for the stay green trait. Both markers TXS584 and WG889 of *Stg2* showed a significant interaction with the marker TXS1090 (closely linked to BNL5.71 on linkage group C) with *F*-values of 19.60 and 20.60, respectively.

A marker from the *Stg3* region, UMC116, showed significant interaction with TXS1695 on linkage group B, whereas UMC5 showed an interaction with pSB416 on the same linkage group for the chlorophyll rating. The marker BNL6.16 on *Stg1* and *Chl1* also interacted significantly with CSU37CE on linkage group C. In all cases the favorable 2-locus combination was AAbb (where A is the B35 allele of the first marker and b is the Tx7000 allele of the second marker). The only exception was for UMC116 x TXS1695, where the B35 allele at both loci was favorable to improving stay green rating. However, the worst combination for all these 2-locus interactions was aabb (Tx7000 alleles in both loci).

Table 3 Percent phenotypic variation explained by different combination of QTLs for stay-green and chlorophyll rating. QTLs were fit into multiple regression models for calculating the % phenotypic variation explained (PVE)

SG3478[a]	QTL combination	LOD score	% PVE
	Stg1+Stg2+Stg3+Stg4	12.70	53.5
	Stg2+Stg3+Stg4	12.19	53.1
	Stg1+Stg2+Stg3	11.28	49.8
	Stg1+Stg2+Stg4	8.37	38.6
	Stg1+Stg3+Stg4	8.27	36.9
	Stg2+Stg3	10.70	49.6
	Stg2+Stg4	7.31	34.8
	Stg1+Stg2	6.85	33.7
	Stg1+Stg3	6.89	31.8
	Stg3+Stg4	5.13	25.2
	Stg1+Stg4	5.10	25.1
CHL34[b]	*Chl1+Chl2+Chl3*	8.56	34.6
	Chl2+Chl3	8.26	33.3
	Chl1+Chl3	6.56	27.2
	Chl1+Chl2	5.68	24.7

[a] Average stay-green rating over seven stress environments during 1993, 1994, 1997 and 1998. The data was log-transformed to ensure normality

[b] Average chlorophyll rating over four stress environments during 1993 and 1994

QTL profiles of the best and poorest stay green RILs

Quantitative trait-loci profiles of the best stay green and poorest stay green lines of the B35 x Tx7000 population were tabulated to confirm the consistency and importance of the identified QTLs (Table 4). The stay green QTL profiles indicated that all four stay green QTLs were present in most of the best stay green lines, but absent in the poorest stay green lines. However, one of the best, RIL # 21 (with a stay green rating of 1.7), has only *Stg2* and *Stg3*, whereas two other RILs # 56 and # 74 with *Stg1* and *Stg2* have stay green ratings of 2.29 and 2.09 respectively, which is higher than the score of RIL # 21. This further indicated that the *Stg2, Stg3* QTL combination was better than the *Stg1 and Stg2* combination. In another exception, we noticed that RIL # 50 harboring *Stg1* and *Stg4* showed the worst stay green rating consistently over all environments (mean of 4.21). This clearly indicated the presence of an epistatic interaction among these QTLs making it closer to the non-stay green category.

In a similar manner we analyzed the QTL profile of seven each of the best and poorest stay green lines of the

Table 4 Quantitative trait locus (QTL) profiles and stay-green response of RILs of the B35 x Tx7000 population

RIL #	Stg1[a]	Stg2	Stg3	Stg4	Mean stay green rating[b]
Best stay green lines					
8	+	+	+	+	2.05
21	–	+	+	–	1.70
40	+	+	+	+	1.75
42	+	+	+	+	1.95
52	+	+	–	+	1.82
56	+	+	–	–	2.29
62	+	+	+	+	1.78
74	+	+	–	–	2.09
77	+	+	+	+	1.99
80	+	+	+	+	1.93
Poorest stay green lines					
18	–	–	–	–	3.20
19	–	–	–	–	3.39
50	+	–	–	+	4.21
63	–	–	–	–	3.33
95	–	–	–	–	3.41
13	–	–	+	-+	3.11
58	+	–	+	–	3.21
81	–	–	–	+	3.15
85	–	+	–	+	3.19
94	–	—	–	–	3.10
B35 (stay green)	+	+	+	+	1.83
Tx7000 (non-stay green)	–	–	–	–	4.42

[a] The presence and absence of QTLs is denoted by '+' and '–' respectively

[b] Mean stay green rating over seven environments during 1993, 1994, 1997,1998; a stay green rating of 1 indicates no leaf and plant death, whereas a rating of 5 indicates complete plant death

B35 x Tx430 RIL population (data not shown). This revealed that five out of the seven best stay green lines have QTL *SGA*, which is equivalent to *Stg2* of the B35 x Tx7000 population, but this is absent in all of the poorest stay green lines. This further reinforces our conviction that *Stg2* is the most important QTL for stay green expression.

