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**Stay-green QTLs effects on water extraction, transpiration efficiency and seed yield
depend on recipient parent background**

*Vincent Vadez^{A,D}, Santosh P Deshpande^A, Jana Kholova^A, Graeme L. Hammer^B,
Andrew K Borrell^B, Harvinder S Talwar^C and C Thomas Hash^A*

^A International Crops Research Institute for the Semi Arid Tropics (ICRISAT),
Patancheru, Greater Hyderabad 502324, Andhra Pradesh, India

^B The University of Queensland, Queensland Alliance for Agriculture and Food
Innovation, Hermitage Research Station, Warwick, QLD 4370, Australia.

^C Directorate of Sorghum Research Directorate of Sorghum Research (DSR),
Rajendranagar Hyderabad 500 030, Andhra Pradesh, India

^D Corresponding author. Email: v.vadez@cgiar.org

Abstract. A stay-green phenotype enhances the adaptation of sorghum to terminal drought conditions, although the underlying physiological mechanisms leading to the expression of stay-green remain unclear. Differences in tillering and leaf area at anthesis, transpiration efficiency (TE), water extraction, harvest index (HI) and yield under both terminal drought and fully-irrigated conditions were assessed in 29 introgression lines (IL) developed targeting stay-green QTLs Stg1, Stg2, Stg3, Stg4, StgA, and StgB in S35 background, and 16 IL developed targeting Stg1, Stg3, Stg4, and StgB in R16 background. Transpiration efficiency was increased by StgB in the R16 background, whereas there was no effect on this trait in the S35 background. Water extraction was increased by Stg1 in the S35 background, whereas there was no effect in the R16 background. StgB modified the proportion of water extracted before and after anthesis in the S35 background. While tillering and leaf area at anthesis were decreased by Stg1 and Stg3 in the S35 background, there was no such effect in R16. By contrast, yield data under fully-irrigated conditions showed higher tiller grain yield in Stg1, Stg2, and Stg3 ILs. While yield differences were mostly explained by harvest index (HI) variation, the substantial yield variation unexplained by HI was closely related to TE in the S35 background ($R^2 = 0.29$), and more so in the R16 background ($R^2 = 0.72$), while it was closely related to total water extracted in the S35 background ($R^2 = 0.41$), but not in the R16 background. These data indicate the potential for several stay-green QTLs to affect traits related to plant water use and capture. However, they also show that these effects depend on the interaction between genetic background and individual QTLs.

Key words: Stay-green QTL, transpiration efficiency, water extraction, root, lysimeters, genetic background

Introduction

Stay-green has been described as the best characterized trait contributing to the adaptation of sorghum to terminal drought conditions, i.e. conditions of un-relieved water deficit that occur during and after flowering (Rosenow and Clark, 1981). Yet, the physiological mechanisms behind the expression of a stay-green phenotype are still not

clear. Early hypotheses proposed a role for nitrogen (N) status of the plants, either allowing more N to be taken up post-anthesis (Rajcan and Tollenaar, 1999), or exhibiting better balance between demand and supply (larger grain size but more N uptake in stay-green), leading to the expression of a stay-green phenotype (Borrell and Hammer, 2000). More recent hypotheses for the explanation of the stay-green trait deal with plant water management (Hammer, 2006). Indeed, having leaves staying green and seemingly active under terminal drought conditions likely requires having access to water at this stage. This could relate to differences in how genotypes manage water resources and/or minimize/optimize water use by the crop canopy as previously described in pearl millet (Kholova et al., 2010a), or to deeper rooting for maximizing water extraction (Vadez et al., 2007a).

At the canopy level, plants can curb water use by reducing the leaf area being developed, or by reducing the conductance of the canopy (Kholova et al., 2010a, 2010b). The latter has been shown to be one of the reasons for the expression of the stay-green phenotype in *Miscanthus* (Clifton-Brown et al., 2002). Hammer (2006) used crop simulation modeling to evaluate the effect of a reduced leaf area on the yield across locations receiving different amounts of rainfall and showed that a leaf area index of 1 would bring a substantial yield benefit in situations where rainfall is below 175 mm. A reduction in the leaf area at anthesis in stay-green genotypes could be a way of reducing pre-anthesis water losses. It has been shown also that higher tolerance could be related to a slower rate of soil water use (Kirkham, 1988). Here, we assess the possible effect of introgressing different stay-green QTLs (all from donor parent B35 = BTx642) on leaf area and tillering at anthesis. Van Oosterom and colleagues (2006) have also hypothesized that stay-green could be the consequence of water saving from having higher water use efficiency. Differences in transpiration efficiency (TE) have been reported under fully-irrigated conditions in sorghum germplasm (Hammer et al., 1997; Xin et al., 2009). However, it has not been determined whether any QTL for stay-green contributes to increasing TE, and whether TE can be modified by the introgression of one or more stay-green QTLs regardless of the recurrent parent background. In addition, the interaction of TE with water regime has been only tested in a few entries (Donatelli et al., 1992; Balota et al., 2008).

There has been limited work on roots in sorghum, except recent report by Singh et al (2010), and until recently this work has been restricted to a morphological evaluation of a limited number of genotypes. For instance, several studies have shown genotypic variations for rooting traits in genotypes contrasting for drought tolerance (Blum et al., 1977; Abd-Ellatif et al., 1978; Jordan et al., 1979; Vadez et al., 2007a). Yet, these studies did not resolve how particular root traits would contribute to water extraction, especially during grain filling, which could link with the expression of a stay-green phenotype. Recent work in wheat indicates that water extraction during grain filling is critical for enhanced seed yield (Manshadi et al., 2006; Kirkegaard et al., 2007). A method has been recently developed that measures water uptake by roots in a lysimetric system where plants are grown in tubes with plant density and available soil volume approximating field conditions (Vadez et al., 2008). Here we report the use of that system to test the effect of individual stay-green QTL introgressions on the amount and timing of water extraction, in two different elite sorghum genetic backgrounds.

While the lysimetric method does not evaluate roots *per se*, it has the advantage of precisely assessing water extraction of genotypes exposed to a range of water regimes (e.g. Ratnakumar et al., 2009), along with yield, HI and TE, i.e. the four components of Passioura's equation (Passioura, 1977): $Y = T \times TE \times HI$. While this equation has been extremely useful to frame an approach to drought, the limitation has been the difficulty to assess all three explanatory parameters on the same plants. While it is easy to measure HI in the field, measuring TE or water extraction there is more complex and prone to error. Conversely, it is difficult to obtain reliable yield data from plants grown in pots, especially because of the limited soil volume and planting density issues, although it has been done recently on a small set of hybrids (Balota et al., 2008). In our study, a lysimetric system is used to assess T, TE and HI in the same plants (Vadez et al., 2008). The data are used to investigate the relative importance of each component on yield, and how individual stay-green QTLs and genetic backgrounds interact to affect any of these components.

In summary, the overall objective of the work was to assess the effect of stay-green introgressions on traits related to water demand and supply, and their interaction with two genetic backgrounds. Specifically we compared: (i) leaf area, tillering, and leaf number at

the time of anthesis; (ii) water-use efficiency; (iii) patterns and quantities of water extracted; and (iv) the contribution of these different traits, and their putative modification by stay-green QTLs, on the yield in two different elite sorghum genetic backgrounds, one a rainy-season adapted caudatum race variety (S35) and the other a postrainy-season adapted durra race variety (R16).

