

Evaluation of Stay-Green QTL Introgression Lines for Drought Tolerance in Sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Stay-green QTL introgression lines (plus the parents and checks) were evaluated during the 2004/05 and 2005/06 post-rainy sorghum cropping season at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, India. The stay-green introgression lines (BC2F2/BC1F3) were field evaluated for their agronomic and stay-green related characters under well watered and water-stress conditions. The introgression lines had high green leaf area (%) and chlorophyll content compared with the senescent lines showing the relationships among these traits. Moreover, many of the introgression lines had grain yields statistically similar to grain yields of the recurrent parent (R16). The introgression lines such as RSG04001 were good both in terms of green leaf area (%) and grain yield indicating the contribution of stay green to grain yield. This has also been evidenced by the highly significant ($R^2 = 0.47$ in 2004/05 and $R^2 = 0.77$ in 2005/06) correlations of relative grain yield to relative green leaf area (%) in the moisture stress environments. Overall, the results showed promise for utilization of the stay-green trait for better grain yield in drought-prone environments.

Key words: drought tolerance, stay-green, MABC, QTL, sorghum

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is globally the fifth most important cereal crop after wheat, maize, rice and barley (FAO, 2006). Sorghum is unique among the major cereals in that its grain is one of the main staples for the world's poorest and most food-insecure people located in the semi-arid tropics. World sorghum grain production was about 57 million metric tons and the total area under sorghum was about 42.7 million hectares during 2004/05 (FAO, 2006).

Improving the drought tolerance of sorghum has been considered as a plant breeding target of utmost importance to minimize yield losses resulting from moisture stress. The most damaging drought is that which occurs during the post-flowering stage (referred to as

“terminal drought”). Genotypes sensitive to this type of drought are characterized by premature leaf and plant death (senescence), stalk collapse and lodging, charcoal rot, and reduced grain number and size. In sorghum, the best characterized form of drought tolerance during this crop growth stage is the so called “non-senescence (stay-green)” trait. This refers to the ability to resist premature plant senescence, retain green leaf area, fill grain normally, and resist lodging under post-flowering drought stress conditions (Rosenow, 1987). Under grain-filling period water limitation, stay-green sorghum types maintain photosynthetically active leaf area better than those that do not possess this trait (Rosenow *et al.* 1983).

Delayed leaf senescence (stay-green) in sorghum has been linked to improved grain yields particularly in environments in

which available water during grain filling is not adequate to support potential transpiration (Borrell *et al.*, 1999, 2000a, b). Yield increases in stay-green types have been attributed directly to the maintenance of photosynthetic capability during grain filling period. During post-anthesis, drought tolerant hybrids containing the stay-green trait maintain more photosynthetically active leaves compared with hybrids not having this. (Rosenow *et al.*, 1983, Borrell *et al.*, 2000a,b). A delayed remobilization of N from the leaves or a remobilization of N from leaves having a larger pool of N in stay-green genotypes would, indeed, maintain photosynthetic capacity for longer, and therefore carbohydrate supply to the developing grain, possibly resulting in higher grain yield. Under post-anthesis drought, grain yield in sorghum is positively correlated with green leaf area at maturity (Borrell *et al.*, 2000b) at mid grain filling (Borrell *et al.*, 1999). Borrell *et al.* (2000b) observed that under terminal moisture deficit conditions stay-green sorghum hybrids produced 47% more post-anthesis biomass than their counterparts.

The complex expression of drought tolerance makes the stay-green trait difficult to study using traditional genetic and physiological methods. Since the stay-green character in sorghum is expressed only in environments with sufficiently severe post-anthesis drought, neither efficiency nor reliability of selection is high when conventional breeding is used to select for this trait. It has been proposed that use of molecular markers identified in quantitative trait loci (QTL) analysis of stress related traits based on carefully managed, replicated, multi-environment tests has the potential to alleviate the phenotypic selection problems associated with the inconsistent and unpredictable onset of moisture stress or the confounding effect of other stresses such as high temperature (McCouch and Xiao, 1998).

Use of molecular markers and QTL analysis has led to a better understanding of the inheritance of the stay-green trait and mapping of quantitative trait loci (QTLs) associated with post-flowering drought resistance in sorghum (Tuinstra *et al.*, 1996, 1997a, 1998; Crasta *et al.*, 1999; Tao *et al.*, 2000; Xu *et al.*, 2000b; Subudhi *et al.*, 2000; Kebede *et al.*, 2001; Sanchez *et al.*, 2002; Haussmann *et al.*, 2002b). Recent studies on the effects of individual stay-green QTL has also shown the contribution of each QTL in reducing leaf senescence (Harris *et al.* 2007).

The ability to manipulate complex traits such as drought tolerance *via* marker-assisted backcrossing (MABC) makes it much easier to introduce such a new trait into a breeding program. MABC increases efficiency in introgression programs by permitting simultaneous foreground selection for introgression of exotic alleles with potential to improve performance for a trait of interest and background selection for the desired recurrent parent genotype in other genomic regions. Hence, MABC for stay-green should greatly enhance the efficiency of selection for this trait (Borrell *et al.*, 2000b).

