Theor Appl Genet (2002) 106:133–142
DOI 10.1007/s00122-002-1012-3

B. I. G. Haussmann · V. Mahalakshmi
B. V. S. Reddy · N. Seetharama · C. T. Hash
H. H. Geiger

QTL mapping of stay-green in two sorghum recombinant inbred populations

Received: 5 November 2001 / Accepted: 5 April 2002 / Published online: 13 September 2002

Abstract The stay-green trait is a reported component of tolerance to terminal drought stress in sorghum. To map quantitative trait loci (QTLs) for stay-green, two sorghum recombinant inbred populations (RIPs) of 226 $F_{3:5}$ lines each were developed from crosses (1) IS9830 × E36-1 and (2) N13 × E36-1. The common parental line, E36-1 of Ethiopian origin, was the stay-green trait source. The genetic map of RIP 1 had a total length of 1,291 cM, with 128 markers (AFLPs, RFLPs, SSRs and RAPDs) distributed over ten linkage groups. The map of RIP 2 spanned 1,438 cM and contained 146 markers in 12 linkage groups. The two RIPs were evaluated during post-rainy seasons at Patancheru, India, in 1999/2000 (RIP 2) and 2000/2001 (RIP 1). The measures of stay-green mapped were the green leaf area percentages at 15, 30 and 45 days after flowering (% GL15, % GL30 and % GL45, respectively). Estimated repeatabilities for % GL15, % GL30 and % GL45 amounted to 0.89, 0.81 and 0.78 in RIP 1, and 0.91, 0.88 and 0.85 in RIP 2, respectively. The number of QTLs for the three traits detected by composite interval mapping ranged from 5 to 8, explaining 31% to 42% of the genetic variance. In both RIPs, both parent lines contributed stay-green alleles. Across the three measures of the stay-green trait, three QTLs on linkage groups A, E and G were common to both RIPs, with the stay-green alleles originating from E36-1. These QTLs were therefore consistent across the tested genetic backgrounds and years. After QTL validation across sites and verification of the general benefit of the stay-green trait for grain yield performance and stability in the target areas, the corresponding chromosomal regions could be candidates for marker-assisted transfer of stay-green into elite materials.

Keywords *Sorghum bicolor* · Drought stress · Molecular marker · QTL · Stay-green

Communicated by F. Salamini

B.I.G. Haussmann · H.H. Geiger (✉)
University of Hohenheim, 350 Institute of Plant Breeding, Seed Science, and Population Genetics, 70593 Stuttgart, Germany
e-mail: geigerhh@uni-hohenheim.de

V. Mahalakshmi · B.V.S. Reddy · N. Seetharama · C.T. Hash
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop, and staple food of millions of people in the semi-arid tropics of Africa and Asia. In these areas, rainfall is low and highly erratic, and drought stress is a major constraint to agricultural production. Dry spells can occur at any time during the growing season, but terminal drought stress is prevalent in many regions and years. The stay-green trait, i.e. delayed leaf senescence during grain ripening under drought stress conditions, has been reported as an important component of terminal drought tolerance in sorghum because it assures normal grain filling under water-limited conditions (Tenkouano et al. 1993; Walulu et al. 1994; van Oosterom et al. 1996; Borrell et al. 1999, 2000a,b; Borrell and Hammer 2000; Thomas and Howarth 2000; Xu et al. 2000). It is often associated with resistance to charcoal stalk rot [*Macrophomina phaseolina* (Tassi) Goid] (Rosenow 1984), lodging (Woodfin et al. 1988) and superior ruminant nutritional quality of grain crop residues (i.e. stover) due to a higher content of basal stem sugars (Duncan 1984; van Oosterom et al. 1996). Because of the better quality of crop residues, manipulation of the stay-green trait is also considered important for the improvement of the stover ruminant nutritional quality of dual-purpose sorghum (Tao et al. 2000). Despite all these studies, the value of the stay-green trait under true dryland conditions is not uncontested (Haussmann et al. 1999). In fact, there is some chance that the stay-green trait in at least some genetic backgrounds is simply due to small panicle size and low panicle grain number, providing a poorly developed sink (Ludlow and Muchow 1990). Consequently, it is most important to consider

Table 1 Summary of recent stay-green mapping studies

Reference	Pop.[a]	Parents	Test sites[b]	Linkage map[c]		Number of QTLs for stay-green
				LGs	Length [cM]	
Tuinstra et al. (1997)	98 RIL	B35, Tx7078	2 E in Mexico and Arizona, irrigated and post-flowering drought	17	1,580 R	6
Crasta et al. (1999)	96 RIL	B35, Tx430	4 E in Texas	14	1,602 K	7
Xu et al. (2000)	98 RIL	B35, Tx7000	5 E in Texas	10	837 H	4
Subudhi et al. (2000)	98 RIL	B35, Tx7000	2 E in Texas added to those of Xu et al. (2000)	10	91 markers added to map of Xu et al. (2000)	5
Tao et al. (2000)	152 RIL	QL41,[d] QL39	5 E in Australia	14	1,871 U	5
Kebede et al. (2001)	125 RIL	SC56, Tx7000	5 E in Texas and Kansas	10	1,355 K	9

[a] Mapping population; RIL = recombinant inbred lines
[b] E = environments (site/season combinations)
[c] LG = linkage groups; R = recombination frequency; K, H = Kosambi and Haldane mapping function, respectively; U mapping function not indicated
[d] QL41: derived from a cross of B35 × QL33

stay-green not alone, but in relation to grain production (Borrell et al. 1999).

Putative QTLs for the stay-green trait have been identified in six recently published studies (Table 1). Four studies used line B35, a BC_1 derivative of the Ethiopian durra sorghum IS12555, as source of stay-green (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000). Tao et al. (2000) exploited the stay-green line QL41, which was derived from a cross of B35 with line QL33. Only Kebede et al. (2001) have previously utilized a stay-green source, line SC56 (derived from a Sudanese caudatum-nigricans sorghum), which is not related to B35. To reduce the risk of genetic vulnerability, breeding programs should not rely on one or few sources of any trait, especially one where there could be unfavorable mechanisms contributing to its expression as is the case with the stay-green component of terminal drought tolerance in sorghum. Therefore, objectives of the present investigation were to identify and map QTLs for the stay-green component of terminal-drought tolerance in two recombinant inbred populations of sorghum derived from stay-green source line E36-1, and to study the relationships between the stay-green trait and grain yield, stover yield, total biomass and harvest index under terminal drought stress in these materials.