Materials and Methods

Soil filling and growth conditions of the lysimeters

Plants were grown in lysimeters, i.e. PVC tubes of 25 cm diameter and 2.0 m length, filled with a sandy-clay loam Alfisol. The tubes were set in 2.0 m deep trenches (see below) so that the top of the cylinder was at the ground level, which avoided sun beaming on the lysimeters. The trenches were set outdoors under natural conditions. Sowing was done on 20th October 2008, at the beginning of the post-rainy sorghum season. During the experimental period, the maximum and minimum temperature ranges were 23.6-34.0°C and 9.5-23.6°C, the minimum and maximum % humidity ranges were 10-87% and 72-98%., and the vapor pressure deficit ranges were 0.4-4.9 kPa, with a mean of 2.5 kPa. PVC end-plate was placed on top of four screws 3 cm from the bottom of the cylinders to prevent the soil from seeping through. The end-plate did not fit the cylinder tightly, allowing water drainage. The Alfisol used to fill the tubes was collected on the ICRISAT farm and was sieved to a particle size less than 1 cm. This enabled the bulk density of the soil profile to be regulated at approximately 1.35, the standard value for an Alfisol. The cylinders were filled with soil in three increments of 40 kg of dry soil. After addition of each 40 kg increment, the soil level in several cylinders was checked to ensure they were similar, and all tubes were watered. A previous assessment of the water needed to fill the profile before drainage determined that the soil water holding capacity of the Alfisol was approximately 20%. Therefore, 8 L of water were added to each 40 kg increment. After adding and watering 40 kg of soil three times, an additional 15 kg of dry soil was added to each cylinder and watered with 3 L. At that stage, the cylinders were almost filled at the desired level, i.e. approximately 5 cm from the top. Finally, all cylinders were topped-up with dry soil to ensure they were filled to the same level. This top-up varied between

500 g and about 1 kg, i.e. less than 1% variation across cylinders for which all the cylinders had a bulk density close to 1.35. A few more liters of water were added to the cylinders resulting in drainage at the bottom. Weighing of the cylinders indicated that all saturated and freely drained cylinders weighed between 164 and 165 kg.

The lysimeters were filled with soil that had been fertilized with di-ammonium phosphate (DAP, 18-46-0 NPK) and muriated potash (0-0-60 NPK), both at a rate of 200 mg kg⁻¹ soil. The soil also contained sieved and sterilized farm manure at a rate of 1:25 to prevent micro-nutrient deficiency. Before growing the sorghum crop, the lysimeters were used for a crop of finger millet. The finger millet crop had received a urea top-dressing of 3 g plant⁻¹. At the end of this crop, only the main root stock from the plants was removed from the top part by softening the top-soil with water and pulling, so that only the top 5 cm of the soil were disturbed. The soil was then tilled superficially with sickles and limited Alfisol top up was added so that the surface level was approximately 5 cm from the lysimeter brim. This was one of the important features of this setup, i.e. to use a deep soil profile undisturbed from previous cropping, except for minimum tillage of the surface. After that, the lysimeters were watered to field capacity, based on their expected weight, so that the sorghum crop was sown on a full profile. A top-dressing of 3 g urea plant⁻¹ was applied at 4 weeks after sowing.

Spatial arrangement of the lysimeters and weighing

The top of the cylinders was equipped with a metal collar and rings that allowed the cylinder to be lifted. Weighing of the cylinders was done by lifting the cylinders with a block-chained pulley, and an S-type load cell (Mettler-Toledo, Geneva, Switzerland) was inserted between the rings of the cylinder and the pulley. The scale, of 200 kg capacity, allowed repeated measurements and gave an accuracy of 20 g on each weighing. The lysimeters were separated from one another by a distance of approximately 5 cm. Therefore the crop of sorghum was planted at a density of about 11 plants m⁻², a plant population very similar to the field planting (row-to-row distance of 60 cm and plant-to-plant spacing of 15-20cm). This allowed an accurate assessment of the water extraction pattern of a crop cultivated in conditions similar to the field. The tubes were arranged in

two sets of two adjacent trenches of 2 m depth and 1.75 m width, each set being separated by a 1.5 m path and each trench within each set being separated by a 20 cm concrete wall. Two trenches were used for the terminal drought stress (DS) treatment and two trenches for the well-watered (WW) treatment. Possible border effects were expected on the south set of trenches (DS) (these were oriented east-west) and those effects were curbed by bordering the most southern trench by two rows of border plants. We also used an Alpha-lattice design to further help separate possible border effects.

Treatments used and traits assessed

The purpose of the trial was to assess the pattern of water use in plants grown under both fully-irrigated conditions (well-watered, WW) and under terminal-drought conditions (drought stress, DS), and three replications were used per treatment and genotype. Four seeds were sown in each cylinder on 20th Oct 2008. Plant stand was thinned to two seedlings per cylinder at 14 days after sowing (DAS) and then to one plant per cylinder at 21 DAS. All plants were kept under fully-irrigated conditions until 28 DAS. This involved cylinders receiving 500 mL twice a week until two weeks after sowing, and then on alternate days until 28 DAS. After the regular irrigation at 28 DAS, the cylinders were covered with a 2-cm layer of low density polyethylene beads to prevent soil evaporation. Preliminary testing indicated that the beads prevented more than 90% of the soil evaporation, so that differences in cylinder mass were primarily due to plant transpiration (data not shown). Weighing of the cylinders was first done at 30 DAS, and then subsequently every two weeks. This gave a total of five weights until harvest for the DS plants and six weights for the WW plants. The first weighing at 30 DAS gave the saturated weight of each cylinder. The cylinders in this experiment were distributed across four trenches, with one trench per day being weighed. The same sequence of weighing was used for each trench so that the time intervals between weighing were the same in all cylinders (usually 14 days).

To maintain the WW plants fully-irrigated and to avoid water drainage after irrigation, the watering to the WW plants was done when the cylinder weight, at the time of weighing, had fallen below 2L from the saturated weight. This ensured that the soil was

kept sufficiently wet to allow maximum growth, while preventing drainage at the bottom. The watering was done every week. The week that plants were not weighed, the water addition of the previous week was used for watering the WW plants. The DS treatment received no water from 28 DAS until maturity, except for 2L that were added to all cylinders at 73 DAS.

Date of booting and flowering were recorded on an individual plant basis. Transpiration was calculated for approximately 2-week intervals between 30 DAS and maturity. This allowed pre- and post-anthesis water use to be calculated. Daily transpiration values were calculated for each plant by dividing the transpiration of each time interval between weighing by the number of days in each interval. Then, pre-anthesis transpiration was the sum of the daily transpiration values until anthesis, plus water used in the first 28 days after sowing that was estimated to be 1.5L for all genotypes. This was based on dry weight estimates for plant biomass of 15 g at 28 DAS, and on the assumption of a TE of 10 g kg^{-1} water transpired at such an early stage of crop development (our unpublished observations). The post-anthesis water use was the sum of the daily transpiration values from anthesis until maturity. At harvest, leaf, stem and panicle weights were taken after drying for 3 days in a forced-air oven set at 70°C . Dried panicles were then threshed to determine grain yield. Harvest index was calculated as the ratio of grain yield divided by the total above-ground biomass (the aggregated dry weight of stems, leaves, and panicles). Transpiration efficiency was calculated as the ratio of the total above-ground biomass to the sum of transpiration values between 30 DAS and maturity. The initial biomass at the time of commencing the transpiration measurements was not taken into account. Although the biomass at that stage was not negligible (circa 15 g plant^{-1}), it was assumed that biomass differences among genotypes at that stage ($\sim 3 \text{ g plant}^{-1}$) were negligible compared to the final biomass ($70\text{-}100 \text{ g plant}^{-1}$), and the TE assessment was valid for a comparative assessment of germplasm.

Tiller number at booting, leaf area of the main stem, number of visible leaves at booting and total leaf area at booting were assessed from an extra set of plants that were grown under fully-irrigated conditions in 27-cm diameter pots, filled with adequately fertilized Alfisol.

Development of stay-green isogenic lines

Stay-green QTL introgression lines, each containing one of six stay-green QTLs (Stg1, Stg2, Stg3, Stg4, StgA and StgB) from donor B35 = BTx642, were generated in a sweet-stemmed medium-duration grain+stover sorghum variety S35 (29 entries) after 3-4 backcrosses. There were five introgression lines (IL) for each of the individual stay-green QTLs, except for Stg3 where only four IL were generated. The S35-background trial included S35 and E36-1 as checks.

Similarly, stay-green introgression lines, each containing one of four stay-green QTLs (Stg1, Stg3, Stg4 and StgB) from donor B35, were generated in R16 genetic background (16 entries) after 3-4 backcrosses with background selection (Kassahun et al., 2010). There were four introgression lines (IL) for each of these stay-green QTLs in this genetic background. The R16-background trial used R16 and several checks: Phule Maulee, CRS1, CRS4, and NTJ-2, which are released post-rainy sorghum varieties. It also included E 36-1 and RSG 04005, the latter being an early generation backcross line (two backcrosses) from which the IL for Stg3, Stg4 and StgB were subsequently derived (Kassahun et al., 2010). Flanking marker information is provided in supplementary Table 1 and 2.