While considerable work has been done regarding the mapping of QTLs associated with stay-green and identification of molecular makers linked to these QTLs (Hash *et al.*, 2003), the transfer of these QTLs into elite cultivars and assessment of the performance of the introgression lines of the stay-green trait in different backgrounds has just been initiated. Hence, the objective of the present study was to evaluate stay-green QTLs introgression lines for grain yield, grain yield related traits, percent green leaf area (stay-green) and leaf chlorophyll content.

Materials and Methods

Plant materials

Nine stay-green QTL introgression lines [three BC₂F₃ (RSG 04001, RSG 04002 and RSG 04003) with a single QTL (*StgB*) and six BC₁F₄ (RSG 04004, RSG 04005, RSG 04007, RSG 04009, RSG 04011 and RSG 04014) with multiple QTLs (*StgB*, *Stg1*, *Stg3* and *Stg4*)] and the parents were used in this study. The introgression lines were previously developed by a marker-assisted backcrossing program using B35 (donor parent) and R16 (recurrent parent). B35 is a BC₁ derivative of landrace germplasm accession IS12555, which is durra sorghum from Ethiopia (Rosenow *et al.*, 1983), and is the best characterized source of stay-green for terminal drought tolerance in sorghum. A number of stay-green QTLs have been identified by different research groups based on mapping populations derived from crosses involving this line. The recurrent parent (R16) is a high yielding but senescent line from India. It is a post rainy season-adapted restorer line and the parent of a released hybrid.

Experimental sites and season

The nine stay-green QTL introgression lines (plus the parents and checks) were evaluated during 2004/05 and 2005/06 at ICRISAT, Patancheru, India. The evaluations were done in the post-rainy sorghum cropping season (October to February) in which crops grow largely on stored soil moisture, predictably experiencing moderate but prolonged terminal drought stress during grain filling, thus, forming an ideal environment for evaluating the stay-green trait (Mahalakshmi and Bidinger 2002). The evaluations included a supplementally irrigated environment and one (2004/05) or two (2005/06) “dryland” environments that were managed to exhaust readily available soil moisture at different times.

All environments were planted into a full soil profile and given irrigation at 25–30 days after emergence to rewet the profile and assure secondary root penetration. The 2004/05 dryland and the 2005/06 early-onset stress dryland environments received no further irrigation; the 2005/06 late-onset stress environment received another light sprinkler irrigation of 25 mm at 54 days after emergence.

Experimental design

Plots for the trials were 4 rows × 9 m (2004/05) or × 4 m (2005/06) in a 4 (blocks per rep) × 5 (entries per block) alpha design with 4 replications in each moisture environment.

Observations and data analysis

Three plants per plot were tagged at flowering and the areas of leaves 1 to 6 (where leaf 1 = the flag leaf) were estimated from leaf length and width measurements, based on an experimentally determined ratio of leaf area to leaf length × width for each genotype. The percent green leaf area (GLA%) of each leaf of each of the tagged plants was estimated visually on a weekly basis from flowering to harvest. Weekly weighted (by leaf size) average GLA/plant was calculated and averaged first on a plot basis and then on a genotype basis.

Chlorophyll content of the leaves was measured in the early stress environment of the 2005/06 trial using a Minolta Chlorophyll Meter, SPAD-502 (Minolta Camera Co. Ltd.) as described by Dwyer *et al.* (1991). The SPAD values were taken at the middle of the leaf lamina of the second and fourth leaves from the top from three plants per plot (same plants that were used for visual scoring), averaged on a plot basis for each leaf. SPAD values provide an indication of the relative amount of total chlorophyll present in plant leaves, based on the amount of light transmitted

by the leaf (area 2×3 mm) in two wavelength regions in which the absorption of chlorophyll is different. Higher SPAD values represent higher total chlorophyll contents and the arbitrary SPAD values can be translated to the actual value of total chlorophyll/ unit area (mg cm^{-2}) using the equation (Xu *et al.*, 2000a):

$$\text{chlorophyll content} = \text{SPAD value} \times 0.003 - 0.048$$

Data on yield related characters (grain dry weight, biomass dry weight, harvest index, and 100-grain weight) was taken on 1 m^2 basis.

The data were analyzed using Genstat Version 8.0 REML, General Linear Mixed Model (Lawes Agricultural Trust).

Results and Discussion

Percent green leaf area (GLA%)

The analysis of variance of the GLA% for the full data set indicated significant effects of moisture environmental, trial within environment and genotype, but (apart from the crop maturity stage) no significant genotype \times environment effects (Table 1). This is consistent with the examples in Fig. 1. Differences between grain filling moisture environments, based

on predicted GLA% from the fitted logistic equations, were very clear as GLA at mid grain filling was approximately 82% in the supplemental irrigated control environment *versus* 47% in the stress environment, and 26% at physiological maturity in the control environment *versus* 3% in the stress environment (Table 2). Similarly, there were large differences between the stay-green donor and recurrent parents (Table 2). B35 had an average of 82% GLA at mid grain filling (95% GLA in the control and 75% in the stress), and an average 46% GLA at physiological maturity (70% GLA in the control and 23% in the stress (data not shown). R16, in contrast, had only an average of 45% GLA and mid grain filling (68% in the control and 30% in the stress) and only 3% GLA at physiological maturity (4% in the control and 0% in the stress).