Materials and methods

Genetic materials

Two recombinant inbred populations (RIPs) of sorghum, each consisting of 226 F_3-derived F_5 lines ($F_{3:5}$ lines), were developed from the crosses IS9830 × E36-1 (RIP 1) and N13 × E36-1 (RIP 2). Line E36-1, the source for the stay-green trait in these RIPs, is a high-yielding breeding line assigned to the guinea-caudatum hybrid race with Ethiopian origin. Line IS9830 is a tall Sudanese feterita belonging to the caudatum race. Line N13 from India is a durra sorghum. The crosses were selfed and 226 F_2 plants per population advanced by single-seed descent to the F_4 generation. The F_4 lines were multiplied by selfing 40 panicles per line, and the resulting F_5 seed was bulked. These F_3-plant derived bulks in F_5 are referred to hereafter as $F_{3:5}$ lines. Each $F_{3:5}$ line represents the gene content of the parental F_3 plant. One $F_{3:5}$ line in RIP 1 proved to be an off-type and was therefore removed from the data set after the field evaluation of the RIPs.

Field trials

The experiments were conducted during the post-rainy seasons (October to March) of 1999/2000 (RIP 2) and 2000/2001 (RIP 1) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17°30′N, 78°16′E, altitude 545 m). This season is ideal for evaluating the expression of adaptive traits for terminal moisture-deficit conditions, as the crop is dependent almost entirely on stored soil moisture and undergoes a long progressive stress under moderate evaporative-demand conditions. Severity and time of onset of stress can be manipulated by a choice of soil texture and/or depth to vary total plant-available water content, and onset of stress can be further manipulated (on shallow soils) by refilling the profile at varying times prior to flowering. The experiments reported here were planted on a shallow (40 to 60 cm) vertic inceptisol (very fine montmorillontic isohyperthermic) overlying a loose, decomposing granite-base material that is permeable to roots but contains limited plant-available water.

Each RIP was sown together with the two respective parental lines in a 19 × 12 lattice design with three replications. The experimental units were 2-row plots, with each row being 4-m long and spaced 0.60-m apart. A basal application of 20 kg ha^{-1} N and 20 kg ha^{-1} P_2O_5 as di-ammonium phosphate was banded before sowing. Seeds were machine-sown and the field irrigated (10 mm) with overhead sprinklers to ensure germination. The crop was successfully thinned 10 days after emergence to about 100,000 plants ha^{-1}. Twenty days after emergence, an additional 45 kg ha^{-1} of N, as urea, was side-dressed and the field given a light (15-mm)-sprinkler irrigation. The temperature regime was very similar in the two seasons (Fig. 1). A slight climatic difference between the 2 test years was that the 2000/2001 season (RIP 1) received 20 mm rain in January, while the 1999/2000 season (RIP 2) received 58 mm rain in February.

The crop was protected from both leaf-feeding insect pests and stem borers using appropriate insecticides. Regular prophylactic measures were taken to prevent leaf rust that is common during this season. Traits assessed in the two trials included the number of sorghum plants, the time from emergence to 50% flowering (d), the number of panicles, the dry panicle yield, the grain yield, the dry stover yield, the total above-ground biomass, the harvest in-

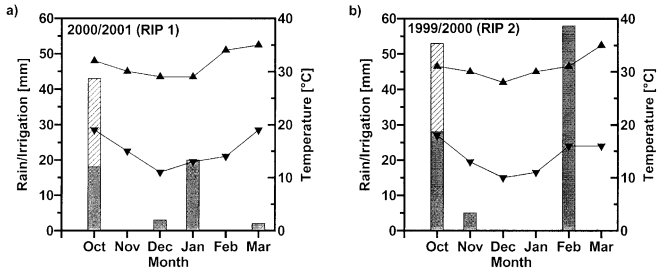

Fig. 1 Amount of rain (*black column*) and irrigation (*hedged column*), as well as minimum (*triangle down*) and maximum (*triangle up*) air temperatures, during the a) 2000/2001 and b) 1999/2000 post-rainy season at Patancheru, India, where RIP 1 and RIP 2, respectively, had been grown

dex, the leaf area per plant and the percentage of green leaf area at 15, 30 and 45 days after flowering, assessed as described below. Panicles and stover were oven-dried at 60 °C for 48 h before weighing. This removes all the moisture.

Estimation of senescence

At the time of emergence of the flag leaf, three representative plants in each plot were tagged; the length and width of the upper six leaves were measured, and the area of each estimated as: leaf length × leaf width × 0.70. Beginning at flag-leaf emergence, the percentage of each of the upper six leaves of each tagged plant remaining green was visually estimated at weekly intervals. The green-leaf area (GL) of each tagged plant was computed by multiplying the percent green-leaf area by the measured area of each leaf, and summing across the six measured leaves. The percentage of green-leaf area (% GL) for each plant, for each week, was calculated by dividing the estimated GL for that week by its measured leaf area at flowering. Plot values for % GL were derived by averaging the three individual plant values for each plot. The weekly % GL data were used to fit an appropriate equation to describe the pattern of leaf senescence during the period of observations. Linear fits were satisfactory for most plots. A second order, or logistic fit, was used when the linear fit was not adequate. In both years, the coefficient of determination for all plots was not less than 90%. The fitted equation for each individual plot were used to estimate the % GL at 15, 30 and 45 days after flowering of the individual entries (% GL15, % GL30 and % GL45, respectively). For more details of the method, see Mahalakshmi and Bidinger (2002).

Genotyping

Marker analyses of the two mapping populations were performed with bulked DNA from 20 plants per $F_{3:5}$ line. AFLP, RFLP and SSR analyses were carried out by commercial laboratories, and RAPD analyses by ICRISAT. RIP 1 was genotyped for 225 marker loci (131 codominantly and 20 dominantly scored AFLPs, 51 SSRs, 17 RFLPs and 6 RAPDs) and RIP 2 for 292 marker loci (122 codominantly and 75 dominantly scored AFLPs, 58 SSRs, 20 RFLPs and 17 RAPDs). The AFLP markers were created using ten *Eco*RI/*Mse* primer combinations. The RFLP markers (about two anchors per linkage group) were selected from the sorghum genetic map published by Boivin et al. (1999). The SSRs had been developed by Brown et al. (1996), Taramino et al. (1997), Kong et al. (2000) and Bhattramakki et al. (2000). Genetic maps were constructed using the software JoinMap 2.0 (Stam and Van Ooijen 1995). The Haldane mapping function was used and linkage groups were named according to common anchor markers with the map of Bhattramakki et al. (2000). After having constructed the maps for each RIP using all marker data (Haussmann et al. 2002), it was found that some (mainly AFLP) markers clustered in certain regions of the genome. In order to obtain maps with a more-uniform marker distribution, certain markers were removed from the data set and new maps computed. These new maps are presented here and were used in the QTL analyses.

