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Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench)

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Abstract Drought is a major constraint in sorghum production worldwide. Drought-stress in sorghum has been characterized at both pre-flowering and post-flowering stages resulting in a drastic reduction in grain yield. In the case of post-flowering drought stress, lodging further aggravates the problem resulting in total loss of crop yield in mechanized agriculture. The present study was conducted to identify quantitative trait loci (QTLs) controlling post-flowering drought tolerance (stay green), pre-flowering drought tolerance and lodging tolerance in sorghum using an F_7 recombinant inbred line (RIL) population derived from the cross SC56×Tx7000. The RIL lines, along with parents, were evaluated for the above traits in multiple environments. With the help of a restriction fragment length polymorphism (RFLP) map, which spans 1,355 cM and consists of 144 loci, nine QTLs, located over seven linkage groups were detected for stay green in several environments using the method of composite interval mapping. Comparison of the QTL locations with the published results indicated that three QTLs located on linkage groups A, G and J were consistent. This is considered significant since the stay green line SC56 used in our investigation is from a different source compared to B35 that was used in all the earlier investigations. Comparative mapping has shown that two stay green QTLs identified in this study corresponded to stay green QTL regions in maize. These genomic regions were also reported to be congruent with other drought-related agronomic and physiological traits in maize and rice, suggesting that these syntenic regions might be hosting a cluster of genes with pleiotropic effects implicated in several drought tolerance mechanisms in these grass species. In addition, three and four major QTLs responsible for lodging tolerance and pre-flowering drought tolerance, respectively, were detected. This investigation clearly revealed the important and consistent stay green QTLs in a different stay green source that can logically be targeted for positional cloning. The identification of QTLs and markers for pre-flowering drought tolerance and lodging tolerance will help plant breeders in manipulating and pyramiding those traits along with stay green to improve drought tolerance in sorghum.

Keywords Pre-flowering drought tolerance · Stay green · Genetic mapping · Comparative mapping

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Introduction

Improving drought tolerance is an important objective in many crop-breeding programs. However, selection for drought tolerance is difficult because of inconsistency in testing environments and interaction between stages of plant growth and environment. The genetic mechanisms that condition the expression of drought tolerance in crop plants are poorly understood. Since drought tolerance is a complex trait controlled by many genes, and is dependent on the timing and severity of moisture stress, it is one of the most-difficult traits to study and characterize.

Drought-stress is a major constraint to sorghum [*Sorghum bicolor* (L.) Moench] productivity worldwide. However, sorghum is one of the most drought tolerant grain crops and is an excellent model for evaluating mechanisms of drought tolerance. Sorghum lines with a distinct phenotypic response to pre-flowering and post-flowering drought-stress have been characterized and excellent sources of resistance to each type of stress have been identified (Rosenow 1993). Pre-flowering drought-stress response in sorghum occurs when plants are under

significant moisture stress prior to flowering, especially from panicle differentiation or shortly thereafter until flowering. This type of stress directly affects panicle size, grain number and grain yield. Post-flowering drought stress causes premature leaf senescence leading to stalk lodging, stalk rot disease and significant yield loss in sorghum (Rosenow and Clark 1995). In recent years, the stay green trait has been recognized as a major mechanism of post-flowering drought-stress tolerance in sorghum (Rosenow et al. 1996).

Lodging is another constraint to sorghum production in mechanized agriculture. It reduces both productivity and grain quality. In sorghum, loss of grains results from the inability to harvest lodged plants, while grain quality can be lowered due to germination or grain-mold on lodged panicles (Rosenow and Clark 1995). Stalk lodging in sorghum is often related to post-flowering drought stress (Rosenow 1977). Lodging that occurs following a water-deficit grain-filling period causes the most grain loss worldwide. Severe moisture stress during the grain-filling stage often results in premature leaf and stalk senescence, leading to stalk death and collapse, and this type of lodging is often associated with stalk rots, especially charcoal rot (*Macrophomina phaseolina*) (Rosenow and Clark 1995).

Rosenow (1984) and Henzell et al. (1992) have reported correlations between stay green and lodging tolerance. During post-flowering drought-stress stay green lines resist premature plant and leaf death, develop grains normally, and resist charcoal rot and lodging (Rosenow and Clark 1981; Tenkuano et al. 1993). These lines maintain photosynthesis during grain filling with the upper canopy leaves remaining active photosynthetically even after physiological grain maturity. Stay green thus reduces the need for translocation of stored assimilates from the stem during grain filling, and extends the period of active assimilation past maturity (Oosterom et al. 1996) resulting in green stems which have good resistance to stalk lodging. The stay green trait has been successfully used in Australia to improve lodging resistance under terminal drought-stress (Henzell et al. 1992).

Understanding the genetic and molecular basis of drought tolerance has been a challenge to plant biologists. Quantitative trait loci (QTLs) associated with stay-green have been identified both in sorghum (Tuinstra et al. 1997; Crasta et al. 1999; Xu et al. 2000) and maize (Beavis et al. 1994). The determination of the consistency of stay green QTLs across different genetic backgrounds would be important in improving drought tolerance in sorghum and other grass species. In sorghum, stalk lodging associated with late-season drought-stress and its relationship to the stay green trait has been studied, but further research is needed to study their association and identify molecular markers linked to these specific traits. Although pre-flowering drought-stress tolerance is found more commonly than post-flowering drought tolerance in sorghum (Rosenow et al. 1996), very little information is available regarding the genetic analysis of this trait. Therefore, this study was undertaken using a RIL population derived from the cross SC56×Tx7000 with the following two objectives: (1) to identify QTLs for pre-flowering drought-stress tolerance and stalk-lodging tolerance, traits associated with late-season drought-stress, and (2) to identify and compare the QTL information for stay green with the earlier results in sorghum, maize and rice. The immediate benefit of this study lies in using the identified markers as diagnostics for several drought tolerance traits for improving drought tolerance through marker-assisted breeding. The identification of consistent stay green QTLs in a population involving a stay green line from a totally different genetic source is significant in the sense that the stay green mechanism might be conditioned by the same group of loci or with little variation. This now warrants a concerted and serious effort to identify the specific genes present in those consistent genomic regions responsible for stay green by positional cloning and functional genomic approaches. Cross-species comparative analysis of genomic regions for drought tolerance traits gave an indication that there may be some orthologous conserved regions for drought resistance in rice, maize and sorghum, which can be targeted for intensive investigation in future to improve drought tolerance.