Statistical analysis

The experimental design was a randomized complete block design with two factors (water regimes) and three replicated plants per factor and genotype. The Residual Maximum Likelihood (ReML) method of Genstat was used to obtain the unbiased estimate of different parameters within each treatment. Two-way ANOVA analysis was also performed to assess the effect of genotype (G), water treatment (W) and genotype-by-water treatment (G×W) interaction for the different traits measured. For the multi-linear regression analysis, a multi-linear model was used in the software STATA (Stata Corp., College Station, TX, USA), where yield was an additive function of HI, TE, total water extraction, water extracted in the post-anthesis period, water extracted in the 59-78

DAS and 78-94 DAS periods, days to flowering, and a constant. The same multi-linear model was used to assess the residual yield variations not explained by HI (see below), therefore excluding HI from the list of explanatory variables. For both of these analyses, entries from each respective background that were used as checks were removed from the analysis.

To compare means between groups of stay-green introgression lines and the recurrent parent, a one-way ANOVA was conducted using the mean value of each introgression line (IL) as a replicate in a group of IL having the same QTL (n=5 for the different stay-green groups in S35 background, except n=4 for Stg3, and n=4 in R16 background). Recurrent parents were replicated twice. This approach was valid because in both backgrounds, the marker information was relatively similar in all IL carrying a given QTL (Suppl. Table 1 and Table 2). For instance, in Stg1 IL in S35 background, all 5 entries tested carried the allele from B35 donor parent, except S35SG entry 06036 at marker locus *Xisp10323*. In few cases (Stg2 and Stg3 in S35 background), the marker information varied between IL, although all entries had at least one marker for the staygreen QTL from donor parent. So, at least a portion of each staygreen QTL in each IL was present and since there is no established relationships between presence/absence of a particular marker and any phenotypic trait, except for the staygreen phenotype that was present in all ILs, there was no *a-priori* bias for the grouping of IL in this way. We debated the possibility to generate more marker data in the vicinity of the QTL regions that showed interest, in order to resolve contrasting phenotypes within QTL introgression. However, this idea would have been limited by the number of entries available in each Stg introgression. Work is on-going to generate such denser marker data, although using more number of putative staygreen QTL introgression lines in each Stg QTL. Only S35SG06020 and K252 and K258, putatively introgressed with Stg3, had recipient parent alleles at staygreen marker loci and were not considered in the analysis of means.

Results

Plant characteristics at time of anthesis under well-watered conditions

Stg1 and Stg3 QTLs significantly reduced tiller numbers in the S35 background. One IL, S35SG06020 with Stg3 had recurrent parent allele at marker loci Xtxp019 and Xtxp298, and had same number of tillers than recurrent S35, suggesting a role of at least one of these loci in tillering. However, none of the stay-green QTLs affected tiller number in the R16 background (Fig. 1a&b). The number of leaves present on the main-stem at anthesis was similar in the two recurrent parent backgrounds and their respective ILs (Fig. 1c&d). Stay-green QTLs did not affect the leaf area of the main-stem in any background, except in the case of Stg1 where the QTL decreased the leaf area in S35 background. Since main stem leaf number was the same for S35 and the Stg1 IL, the lower main-stem leaf area in Stg1 was presumably due to smaller leaves (or at least some of the leaves being smaller, as it could also be observed) (Fig. 1e&f). The total leaf area at anthesis was lower in the Stg1 and Stg3 ILs in the S35 background, while no stay-green QTL had any effect on the total leaf area at anthesis in the R16 background, although there was a trend for reduced leaf area in the Stg3 and StgB ILs (Fig. 1g&h).

All ILs in the S35 background flowered in 52-61 days after sowing (DAS) and 52-65 DAS under DS and WW conditions, respectively (Table 1). One Stg1 IL and two Stg2 ILs flowered earlier than S35. In the R16 background, all ILs flowered in 59-64 DAS and 58-64 DAS under DS and WW conditions, respectively, with significant differences, although there was no clear explanation on the basis of the marker data in either case, and there was no significant effect of any stay-green introgression group on flowering (data not shown).

Transpiration efficiency

ANOVA of TE indicated that the water treatment (W) had a significant effect on TE in the S35 background, with the drought treatment exhibiting significantly higher TE than the fully-irrigated control, but this was not observed in the R16 background. Also, TE was mostly influenced by the genotype (G), with no significant genotype-by-treatment (G×W) interaction observed in either of the recurrent parent or stay-green QTL genetic backgrounds. Overall, entries in the S35 background had higher TE (5.29 g kg⁻¹) than those in the R16 background (4.50 g kg⁻¹). In the S35 background, TE varied from 4.35 to

6.08 g kg⁻¹ water transpired, with S35 having a value of 5.52 g kg⁻¹. As such, no set of ILs for any particular stay-green QTL in this background had any significant effect on the TE values. Yet, in this background the means of Stg3 and StgB were 5.59 and 5.65 g kg⁻¹, with most StgB individual entries being at the top of the rankings. For StgB QTL, there was no relation between TE value and presence/absence of donor allele at locus Xtxp8. By contrast, most Stg1 ILs in S35 background were at the bottom of the ranking with a mean of 4.93 g kg⁻¹ across Stg1 ILs (Fig. 2a). Heritability for TE under DS was 55%. In the R16 background, TE varied from 3.69 to 5.49 g kg⁻¹, i.e. a slightly broader range than in the S35 background, with the recurrent background R16 having a value of 4.33 g kg⁻¹ (markedly lower than S35). Only one StgB IL had a significantly higher TE than R16 (Fig. 2b), although most StgB ILs also had TE values above R16 and the mean of the four StgB ILs was 4.79 g kg⁻¹, significantly above the TE of R16. Heritability for TE under DS was 58%.

Water extraction

Beside an obvious W effect on total water used, the ANOVA showed that both G and G×W had significant effects in both backgrounds, although the G effect was predominant in the R16 background. Overall, entries in the R16 background had higher total water extraction (13,900 g kg⁻¹) than in the S35 background (13,140 g kg⁻¹) (Table 2). In the S35 background, the total water extracted from the soil profile between 30 DAS and maturity varied from 10,880 to 14,640 g plant⁻¹. S35 extracted 12,800 g plant⁻¹ of water and was therefore situated in the lower quartile of genotypic rankings. Four of five Stg1 ILs in S35 background were in the top quartile of genotypic rankings, with an overall mean water extraction for these Stg1 ILs of 14,080 g plant⁻¹, significantly higher than S35 by about 1.2 L (Fig. 3a). All StgA ILs in the S35 background were situated in the lower half of the genotypic rankings. Heritability for water extraction in the S35 background was 72%. In the R16 background, total water extracted in ILs varied from 11,000 to 14,820 g plant⁻¹, with R16 itself reaching 14,340 g plant⁻¹. No introgression line had any higher water extraction capacity than R16, neither individually or as a group of ILs. Heritability for water extraction in the R16 background was 78%.. Two durra race local

checks, Phule Maulee and CRS4 had the highest water extraction, at 15,180 and 15,260 g plant⁻¹, respectively, while caudatum race NTJ-2, a popular postrainy season-adapted sweet and grain+stover sorghum variety, had poor water extraction capacity (Fig. 3b). This information confirmed another study that showed the superiority of durra race, but the poor capacity of caudatum race, for water extraction (Vadez et al., personal communication).