Statistical analyses

Phenotypic data were analyzed using the software package PLABSTAT (Utz 1998). Estimates of operative repeatability (i.e. heritability in a replicated trial) were computed, using the following formula:

$$\text{Operative Repeatability} = \sigma_t^2 / \left(\sigma_t^2 + \sigma_e^2/R\right),$$

where σ^2_t and σ^2_e are the estimated treatment and error components of variance, respectively, and R the number of replications. It should be noted that the "true" heritabilities are lower, since repeatabilities are overestimated due to genotype × environment interaction. Phenotypic correlations among traits were computed according to standard procedures.

QTLs were detected using the method of composite interval mapping (Jansen and Stam 1994; Utz and Melchinger 1994; Zeng 1994) and the software PLABQTL Version 1.1 (Utz and Melchinger 2000). A purely additive model was employed since it is impossible to reliably estimate the remaining dominance effects in $F_{3:5}$ lines (H.F. Utz, personal communication). The critical LOD thresholds as determined by PLABQTL using the Bonferroni chi-square approximation (Zeng 1994) were 2.67 for RIP 1, and 2.72 for RIP 2 (DF=2). Both values approximately refer to comparison-wise and experiment-wise error rates of α = 0.002 and 0.25, respectively. QTLs with a LOD score between 2.5 and 2.7 are also being reported and may be regarded as suggestive. Individual cofactor sets were selected via stepwise regression for each trait and data set by PLABQTL, and subsequently extended or reduced by maximizing the R^2 value. Final selection was for the model that minimized Akaike's information criterion (AIC), a measure of the goodness-of-fit of the regression model (Jansen 1993). QTL positions were determined at the local maxima of the LOD-curve plot in the region under consideration. Adjacent QTLs on the same chromosome were considered different when the curve had a minimum between peaks that was at least 1 LOD unit below either peak or when the support intervals were non-overlapping.

The proportion of phenotypic variance explained by a single QTL was obtained by the square of the partial correlation coefficient (R^2). Estimates of the additive effects of the QTL, and the total genetic variance explained by all QTLs, were obtained by fitting a model including all putative QTLs for the respective trait.

A five-fold cross-validation was employed using the best-fitting model to determine how QTL estimates depended on genotype sampling. In such a validation, QTL positions and effects are estimated with 4/5th of the data, and with the remaining 1/5th a validation is performed. This can be done five-times, such that each fifth of the data is used in validation (Utz and Melchinger 2000). Since in the present study the number of genotypes in the validation run was rather small (45), the five-fold cross-validation was used to check how often a QTL was detected in the five calibration runs with 4/5th of the data (180 $F_{3:5}$ lines).

Results

Field data

Environmental means for grain yield were 267 and 199 g m^{-2} in the two years (Table 2). Since the two RIPs were evaluated in different years they are not directly comparable. However, it may be noted that, on average,

Table 2 Means, significance of genetic differences among the $F_{3:5}$ sorghum lines (Signif.), estimated operative repeatabilities (Rep.), and coefficients of error variation (CV%) for Recombinant Inbred Population (RIP) 1 and RIP 2 for various traits assessed in Patancheru, India, in the post-rainy seasons 2000/2001 (RIP 1) and 1999/2000 (RIP 2)

Trait	RIP 1 (IS9830 × E 36-1)				RIP 2 (N13 × E 36-1)			
	Mean	Signif.	Rep.	CV%	Mean	Signif.	Rep.	CV%
Time to flowering (d)	54.3	**	0.95	2.7	69.8	**	0.94	2.8
Panicles m^{-2}	11.4	**	0.55	13.3	13.4	**	0.70	17.6
Panicle yield (g m^{-2})	334	**	0.67	17.2	259	**	0.70	22.3
Grain yield (g m^{-2})	267	**	0.69	18.3	199	**	0.67	23.5
Stover yield (g m^{-2})	343	**	0.84	17.8	412	**	0.78	18.1
Biomass yield (g m^{-2})	678	**	0.73	14.9	672	**	0.53	17.8
Harvest index (%)	39.8	**	0.85	10.2	29.8	**	0.89	11.8
Leaf area per plant	16.6	ns	–	16.4	14.0	**	0.49	22.7
Green-leaf area 15d[a](%)	74.1	**	0.89	6.0	73.6	**	0.91	5.0
Green-leaf area 30d[a](%)	55.1	**	0.81	10.7	55.7	**	0.88	8.4
Green-leaf area 45d[a](%)	36.1	**	0.78	22.3	37.7	**	0.85	16.6

[a] Percentages of green-leaf area at 15, 30 and 45 days after flowering
**Genotypic differences significant at the 0.01 probability level; ns non-significant

RIP 1 flowered relatively about 2 weeks earlier than RIP 2, a behavior which we know also from trials conducted in West Africa where both RIPs were planted side by side (Haussmann, unpublished data). The mean percentage green leaf area decreased from 74% at 15 days after flowering to 36–38% at 45 days after flowering in both RIPs. Genetic variation among the $F_{3:5}$ lines within each RIP was highly significant for all traits, except for the leaf area per plant in RIP 1. Estimated operative repeatabilities ranged from 0.55 to 0.95 in RIP 1, and from 0.49 to 0.94 in RIP 2, for the traits with significant genetic variation. Coefficients of error variation were low or moderate for all traits.

Frequency distributions of the three stay-green traits in RIPs 1 and 2 were unimodal and approximately normal (Fig. 2); only the distribution of % GL15 in RIP 1 had a slightly negative kurtosis ($P = 0.05$). The two parent lines of each RIP differed significantly for % GL30 and % GL45. For % GL15, the difference between the parents of RIP 2 (N13 and E36-1) was significant, while that for the parents of RIP 1 (IS9830 and E36-1) was not significant. Transgressive segregation occurred in both directions for all three measures of the stay-green trait in both RIPs, except for % GL45 in RIP 1.