The stay-green QTL introgression lines were generally intermediate between the parents, maintaining an average (across moisture environments) of 60% GLA and mid grain filling (range of 50 - 73%) and 10% GLA at maturity (range of 2-20%) (Table 2). In the individual moisture treatments, the QTL introgression lines at mid grain filling had 70% to 94% GLA *versus* 68% in R16 in the control environment and between 36% and 59% in the stress environment *versus* 30% for R16 (data not shown).

Table 1. Mean squares for genotype, moisture environment and genotype \times moisture environment effects on percent green leaf area at mid grain filling, 2/3 grain filling and physiological maturity for stay-green introgression lines from irrigated (control) and post-flowering stress environments

Source of variation	Df	Percent green leaf area		
		Mid grain filling	2/3 grain filling	Maturity
Environment	1	16408***	26874***	6718***
Trial (environment)	3	525***	1326***	33
Genotype	10	596***	843**	743***
Genotype \times Environment	10	46	130	250***

df = degrees of freedom;***, ** = Significant at $P < 0.001$ and $P < 0.01$, respectively

Similar figures for physiological maturity were 5-48% GLA in the control for the introgression lines *versus* 4% for R16 and 0-3% in the stress environment, *versus* 0% for R16. On average, among the nine introgression lines significantly ($P < 0.05$) higher GLA% than R16 were noted for six lines at mid grain filling, five lines at 2/3 grain filling and two lines at maturity (Table 2). The best of the introgression lines for the first two-thirds of grain filling were RSG 04001 and RSG 04003, with $> 40\%$ mean GLA at 2/3 grain filling, and the best during the last third period of grain filling were RSG 04002, RSG 04003 and RSG 04013, with 10-20% mean GLA at maturity (Table 2).

In the non-stress environment, there were no evident differences in the rate of decline in GLA% among genotypes: however, in the absence of stress, the results showed that the final GLA% at maturity was mainly a function of the time of onset of stress.

In contrast, in the post-flowering drought stress environments, the decline in GLA% began about 15 days after flowering in all genotypes, but the rates of decrease varied among genotypes. B35 had the slowest rate of decrease, and, thus, reached maturity with a substantial percent green leaf area (about 40%). R16 had the greatest rate of leaf senescence under stress and consequently reached zero GLA% well before crop maturity. Most of the QTL

introgression lines were again intermediate, but still had effectively no green leaf left at maturity. From these comparisons, it appears that post flowering moisture stress overrides the normal (in the absence of stress) differences in the time of onset of stress between a stay-green and a senescent line. But the stay-green trait still results in a reduced rate of senescence, and consequently greater GLA% during the critical late stages of grain filling when final grain size is determined. Van Oosterom *et al.* (1996) concluded that the inheritance of onset of senescence was additive whereas the inheritance of the rate of senescence was completely dominant for the slow rate over a fast rate. If it can be assumed that the presence of a partial complement of the total B35 set of stay-green QTLs in the introgression lines is analogous to a genetically heterozygous condition for the trait, the conclusions of van Oosterom *et al.* (1996) describe our findings on the differences in the onset of leaf senescence in the absence of stress but not the differences in rate of senescence under stress (Fig.1). Green leaf area duration, being the sum of an additively and a dominantly inherited trait, displayed partial dominance for a large green leaf area duration in the data of van Oosterom *et al.* (1996). Walulu *et al.* (1994) also suggested that a major gene in B35 influencing leaf area duration exhibits varied levels of dominant gene action depending on the evaluation environment.

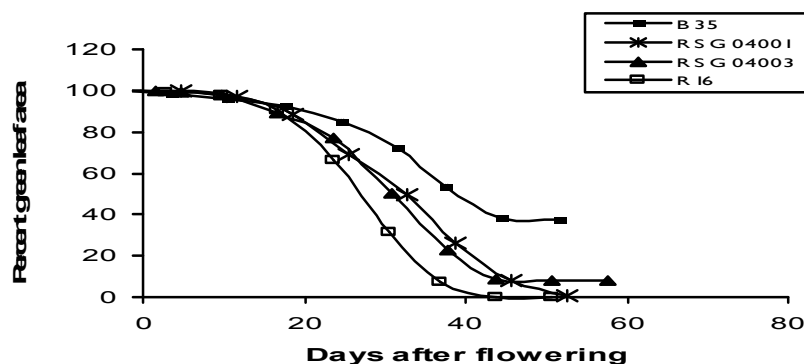


Fig. 1. Leaf senescence patterns for R16, B35, RSG 04001 and RSG 04003 in the late-onset stress dryland environment, 2005/06

Table 2. Percent green leaf area at mid grain filling, 2/3 grain filling and physiological maturity by moisture environment and R16 stay-green QTL introgression line. (The RSG > R16 is the number of QTL introgression lines that are significantly ($P < 0.05$) greater than R16)

Parameters	Mid grain filling	2/3 grain filling	Physiol. Maturity
Environments			
Irrigated control	82.0	60.7	25.90
Post-flowering stress	46.7	15.6	3.30
s.e.d	2.31	2.21	2.17
Genotypes			
B35	82.0	65.0	45.80
R16	45.2	17.0	2.80
RSG 04001	67.4	40.6	7.80
RSG 04002	65.6	34.8	10.20
RSG 04003	72.8	43.8	19.20
RSG 04004	54.8	26.8	9.00
RSG 04005	55.8	33.0	7.60
RSG 04007	55.4	26.2	7.60
RSG 04009	56.2	24.8	2.40
RSG 04011	50.0	24.0	6.80
RSG 04013	63.6	34.2	16.40
s.e.d.	5.31	5.07	4.98
RSG > R16	6	5	2