In general, pre- and post-anthesis water use was not very different among groups of ILs and recurrent parents, although some differences are noteworthy. StgB ILs in the S35 background had a higher pre-anthesis water use than their recurrent parent (Fig. 4). Interestingly, among the five IL with StgB QTL in S35 background, one had donor allele at locus Xtxp8, and this IL had lower pre-anthesis water use and higher post-anthesis water use than the other ILs carrying StgB QTL, suggesting the importance of that locus in the control of the balance of pre- and post-anthesis water use. StgB ILs had a lower post-anthesis water use than the recurrent parent in the S35 background, and there was a trend for Stg1 ILs to have higher post-anthesis water use than S35 (Fig. 4a&b). In the R16 background, none of the other ILs made any difference to post-anthesis water use (Fig. 4c&d). There was no treatment effect on pre-anthesis water use in either of the backgrounds and differences were mostly explained by a G effect (Table 2). Besides a strong treatment effect on post-anthesis water use, differences were explained by both G and G×W effects, the latter being predominant in the S35 background (Table 2). There was a positive relationship between pre-anthesis water use and leaf area at anthesis ($R^2 = 0.18$), and similarly a negative relationship between post-anthesis water use and the leaf area at anthesis ($R^2 = 0.17$). There was also a negative relationship between pre- and post-anthesis water use in S35 ($R^2 = 0.51$) and R16 background ($R^2 = 0.87$).

Grain and stover yield under DS and grain yield in tillers and main panicle under WW

In both recurrent parent backgrounds, besides a strong treatment effect, there was a G effect that influenced grain yield, whereas the G×W interaction was not significant (Table 1). Under DS, grain yields reached 22.19 and 24.02 g plant⁻¹ in recurrent parents S35 and R16, respectively. Grain yield was decreased in Stg1 and Stg2 ILs in the S35

background. At the Stg2 QTL loci, neither presence/absence of donor allele nor grain color differences could simply explain the yield differences. In the R16 background, none of the ILs contributed to a grain yield increase, nor did they reduce yield (Fig. 5a&b). Stover yield was increased in Stg3 and StgB ILs in the S35 background. Stover yield was not significantly changed in the R16 background, although there was a trend for an increase in stover and total biomass (grain plus stover yield) in the StgB ILs in both backgrounds (Fig. 5e&f).

Harvest index was also influenced by a large G effect, whereas the G×W interaction was not significant in either recurrent parent background. There was no treatment effect on HI in the S35 background, with the mean HI under DS and WW conditions equivalent. By contrast, there was a significant T effect on HI in the R16 background, with a decrease in the mean HI under DS by about 30% (Table 1). Similar results were obtained for the panicle harvest index (PNHI).

The main-panicle yield under WW conditions was severely reduced in the Stg1 ILs in the S35 background, except in IL S35SG06032 and in spite of the presence of donor allele at all four marker loci of that Stg1 QTL. In the R16 background, the main-panicle grain yield was not significantly modified in stay-green ILs (Fig. 6a&b). The tiller grain yield data gave the opposite picture, with tiller grain yields being higher than their recurrent parent in Stg1, Stg2, and Stg3 ILs in the S35 background. This was despite the lower tiller number in Stg1 and Stg3 IL in the potted trial at anthesis, which may have been an artifact of the pot effect. The tiller grain yield of recurrent parent R16 was almost nil and only slightly higher ($\sim 5 \text{ g plant}^{-1}$) in stay-green ILs in this background (Fig. 6c&d).

Relationship between grain yield and component traits

One purpose of this work was to assess the role of TE, water extraction (the T component of Passioura (1977)) and HI on grain yield under terminal stress. The harvest index varied largely across the different entries and, as expected, yield and HI were strongly related. However, in each background, a substantial part of the grain yield variations remained unexplained by HI, especially at HI above 0.3. Therefore, in each of the backgrounds, the predicted grain yields were calculated from the regression equation between grain yield

and HI (Fig. 6a). These relationships were highly significant, although less so in the case of R16 ($R^2 = 0.71$). The residuals of the relationship between grain yield and HI under DS were calculated by subtracting the predicted grain yield according to previous work (Vadez et al., 2007b) from the observed values. These residuals were plotted against TE (Fig. 6b) and against the total water extracted from the soil profile (Fig. 6c). The regression curves indicated that the grain yield variations not explained by HI were significantly correlated with TE. However, this association was weaker in the S35 background ($R^2 = 0.29$) than in the R16 background ($R^2 = 0.72$). Similarly, the residual grain yield not explained by HI was regressed against the total water extracted and showed a highly significant and close relationship in the S35 background ($R^2 = 0.41$). Interestingly, there was also a highly significant correlation between these residuals and the water extracted during the period 45-59 DAS ($R^2 = 0.31$ – data not shown), which corresponded to booting-flowering stage. By contrast, in the R16 background there was no relationship between the residual grain yield unexplained by HI and the total water extraction (Fig. 6c). It was also clear from the scattered diagram that there was larger variation in the S35 background values of water extraction than in the R16 background.

These data were confirmed by a multi-linear regression analysis, where HI, TE and total water used were the factors having the most important weights in the regression.

However, in the S35 background, the total water used had a t-value similar to the t-value for TE (Table 3), and both were about one-third the t-value for HI. In contrast, in the R16 background, the t-value was almost five-fold higher for TE than for total water use, and there the t-values for HI and TE were close to one another. In addition, in the S35 background, the water extracted during the period 59-78 DAS, a period corresponding to grain filling, had a significant and negative slope in the regression. In the R16 background, no other variable had any effect on grain yield (Table 3).

The same multi-linear regression model was used to explain the grain yield variations unexplained by HI. In the S35 background, the model explained 83% of the variation (Table 4). TE and the total water use were the only two variables having a significant effect on the residuals, and the total water used had a higher t-value than TE. In the R16 background, the model explained 88% of the variation and TE had a significant and positive influence on grain yield, along with the post-anthesis water use (Table 4).

Discussion

Results showed that stay-green QTLs affected a number of water-related traits under terminal drought conditions in sorghum, although the significance and magnitude of the effects depended critically on the genetic background. Stg1 and to some extent Stg3 increased water extraction in S35 background. Stg B increased TE in R16 background. The S35 background exhibited relatively high TE and none of the stay-green QTL further increased TE in this background. The R16 background exhibited a relatively higher capacity for water extraction from the soil profile than most line tested, and none of the stay-green QTLs further increased water extraction in this background. While the harvest index had a strong influence on grain yield in both genetic backgrounds, the substantial residual grain yield variation not explained by differences in HI was closely related to TE, but not to total water extraction, in the R16 background, while it was closely related to both TE and total water extraction in the S35 background.

Differences in tillering (late tillering in the S35 background)

Stay-green QTL introgressions had different effects in two genetic backgrounds. Tillering was initially lower in Stg1 and Stg3 ILs in the S35 background, while no effect on tillering was observed in the R16 background. However, this might have been an effect of pots in the plants that were harvested at anthesis to assess tillering. Indeed, at maturity under fully-irrigated conditions, tiller grain yield represented the highest proportion of grain yield in the S35 background, with a similar tendency in R16 background. Tillering was not recorded under DS but was very limited. Hence, the stay-green QTL increased tillering under non-stress conditions. This is surprising since the donor parent has mostly one main culm, suggesting the ability of the ILs to tiller must be related to an effect of the QTL on the supply/demand balance, which is known to influence tillering (Kim et al., 2010a, 2010b). IL S35SG06020 had same tiller number as S35 and also recurrent parent S35 allele at marker loci Xtxp019 and Xtxp298, which suggests that these two loci are involved in reducing tillering.

TE differences in R16 background only

Differences in TE have been reported in sorghum, although generally under well-watered conditions (Hammer et al., 1997; Xin et al., 2009). Other reports also show the existence of genotypic variation in TE under differing water regimes (Donatelli et al., 1992; Balota et al., 2008) in a work that included Tx7078, a genotype with low TE and assumed to be tolerant to pre-flowering drought (Tuinstra et al., 1998). However, little prior assessment of TE in sorghum stay-green materials has been made and none of the previous reports show an evaluation of TE until maturity, except Borrell et al. (2000) and Balota et al. (2008). Borrell et al. (2000) showed higher TE in one line having B35 as its source of stay-green. They also reported that differences among nine sorghum genotypes in biomass production under terminal water deficit were associated with variation in transpiration and TE for both the A35 and RQL12 sources of stay-green, although these relationships were highly dependent on the genetic background in which they were evaluated. In that study, A35 (the male-sterile line counterpart of stay-green donor B35) increased TE relative to AQL39 in two out of three genetic backgrounds. In our study, TE was improved in the StgB QTL ILs in the R16 background, and this was likely related to the fact that R16 had relatively low TE compared to the other lines tested, and also compared to other germplasm (our unpublished data). There was also a trend for higher TE in 3 out of 4 StgB QTL ILs compared with the recurrent parent in the S35 background. According to Fig. 7b, one unit increase in TE would contribute to an increase of 3 g grain plant⁻¹ which could be extrapolated to 330 kg ha⁻¹ of grain, or about 16% of the existing mean yield under terminal stress. These differences in TE are still unexplained and work is on-going to investigate traits that might be related to this, in particular leaf conductance aspects.