In both RIPs, coefficients of phenotypic correlation were high between % GL15 and % GL30, and between % GL30 and % GL45 (Table 3). Only moderate correlations occurred between % GL15 and % GL45. High grain yield was associated with stay-green in RIP 2 whereas the relationship between the two traits was non-significant in RIP 1. Besides stay-green, escape through early flowering was important for grain yield performance in RIP 2 (which is the relatively later flowering of the two populations). In RIP 1, there was only a low correlation between grain yield and early anthesis. In both RIPs, high harvest index was associated with early flowering and high % GL15. High stover yield was moderately correlated with late flowering and lower values of % GL15 in both RIPs.

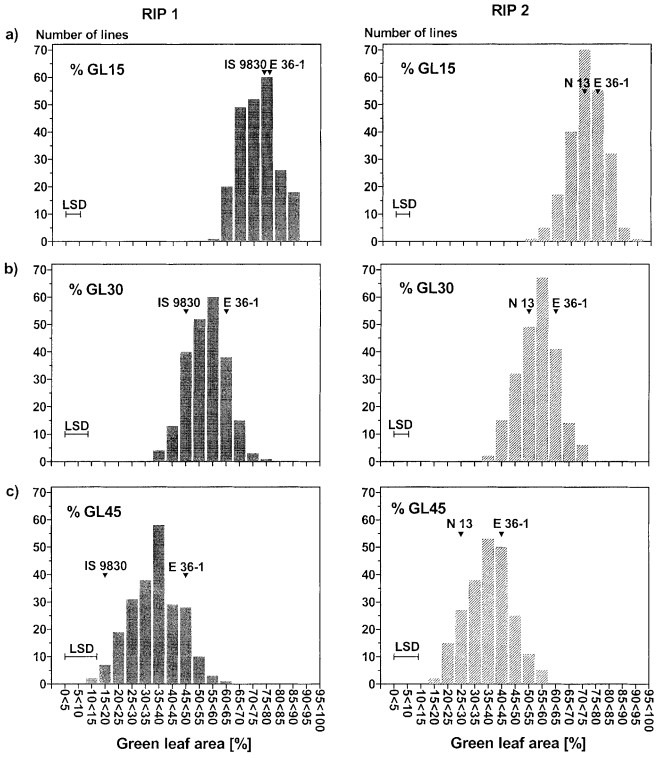

Fig. 2 Frequency distributions of the $F_{3:5}$ lines of RIP 1 (IS 9830 × E 36-1) and RIP 2 (N 13 × E 36-1) for the percentage of green-leaf area at a) 15, b) 30 and c) 45 days after flowering (% GL15, % GL30, and % GL45, respectively). "▼" marks the classes of the respective parent lines. LSD = least significant difference at $P = 0.05$

Linkage maps

The genetic map of RIP 1 had a total length of 1,291.2 cM, with 128 markers (AFLPs, RFLPs, SSRs and RAPDs) distributed over ten linkage groups (Fig. 3a). The average and maximal distances between two individual markers were 10.1 and 38.8 cM, respec-

Table 3 Coefficients of phenotypic correlation among the investigated traits in Recombinant Inbred Population (RIP) 1 (IS 9830 × E 36-1) and RIP 2 (N 13 × E 36-1)

RIP	Correlated traits → ↓	Grain yield	Stover yield	Biomass	Harvest index	Time to flowering	% GL15	% GL30
1	Stover yield	0.13						
	Biomass yield	0.60**	0.86**					
	Harvest index	0.54**	–0.74**	–0.33**				
	Time to flowering	–0.13*	0.54**	0.37*	–0.58**			
	% GL15[a]	0.09	–0.38**	–0.26**	0.42**	–0.72**		
	% GL30	0.05	–0.07	–0.05	0.13	–0.37**	0.86**	
	% GL45	0.01	0.17*	0.12	–0.12	–0.04	0.60**	0.92**
2	Stover yield	–0.19**						
	Biomass yield	0.44**	0.80**					
	Harvest index	0.79**	–0.72**	–0.18**				
	Time to flowering	–0.60**	0.53**	0.11	–0.73**			
	% GL15	0.47**	–0.29**	0.02	0.51**	–0.64**		
	% GL30	0.33**	–0.15*	0.07	0.34**	–0.41**	0.93**	
	% GL45	0.20**	–0.02	0.10	0.17	–0.19**	0.79**	0.96**

[a] % GL15, % GL30, % GL45 = percentage of green leaf area at 15, 30 and 45 days after flowering, respectively
*, ** Significant at the 0.05 and 0.01 probability levels, respectively