If leaf senescence is driven by a demand for nitrogen from the developing grain, then the senescence patterns of B35 under the two moisture environments suggest that it may continue to take up soil nitrogen after flowering as long as soil water is plentiful, and thereby delay senescence until well into grain filling. But when soil water supplies are limited, soil N uptake is reduced and it begins breaking down chlorophyll and translocating N from its leaves much earlier in the grain filling period. However its slower rate of leaf senescence (compared to that of R16) even under limited soil water supply suggests that it either still accesses some soil N or that it has a greater N pool in the vegetative parts to draw on, thereby reducing the demand from the leaves and delaying senescence. There are reports in the literature of both effects in stay-green hybrids. For example, Rajcan and Tollenaar (1999b) found that the stay-

green maize hybrid Pioneer 3902 accumulated 60% of total plant N after silking compared to only 40% in the case of the senescent hybrid Pride 5. Borrell and Hammer (2000) reported that from as early as 27 days after emergence stay-green sorghum hybrids had higher percentage of total plant N in the leaves compared with their senescent counterparts. This resulted in a higher specific leaf N in stay-green genotypes at anthesis, mid grain-filling and maturity, which they associated with the reduced rates of leaf senescence.

The finding that most of the R16 derivatives were more stay-green than R16 during the first two-thirds of grain filling, indicates that the transfer of one or more putative stay-green QTLs into R16 did reduce leaf senescence in most of the backcross derivatives. So, it appears that the introgressed stay-green QTLs were well expressed. Ultimately, it would be

useful to assess the contribution of individual QTLs transferred from B35 to R16. While most of the derived lines, some of which had only one to three stay-green QTLs, were less senescent than R16, none approached the degree of non-senescence of B35. The higher degree of stay-green in B35 could be a result of either an additive effect of multiple QTLs, a direct effect of specific QTLs, or a result of complex interactions between favorable alleles at these QTLs. But these effects were not replicated in any of the individual derivatives. Sanchez *et al.* (2002), in reporting the phenotypic contribution of QTLs to the expression of stay-green, indicated that each QTL had a different level of contribution to the expression of the trait, and that the combined effects of the QTLs enhanced stay-green expression, although they were not fully additive. They reported that *Stg1*, *Stg2*, *Stg3*, and *Stg4* individually explained 17.1%, 24.5%, 10.7%, and 11.1% of the phenotypic variance of the stay-green expression, respectively, whereas the four QTLs together explained 42.5% of the phenotypic variance. Harris *et al.* (2007), using near isogenic lines (NIL) for each QTL, also reported the contribution of individual QTL in reducing leaf senescence indicating higher contribution by *Stg2*. This suggests that it may be necessary to pyramid a number of QTLs in the background of a highly senescent line such as R16 in order to achieve the level of expression of non-senescence present in B35. However, the expression of a stay-green QTL in a different (R16) genetic background may not be the same as in B35 background, either because of different kinds of gene interactions or because of a greater sink demand for N in the higher yielding background. This latter effect would mean that it would be very difficult to achieve the characteristically late onset and/or slow rate of leaf senescence of B35 (with its relatively small sink size) in a background with a greater sink size.

Leaf chlorophyll content

In order to determine if the introgression of the B35 stay-green QTL into the R16 background also affected chlorophyll concentration, we made regular SPAD measurements on leaves 2 and 4 of the introgression lines in the 2005/06 early-onset stress environment, from flowering to about 35 days after flowering.

The analysis of the full SPAD data set confirmed that the chlorophyll content was affected by genotype as well as leaf number and date of observation, and all interactions of these factors. B35 and R16 were significantly different over the whole course of the measurements, and the majority of the QTL introgression lines had significantly higher SPAD readings over the first 20 days of grain filling. For example, the mean SPAD reading 5 days after flowering of the introgression lines was 46 whereas the donor parent (B35) and the recurrent parent (R16) had readings of 51 and 32, respectively (Table 3). At this stage, all nine introgression lines had significantly higher readings than R16 and all but three were statistically equal to B35 (Table 3). At about 29 days after flowering, when the senescence was more advanced in the introgression lines, three of the nine were still significantly superior to R16, although all by this time had SPAD readings that were less than that of B35. Clearly the introgression of the B35 alleles at one or more of stay-green QTLs increased the chlorophyll content of the resulting lines to levels above those of R16, especially before leaf senescence became a confounding factor. But, as in the case of leaf senescence itself, this was not to the level of the donor parent. Similar results were reported by Xu *et al.* (2000a) where B35 showed a much higher chlorophyll content (40.1%) than that of the senescent parent Tx7000 (16.2%) at physiological maturity but in this case the RIL population progenies exhibited transgressive segregation, with chlorophyll values ranging from 9.5% to 59.3%.