The possibility that variation in TE may be associated with rooting differences has been considered, but appears to be an unlikely option. The range of variation in TE was about 2 g kg⁻¹ water in the R16 background. Using a mean total water use of 14 L, increasing TE by 2 g kg⁻¹ water with root growth would require a root growth differential of 28 g/plant, which roughly equates to a doubling in the root:shoot ratio, assumed to be in the

range of 40% in sorghum (our unpublished data). This is extremely unlikely, especially because of the limited difference in water extraction among ILs in the R16 background.

Differences in water extraction

There has been surprisingly no work directly focused on testing the relationship between the expression of stay-green and the role of rooting traits, except for preliminary work (Vadez et al., 2007a) that showed differences in rooting in early generation stay-green ILs and donor B35 compared to R16. Tuinstra et al. (1998) also referred to differences in water status explaining differences in stay-green expression, but didn't mention a possible involvement of rooting. This is surprising, since higher N uptake has often been reported in stay-green lines of sorghum (Borrell and Hammer, 2000; van Oosterom et al., 2010) and of maize (Rajcan and Tollenaar, 1999), although no attempt to link this N uptake with water uptake has apparently been made. Differences in rooting have been reported in sorghum, showing up to 40 cm increased rooting depth in drought tolerant sorghum lines (Salih et al., 1999). Similarly, differences in rooting have been reported in other crops where a stay-green phenotype gets expressed. For example, Manschadi et al. (2006) showed that the drought tolerant wheat genotype SeriM82 had more uniformly distributed roots, although they did not contribute to higher water extraction. Furthermore, Christopher et al. (2008), in another study on SeriM82, suggested that small differences in water use before anthesis, or greater water extraction from depth after anthesis, could underlie the stay-green phenotype in wheat. It has also been shown in rice that stay-green lines have a higher profusion of roots under water stress, leading to higher stay-green scores (Hoang et al., 2009). Similar work testing the relation between roots and drought tolerance has been done in maize (Hund et al., 2009; Landi et al., 2007). However, none of that previous work, except Manschadi et al. (2006) and Christopher et al. (2008), attempted to assess water extraction differences. In our study, an improvement of up to 2 L plant⁻¹ in one Stg1 IL is reported in the S35 background, but no improvement was achieved in the R16 background. This is likely related to a fairly high water extraction capacity in R16 (our unpublished work). Based on Fig. 6c, a 2 L difference in water extraction would represent a yield increase close to 4 g plant⁻¹, equivalent to 400 kg

ha⁻¹, about 20% of the usual mean grain yield under terminal stress. However, the higher water extraction of that one Stg1 IL did not convert in a higher grain yield but only in a higher stover dry weight. It is estimated that about 19 L of water are available for transpiration in the cylinders. Therefore a 2 L difference in extraction in by the Stg1 IL would correspond to a difference in root extraction depth of about 20 cm. Larger rooting depth differences were reported earlier in stay-green material (Vadez et al., 2007a) and call for more work on the root development and/or rooting profile in relation to water extraction.

Yield and relationships with TE and HI – Influence of genetic background

It has been previously stated that, in general, TE and water use are negatively correlated (Blum, 2005, 2009), for instance in groundnut (Wright et al., 1994). We found no such relationships in any of the genetic backgrounds and conclude that the TE-water use and TE-HI relationships may not be mutually exclusive, contrary to previous thinking. In fact, the data showed clearly that improvement of TE was possible in an R16 background that already exhibited a high capacity to extract water, without compromising this capacity in introgression lines. Similarly, it was possible to enhance water extraction capacity in the S35 background, without compromising the high TE baseline in that genetic background.

Transpiration efficiency and water extraction had an important bearing on yield differences that were not explained by the harvest index. However, the magnitude of that influence was conditioned by the genetic background. For instance, water extraction was improved by the Stg1 QTL and, to some extent, by the Stg3 and StgB QTLs in S35 background. Therefore, in that background both TE and water extraction explained the residual grain yield variation unexplained by HI. Similarly, TE was improved by the StgB QTL in R16 background. There, only TE had an influence on the residual grain yield variation under drought stress that was not explained by harvest index. The present results contrast with previous observations that four stay-green QTLs all showed consistency across different genetic backgrounds (Subudhi et al., 2000). It is clear from this work that stay-green is possibly related to different components such as TE and/or water extraction capacity, themselves likely to be an aggregate of different mechanisms.

Here we tested more specific traits (i.e. water extraction and TE), rather than an integration of these into an overall stay-green phenotype. It can be illustrated here by the trend for increased total biomass with StgB (grain + stover) in both S35 and R16 backgrounds, although this was likely related to an increase in water extraction in the S35 background and to an increase in TE in R16 background. This highlights the need to better understand the role of each QTL in controlling the expression of a stay-green phenotype, and their interactions with the recipient genetic background. This will assist plant breeders to select the best combinations of Stg QTLs to pyramid in a particular genetic background. It is also critical to decipher the mechanisms underlying the different QTLs so their effects via specific mechanisms (e.g. Kholova et al., 2010a,b) can be predicted using crop simulation modeling (Chenu et al., 2009; Sinclair et al., 2010).

Conclusions

This work showed that different stay-green QTLs affected different traits such as transpiration efficiency and the capacity to extract water. However, the effect of QTLs on these traits was dependent on the value of these traits in the recipient background. This shows the importance of precisely deciphering the mechanisms underlying drought tolerance QTLs, like stay-green here, and their value in recipient backgrounds, before undertaking costly introgression work. However, this study also shows that completing at least some exploratory QTL introgression work can provide powerful genetic tools that can contribute to more precisely deciphering these mechanisms and assessing the variation in values of different QTLs across a range of diverse genetic backgrounds.

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Table 1: Trial means, range of expected means, standard error of differences (SED) within treatment, and Wald statistics and F-probability for genotype effect (G), treatment effect (W) and genotype-by-treatment (G×W) interaction related to time to flowering (d), grain dry weight and total dry weight (g plant⁻¹), harvest index (HI), transpiration efficiency (TE, g kg⁻¹) and panicle harvest index (PNHI).

		50% FI		Grain yield		Total DW		Harvest index		TE		PNHI	
		WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
S35 Background													
Mean		58	58	37.00	22.19	122.96	69.24	0.30	0.29	4.64	5.29	0.79	0.76
Min		52	52	15.96	6.22	74.72	54.71	0.16	0.09	3.70	4.35	0.48	0.46
Max		65	61	72.24	35.29	180.38	84.42	0.43	0.44	5.62	6.08	1.00	0.92
SED		2.62	1.77	14.64	8.56	20.55	8.90	0.08	0.10	0.58	0.54	0.09	0.10
G	F-value	5.22		2.38		2.98		2.62		1.54		2.02	
	Prob	0.001		0.001		0.001		0.001		0.05		0.004	
W	F-value	0.01		51.33		371.3		0.00		40.1		4.08	
	Prob	ns		0.001		0.001		ns		0.001		0.05	
G×W	F-value	1.40		1.15		2.48		1.05		0.88		1.45	
	Prob	ns		ns		0.001		ns		ns		ns	
R16 Background													
Mean		61	60	46.75	24.02	113.18	62.70	0.41	0.28	4.52	4.50	0.76	0.59
Min		55	53	4.66	18.45	51.36	39.34	0.09	0.00	3.66	3.69	0.55	0.00
Max		75	71	76.80	30.52	167.25	80.42	0.50	0.42	6.64	5.49	0.93	0.84
SED		1.66	1.7	11.61	7.03	20.30	8.36	0.06	0.08	0.67	0.60	0.07	0.09
G	F-value	11.2		3.68		4.19		4.76		2.02		2.32	
	Prob	0.001		0.001		0.001		0.001		0.008		0.002	
W	F-value	0.34		149.3		264.8		61.26		0.00		27.58	
	Prob	ns		0.001		0.001		0.001		ns		0.001	
G×W	F-value	0.77		1.45		2.02		1.28		0.89		1.01	
	Prob	ns		ns		0.008		ns		ns		ns	

Table 2: Trial means, range of expected means, standard error of differences (SED) within treatment, and Wald statistics and F-probability for genotype effect (G), treatment effect (T) and genotype-by-treatment (G×W) interaction related to total water use, pre-anthesis water use, post anthesis water use, and water used in the 45-59 DAS, 59-78 DAS and 78-94 DAS period (L plant⁻¹).