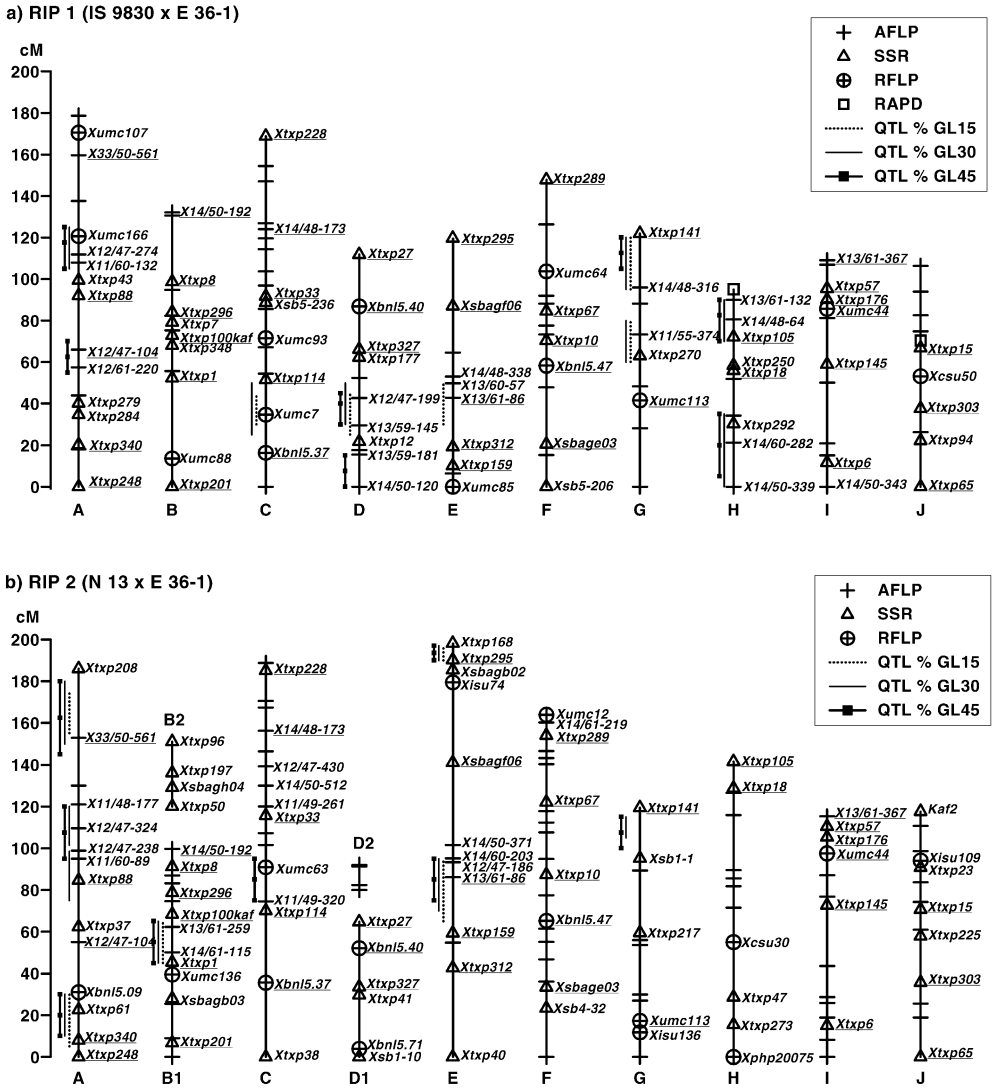

Fig. 3 Genetic linkage maps of a) RIP 1 and b) RIP 2. RFLP and SSR marker names are indicated on the right of each linkage group. AFLP markers names are only given where AFLPs were common to both RIPs or next to a QTL for the stay-green trait. *Underlined markers* are common to both RIPs. *Vertical lines* on the left of the linkage groups indicate support intervals of QTLs for the percentage of green-leaf area at 15, 30 and 45 days after flowering (% GL15, % GL30, and % GL45, respectively)

Table 4 Linkage group position (LG, Pos.), flanking marker interval, individual LOD score, partial coefficient of determination (R^2), and estimated additive effects (a_i) of the QTLs detected in Recombinant Inbred Population 1 (IS 9830 × E 36-1) for the percentage of green leaf area at 15, 30 and 45 days after flowering (% GL15, % GL30, and % GL45, respectively). C indicates the number of calibration runs in which the respective QTL was detected during the five-fold cross-validation

Trait	QTL	LG	Pos. cM	Marker interval		LOD	R^2	a_i[b]	C
% GL15	1	C	40	*umc7*;	*txp114*	14.9	26.3	–4.4	5
	2	D	35	*13/59–145*;	*12/47–199*	3.1	6.1	–2.0	2
	3[a]	E	45	*13/61–86*;	*14/60–57*	2.6	5.2	1.5	2
	4	G	70	*txp270*;	*11/55–374*	3.5	7.0	2.3	5
	5	G	105	*14/48–316*;	*txp141*	3.3	6.6	3.3	3
	Percentage genetic variance explained in total[c]:						38.0		
% GL30	1[a]	A	115	*12/47–274*;	umc166	2.6	5.2	1.6	3
	2	C	35	*umc7*;	*txp114*	6.4	12.4	–2.7	5
	3	D	40	*13/59–145*;	*12/47–199*	2.8	5.5	–3.0	2
	4	G	65	*txp270*;	*11/55–374*	2.9	5.9	1.7	2
	5[a]	G	110	*14/48–316*;	*txp141*	2.9	5.9	2.2	2
	6	H	20	*14/50–339*	*14/60–282*	2.6	5.9	1.7	2
	7	H	80	*txp105*;	*14/48–64*	2.6	5.3	–1.7	1
	Percentage genetic variance explained in total[c]:						42.4		
% GL45	1	A	60	*12/61–220*;	*12/47–104*	4.5	8.8	3.0	5
	2[a]	A	115	*12/47–274*;	*umc166*	2.9	5.8	2.3	3
	3	D	10	*14/50–120*	*13/59–181*	3.0	6.2	–4.4	3
	4	D	40	*13/59–145*;	*12/47–199*	2.6	5.1	–3.7	1
	5[a]	G	120	*14/48–316*;	*txp141*	3.0	6.0	2.4	2
	6	H	20	*14/60–282*;	*txp292*	2.6	6.0	2.2	2
	7	H	80	*txp105*;	*14/48–64*	3.3	6.6	–2.4	4
	Percentage genetic variance explained in total[c]:						41.0		

[a] QTLs common with RIP 2 (see Table 5)
[b] Positive effect: stay-green allele from E 36-1; negative effect: stay-green allele from IS 9830
[c] Estimate obtained from a simultaneous fit of all putative QTLs affecting the respective trait, adjusted according to Hospital et al. (1997)

tively. Large gaps (>25 cM) occurred in linkage groups B (2 gaps), D (1), E (2), F (1) and G (2).

The map of RIP 2 spanned 1,438.1 cM and contained 146 markers in 12 linkage groups (Fig. 3b). Linkage groups B and D were split in two parts. The average and maximal distances between two individual markers were 9.8 and 42.7 cM, respectively. Large gaps (>25 cM) occurred on linkage groups A (1 gap), C (2), D (1), E (4), G (2) and I (1).

The two maps had 42 markers in common. The order of common markers was completely conserved, except for the neighboring marker loci *Xtxp159* and *Xtxp312* on linkage group E, whose positions were exchanged in the two maps.

QTLs for stay-green

In RIP 1, composite interval mapping detected five QTLs for % GL15 and seven each for % GL30 and % GL45; these explained 38%, 42% and 41% of the genetic variation, respectively (Table 4 and Fig. 3a). The number of cofactors employed was 11, 11 and 13 for the three traits, respectively. Two QTLs on linkage groups D (at 35–40 cM) and G (105–120 cM) were common to all three traits. Another two QTLs on linkage groups C (35–40 cM) and G (65–70 cM) were common between % GL15 and % GL30, while one QTL on linkage group A (115 cM) and two QTLs on linkage group H (20–25 cM and 80 cM) were common between % GL30 and % GL45. Individual coefficients of determination, i.e. the percentage of phenotypic variance explained by the individual QTL, ranged from 5.1% to 26.3%. Both parent lines contributed high stay-green alleles. Surprisingly, the major QTL allele for high % GL15 and % GL30 on linkage group C at 35–40 cM was contributed by the non-stay-green line IS9830.