Discussion

Consistency of stay green QTLs

Plant responses to water stress are clearly influenced by the timing and intensity of stress (Ludlow and Muchow 1990), indicating a genotype x environment interaction which makes the genetic analysis of such traits more complicated. Therefore, in order to minimize environmental error and to arrive at a meaningful conclusion, mean values over several environments is most ideal. The higher the number of environments, the more reliable is the data, and the result from QTL mapping. In this study, we analyzed the stay green data over two environments but the most significant result was obtained by combining these two environments with earlier data collected over five environments, and analyzing with a more comprehensive and expanded molecular map for QTL identification. We further confirmed all four stay green QTLs identified earlier (Xu et al. 2000) explaining 53.5% of the phenotypic variation. With regard to the chlorophyll content, three *Chl* QTLs were identified using the same data over four environments in place of the two earlier identified QTLs. These three QTLs coincided exactly with the *Stg1*, *Stg2*, and *Stg3*, respectively. This result increased our confidence in arriving at the conclusions for the localization of the stay green QTLs. It is hypothesized that probably the factors responsible for the maintenance of stability and the continuity of chlorophyll synthesis confer stay green in crop plants.

Stay green is a classic example of a quantitative trait showing continuous variation (Crasta et al. 1999). Previous studies indicated that between 4 (Xu et al. 2000) and 6–7 (Tuinstra et al. 1997; Crasta et al. 1999) genes/QTLs are responsible for the expression of this trait. The total phenotypic variation explained by these QTLs ranged from 46% (Xu et al. 2000) to 66.5% (Crasta et al. 1999) and 52.6% (Tuinstra et al. 1997). In both studies, the same stay green donor B35 was used in mapping stay green QTLs. After comparing our results with the above previously reported results, we concluded that all four QTLs identified in this study are consistent over environments or genetic backgrounds. *Stg1* and *Stg3* might be same QTLs as those identified by Tuinstra et al. (1997) (Fig. 1). However, the major QTL *Stg2* was not identified in their study, which might be due to incomplete genome coverage in this region. Quantitative trait loci *Stg2* and *Stg4* were consistent in both the B35 x Tx7000 and B35 x Tx430 populations (Crasta et al. 1999). The parents, B35 and Tx7000, used by us are the most contrasting parents employed so far for stay green QTL mapping. The field performance of both Tx430 and Tx7000 in post-flowering stress environments indicated that Tx430 (with an SG score of 3.4) (Crasta et al. 1999) is less susceptible compared to Tx7000 (with an SG score of 4.9) (Xu et al. 2000). A similar case is Tx7078, the non-stay green line used by Tuinstra et al. (1997). Thus, we believe that the QTL information generated from this RIL population that was evaluated over seven environments should be most informative to determine the most important QTL responsible for this trait for future investigation. Before we undertake any fine-mapping activity, which is usually followed by the physical mapping and cloning of genes, we need to justify the importance of the QTLs. Although most stay green QTLs were found to be consistent, the most important QTL is *Stg2*. This QTL was consistent over all the environments, as well as in different genetic backgrounds, and explained the highest percent of phenotypic variation (~30%) in this study as well as that of Crasta et al. (1999).

Interaction among the QTLs and markers influencing the stay green rating

While practicing marker-assisted selection (MAS), knowledge regarding QTL interaction is helpful for the effective manipulation of this trait. Identifying QTLs based on means over several environments seems to provide the best strategy for detecting QTLs for use in a marker-assisted selection program. From this investigation, we noticed that *Stg2* and *Stg3* interacted together most favorably explaining 49.8% of the phenotypic variation. Those two QTLs were also identified with a higher LOD score, and the additive effect of alleles was also higher compared to other two QTLs. The stay green QTLs *Stg2*, *Stg3*, and *Stg4* together explained the same amount of phenotypic variation as the four QTLs together. This indicated that introgression of the QTL *Stg1*, whose contribution was more, may not be having any significant advantage when combined with other QTLs. Therefore, theoretically, incorporation of both *Stg2* and *Stg3* should improve stay green expression. The other important finding is the detection of significant 2-locus interactions for this trait. Many markers in the *Stg2* region interacted significantly with the markers BNL5.71 or TXS1090 (both are closely linked on linkage group C). The most significant interaction is that of TXS584 which is the closest marker to *Stg2*. When introgressing B35 alleles in the *Stg2* region, it is necessary to ensure Tx7000 alleles in the BNL5.71/TXS1090 region to improve post-flowering drought tolerance. Another important 2-locus interaction noted was UMC116 (linked to *Stg3* on linkage group D) with TXS1695 (linkage group B). Similarly, when we analyzed the chlorophyll rating, the *Chl2*-linked markers also interacted with TXS1090 in a significant manner further reinforcing our belief. We found that two more markers for *Stg1* interacted significantly with TXS1224 and CSU37CE of linkage group C.