Water use		Total		Pre-anthesis		Post-anthesis		45-59 DAS		59-78 DAS		78-94 DAS	
		WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
S35 Background													
Mean		26385	13142	9084	8850	18642	5791	311	276	337	175	333	137
Min		18333	10680	6545	7076	11830	2647	227	100	241	0	197	87
Max		39020	14647	13391	12605	32700	7911	436	508	503	248	564	172
SED		3538	840	1226	1031	3345	1146	45.7	140.1	59.5	60.7	75.2	24.8
G	F-value	2.44		3.79		1.99		0.79		1.60		1.37	
	Prob	0.001		0.001		0.005		ns		0.04		ns	
W	F-value	802.2		1.46		1100		3.55		221.2		535.4	
	Prob	0.001		ns		0.001		ns		0.001		0.001	
G×W	F-value	2.49		2.40		2.84		0.84		1.46		1.62	
	Prob	0.001		0.001		0.001		ns		ns		0.04	
R16 Background													
Mean		24772	13894	10478	10376	15754	5045	353	352	368	193	303	102
Min		14087	10193	5120	6897	7940	3011	131	131	166	88	148	83
Max		35570	15267	17751	13675	21565	7223	471	521	542	292	513	142
SED		3487	822	1162	1086	3134	1041	758	1429	1205	1139	909	185
G	F-value	4.60		7.00		2.09		3.50		2.52		2.92	
	Prob	0.001		0.001		0.006		0.001		0.001		0.001	
W	F-value	480.1		0.81		628.3		0.02		210.8		594	
	Prob	0.001		ns		0.001		ns		0.001		0.001	
G×W	F-value	2.84		2.10		1.99		0.96		2.28		4.30	
	Prob	0.001		0.005		0.009		ns		0.002		0.001	

Table 3: Multi-linear regression between grain yield and several explanatory variables: harvest index, transpiration efficiency, total water extracted, post-anthesis water use, water used in the 59-78 DAS and 78-94 DAS periods, and days to flowering.

Factors	Coefficient	SE	t-value	P > t
S35 background - R² = 0.99				
Harvest index	68.6	2.08	32.98	0.000
Transpiration efficiency	4.03	0.42	9.46	0.000
Total water extracted	0.00174	0.00018	9.69	0.000
Post-anthesis water use	0.00090	0.00056	1.59	ns
Water use between 59-78 DAS	-0.00112	0.00054	-2.05	0.05
Water use between 78-94 DAS	-0.00068	0.00090	-0.76	ns
Days to 50% flowering	0.207	0.153	1.36	ns
Constant	-56.2	9.2	-6.10	0.000
R16 background - R² = 0.99				
Harvest index	63.85	1.03	61.90	0.000
Transpiration efficiency	5.09	0.10	48.30	0.000
Total water extracted	0.00168	0.00016	10.53	0.000
Post-anthesis water use	-0.00018	0.00042	-0.43	ns
Water use between 59-78 DAS	0.00023	0.00039	0.59	ns
Water use between 78-94 DAS	0.00013	0.00058	0.22	ns
Days to 50% flowering	-0.044	0.124	-0.36	ns
Constant	-44.2	8.5	-5.19	0.000

Table 4: Multi-linear regression between the residual grain yield variations not explained by HI and several explanatory variables: transpiration efficiency, total water extracted, post-anthesis water use, water used in the 59-78 DAS and 78-94 DAS periods, and days to flowering. The regressions were run separately in each genetic background (S35 and R16).

Factors	Coefficient	SE	t-value	P > t
S35 background - R² = 0.83				
Transpiration efficiency	3.24	0.55	5.90	0.000
Total water extracted	0.00187	0.00025	7.41	0.000
Post-anthesis water use	0.00111	0.00080	1.39	ns
Water use between 59-78 DAS	-0.00132	0.00077	-1.73	0.097
Water use between 78-94 DAS	-0.00131	0.00120	-1.09	ns
Days to 50% flowering	0.178	0.202	0.30	ns
Constant	-51.73	12.50	-4.14	0.000
R16 background - R² = 0.88				
Transpiration efficiency	4.38	0.48	9.09	0.000
Total water extracted	0.00104	0.00068	1.53	ns
Post-anthesis water use	0.0049	0.00188	2.62	0.022
Water use between 59-78 DAS	-0.00450	0.00170	-2.64	0.022
Water use between 78-94 DAS	-0.00637	0.00258	-2.46	0.030
Days to 50% flowering	1.38	0.56	2.47	0.029
Constant	-118.7	39.3	-3.02	0.011

Figure captions

Fig. 1. Tiller number per plant (a, b), number of remaining leaves on main stem (c, d), leaf area of the main stem (cm², e, f), and total leaf area (cm², g, h) in different stay-green introgression lines in S35 (a, c, e, g) and R16 (b, d, f, h) backgrounds grown under fully-irrigated conditions in 27-cm diameter pots until booting time. For the stay-green QTL introgressions, the data are the means (\pm SE) of all ILs carrying the same stay-green QTL (alleles of donor B35 = BTx642) in each respective background (n = 4 and 5). Stars above a given bar indicate that the mean was significantly different (P<0.05) from the recurrent parent (S35 or R16).

Fig. 2. Transpiration efficiency (g kg⁻¹ water transpired) under terminal water stress conditions in a set of introgression lines each carrying an individual stay-green QTL in S35 (a) and R16 (b) backgrounds, plus checks. Data are the mean of three replicated plants per genotype. Bar indicates LSD (P<0.05).

Fig. 3. Total water extracted from the lysimeter soil profile (g plant⁻¹) under terminal water stress conditions in a set of introgression lines each carrying an individual stay-green QTL in S35 (a) and R16 (b) backgrounds, plus checks. Data are the mean of three replicated plants per genotype. Bar indicates LSD (P<0.05).

Fig. 4. Water used during the pre-anthesis period (g plant⁻¹; a, b) and during the post-anthesis period (g plant⁻¹; c, d) in different groups of stay-green introgression lines in S35 (a, c) and R16 (b, d) backgrounds under terminal water stress conditions. For the stay-green introgressions, the data are the means (\pm SE) of all introgression lines carrying the same stay-green QTL (alleles of donor B35 = BTx642) in each respective background (n = 4 and 5). Stars above a given bar indicate that the mean was significantly different (P<0.05) from the recurrent parent (S35 or R16).

Fig. 5. Grain yield (g plant⁻¹; a, b), stover yield (g plant⁻¹; c, d), and stover + grain yield (g plant⁻¹; e, f) in different groups of stay-green introgression lines in S35 (a, c, e) and

R16 (b, d, f) backgrounds under terminal water stress conditions. For the stay-green introgressions, the data are the means (\pm SE) of all introgression lines carrying the same stay-green QTL (alleles of donor B35 = BTx642) in each respective background (n = 4 and 5). Stars above a given bar indicate that the mean was significantly different ($P < 0.05$) from the recurrent parent (S35 or R16).

Fig. 6. Main head grain yield (g plant^{-1} ; a, b), and tiller grain yield (g plant^{-1} ; c, d) in different stay-green introgression lines in S35 (a, c) and R16 (b, d) background grown under fully-irrigated conditions. For the stay-green introgressions, the data are the means (\pm SE) all introgression lines carrying the same stay-green QTL (alleles of donor B35 = BTx642) in each respective background (n = 4 and 5). Stars above a given bar indicate that the mean was significantly different ($P < 0.05$) from the recurrent parent (S35 or R16).