Materials and methods

Plant material

A population of 125 recombinant inbred lines (RILs), obtained by F_7 generations of selfing of the F_1 between parental lines SC56×Tx7000, was used. SC56, a caudatum-nigricans from Sudan, is a post-flowering drought-tolerant (stay green) and lodging-tolerant line, but susceptible to pre-flowering drought stress. Tx7000 is a high yielding elite line widely used in sorghum breeding programs in the United States and is tolerant to pre-flowering drought stress. Tx7000, however, is susceptible to post-flowering drought stress and lodging.

Field trials and phenotyping

The 125 F_7 RILs and the two parents were grown in eight environments. The location and year combinations were considered as different environments. These were Lubbock (limited irrigation) (1997, 1998), Lubbock dryland (1997, 1999), Crossbyton (1997), Plainview (1998), and Halfway (1998), Texas, and Galveston, Kansas (1998), that are abbreviated as LL97, LL98, LD97, LD99, CB97, PV98, HW98, and GK98, respectively, throughout the text. The field layout was a randomized complete block design with two replications and single plots 4.9-m long and 1.0-m apart.

Visual ratings of stay green expression were recorded on a scale of 1 to 5 based on the degree of leaf and plant death at physiological maturity on a plot basis. Score 1 indicates no senesced leaves whereas 5 indicates complete plant death. The stay green trait was evaluated under five environments: HW98, LD97, GK98, CB97 and LL98. Trials at these sites, except at Lubbock dryland, Crossbyton and Galveston, Kan., were irrigated adequately up to flowering stage and irrigation was withdrawn just before anthesis to allow moisture stress to develop during the grain-development stage. Lubbock dryland was meant to be an experiment site to evaluate pre-flowering drought stress. However, the plants still survived through the pre-flowering drought stress and we were able to evaluate the plants for both pre-flowering and post-flowering drought stresses.

Similarly, the pre-flowering stress rating was done on a 1 to 5 scale, where 1 is tolerant and 5 is very susceptible. Ratings were based on leaf rolling, uncharacteristic leaf erectness, leaf bleaching, leaf tip and margin burn, delayed flowering, 'saddle effect' in which only end plants next to alleyways produce panicles, poor panicle exertion, panicle blasting and floret abortion, and reduced panicle size. All these traits were combined into a single overall drought susceptibility rating (Rosenow et al. 1996). This trait was evaluated under three environments: LD97, LD99 and CB97.

The scoring scale for lodging ranged from 0 (all plants in the plot completely upright) to 100 (all plants in the plots completely lodged). Lodging evaluation was done by allowing the plants to remain in the field after maturity and throughout the winter, to apply uniform lodging pressure. This subjected all lines to freezing temperatures, which kill stem tissue, and to external factors such as wind and snow, which exert a bending force. Lodging was evaluated only under one environment: LL98.

Data on flowering was recorded as the number of days from planting to when 50% of the plants in each plot flowered. This trait was evaluated in three environments: PV98, LL97 and LL98. Plant height was measured in centimeters from the base of the plant to the tip of the panicle. Ten representative plants were sampled in each plot for this trait.

RFLP analysis

DNA was isolated from leaf tissue of greenhouse-grown seedlings of parental lines and 125 F_7 RILs (Saghai-Maroof et al. 1984). The genomic DNA was digested using five restriction endonucleases (*Bam*HI, *Eco*RI, *Eco*RV, *Hind*III and *Xba*I). Restriction fragment length polymorphism analysis was done following Gardiner et al. (1993) using sorghum genomic clones (txs and psb probes obtained from Drs. G. Hart and A. Paterson, respectively, of Texas A & M University), maize cDNA and genomic clones from the University of Missouri-Columbia, and the cDNA clones available in our laboratory. The probes that showed polymorphisms between the parental lines were used to probe the DNA blots from the 125 RILs. For each marker the RILs were scored as 'A' or 'B' for presence of the parental band of the female parent (SC56) or male parent (Tx7000), respectively, or 'H' for heterozygote, 'C' for non-female parent, 'D' for non-male parent, or '–' for missing data.

Data analysis

A Macintosh version of the MAPMAKER program (version 2.0) (Lander et al. 1987) using the Kosambi function and the RIL option was employed for linkage analysis. A LOD score of 4.0 and maximum recombination of 40% was used for two-point analysis and a LOD score of 3.0 was subsequently used for all three-point and multi-point analysis. Chi-square values were calculated to examine if the observed allelic and genotypic frequencies of the marker loci deviated from the expected ratios.

QTL analysis was performed using the software-package PLABQTL (Utz and Melchinger 1996) based on composite interval mapping (CIM). Co-factors were assessed by the procedure Cov SELECT. The critical LOD value of 3.41, as determined by the above program based on Bonferroni chi-square approximation, was selected for declaring the presence of a QTL. With such a threshold, the probability that even a single false-positive QTL would be detected anywhere in the sorghum genome is approximately 0.05. The QTLs detected with a LOD score of >2.5 were also reported, which can be viewed as suggestive QTLs. The proportion of total phenotypic variance explained collectively by all identified QTLs for each trait was obtained by fitting the multiple regression models containing all QTLs for that trait in PLABQTL under composite interval mapping. QTLs were designated with the abbreviation for the trait name and the linkage group. For example, stay green, pre-flowering drought tolerance, lodging, plant height and flowering time were designated as *Stg*, *Pfr*, *Ldg*, *Pht* and *Flr*, respectively, and a QTL for stay green on linkage group A was designated as *Stg A*.

Data were analyzed using the SAS statistical program (SAS Institute 1989). The Proc GLM procedure was used to test differences between RILs in each environment and over environments, assuming a random statistical model. Pearson's correlation coefficients were calculated to determine relationships between the various traits under study. Broad-sense heritability was estimated for the traits on a family mean basis using the estimated variance components.