In RIP 2, five QTLs were detected for % GL15, and eight each for % GL30 and % GL45 (Table 5 and Fig. 3b). The number of cofactors employed was 20, 18 and 19 for the three traits. In total, the QTLs detected explained 31%, 37% and 36% of the genetic variation for % GL15, % GL30 and % GL45, respectively. Five QTLs on linkage groups A (at 20 cM and 165 cM), B1 (50–55 cM) and E (75–85 cM and 195 cM) were common between all three traits. In addition, two QTLs on linkage groups A (110–115 cM) and G (115 cM) were common between % GL30 and % GL45. Individual coefficients of determination ranged from 4.9% to 23.0%. Both parents contributed high stay-green alleles. Again, a major QTL allele for high stay-green on linkage group A at 165 cM was derived from the non-stay-green parent, line N13, in this case.

The five-fold cross-validation showed that genotype-sampling effects had an effect on QTL detection in the present materials. QTLs that were detected in only one out of the five calibration runs may be less reliable. However, when the LOD curves in the calibration runs were inspected (data not shown), it was found that peaks were present in most cases, but did not exceed the threshold of 2.5.

Table 5 Linkage group position (LG, Pos.), flanking marker interval, individual LOD score, partial coefficient of determination (R^2) and estimated additive effects (a_i) of the QTLs detected in Recombinant Inbred Population 2 (N 13 × E 36-1) for the percentage of green leaf area at 15, 30 and 45 days after flowering (%GL15, %GL30, and %GL45, respectively). C indicates the number of calibration runs in which the respective QTL was detected during the five-fold cross-validation

Trait	QTL	LG	Pos. cM	Marker interval		LOD	R^2	a_i[b]	C
% GL15	1	A	20	*txp340*;	*txp61*	3.1	6.2	1.8	2
	2	A	165	*33/50–561*;	*txp208*	12.0	23.0	–4.5	5
	3	B1	50	*txp1*;	*14/61–115*	2.5	4.9	–1.4	1
	4[a]	E	75	*txp159*;	*13/61–86*	6.8	13.0	3.1	5
	5	E	195	*txp295*;	*txp168*	2.8	5.6	–1.6	1
	Percentage genetic variance explained in total[c]:						31.4		
% GL30	1	A	20	*txp340*;	*txp61*	3.0	5.9	1.9	2
	2	A	90	*txp88*	*11/60–89*	4.8	9.3	2.4	4
	3[a]	A	110	*12/47–324*;	*11/48–177*	2.9	5.7	1.9	2
	4	A	165	*33/50–561*;	*txp208*	6.2	12.7	–3.3	5
	5	B1	50	*14/61–115*;	*13/61–259*	3.0	5.8	–1.6	2
	6	E	80	*13/61–86*;	*12/47–186*	7.5	14.1	3.3	5
	7	E	195	*txp295*;	*txp168*	3.4	6.7	–1.9	4
	8[a]	G	115	*sb1–1*;	*txp141*	2.7	5.5	1.7	1
	Percentage genetic variance explained in total[c]:						36.5		
% GL45	1	A	20	*txp340*;	*txp61*	3.0	5.9	2.1	3
	2[a]	A	115	*12/47–324*;	*11/48–177*	4.1	8.0	2.9	2
	3	A	165	*33/50–561*;	*txp208*	4.0	8.3	–3.1	3
	4	B1	55	*14/61–115*;	*13/61–259*	4.9	9.5	–2.5	5
	5	C	90	*11/49–320*;	*umc63*	2.8	5.6	2.0	2
	7	E	85	*txp159*;	*13/61–86*	7.6	14.3	3.4	5
	8	E	195	*txp295*;	*txp168*	2.9	5.8	–1.9	2
	9[a]	G	115	*sb1–1*;	*txp141*	2.6	5.2	1.9	2
Percentage genetic variance explained in total[c]:							36.3		

[a] QTLs common with RIP 1 (see Table 4)
[b] Positive effect: stay-green allele from E36-1; negative effect: stay-green allele from N 13
[c] Estimate obtained from a simultaneous fit of all putative QTLs affecting the respective trait, adjusted according to Hospital et al. (1997)

Considering % GL15, % GL30 and % GL45 simultaneously, three QTLs in linkage groups A, E and G were common to both RIPs, with the high stay-green alleles deriving from the stay-green source-line E36-1. Since the two RIPs were tested in different years, these three QTLs may be considered as consistent not only across the two genetic backgrounds, but also across the 2 experimental years. Individual coefficients of determination for these QTLs ranged from 5.2% to 6.0% in RIP 1, and from 5.2% to 13.0% in RIP 2.

Discussion

Knowing that sorghum grain yield may range from below 80 to above 1,000 g m^{-2}, the environmental means for grain yield in the present study (267 g m^{-2} in RIP 1, and 199 g m^{-2} in RIP 2) may be considered as moderate drought-stress conditions in both experimental years. The ability to maintain green leaf area was moderately positively correlated with grain yield in RIP 2, but had no effect on the grain yield performance in RIP 1. The earlier flowering of RIP 1 (as compared to RIP 2) and the 20-mm January rain received by RIP 1 could have reduced the severity of the post-flowering drought-stress experienced by the crop, thus reducing the potential impact of stay-green on grain yield performance. In contrast, the 58-mm rain received by RIP 2 in February was too late to have any impact. Alternatively, rapid growth and, thus, high sink strength of the panicles of the highest yielding lines of RIP 1 could have competed with the leaves for remobilizable nitrogen, leading to earlier senescence of the leaves. This may explain the fact that there was no advantage of the stay-green trait in RIP 1. In contrast, Borrell et al. (2000b) reported a correlation of $r = 0.75$ ($P = 0.01$) between the green leaf area at maturity and the grain yield under terminal (pre- and post-flowering) water stress. Their study involved nine closely related hybrids varying in the rate of leaf senescence; these were evaluated under three water regimes at one location in northeastern Australia. Mean grain yields in the non-stress, post-flowering and terminal drought treatments were 1,030, 1,007, and 849 g m^{-2}, respectively. Considering the mean grain yield of sorghum in Africa and Asia of 80 and 120 g m^{-2}, respectively, the treatments in the study of Borrell et al. (2000a,b) may be regarded as non-stress conditions. In field trials involving 48 hybrids and their 24 parent lines evaluated in eight site/season combinations in a semi-arid area of Kenya, with environmental means for grain yield ranging from 167 to 595 g m^{-2}, Haussmann et al. (1999) reported a negative genetic correlation coefficient ($r = –0.47$, exceeding twice its standard error) between the number of green leaves at 95 days after sowing and grain yield in the hybrids. In their study, therefore, those hybrids that quickly translocated all assimilates to the grains were