Fig. 7. Relationship between grain yield under terminal water stress and the harvest index (HI) (a), relationship between the residual yield variations unexplained by HI and transpiration efficiency (TE) (b), and relationship between the residual yield variations unexplained by HI and the total water extracted from the soil profile (c) in S35 (closed symbols) and R16 (open symbols) background. Data are the mean of 3 replicated lysimeter-grown plants per genotype.

Supplementary Table 1. Marker information in different staygreen QTL chromosome portions in S35 background.

Entry	Stg1	Xtxp285	Xisp10361	Xtxp034	<u>Xisp10323</u>	
06032	<i>stg1</i>	B	B	B	B	
06032a	<i>stg1</i>	B	B	B	B	
06036	<i>stg1</i>	B	B	B	-	
06039	<i>stg1</i>	B	B	B	B	
06040	<i>stg1</i>	B	B	B	B	
	Stg2	Xtxp120	<u>Xisp10307</u>	XSbAGF08	Pericarp	
06025	<i>stg2</i>	B	B	Mono	<u>Yellow & White</u>	
06026	<i>stg2</i>	B	B	Mono	<u>Red & Yellow</u>	
06028	<i>stg2</i>		B	H	White	
06029	<i>stg2</i>	A	B		Red: Y/Y R/R	
06030	<i>stg2</i>	A	B		Red: Y/Y R/R	
	Stg3	<u>Xtxp019</u>	<u>Xtxp298</u>		Mesocarpe Thickness	
06014	<i>stg3</i>	B	A		Thick: z/z	
06015	<i>stg3</i>	A	B		Thick: z/z	
06016	<i>stg3</i>	H	B		Thin: Z/Z	
06020	<i>stg3</i>	A	A		Thick: z/z	
	Stg4	<u>Xisp10257</u>	<u>Xtxp15</u>	<u>Xtxp14</u>	<u>Xtxp299</u>	<u>Xtxp225</u>
06021a	<i>stg4</i>	B	Mono			B
06021b	<i>stg4</i>	B	Mono			B
06022	<i>stg4</i>	B	Mono			B
06023	<i>stg4</i>	B	Mono			B
06024	<i>stg4</i>	B	Mono			B
	StgA	<u>Xisp10204</u>	<u>Xtxp357</u>	<u>Xtxp43</u>	<u>Xtxp149</u>	<u>Xtxp88</u>
06003	<i>stgA</i>	NP	B	-	B	B
06005	<i>stgA</i>	NP	B	-	<u>B</u>	B
06007	<i>stgA</i>	NP	H	B	<u>B</u>	-
06008	<i>stgA</i>	NP	B	-	<u>B</u>	-
06010	<i>stgA</i>	NP	B	-	<u>B</u>	-
	StgB	<u>Xtxp100</u>	<u>Xtxp7</u>	<u>Xtxp207</u>	<u>Xtxp296</u>	<u>Xcup26</u>
07001	<i>stgB</i>	A		B		
07002	<i>stgB</i>	A		B		
06011b	<i>stgB</i>	A		B		
06012	<i>stgB</i>	A		B		
06013	<i>stgB</i>	A		B		

Supplementary Table 2. Marker information in different staygreen QTL chromosome portions in R16 background.

Entry	Stg1	<i>Xtxp285</i>	<i>Xisp10361</i>	<i>Xtxp034</i>	<u>Awns: A/a</u>	
K 225	<i>stg1</i>	B	B	B	Awns	
K 228	<i>stg1</i>	B	B	B	Awns	
K 352	<i>stg1</i>	B	B	B	Awns	
K 354	<i>stg1</i>	B	B	B	Awns	
	Stg3	<i>Xtxp019</i>	<i>Xtxp298</i>	<i>Xgap084</i>	<u>Mesocarp thickness</u>	
K 253	<i>stg3</i>	B	B	B	not scored	
K 260	<i>stg3</i>	B	B	B	Thick: z/z	
K 252	<i>stg3</i>	A	A	A	not scored	
K 258	<i>stg3</i>	A	A	A	not scored	
	Stg4	<i>Xisp10257</i>	<i>Xtxp014</i>	<i>Xtxp225</i>		
K 279	<i>stg4</i>	B	B	B		
K 287	<i>stg4</i>	B	B	B		
K 472	<i>stg4</i>	B	B	B		
K 648	<i>stg4</i>	B	B	B		
	StgB	<i>Xtxp100</i>	<i>Xtxp007</i>	<i>Xtxp296</i>	<i>Xisep0841</i>	
K 214	<i>stgB</i>	B	B	B	B	
K 219	<i>stgB</i>	B	B	B	B	
K 359	<i>stgB</i>	B	H	-	B	
K 369	<i>stgB</i>	B	B	-	B	

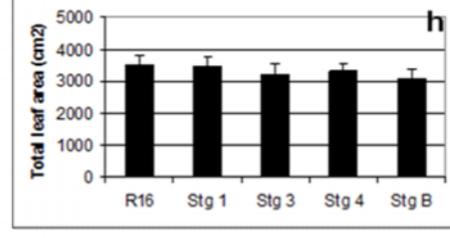
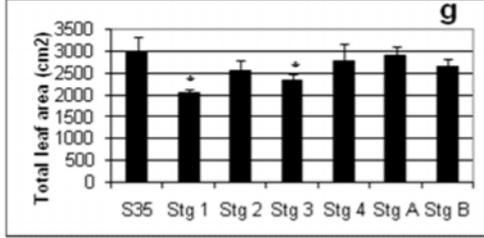
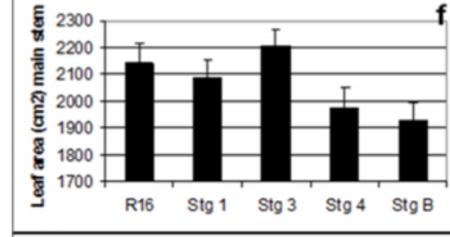
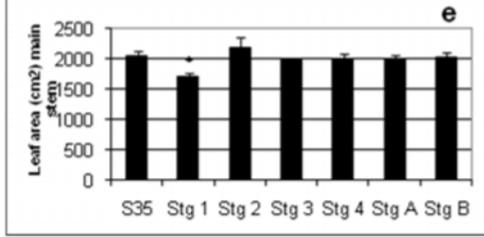
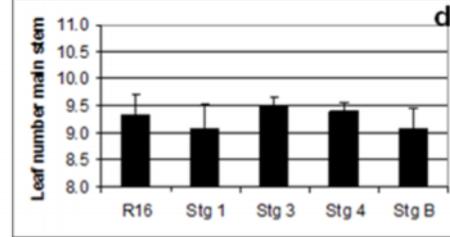
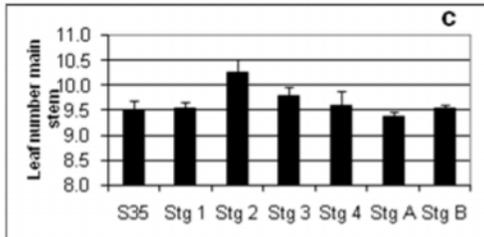
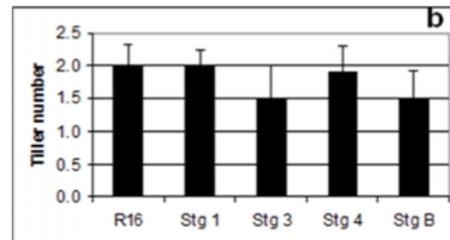
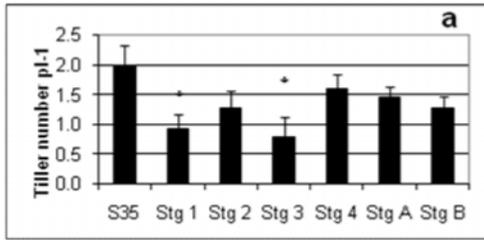