Results

Phenotypic trait analysis

The results obtained for the two parental lines and RILs with respect to all the traits are summarized in Table 1. Most of the traits showed moderate to high heritability. The stay green rating for the stay green parent, SC56, was significantly higher than that of the senescent parent, Tx7000, with a mean of 2.1, whereas the mean rating for the senescent parent was 3.7. The mean stay

Table 1 Means of parents SC56 and Tx7000 and 125 RILs, with their mean square, range and broad-sense heritability (h^2) estimates under different environments for stay green, lodging, pre-flowering drought tolerance, flowering time and plant-height

Trait	Environment[a]	Parental mean		Mean RILs	Mean square	Range	h^2
		SC56	T×7000				
Stay green (1–5 Scale)	HW98	2.2**	4.1**	2.5	1.0**	1.2–4.5	0.73
	LD97	2.0**	4.0**	2.2	0.8**	1.1–4.3	0.83
	GK98	2.0**	4.1**	2.2	0.3**	1.3–4.8	0.58
	CB97	2.2**	3.5**	2.5	0.3**	1.3–3.5	0.71
	LL98	2.0*	2.8*	2.9	0.7**	1.5–5.0	0.68
Lodging (%)	LL98	5**	31**	11	445.5**	0–100	0.68
Pre-flowering drought tolerance (1–5 Scale)	LD97	3.1**	1.6**	2.7	1.1**	1.3–4.8	0.77
	CB97	4.5**	2.7**	3.0	2.0**	1.2–5.0	0.76
	LD99	3.9**	2.4**	2.9	0.74**	1.5–5.0	0.68
Flowering time (days)	LL98	70**	62**	66	79.4**	57–83	0.87
	LL97	59**	60**	60	80.2**	34–82	0.86
	PV98	77**	71**	73	114.7**	59–89	0.95
Plant-height (cm)	LL98	39^{NS}	39^{NS}	38	68.7**	23–61	0.85
	LL97	37^{NS}	37^{NS}	37	40.3**	22–59	0.73

*, ** Significant at 0.05 and 0.01 probability levels respectively; ^{NS} Not significant

[a] HW98: Halfway 98, LD97: Lubbock dryland 97, LD99: Lubbock dryland 99; GK98: Galveston, Kansas 98, CB97: Crossbyton 98, LL98: Lubbock Limited 98; LL97: Lubbock Limited 97; PV98: Plainview 98

green rating for the RILs was 2.5 with a range of 1.1–4.8. A highly significant difference was observed among the RILs for this trait.

The lodging-tolerant parent, SC56, showed a significantly lower mean lodging value (5%) than the susceptible parent Tx7000 (31%). The RILs showed a highly significant difference among each other for this trait with a mean value of 11% and a range of 0–100%. Tx7000 (the pre-flowering drought-tolerant parent) had a significantly lower mean score (2.2) for pre-flowering drought stress compared to the susceptible parent SC56 (3.8). The RILs showed a mean value of 2.9 with a range of 1.2–5.0. Highly significant differences were observed among the RILs for this trait.

The two parental lines differed significantly as regards to days to flowering. Averaged over environments the stay green parent SC56 flowered 5 days later than the non-stay green parent Tx7000. Mean flowering time for the RILs was 66 days with a range of 34–89 days. The RILs were significantly different among each other for this trait. For plant height, although parents did not differ significantly, a wide range of variation was observed with a range of 22–61 cm indicating the occurrence of transgressive segregation.

Analysis of variance revealed significant differences between the RILs for each trait. Estimates for σ_g^2 and for σ_{ge}^2 indicated that both genotypic effects and genotype×environment interactions were highly significant ($p<0.0001$) for all traits that were evaluated in multiple environments (Table 2). Therefore, phenotypic data were not averaged over environments. Heritability of the different traits ranged from 0.72 to 0.84, which is comparable with the estimates obtained on a single-environment basis. High-heritability estimates observed in most traits indicated less environmental influence over these traits.

Phenotypic correlation coefficients were estimated among different traits. Stay green showed a highly significant correlation with lodging ($r=0.221$, $P<0.01$), pre-flowering drought stress ($r=-0.448$, $P<0.01$) and days to flowering ($r=-0.437$, $P<0.01$) (Table 3). Lodging also showed a highly significant negative correlation with pre-flowering drought stress and flowering time, whereas pre-flowering drought stress showed a highly significant positive correlation with days to flowering ($r=0.698$, $P<0.001$). Plant height showed a significant correlation only with flowering time.

Table 2 Estimates of variance components and heritabilities (h^2) over environments for stay green (*Stg*), pre-flowering drought-stress tolerance (*Pfr*), flowering time (*Flr*) and plant-height (*Pht*) for 125 RILs from the cross SC56×Tx7000

Trait	σ_g^2	σ_{ge}^2	h^2	Environments
Stg	0.14***	0.07***	0.84	5
Pfr	0.41***	0.27***	0.72	3
Flr	19.78***	7.96***	0.82	3
Pht	17.70***	5.06***	0.78	2

*** Significant at 0.001 probability level

Table 3 Phenotypic correlation coefficients averaged over environments among stay green (*Stg*), lodging tolerance (*Ldg*), pre-flowering drought-stress tolerance (*Pfr*), flowering time (*Flr*) and plant-height (*Pht*) for 125 RILs from the cross SC56×Tx7000

Trait	Stg	Ldg	Pfr	Flr
Ldg	0.221**			
Pfr	–0.448***	–0.256**		
Flr	–0.437***	–0.229**	0.698***	
Pht	-0.158^{NS}	0.133^{NS}	0.066^{NS}	0.203**

, * Significant at 0.01 and 0.001 probability levels, respectively
^{NS} Not significant

Linkage map

A total of 170 RFLP probes were used to survey polymorphisms between the parents SC56 and Tx7000. One hundred and forty four markers were placed on the linkage map with ten linkage groups covering a distance of 1,355 cM (Kosambi function) (Fig. 1). Linkage groups ranged from 45.7 cM to 225.2 cM with an average distance of 9.4 cM between loci. These linkage groups were designated as A to J and match the same linkage groups as those of Xu et al. (2000). On average, 46.9% of the genome was homozygous for SC56 alleles, 47.5% of the genome was homozygous for Tx7000 alleles, and 5.6% of the genome was heterozygous indicating a 1:1 ratio of the transmission of parental alleles.