Table 6 Linkage group names in the present study and corresponding linkage groups in other stay-green mapping studies

Reference	Linkage group									
This paper (as Bhattramakki et al. 2000)	A	B	C	D	E	F	G	H	I	J
Tuinstra et al. (1997)[a]	F	B	G	N	D	K	E	?	?	H
Tao et al. (2000)	C	B	A	F	J	G	I	E	D	H
Crasta et al. (1999), Xu et al. (2000), Subudhi et al. (2000), Kebede et al. (2001)	G	D	A	C	E	I	B	H	F	J

[a] Only eight of the 15 linkage groups of the RAPD-based map of Tuinstra et al. (1997) could be aligned

Table 7 Linkage group positions of putative stay-green QTLs identified in various studies relative to the linkage group names assigned in our study, and respective donors of the stay-green alleles

Reference and parents of mapping population	Positions of stay-green QTLs relative to our linkage groups										
	A	B	C	D	E	F	G	H	I	J	?
This study: %GL45[b] E36-1 × IS 9830	2×P1[a]			2×P2[a]			P1	P1, P2			
This study: %GL45[b] E36-1 × N13	2×P1, P2	P2	P2		P1, P2		P1				
Tuinstra et al. (1997)[c] B35 × Tx7078	P?	2×P?	P?							P?	I:P?
Crasta et al. (1999) B35 × Tx430	P1	P1	P1			P1, P2	P2			P1	
Xu et al. (2000) B35 × Tx7000		P1	2×P1							P1	
Subudhi et al. (2000) B35 × Tx7000		P1	2×P1		P1					P1	
Kebede et al. (2001) SC56 × Tx7000	P1	P2	P1	P1, P2	P1		P2		P2	P1	
Tao et al. (2000) QL41 × QL39	P2	P1	P2			P1	P1				

[a] P1: QTL with the stay-green allele coming from stay-green parent (E 36-1, B35, SC56, or QL41); P2: QTL with the stay-green allele coming from the non-stay-green parent (IS 9830, N 13, Tx7078, Tx430, Tx7000, or QL39). 2× P1: two QTLs from P1 on the respective linkage group
[b] Only the QTLs for green leaf area at 45 days after flowering are included here
[c] ? = Donor parent of stay-green alleles not indicated

highest yielding, while the stay-green types were inferior. In the study of Tuinstra et al. (1997), two QTLs were identified with major effects on both stay-green and grain yield under post-flowering drought, suggesting a positive relationship between the two traits at stress sites in Mexico (mean grain yield 120 g m^{-2}) and Arizona (467 g m^{-2}). More data is needed on the relationship between stay-green and grain yield, especially under severe terminal drought stress (E. Weltzien, personal communication). Unfortunately, none of the sorghum stay-green trait mapping-studies published recently report this correlation in the tested recombinant inbred populations.

The total lengths of the two genetic linkage maps presented here are within the range reported for sorghum in the literature (Haussmann et al. 2002). The shorter map for RIP 1 (1,291 cM, versus 1,438 cM in RIP 2) is probably due to a lower genetic distance between IS9830 and E36-1 (the parent lines of RIP 1) as compared to N13 and E36-1 (the parent lines of RIP 2). Because of the lower genetic distance, a lower number of polymorphic markers were identified for RIP 1 and this hindered the completion of some of the linkage groups. Relative to the sorghum genetic map published recently by Bhattramakki et al. (2000), about 230 and 120 cM are still missing in the maps of RIPs 1 and 2, respectively. In RIP 1, missing regions are suspected in linkage groups B (70 cM), C (10 cM), D (47 cM), E (45 cM), G (15 cM), H (40 cM) and J (7 cM). In RIP 2, missing regions are probable in linkage groups A (34 cM), B (35 cM) and D (53 cM). Some of these missing regions correspond to mapped positions of stay-green QTLs previously reported in sorghum. For example, Crasta et al. (1999) mapped a minor stay-green QTL to a region located between *Xtxp141* and the upper end of linkage group G (their linkage group B). This region is beyond the reach of our maps.

Between five and eight QTLs were detected for the three stay-green traits evaluated in the present study. This range corresponds well to the number of QTLs identified in other stay-green mapping experiments (Table 1). Contrastingly, earlier classical genetic studies suggested one to three major genes being involved in the inheritance of stay-green in B35, SC599-11*E* and BTx378 (Tenkouano et al. 1993), and B35 (Walulu et al. 1994). The partially different QTLs identified for % GL15, % GL30, and % GL45 may reflect a partially independent inheritance of different components of stay-

green, like the initial green leaf area at flowering, the time of onset of senescence, and the average or maximum rate of senescence. Further data analysis is required to prove this hypothesis. An independent inheritance of the mentioned components of stay-green had also been suggested by van Oosterom et al. (1996).

By using the same stay-green source in two different mapping populations and by testing the two populations in different years, we were able to validate the stay-green QTLs from line E36-1 across genetic backgrounds and years. Three QTLs on linkage groups A, E and G were common to both RIPs across the three traits, with the stay-green allele deriving from line E36-1. These QTLs could be potential candidates for marker-assisted transfer of stay-green into locally adapted materials. Possible QTL × environment interaction effects could not be estimated in the present single-environment experiments. The QTLs therefore need further validation in different test environments (sites and/or years), especially under severe drought-stress conditions, before applied marker-assisted selection is initiated. However, exploratory marker-assisted backcrossing of these putative QTLs into a small number of genetically diverse, agronomically elite, drought-sensitive genetic backgrounds, could also contribute to their validation. It would also allow an estimation of the overall benefit of individual stay-green genes for grain yield and other agronomic traits in the target areas.