Implications and future prospect of cloning the stay green QTLs

Due to the advent of molecular-marker technology, it is now possible to make a systematic search of the whole genome to identify regions associated with traits of interest that are quantitative in nature. Many studies involving quantitative traits (Paterson et al. 1988; Dorweiler et al. 1993; Doebley et al. 1995) revealed that few major QTLs contribute to the major portion of the total phenotypic variation. These QTLs and some of lesser effect (minor QTLs), together with the environment, are responsible for the expression of most quantitative traits. In most current studies involving complex traits, due to environmental and epistatic influences the QTLs are mapped with only low resolution (>10 cM intervals in the genome) (Tanksley 1993). Such large chromosomal regions most likely contain clusters of genes spanning millions of base pairs of DNA, so it is difficult to ascertain whether individual effects are due to a single gene or a group of genes. Therefore, mapping of these QTLs down to sub-centimorgan intervals and as Mendelian factors is a prerequisite before initiating the map-based cloning of these factors. Alpert and Tanksley (1996) for the first time mapped a consistent fruit-weight QTL, *fw*2.2, accounting for 5–30% of the phenotypic variance (Paterson et al. 1991; Alpert et al. 1995) with great precision and delimited it to a 150-kb cloned DNA of tomato. Similarly, Yamamoto et al. (1998) mapped the heading-date QTLs in rice as Mendelian factors. With the importance and consistency of *Stg2* and other QTLs for the stay green trait ascertained through the present paper, we are now developing a large segregating population involving near-isogenic lines (NILs) for precision mapping. Fine mapping of the major QTL *Stg2* may also help in understanding the evolutionary relationship between QTLs in different species. It was observed that though *Stg1* alone accounts significantly for phenotypic variation, it does not act favorably in combination with other stay green QTLs. Further research is needed to determine how the stay green QTLs alone, or in different combinations, express themselves in a near-isogenic background under post-flowering stress environments. This may improve our general understanding regarding genetic interaction among quantitative trait loci, which at present is poorly understood.

Recent comparative molecular mapping studies revealed an unexpected closeness with respect to gene content and DNA sequence among large members of the grass family (Bennetzen and Freeling 1993; Moore et al.1995). Further investigation may lead to discovery of orthologous QTLs for this useful trait in other related grass species, where the drought-tolerance phenomenon in general and stay green in particular is not well understood. This latter trait is very important in many grass relatives, which are subjected to stress environments during their growth stage. Discovery of a major, conserved locus controlling the stay green trait may not only facilitate breeding for this character in sorghum but may ultimately lead to the molecular cloning of this important locus. This investigation will have great impact in the future, opening the door to an understanding of the molecular biology of drought-induced senescence in general and, potentially, to the genetic engineering of drought tolerance. Therefore, an understanding of the stay green phenomenon in sorghum at the molecular level will have a tremendous impact for improving drought tolerance in other grass species as well.

Acknowledgements We thank Dr. G. Hart, Dr. A. Paterson (Texas A & M University), Dr. Ed Coe (University of Columbia, Missouri), and Dr. S. McCouch and Dr. M. Sorrells (Cornell University) for providing the probes for RFLP mapping. Thanks are also due to Mr. Charles Woodfin (Texas A & M University Agricultural Research and Extension Center, Lubbock) for his help in conducting the field experiments. This work was supported by a grant from the U.S. Department of Agriculture Plant Genome Program (Grant No. 96-35300-3722). This is contribution number T-4–474 of the College of Agricultural Sciences and Natural Resources, Texas Tech University.