Table 3. SPAD reading (chlorophyll content) for leaves two and four (from the panicle) for the parents and the R16 stay-green introgression lines during grain filling in the early onset post-flowering stress environment during 2005/06 (The RSG > R16 is the number of QTL introgression lines that are significantly ($P < 0.05$) greater than R16)

Genotype	Time from flowering				
	5 days	15 days	22 days	29 days	36 days
B35	51	50	46	39	22
R16	32	30	23	14	4
RSG 04001	43	39	36	28	12
RSG 04002	47	34	29	18	5
RSG 04003	47	37	31	17	5
RSG 04004	52	49	43	30	13
RSG 04005	43	39	27	17	7
RSG 04007	40	34	29	14	6
RSG 04009	51	44	38	22	6
RSG 04011	46	38	31	15	2
RSG 04013	46	37	30	16	6
s.e.d.	3.3	3.3	3.3	3.3	3.3
RSG > R16	9	7	6	3	2

The changes in GLA% and SPAD reading of leaf 2 over the first 30 days of grain filling were very similar, in terms of both the onset and rate of decline of both parameters (Fig. 2). The most noticeable difference between the two measurements was the differences among genotypes both before and after the onset of senescence in the case of the SPAD readings, where differences were only evident after the onset of senescence in the case of GLA%. Not only did the onset of the decline in the

SPAD reading begin later in B35 compared to R16, but the B35 values were approximately 25% greater than those of R16 before the onset of the decline. The SPAD data for two QTL introgression lines presented in Fig. 2 indicate that they fell between their recurrent and donor parents in terms of leaf chlorophyll, both before and after the onset of leaf senescence. This indicates that the B35 alleles they contain also affected leaf chlorophyll content.

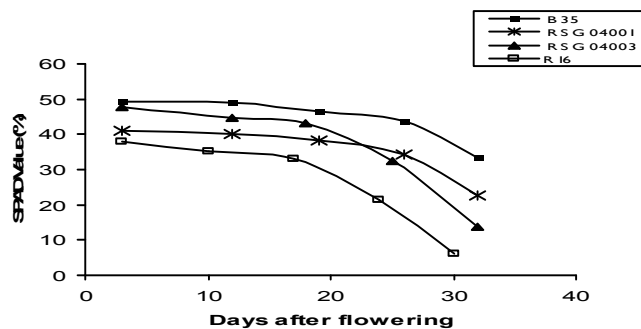


Fig. 2. SPAD values for leaf two of R16, B35, RSG 04001 and RSG 04003 in the early-onset stress dryland environment, 2005/06

In our study, chlorophyll content and GLA% (visual scoring), based on the data from the second leaf at 30 days after flowering (when leaf senescence had begun) showed very high correlation ($r^2 = 0.93$, $P \leq 0.001$) in the early-onset stress environment. This suggests strong relationship between leaf chlorophyll content and green leaf area. Xu *et al.* (2000a) also reported significant correlation ($r^2 = 0.82$, $P \leq 0.001$) between stay-green rating and chlorophyll content (SPAD reading). They also reported that the QTLs detected for chlorophyll content and stay-green were overlapping.

Borrell and Hammer (2000) reported strong association between leaf nitrogen content at anthesis and grain yield under drought. They also suggested that this strong association could be used to screen genotypes for drought tolerance in sorghum breeding programs by measuring leaf N content at anthesis. Chapman and

Barreto (1997) have shown that SPAD chlorophyll meter can be used to estimate leaf N content in maize. Studies in sorghum have also shown good correlations between SPAD and specific leaf nitrogen (Borrell and Hammer, 2000). In addition, the chlorophyll meter could be used to rate stay-green in breeding lines during the latter half stage of grain filling (Borrell *et al.*, 1999).

Yield and yield related characters

In the 2004/05 trial, genotypes were significantly different ($P \leq 0.001$) for all characters including grain yield both in the individual moisture regime (control and stress) environments and across these two environments (Table 4). Similarly, in the 2005/06 trial, genotypes were significantly different ($P \leq 0.001$) for all characters (Table 5) both in individual as well as across the three environments (control, early-onset stress and late-onset stress).

Table 4. Combined analysis of variance for two environments for R16 stay-green QTL introgression lines in 2004/05

Source of variation	Grain dry weight (g m ⁻²)	biomass dry weight (g m ⁻²)	Harvest Index (%)	100 grain weight (g)
Genotype (G)*	126.38	265.37	267.04	578.46
P value	<0.001	<0.001	<0.001	<0.001
SED(±)	20.31	36.39	1.374	0.077
CD (0.05)	39.81	71.32	2.69	0.2
Environment (E) *	39.81	71.32		
P value	<0.001	<0.001	2.69	150.45
SED (±)	10.78	13.85	0.002	<0.001
CD (0.05)	21.13	27.15	0.992	0.049
G x E *	41.09	23.56		
P value	0.002	0.214	87.83	95.75
SED (±)	30	52.03	<0.001	<0.001
CD (0.05)	58.8	NS	2.138	0.1172

NS = non significant

Table 5. Combined analysis of variance from three environments for R16 stay-green QTL introgression lines during 2005/06

Source of variation	Grain dry weight (g m ⁻²)	Biomass dry weight (g m ⁻²)	Harvest index (%)	100 grain weight (g)
Genotype (G)*	197	264.99	683.01	243.81
P value	<0.001	<0.001	<0.001	<0.001
SED (±)	16.75	33.16	1.347	0.116
CD (0.05)	32.83	64.99	2.64	0.227
Environment (E)*	29.36	15.18	86.39	283.18
P value	<0.001	<0.001	<0.001	<0.001
SED (±)	15.48	31.72	0.67	0.041
CD (0.05)	30.34	62.17	1.32	0.081
G x E*	76.71	68.31	67.72	94.33
P value	<0.001	0.002	0.002	<0.001
SED (±)	33.64	66.98	2.49	0.235
CD (0.05)	65.93	131.28	4.89	0.461

In the control environment of 2004–05 trial, mean grain dry weight values for individual entries ranged from 243.7 to 381.2 g/m² while the average across entries was 312 g/m² (Table 6). In contrast, in the stress environment, the individual genotype means ranged from 189.9 to 321.3 g/m² with an average of 257.2 g/m² among the entries (17.6% less than the control) indicating that the stress was not severe as the relative differences between the control and stress environments was not high.