Figure 1

Figure 4

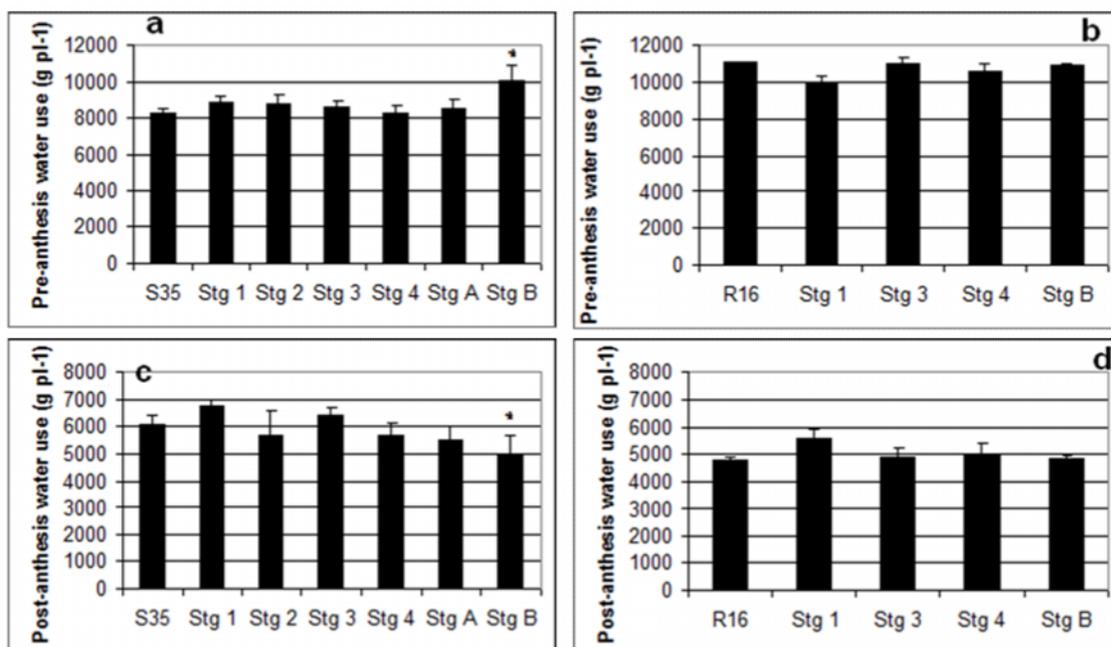


Figure 5

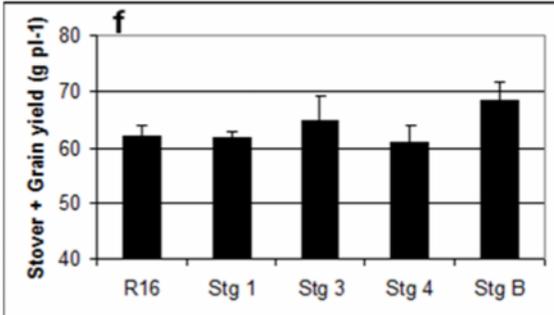
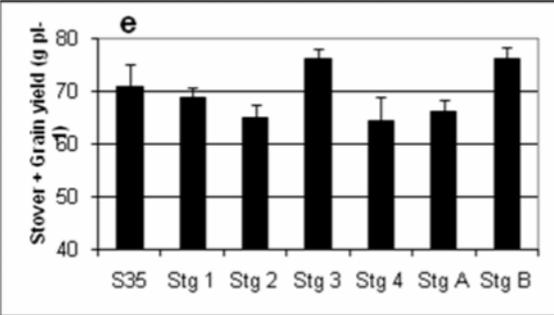
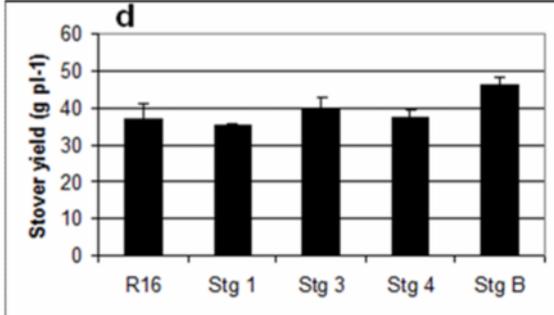
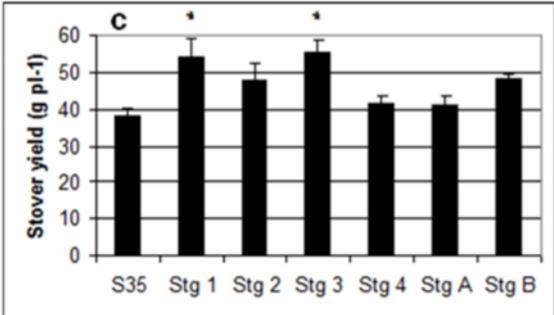
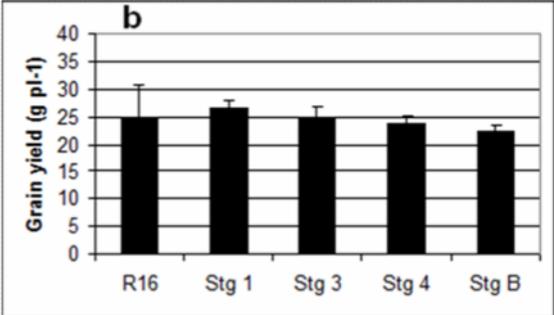
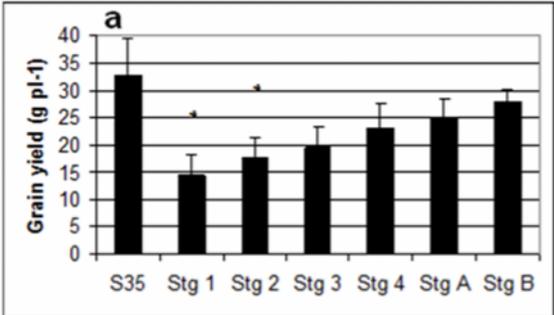


Figure 6

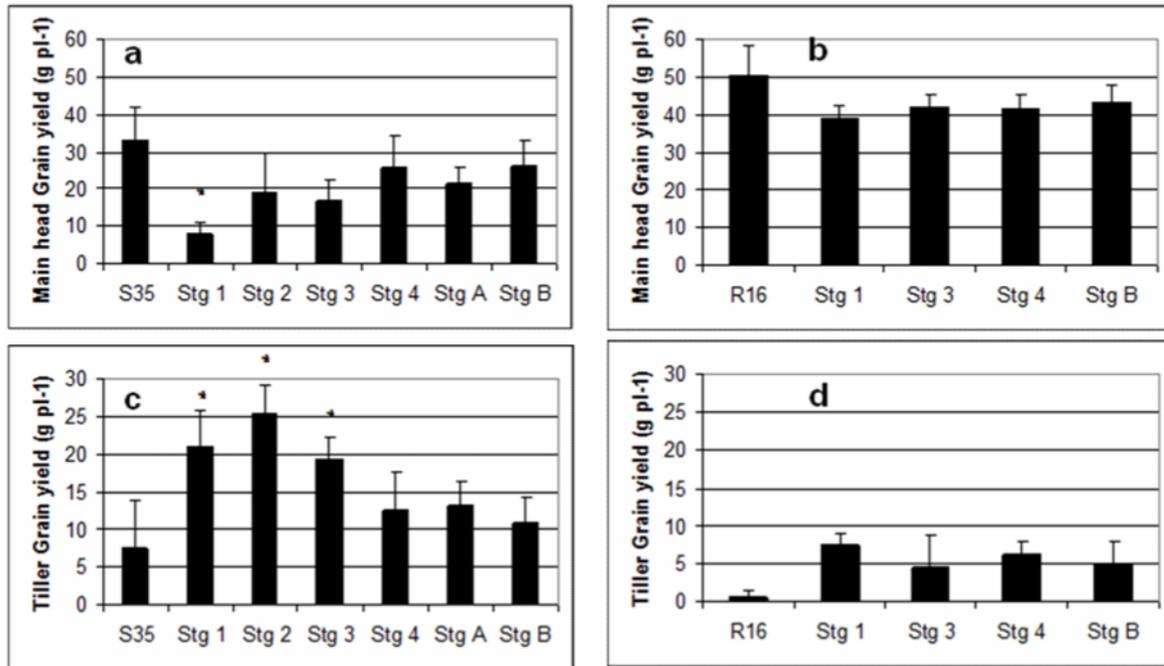


Figure 7