Via common markers and comparison with other genetic linkage maps of sorghum (Boivin et al. 1999; Peng et al. 1999; Subudhi and Nguyen 1999; Bhattramakki et al. 2000; Kong et al. 2000), our maps could be aligned with those of the previous stay-green mapping studies (Table 6). Only seven of 15 linkage groups in the RAPD-based map of Tuinstra et al. (1997) could not be related to any linkage group in our maps. After the alignment of the linkage maps, linkage-group positions of putative stay-green QTLs identified in the various studies were related to the positions of QTLs for % GL45 identified in our study (Table 7). Across all studies, the pattern of QTL appearance is complex, with stay-green QTLs appearing on all ten linkage groups of sorghum. Because of very few common markers (and small sample sizes), it is difficult to further align the maps and compare the QTL positions more precisely. However, it seems that most QTLs for stay-green differ among the various stay-green donor parents lines. Thus:

(1) A stay-green QTL was detected by Tuinstra et al. (1997) near marker locus *XtF16/68* on our linkage group B = their linkage group B, by Crasta et al. (1999) near marker locus *Xtxs1537* on our linkage group B = their linkage group D2, and by Subudhi et al. (2000) near marker locus *Xtxs1845* on our linkage group B = their linkage group D2. This particular stay-green QTL, which is not the same as *Stg3* (identified by Xu et al. 2000) that also maps to this linkage group, has only been detected in mapping populations based on stay-green source B35.

(2) Stay-green QTL *Stg4* was detected from source B35 by Crasta et al. (1999) near marker locus *Xtxs713* on our linkage group J = their linkage group J, by Xu et al. (2000) at this same position, and by Subudhi et al. (2000) at this same position. Similarly, a stay-green QTL was also detected at this same position from source SC56 by Kebede et al. (2001). However, we have detected no stay-green QTL mapping to this linkage group in our studies of two RIL sets based on stay-green source E 36-1.

In the present, as well as in several previous stay-green mapping studies in sorghum (Crasta et al. 1999; Tao et al. 2000; Kebede et al. 2001), transgressive segregation was present and both parent lines contributed alleles for stay-green. Surprisingly, even major QTLs for stay-green were contributed by the non-stay-green parents IS9830 (QTLs for % GL15 and % GL30 on linkage group C) and N13 (QTLs for % GL15, % GL30 and % GL45 on linkage group A at 160–165 cM) in the present study. This indicates some hidden genetic variability for the trait that could be exploited by breeders, where the trait proves to be useful.

Conclusions

Quantitative trait loci and the corresponding flanking molecular markers for stay-green have been identified and verified across environments, years, and/or different genetic backgrounds in the present as well as in other recently published studies. Consistently identified QTLs could be candidates for marker-assisted transfer into locally adapted cultivars. But before marker-assisted selection for stay-green is employed widely in breeding programs targeting extreme drought-stress environments, e.g. in Sub-Saharan Africa, the benefit of stay-green for yield performance and stability should be proven in these target areas. A small-scale marker-assisted backcrossing program to transfer genomic regions contributing to this trait from source lines B35 and E36-1 into a small number of genetically diverse sorghums currently cultivated in these target areas would be a useful step in assessing the potential utility of the stay-green trait for these traditional sorghum production environments.

Acknowledgements The technical assistance of Mr. M. Kistaiah and Mr. P. Shivaiah during the evaluation of the materials as well as the statistical advice of Dr. H.F. Utz are highly appreciated. The tested mapping populations were developed and genotyped within the BMZ (German Federal Ministry for Economic Cooperation and Development)-financed project "Striga resistance in sorghum" (Grant No. 94.7860.3–01.100). Many thanks are also due to the German Research Foundation (DFG) who supported B.I.G. Haussmann during the final data analysis.

References

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

Boivin K, Deu M, Rami JF, Trouche G, Hamon P (1999) Towards a saturated sorghum map using RFLP and AFLP markers. Theor Appl Genet 98:320–328

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

Borrell AK, Bidinger FR, Sunitha K (1999) Stay-green trait associated with yield in recombinant inbred sorghum lines varying in rate of leaf senescence. Int Sorghum and Millets Newslett 40:31–34

Borrell AK, Hammer GL, Douglas ACL (2000a) Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. Crop Sci 40:1026–1037

Borrell AK, Hammer GL, Henzel RG (2000b) Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Sci 40:1037–1048

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzales-Candelas F, Kresovitch 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

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

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. Proc Workshop held in Bellagio, Italy, 27 Nov–2 Dec 1983. ICRISAT, Patancheru, AP, India, pp 99–110

Haussmann BIG, Obilana AB, Ayiecho PO, Blum A, Schipprack W, Geiger HH (1999) Quantitative-genetic parameters of sorghum [*Sorghum bicolor* (L.) Moench] grown in semi-arid areas of Kenya. Euphytica 105:109–118

Haussmann BIG, Hess DE, Seetharama N, Welz HG, Geiger HH (2002) Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps. Theor Appl Genet http://dx.doi.org/10.1007/s00122-002-0900-x

Hospital F, Moreau L, Lacoudre F, Charcosset A, Gallais A (1997) More on the efficiency of marker-assisted selection. Theor Appl Genet 95:1181–1189

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

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

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

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yield in water-limited environments. Adv Agron 43:107–152

Mahalakshmi V, Bidinger FR (2002) Evaluation of putative stay-green sorghum germplasm lines. Crop Sci 42:965–974

Oosterom E van, Jayachandran R, Bidinger FR (1996) Diallel analysis of the stay-green trait and its components in sorghum. Crop Sci 36:540–555

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 Workshop held in Bellagio, Italy, 27 Nov–2 Dec 1983. ICRISAT, Patancheru, AP, India, pp 209–217

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO Wageningen, The Netherlands

Subudhi PK, Nguyen HT (2000) Linkage group alignment of sorghum RFLP maps using a RIL mapping population. Genome 43:240–249

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

Tenkouano A, Miller FR, Frederiksen RA, Rosenow DT (1993) Genetics of nonsenescence and charcoal rot resistance in sorghum. Theor Appl Genet 85:644–648

Thomas H, Howarth CJ (2000) Five ways to stay-green. J Expl Bot 51:329–337

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

Utz HF (1998) PLABSTAT: a computer program for the statistical analysis of plant breeding experiments. Version 2N. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Utz HF, Melchinger AE (1994) Comparison of different approaches to interval mapping of quantitative trait loci. In: van Ooijen JW, Jansen J (eds) Biometrics in plant breeding: applications of molecular markers. Proc 9th Meeting of the EUCARPIA Section Biometrics in Plant Breeding, 6–8 July 1994, CPRO-DLO, Wageningen, The Netherlands, pp 195–204

Utz HF, Melchinger AE (2000) PLABQTL, a computer program to map QTL. Version 1.1 from 29 May 2000. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany

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

Woodfin CA, Rosenow DT, Clark LE (1988) Association between the stay-green trait and lodging resistance in sorghum. Agronomy abstracts. ASA, Madison, Wisconsin: 102

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 43:461–469

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468