In the 2005/06 trial, mean grain dry weights/m² of individual entries ranged from 178.5–315.1 g/m² with an average of 252.2 g/m² among entries in the control environment (Table 7). In the early-onset stress environment, the individual entry means ranged from 94.5–213 g/m², with an average value of 171.8 g/m² across entries (32% less than the control). In the late-onset stress environment, individual entry means ranged from 146.3–286.8 g/m², with an average of 217.7 g/m² across entries (15% less than the control). The stress in the late-onset stress environment was not severe and hence the mean grain yield difference between control and the late-onset stress environments in this 2005–06 trial was not high. There was also huge

variation between the two years as the average grain yield (257 g/m²) in the stress environment of 2004/05 was much higher than that of either the early-onset stress and the late-onset stress environments (171.8 g/m² and 217.7 g/m², respectively) in 2005/06 and was comparable to that of the 2005/06 non-stress control environment (252.2 g/m²). These variations were not unexpected as the timing and intensity of stress varies from year to year. In addition, the rate of fertilizer applied was more in 2004/05 than in 2005/06 which contributed for the higher performances of genotypes in 2004/05 and brought the difference between the two years. Moreover, the 2004/05 trials received about 50 mm of unseasonal post-flowering rainfall, which again might have contributed to the better performances of entries. These differences could also be explained by the fact that due to late rains sowing was done late in 2005/06, and the plants may not have reached full canopy closure at a time of the year when light duration and intensity, as well as temperatures are lower, causing the crop to have had lower light interception and growth rates.

Yield reduction under conditions of terminal drought stress compared with a non-stressed control can occur both due to a decrease in numbers of panicles per plant and a decrease in 100-grain weight or grain number per panicle (Mahalakshmi *et al.*, 1987). This expected trend of reduction in panicle numbers and 100-grain weight in the stress environments compared with the non-stress (control) environments was also observed in our studies, especially for 100-grain weight in both 2005/06 stress environments (Tables 6 and 7). This indicates that the yield decrease in the stress was due to both lower number of panicle/m² and reduced 100-grain weight in the early stress environment, where the onset of stress was early and its intensity was severe. In 2004/05, the mean 100-grain weight across the entries was 2.95 g while the mean individual entry values ranged from 2.25-3.33 g in the non-stress (control) environment (Table 6). In the stress environment, the average 100-grain weight across the entries was 2.35 g while the mean values for individual entries ranged from 1.71-2.81 g. In 2005/06, mean of individual entries ranged from 2.12-3.28 g while the average across entries was 2.73 g in the control environment (quite similar in fact to the values observed in the previous year) (Table 7). In the 2005/06 early-onset

stress environment, mean 100-grain weight values of individual entries ranged from 1.55-2.61 g while the average across entries was 2.05 g. But in the 2005/06 late-onset stress environment, mean 100-grain weight values of individual entries ranged from 1.62-2.93 g while the average across entries was 2.29 g. These data indicate clearly that though the grain yield decrease under relatively severe early-onset or milder late-onset stress was fairly low, filling of grain was the most affected process such that 100-grain weight was reduced in the stress environments due to lack of proper grain filling which in turn affected grain yield.

In the 2004/05 trial, all eight backcross derivatives of R16 were statistically similar to R16 for biomass yield in the control environment and six of them were statistically similar to their recurrent parent for grain yield as well (Table 6). A similar pattern was observed in the stress environment although there was some indication that several of the derivatives were more sensitive to shortage of water during grain filling than was R16. Four of the derivatives were statistically similar to R16 in terms of both grain and biomass yields.

Table 6. Summary of mean performances of stay-green QTL introgression lines in 2004/05