Detection of QTLs

For QTL detection and localization, only composite interval mapping (CIM) is reported for all the traits, since results obtained with CIM were quite close to those obtained with simple interval mapping (SIM) (Rami et al. 1998). The CIM increases the efficiency of QTL mapping by reducing the effect of the variance of each genotypic class at positions other than those being tested. Nine QTLs were identified that influenced stay green over five environments (Table 4 and Fig. 1). Most of the QTLs explained phenotypic variation of 10–15%, with LOD scores ranging from 2.63 to 4.21. In only one environment, GK98, *Stg G* explained a phenotypic variation of 22.6% with a LOD of 5.95 and this QTL was consistent, being identified in four environments. Similarly, *Stg J* was consistent in three environments, while other stay green QTLs were identified in a single environment. In the case of the four QTLs on linkage groups C, B, D and F, Tx7000 alleles contributed towards stay green expression while the SC56 alleles were responsible in the other QTL loci. In each environment, where multiple QTLs for stay green were reported, all the QTLs together

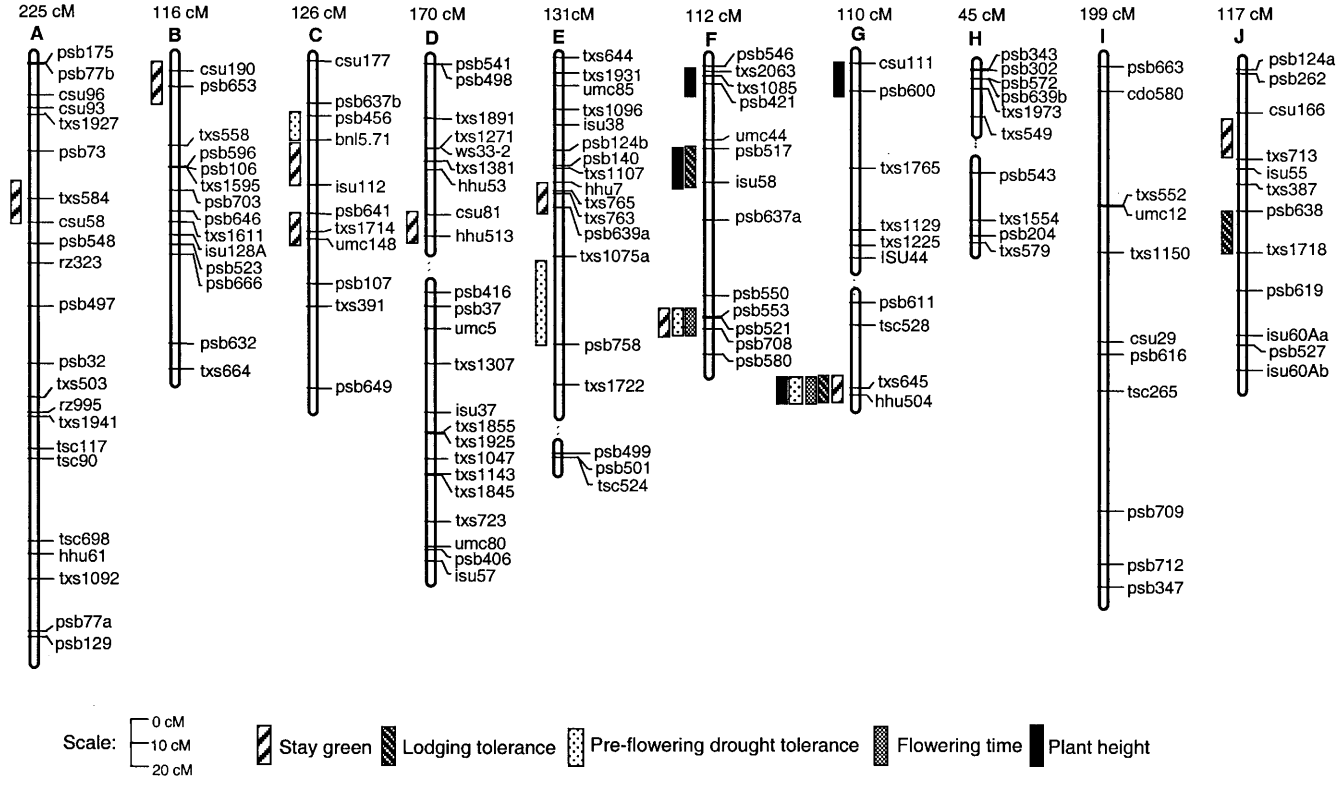

Fig. 1 RFLP linkage map of sorghum showing positions of quantitative trait loci (QTLs) influencing stay green, lodging tolerance, pre-flowering drought-stress tolerance, flowering time and plant height under drought-stress environments. The map was developed using the F_7 RIL population of the cross SC56×Tx7000. Total chromosome length in centiMorgans (Kosambi function) is indicated at the top of each linkage group

explained around 25% of the phenotypic variation indicating strong interaction among the QTLs.

The data on lodging could be collected only in one environment (LL98) and three QTLs were detected on linkage groups F, G and J (Table 4 and Fig. 1). The phenotypic variation explained by individual QTLs ranged from 14.6 to 19.1% with a LOD score of 3.87 to 5.21, and in each case the contribution was from the stay green parent SC56, as expected, unlike the stay green trait described above.

Four QTLs were found to be associated with pre-flowering drought-stress tolerance, which was evaluated over three environments (Table 4). The phenotypic variance explained by individual QTLs ranged from 11.9 to 37.7% with a LOD score range of 3.14 to 9.88. The QTL *Pfr F* was consistent over all three environments and the SC56 allele contributed towards pre-flowering drought tolerance at this locus whereas *Pfr G* was consistent in two environments and the tolerance allele was from Tx7000. For the other two QTLs identified, the source of contribution was SC56 in the case of *Pfr E* and Tx7000 in the case of *Pfr C*.

Two QTLs, *Flr F* and *Flr G*, were detected for flowering time, with the former explaining around 36% of the phenotypic variation (LOD value 10.59) (Table 4). The QTL *Flr G* explained up to 19.2% of the phenotypic variation with a LOD score of 4.95 and the alleles for earlier flowering were inherited from Tx7000, in contrast to the SC56 allele promoting earliness at the *Flr F* region. Two QTLs for plant-height, *Pht G.1* and *Pht F.1*, were common in both the environments LL97 and LL98, and were identified with LOD scores of 3.2 to 6.3 and a phenotypic variation range of 12.5 to 24.2%. Two other QTLs for plant-height were also detected, one each on the same linkage groups G and F. The increasing-height QTL alleles were contributed from Tx7000 at *Pht F.1* and *Pht G.2* and, in the other two QTL regions, the SC56 alleles increased height.

Evidence of pleiotropism

Since all the five traits studied in this investigation are physiologically related, it is of interest to examine the genetic relationships between them. The simple correlation studies reported above showed that most of the traits were related to each other, except for plant-height which showed a significant positive correlation with only flowering time. We noticed three genomic regions, two on linkage group F and one on linkage group G, where the QTLs for different traits overlapped. The QTLs *Ldg F* and *Pht F.1* mapped to approximately the same chromosomal location. Similarly, *Stg F, Pfr F* and *Flr F* were mapped to identical genomic regions, and so also were the QTLs *Pht G.2, Pfr G, Flr G, Ldg G* and *Stg G* on linkage group G.