References

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw*2.2: a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Alpert KB, Grandillo S, Tanksley SD (1995) fw2.2: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. Theor Appl Genet 91:994–1000

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important species. Plant Mol Biol Rep 9:208–218

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, colinearity and compatibility. Trends Genet 9:259–261

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptation to environmental stresses. Plant Cell 7:1099–1111

Borrell AK, Hammer GL, Henzell RG (2000) Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Sci (in press)

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

Dodd JL (1980) The photosynthetic stress-translocation balance concept of sorghum stalk rots. In: Sorghum diseases, a world review. Proc Intl Wkshp on Sorghum Diseases, sponsored jointly by Texas A & M university (USA) and ICRISAT, Patancheru, AP, 502324, India, pp 300–305

Doebley J, Stec A, Gustus C (1995) *Teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Dorweiler J, Stec A, Kermicle J, Doebley J (1993) *Teosinte glume architecture1:* a genetic locus controlling a key step in maize evolution. Science 262:233–235

Duncan RR (1984) The association of plant senescence with root and stalk diseases in sorghum. In: Mughogho LK (ed) Sorghum root and stalk rots, a critical review. ICRISAT, Patancheru, AP, India, pp 99–110

Duncan RR, Bockholt AJ, Miller FR (1981) Descriptive comparison of senescent and nonsenescent sorghum genotypes. Agron J 73:849–853

Haldane JBS (1919) The combination of linkage values, and the calculation of distance between the loci of linked factors. J Genet 8:299–309

Henzell RG, Dodman RL, Done AA, Brengman RL, Mayers PE (1984) Lodging, stalk rot, and root rot in sorghum in Australia.

In: Mughogho LK (ed) Sorghum root and stalk rots, a critical review. Proc Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases, Bellagio, Italy, 27 Nov–2 Dec 1983. ICRISAT, Patancheru, AP, India, pp 225–235

Katsanos RA, Pappelis AJ (1965) Seasonal trends in density and cell death in sorghum stalk tissue. Phytopathology 55:97–99

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–189

Lander ES, Green P, Abrahamson A, Barlow A, Daly WJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43:107–153

McBee GG (1984) Relation of senescence, nonsenescence, and kernel maturity to carbohydrate metabolism in sorghum. In: Mughogho LK (ed) Sorghum root and stalk rots, a critical review. Proc Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases, Bellagio, Italy, 27 Nov–2 Dec 1983. ICRISAT, Patancheru, AP, India, pp 119–129

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Nelson JC (1997) QGENE:software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Oosterom EJ van, Jayachandran R, Bidinger FR (1996) Diallel analysis of the stay green trait and its components in sorghum. Crop Sci 36:549–555

Paterson AH, Damon S, Hewitt JD, Zamir D, Robinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127: 181–197

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete map of restriction fragment length polymorphisms. Nature 335:721–726

Rosenow DT (1984) Breeding for resistance to root and stalk rots in Texas. In: Mughogho LK (ed) Sorghum root and stalk rots, a critical review. Proc Consultative group discussion of research needs and strategies for control of sorghum root and stalk diseases, Bellagio, Italy, 27 Nov –2 Dec 1983, ICRISAT, Patancheru, AP, India, pp 209–217

Rosenow DT (1987) Breeding sorghum for drought resistance. In: Menyonga JM, Bezuneh T, Youdeowei A (eds) Food and grain production in semi-arid Africa. Proc Intl Drought Symp, Sponsored by OAU/STRC-SFGRAD, May 19–23, 1986. Kenyatta Conf Center, Nairobi, Kenya, pp 83–89

Rosenow, DT, Clark LE (1981) Drought tolerance in sorghum. In: Proc 36th Annual Corn and Sorghum Res Conf, 9–11 Dec 1981, Chicago, Illinois, pp 18–30

Rosenow DT, Woodfin CA, Clark LE (1988) Breeding for the stay green trait in sorghum. In: Agronomy abstracts ASA, Madison, Wisconsin, p 94

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, a biometrical approach, 2nd edn McGraw-Hill Book Co, New York

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27: 205–233

Thomas H, Smart CM (1993) Crops that stay green. Ann Appl Biol 123:193–219

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

Walulu RS, Rosenow DT, Wester DB, Nguyen HT (1994) Inheritance of the stay green trait in sorghum. Crop Sci 34:970–972

Wanous MK, Miller FR, Rosenow DT (1991) Evaluation of visual rating scales for green leaf retention in sorghum. Crop Sci 31:1691–1694

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000) Molecular mapping of QTLs conferring stay green in grain sorghum (*Sorghum bicolor* L. Moench). Genome (in press)

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (1998) Fine mapping of quantitative trait loci *Hd-1*, *Hd-2*, and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44