Genotype	Grain dry weight. (g m ⁻²)		Biomass dry weight (g m ⁻²)		Harvest Index (%)		100-grain weight (g)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
RSG 04001	363.9*	271.4	672.3	556.5	54.2*	48.9	2.63*	2.02*
RSG 04002	251.5	197.5*	510.*	444.5*	49.0	44.6	3.26	2.27*
RSG 04003	258.8	189.9*	517.6*	463.8	49.9	41.2	3.25	2.24*
RSG 04004	318.3	225.2	607.9	510.3*	52.3*	44.2	2.83*	2.02*
RSG 04005	285.0	233.4	583.7	583.5	48.7	39.8*	3.33	2.44*
RSG 04007	307.0	256.6	630.3	570.4	48.7	44.9	3.28	2.46*
RSG 04008	318.9	298.6	717.7	680.2*	44.5	43.7	3.13	2.63
RSG 04009	356.4*	205.7*	651.9	463.6*	54.8*	44.4	2.44*	1.71*
RSG 04011	296.5	204.6*	582.3	469.6*	50.8*	43.3	2.48*	2.00*
RSG 04012	243.7	255.9	702.6	718.5*	34.6	35.5*	2.57*	2.35*
RSG 04013	360.5	262.1	725.9	575.4	49.6	45.4	3.22	2.40*
R16	300.8	261.5	643.2	586.1	46.7	44.4	3.2	2.7
E36-1	358.6	320.3	824.9	711.2	43.5	44.9	3.2	2.8
B35	278.2	238.3	616.2	513.0	45.0	46.4	2.7	2.4
NTJ-2 (check)	381.2	311.9	757.0	671.3	50.3	46.5	3.07	2.26
SPV422 (check)	323.9	315.8	857.0	786.7	37.8	40.1	2.91	2.57
ICSV700 (check)	304.9	275.1	723.0	656.4	42.2	41.9	2.25	1.83
Maximum	381.2	320.3	824.9	786.7	54.8	48.9	3.33	2.81
Minimum	243.7	189.9	510.0	444.5	34.6	35.5	2.25	1.71
Mean	312.3	257.2	670.4	588.6	46.9	43.8	2.95	2.35
SED(±)	31.21	23.87	58.56	39.28	1.68	2.2	0.09	0.13
CD (0.05)	61.17	46.79	114.78	76.99	3.3	4.32	0.17	0.25

* Significantly different from the recurrent parent- R16 at $P \leq 0.05$

In the 2005/06 trial, six R16 derivatives in the control environment, ten in the early-onset stress and seven in the late-onset stress had grain yields statistically similar to that of R16 (Table 7). Nearly all of the other backcross derivatives of R16 were inferior to their elite parent in all of these screening environments. This suggests that there may be negative linkage drag associated with one or more of the stay-green QTLs from donors B35. This clearly indicates that further backcross generations should be attempted to try to reduce this. The biomass yields of three derivatives in the control environment, eight in the early-onset stress and five in the late-onset stress were also statistically similar to R16. But none of the backcross derivatives were better than R16 for their grain and biomass yields in any of these three environments (control, early-onset stress and late-onset stress). Exceptions were two derivatives (RSG 05001 and RSG 05006) which produced higher grain yield than R16 (but

not statistically significant) in the late-onset stress environment (Table 7).

The performance differences observed generally reflected the differences between R16 and the donor parent B35, and it is not unexpected in the early backcross generations. At these early generations of backcrossing, the genomic contribution of B35, which is agronomically poor, is still high and the backcross derivatives are not expected to perform like R16. The fact that many of the derivatives performed statistically equal to R16 gives us hope that a few more rounds of backcrosses should be sufficient to remove the undesirable alleles from B35. This is evidenced by the better performance of one of the backcross derivatives (RSG 04001/RSG 05001, which, nonetheless, were shorter than elite recurrent parent R16), and it was one backcross generation ahead (BC2F3) of many others when evaluated.

Table 7. Summary of mean performances of stay-green QTL introgression lines during 2005/06

Genotype	Grain dry weight (g m ⁻²)			Biomass dry weight (g m ⁻²)			Harvest index (%)			100 grain weight (g)		
	Cont.	ES	LS	Cont.	ES	LS	Cont.	ES	LS	Cont.	ES	LS
RSG 05001	302.3	190.5	286.8	602.9	462.3	572.1	50.3	41.6	50.0*	2.5	1.8*	1.9*
RSG 05002	217.1*	94.5*	146.3*	455.8*	241.9*	351.9*	47.3	37.9	41.9	3.3*	2.0	2.3
RSG 05003	178.5*	143.5	152.8*	387.2*	346.8*	341.5*	45.7	40.7	43.2	3.2	2.2	2.6
RSG 05004	255.4	196.0	221.1	494.8*	436.8	455.9	51.5	45.7	48.9	2.5	1.8*	1.9*
RSG 05005	196.7*	157.9	175.0*	452.1*	451.5	381.9*	43.3*	35.4*	39.9	3.0	2.3	2.7
RSG 05006	238.1*	196.9	278.1	449.8*	411.9	557.2	52.9	47.2*	49.8	2.6	2.1	2.4
RSG 05007	236.5*	134.3*	164.1*	503.0*	375.1	396.2*	47.0	35.3*	40.8	3.0	2.1	2.2
RSG 05008	236.3*	187.3	258.0	525.8	462.6	579.6	45.3	39.8	44.4	2.5	2.3	2.8
RSG 05009	234.1*	165.1	214.4	460.4*	366.2	423.8*	54.5*	44.5	50.0*	2.4	1.7*	1.9*
RSG 05010	253.2	158.6	194.5	501.3*	336.0*	393.6*	51.1	47.5*	49.2	2.1*	1.7*	1.7*
RSG 05011	263.9	148.4	184.4*	507.3*	343.9*	416.0*	52.0	42.5	43.6	2.3*	1.6*	1.6*
RSG 05012	215.3*	172.0	207.1	562.7	486.6	546.6	38.4*	35.5*	37.6*	2.6	2.4	2.4
RSG 05013	218.7*	185.7	198.9	690.2	596.2*	614.0	32.1*	31.0*	32.2*	2.5	2.3	2.4
RSG 05014	298.9	164.3	244.2	645.3	373.8	523.9	46.3	43.7	46.6	3.2	1.9	2.3
RSG 05015	313.3	193.8	218.3	576.3	414.5	459.2	54.7*	46.9*	48.0	2.8	1.6*	2.0*
B35	255.0	154.7	182.5*	550.3	385.5	429.4*	46.7	39.0	43.0	2.7	2.3	2.4
E 36-1	267.3	201.4	250.8	652.2	530.0	605.1	39.5*	38.8	41.5	3.0	2.6	2.9
R16	315.1	188.6	247.1	649.8	459.3	546.6	48.4	41.4	44.8	2.8	2.1	2.5
IRAT 204	234.8*	213.3	281.5	432.9*	438.1	515.7	54.3*	48.6*	54.5*	2.7	2.0	2.3
Maximum	315.1	213.3	286.8	690.2	596.2	614.0	54.7	48.6	54.5	3.3	2.6	2.9
Minimum	178.5	94.5	146.3	387.2	241.9	341.5	32.1	31.0	32.2	2.1	1.6	1.6
Average	252.3	171.8	217.7	537.5	418.9	482.8	47.5	41.2	44.7	2.7	2.1	2.3
SED (±)	33.62	26.74	30.92	72.09	53.05	54.15	2.42	2.28	2.64	0.21	0.18	0.22
CD (5%)	65.90	52.41	60.60	141.30	103.98	106.13	4.73	4.46	5.18	0.41	0.35	0.42