Table 4 Quantitative trait loci (QTLs) associated with stay green, lodging tolerance, pre-flowering drought-stress tolerance, flowering time and plant-height in sorghum F_7 RILs from the cross SC56×Tx7000 under drought-stress environments

Trait	Environment[a]	QTLs	Flanking markers	LG[b]	Allelic effects[c]	LOD	R^2(%)[d]	Source
Stay-green	CB97	*Stg J*	csu166–txs713	J	0.181	4.21	15.4	SC56
		Stg C.2	psb641–txs1714	C	–0.216	4.11	15.1	Tx7000
		Stg G	txs645–hhu504	G	0.131	3.06	12.1	SC56
		Stg C.1	bnl5.71–isu112	C	0.197	3.63	13.4	SC56
		Total				6.53	22.8	
	LD97	*Stg B*	csu190–psb653	B	–0.206	3.65	13.8	Tx7000
		Stg E	txs765–txs763	E	0.368	3.53	13.0	SC56
		Stg G	txs645–hhu504	G	0.171	2.76	11.0	SC56
		Stg D	csu81–hhu513	D	–0.301	2.66	9.9	Tx7000
		Total				7.22	24.7	
	GK98	*Stg G*	txs645–hhu504	G	0.348	5.95	22.6	SC56
	HW98	*Stg A*	txs584–csu58	A	0.146	2.63	10.2	SC56
		Stg G	txs645–hhu504	G	0.139	2.81	11.4	SC56
		Stg J	csu166–txs713J		0.171	3.66	13.9	SC56
		Stg F	psb521–psb708	F	–0.137	2.86	11.4	Tx7000
		Total				7.42	26.1	
	LL98	*Stg J*	csu166–txs713	0.137	0.137	4.14	15.5	SC56
Lodging tolerance	LL98	*Ldg G*	txs645–hhu504	G	5.382	4.45	17.4	SC56
		Ldg F	psb517–isu58	F	6.484	5.21	19.1	SC56
		Ldg J	psb638–txs1718	J	6.130	3.87	14.6	SC56
		Total				17.8		
Pre-flowering drought tolerance	LD97	*Prf F*	psb553–psb521	F	0.326	5.22	18.7	SC56
	CB97	*Prf F*	psb521–psb708	F	0.432	6.31	22.2	SC56
		Prf E	txs1075a-psb758	E	0.378	3.14	11.9	SC56
		Prf G	txs645–hhu504	G	–0.343	3.28	15.0	Tx7000
		Total				7.56	25.7	
	LD99	*Prf F*	psb521–psb708	F	0.452	6.36	25.2	SC56
		Prf G	txs645–hhu504	G	–0.562	9.88	37.7	Tx7000
		Prf C	psb456–bnl5.71	C	–0.445	4.66	19.3	Tx7000
		Total				9.47	34.8	
Flowering time	LL97	*Flr F*	psb521–psb708	F	3.985	10.94	35.2	SC56
	LL98	*Flr F*	psb521–psb708	F	3.883	8.41	29.2	SC56
		Flr G	txs645–hhu504	G	–2.848	4.95	19.2	Tx7000
		Total				8.50	29.3	
	PV98	*Flr F*	psb521–psb708	F	4.848	10.59	35.8	SC56
		Flr G	txs645–hhu504	G	–2.423	3.42	13.9	Tx7000
		Total				9.45	32.4	
Plant height	LL97	*Pht G.1*	psb600–csu111	G	–1.958	6.30	24.2	SC56
		Pht F.2	psb421–umc44	F	–1.375	3.45	12.7	SC56
		Pht F.1	psb517–isu58	F	1.569	3.38	12.5	Tx7000
		Pht G.2	txs645–hhu504	G	1.279	3.12	12.4	Tx7000
		Total				10.69	34.4	
	LL98	*Pht G.1*	psb600–csu111	G	–1.859	3.32	13.8	SC56
		Pht F.1	psb517–isu58	F	2.263	4.15	15.6	Tx7000
		Total				5.01	18.5	

[a] HW98: Halfway 98, LD97: Lubbock dryland 97, LD99: Lubbock dryland 99; GK98: Galveston, Kansas 98, CB97: Crossbyton 98, LL98: Lubbock Limited 98; LL97: Lubbock Limited 97; PV98: Plainview 98

[b] LG stands for linkage groups

[c] Allelic effect of Tx7000

[d] Percentage phenotypic variation explained

Flowering time is believed to have a great influence on both stay green and pre-flowering drought tolerance since most late-maturing types behave like stay green, complicating the stay green scoring. In the case of the txs645-hhu504 region, the alleles from SC56 improved lodging, stay green and pre-flowering drought tolerance, whereas substitution for the Tx7000 allele at this locus increased plant-height and induced earliness. Similarly, in the psb553–psb521 region on linkage group F, SC56 alleles contributed to early flowering while Tx7000 alleles improved stay green and pre-flowering drought tolerance.

Discussion

Comparison of stay green QTLs using different sources of stay green

Prior to this investigation, stay green QTLs have been mapped in three different RIL populations (Tuinstra et al. 1997; Crasta et al. 1999; Xu et al. 2000). In all these reports, the stay green line B35 was used as the stay green source. B35 is a BC_1 derivative of IS12555, a durra sorghum race from Ethiopia that responds distinctly differently to drought at pre-flowering and post-flowering stages compared to Tx7000. However, in this current investigation, we used another very well recognized stay green line SC56, which is a caudatum-nigricans race from Sudan. This line is tolerant to stalk lodging but susceptible to pre-flowering drought stress like B35. This study allowed us to compare the genetic analysis of this trait using different sources of stay green. Comparison of the stay green QTL locations with the earlier reports indicated that the stay green QTLs *Stg A*, *Stg G* and *Stg J* of the current map were consistent with the QTLs identified earlier in different populations (Tuinstra et al. 1997; Crasta et al. 1999; Xu et al. 2000) (Fig. 2). *Stg A*, and *Stg J* were located in identical genomic locations in the B35×T×430 and B35×Tx7000 populations, and *Stg G* might be the same QTL as reported by Crasta et al. (1999) and Tuinstra et al. (1997) on linkage groups G and F, respectively. At all these loci, the SC56 allele improved the stay green rating. Both *Stg G* and *Stg J* were highly consistent in different environments and *Stg A* was the major QTL for stay green identified by both Crasta et al. (1999) and Xu et al. (2000). No congruency was observed for the rest of the stay green QTLs in the earlier reports. These QTLs may be considered of lesser importance because of inconsistency and, also, in 4 out of the 6 remaining QTLs, the non-stay green line Tx7000 contributed to stay green expression.

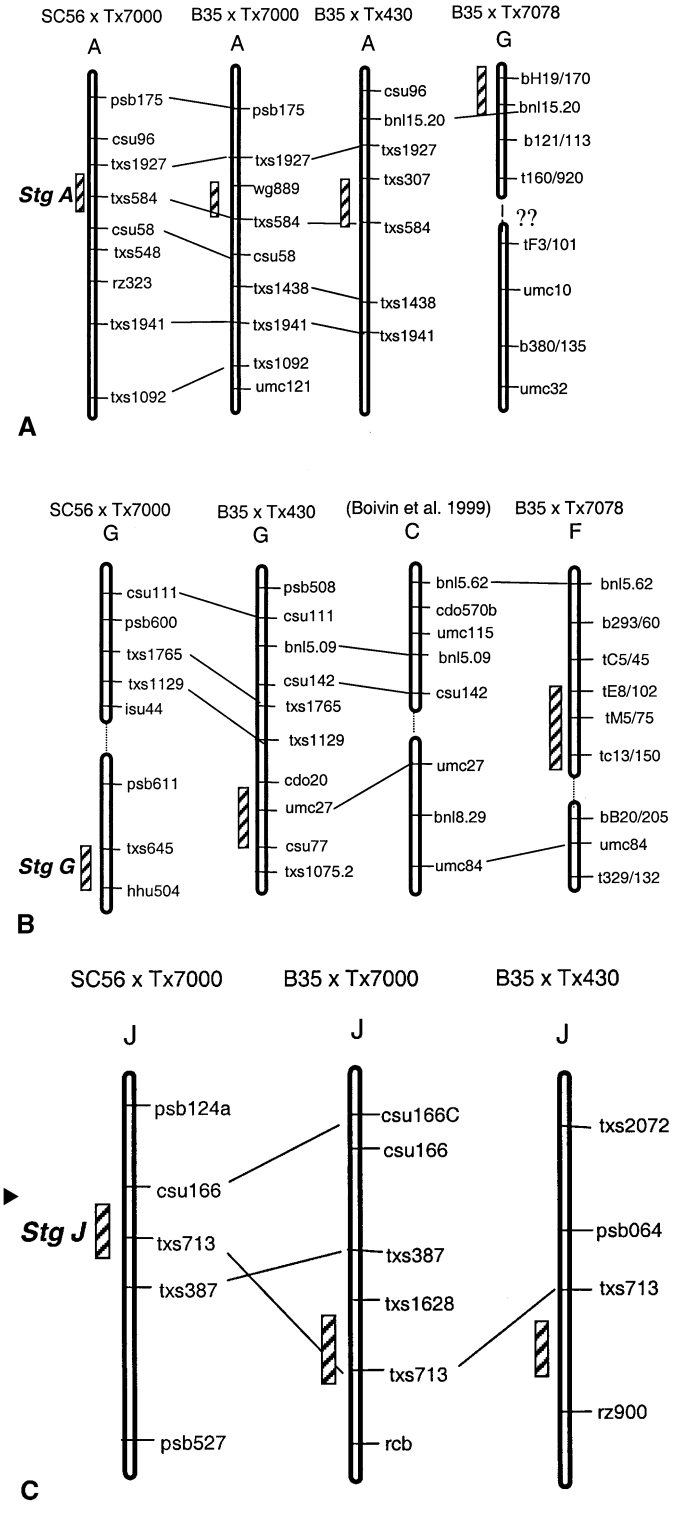

Fig. 2 Comparative map location of stay green QTL locations on linkage groups A, G and J with the earlier published results of Xu et al. (2000), Crasta et al. (1999) and Tuinstra et al. (1997). Maps are drawn with approximate locations of markers. The map of Boivin et al. (1999) was used to work out the correspondence of linkage group G with linkage group F of Tuinstra et al. (1997). The recombinant inbred line (*RIL*) populations used for mapping the stay green trait by Xu et al. (2000), Crasta et al. (1999) and Tuinstra et al. (1997) are developed from the crosses B35×Tx7000, and B35×Tx430, B35×Tx7078 respectively, where B35 is the stay green source in contrast to SC56 of the present investigation. Stay green QTL locations are indicated by hatched bars on the left of each linkage group

Comparative mapping of QTLs for stay green and other drought tolerance traits in sorghum, maize and rice

As sorghum and maize share a high level of homology, the present study allowed us to compare syntenic regions putatively linked to drought tolerance traits in these two species. The QTL *Stg A*, which was identified as the major stay green QTL in earlier studies (Crasta et al. 1999; Xu et al. 2000), showed correspondence to a maize stay green QTL on chromosome 8 (Beavis et al. 1994) (Fig. 3a). Correspondence between these two QTL locations was determined using a sorghum genetic map developed by Boivin et al. (1999). In the same genomic region, a QTL for chlorophyll content was mapped by Xu et al. (2000). A QTL regulating nodal root number was reported to be located at this stay green QTL region in maize (Lebreton et al. 1995). Maize clones encoding heat-shock proteins, specifically hsp70, were also localized in this region in both maize (Davis et al. 1999) and sorghum (Xu et al. 2000). Drought tolerance studies in rice indicated that the corresponding genomic regions were associated with the root-penetration index and the total root number (Ray et al. 1996) (Fig. 3a). These results suggest that this genomic region in sorghum, maize and rice may be responsible for traits of importance to improve drought tolerance, either through pleiotropic effects or tight linkage of a cluster of important genes involved in drought tolerance.

Another stay green QTL, *Stg B*, identified in this study showed correspondence with the maize stay green QTL on chromosome 9 (Beavis et al. 1994) (Fig. 3b). This chromosomal region was reported to have an influence on drought-related traits such as leaf abscisic acid (ABA) content (Lebreton et al. 1995). This region also contained genes encoding hsp70 (Davis et al. 1999), and the corresponding region in rice chromosome 6 harbored QTLs for root penetration ability and total root numbers, which are important factors for improving drought tolerance (Ray et al. 1996) (Fig. 3b).