Cont. = Control, ES = Early stress and LS = Late stress. * Significantly different from the recurrent parent-R16 at $P \leq 0.05$. Note: Entry names like RSG04 XXX in 2004/05 have been renamed as RSG05 XXX. e.g. RSG04001 in 2004/05 is renamed as RSG05001 in 2005/06 so as to differentiate entries by year.

Relationships of leaf senescence and yield

Delayed leaf senescence (stay-green) in sorghum has been associated with improved grain yield, particularly in environments in which available water during grain filling is not adequate to support potential transpiration (Borrell *et al.*, 2000b). The assumption behind the introgression of the stay-green trait into a senescent line like R16 is that this will have a positive effect on grain yield under conditions of moisture stress during grain filling by delaying leaf senescence and increasing carbon availability for grain filling, thereby improving grain size and grain yield.

In the present study, there were significant correlations of relative (dryland environment as a fraction of the supplementally irrigated environment) GLA% at 2/3 of the grain filling period to relative grain filling in two of the three comparisons. Percent green leaf area accounted for 56% ($r^2 = 0.56$, $P < 0.05$) of the variation in relative 100-grain weight in the dryland environment in 2004/05 and 32% ($r^2 = 0.32$, $P < 0.10$) of the variation in relative 100 grain weight in the late-onset stress dryland environment in 2005/06. In the more severe early onset stress environment of 2005/06, there was no relationship of relative GLA% at 2/3 grain filling and relative 100-grain weight largely because few of the introgression

lines had a significant green leaf area remaining at that point. Relative, rather than absolute, values of both traits were used as there were considerable differences in yielding ability and plant type among the introgression lines, which reflected differences in the agronomic value of the parents since the lines were only BC₁ or BC₂ generation, and were not related to stay-green or its effects. Relative values are an appropriate measure of the ability of a genotype to maintain its normal or non stress phenotype in the stress environments.

The relationship of higher relative GLA% and a greater degree of grain filling translated into a relationship between relative GLA% and relative grain yield in both the 2004/05 and the 2005/06 late-onset stress dryland environments. Difference in QTL introgression lines in GLA% at two-thirds of grain filling accounted for 34% ($r^2 = 0.34$, $P < 0.10$) of the differences in relative grain yield in the 2004/05 dryland environment and 76% ($r^2 = 0.76$, $P < 0.01$) of the differences in the late-onset stress dryland environment in 2005/06. Similarly, differences in chlorophyll content (SPAD values) accounted for a significant proportion of the differences in grain yield ($r^2 = 0.42$, $P < 0.05$) at physiological maturity in the early onset stress environment of 2005/06. These relationships reflect the importance of maintaining a functional leaf area into the last stages of the grain filling period, to allow a genotype to fill its grain to its maximum genetic potential grain size, and thereby increase yields to the ceiling set by the actual grain numbers established at flowering. In a similar study of nine sorghum genotypes varying in stay-green, Borrell *et al.* (1999) found that grain yield was correlated positively with green leaf area at maturity ($r = 0.75$, $P < 0.05$) and negatively with rate of leaf senescence ($r = 0.74$, $P < 0.05$), thus, supporting the hypothesis that sorghum genotypes possessing the stay-green trait have a

significant yield advantage under post-anthesis drought stress conditions compared with genotypes not possessing this trait. They also reported that stay-green did not constrain yield when water was not limiting since no differences in grain yield were observed among 8 of the 9 genotypes under fully-irrigated control conditions. When water was limiting during grain growth, yield accumulation in stay-green genotypes was largely dependent on photo-assimilation in the remaining green leaves. Lower grain yields in the intermediate and senescent genotypes were associated with retention of fewer green leaves, although this reduction was to some extent offset in the intermediate hybrid by utilization of stem reserves for grain filling (Borrell *et al.*, 1999).

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