We discovered that QTLs influencing drought-stress-related traits such as stomatal conductance, leaf ABA content, xylem ABA content, root pulling force, nodal root number and the drought sensitivity index in maize (Lebreton et al. 1995; Tuberosa et al. 1998; Sanguineti et al. 1999) mapped to the identical position corresponding to the stay green QTL on chromosome 1 of maize (Beavis et al. 1994) (Fig. 3c). In rice, the corresponding region on chromosome 3 was associated with root traits and stomatal behavior related to drought-stress (Ray et al. 1996; Price et al. 1997) (Fig. 3c). This region corresponded to the stay green QTL on linkage group F (Tuinstra et al. 1997) and was associated with yield stability and grain yield under pre-flowering and post-flowering drought-stress conditions, respectively, in sorghum (Tuinstra et al. 1998) and in maize (Frova et al. 1999). Tuinstra et al. (1997) earlier suggested the presence of tolerance genes at this locus due to the association of this QTL with grain yield under post-flowering drought conditions. They further suggested that some underlying tolerance mechanism controls the expression of stay green under post-flowering drought and yield in both drought and non-drought environments.

The QTL affecting flowering-time, specifically *Flr F*, showed association with *Pfr F* (Fig. 1). This result is consistent with that of Lin et al. (1995) in sorghum where the marker psb521 is common to these corresponding QTLs on the two maps. This flowering-time QTL alone accounted for up to 35% of the phenotypic variance. A strong phenotypic correlation between flowering time and plant-height in sorghum was previously reported (Lin et al. 1995) as observed in the present study (Table 2). No height QTL could be mapped in this region but, in the *Flr G* region, a plant-height QTL overlapped with one for flowering. This association is explained by the fact that flowering terminates the apical growth, which is true in most species of the grass family. Comparative mapping for plant-height across species revealed that a QTL region on linkage group F of the present study is syntenic to a region on maize chromosome 2 (Veldboom and Lee 1996). In sorghum, *Pht G.1* and *Pht F.1* corresponded to the plant-height QTL on linkage group C of Lin et al. (1995) and linkage group B of Pereira and Lee (1995), respectively (Fig. 4).

Mapping of lodging and pre-flowering drought tolerance

A positive relationship was found between stay green and lodging tolerance under post-flowering drought-stress conditions. In this study a significant phenotypic correlation was observed between lodging and stay green ($r=0.221$, $P<0.01$). Stay green and the lodging QTL overlapped on one genomic region in linkage group G (Fig. 1). At this locus, the lodging tolerance allele was derived from the stay green parent. Earlier reports indicated that lodging is associated with plant-height (Pinthus 1967; Keller et al. 1999). In the present study two lodging QTLs, *Ldg F* and *Ldg G*, overlapped with plant-height QTLs. However, no phenotypic correlation was observed between these two traits. The *Ldg F* and *Pht F.1* region was found to be syntenic to a plant-height and stay green QTL region on maize chromosome 2 (Beavis et al. 1994; Vedloom and Lee 1996) (Figs. 1 and 4). This may be due to the fact that this type of lodging was induced by a post-flowering drought-stress, the tolerance for which was contributed by the SC56 allele.

Pfr G is a major QTL influencing pre-flowering drought-stress tolerance, accounting for 15% and 37.7% of the phenotypic variance under the two environments, and at this locus the allele for pre-flowering drought-stress tolerance was from the tolerant parent Tx7000. *Pfr F* and *Pfr G* overlapped with QTLs influencing flowering time on linkage groups F and G (Fig. 1) explaining the strong phenotypic correlation observed between these two traits (Table 2). This association is probably due to the effect of pre-flowering drought stress on flowering time (Rosenow et al. 1996).

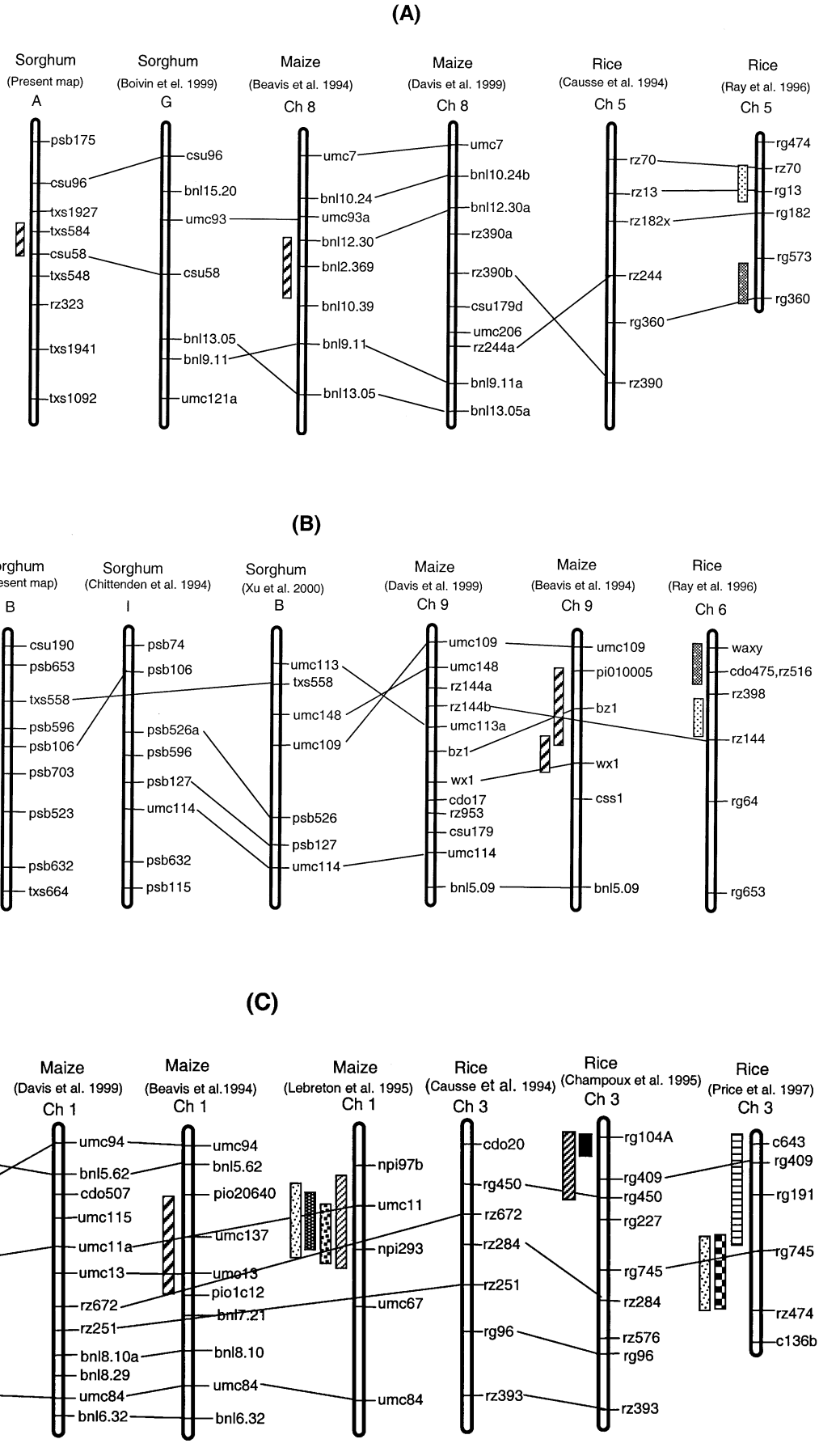

Fig. 3A–C Comparison of the map locations of several drought tolerance traits in rice and maize corresponding to stay green QTL regions in sorghum and maize. Location of the genomic regions in rice and maize were inferred relative to linked DNA markers. (**A**) Corresponding regions in maize and rice harboring stay green and drought-related root traits, respectively, relative to the stay green QTL *Stg A* of the present investigation. (**B**) Genomic regions harboring drought-related traits of rice and stay green QTLs of maize corresponding to the stay green QTL *Stg B*. (**C**) Genomic regions harboring drought-related traits in maize and rice corresponding to the stay green QTL on chromosome 1 of maize (Beavis et al. 1994) that is syntenic to the stay green QTL on linkage group F (Tuinstra et al. 1997)

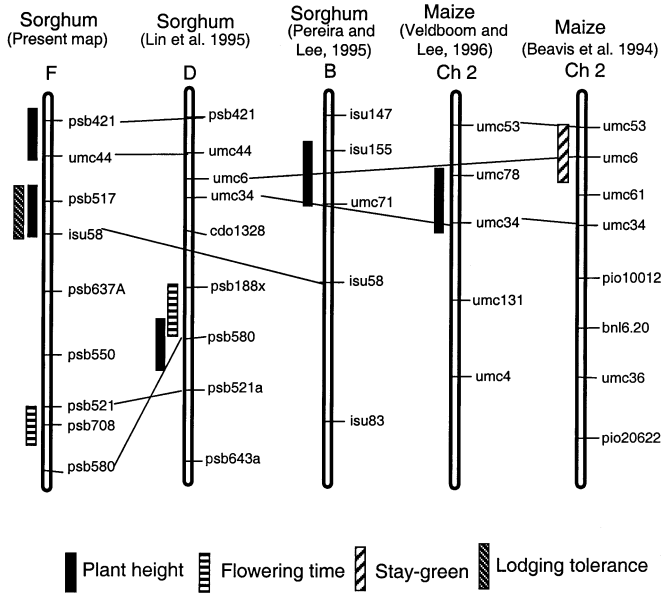

Fig. 4 Comparative map locations of QTLs for plant-height and flowering time in sorghum (Lin et al. 1995) and maize (Pereira and Lee 1995; Vedloom and Lee 1996). A plant-height QTL overlapped with a lodging-tolerance QTL in the present study and this region is syntenic to a stay green QTL region on chromosome 2 (Beavis et al. 1994)

Strategy for improving drought tolerance using QTL information

Drought-stress is an unpredictable event and its timing and intensity vary during different stages of crop growth. Therefore, progress from classical plant breeding programs aimed at improving drought tolerance has been very slow in any crop. The identification of QTLs affecting important drought tolerance traits is an important step in the use of molecular markers for crop improvement and in understanding the genetic factors that determine these traits. A significant finding of the present study is that three stay green QTLs (*Stg A*, *Stg G* and *Stg J*) were consistent across sorghum populations developed from two sources of stay green with different genetic backgrounds. Two of these QTLs (*Stg A*, *Stg G)* and a new one from SC56 *(Stg B)* have shown correspondence to stay green QTL regions in maize. This cross-species information increases our confidence about the existence of QTLs affecting stay green expression in these genomic regions and implies the same type of mechanism in both species. Moreover, these genomic regions were also associated with several drought-related agronomic and physiological traits in maize and rice, suggesting that these syntenic regions could have been conserved during evolution for drought tolerance in these species. It remains to be tested whether these genomic regions have pleiotropic effects or if there are clusters of tightly linked genes for many drought-related traits in these regions. Detailed characterization of these genomic regions through the development and evaluation of near-isogenic lines will definitely lead to an improved understanding of drought tolerance and might set the stage for the positional cloning of drought tolerance genes. These specific genomic regions associated with the stay green and several drought-related traits should also be targeted for marker-assisted introgression for the development of drought-tolerant sorghum and other grass species.

Another important finding is the mapping of QTLs with a major effect for lodging tolerance and pre-flowering drought tolerance. Since the determination of the map location of QTLs for lodging tolerance was based on data for only 1 year, the utility of the loci identified will depend on the level of expression in multiple environments and different genetic backgrounds. However, to our knowledge this is the first report on the mapping of QTLs influencing lodging tolerance in sorghum. In Australia the stay green trait has been successfully used to improve lodging-tolerance under post-flowering or terminal drought-stress conditions (Henzell et al. 1992). There is evidence that SC56, the lodging-tolerant parent, possesses some lodging tolerance independent of drought-related lodging. This suggests that the lodging tolerance QTL, which did not coincide with stay green QTL regions, could be utilized in selecting a non-stress type of lodging tolerance that can be useful under many production conditions. Interaction between the timing of stress and the stage of plant growth limits the success for drought tolerance management in any crop. Sorghum genotypes that are drought-tolerant during one growth stage are susceptible to stress at other times (Rosenow and Clark 1981). Incorporation of genes for both stay green and pre-flowering drought tolerance is likely to improve the ability of future sorghum cultivars to withstand drought at different stages of growth. Most of the QTLs have an independent location except for two genomic regions on linkage groups F and G, where QTLs for several traits overlapped. Further studies may be directed to understand the genetic basis of such association, which might be due to tight linkage or the sharing of common genes. Therefore, the pyramiding of favorable alleles for pre-flowering drought tolerance, stay green and lodging tolerance through marker-assisted selection may help in breeding more drought-tolerant sorghum cultivars in the near future.

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