

CROP PHYSIOLOGY & METABOLISM

Does Maintaining Green Leaf Area in Sorghum Improve Yield under Drought? I. Leaf Growth and Senescence

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ABSTRACT

Production of sorghum [*Sorghum bicolor* (L.) Moench], an important cereal crop in semiarid regions of the world, is often limited by drought. When water is limiting during the grain-filling period, hybrids possessing the stay-green trait maintain more photosynthetically active leaves than hybrids not possessing this trait. To improve yield under drought, knowledge of the extent of genetic variation in green leaf area retention is required. Field studies were undertaken in north-eastern Australia on a cracking and self-mulching gray clay to determine the effects of water regime and hybrid on the components of green leaf area at maturity (GLAM). Nine hybrids varying in stay-green were grown under a fully irrigated control, postflowering water deficit, and terminal (pre- and postflowering) water deficit. Water deficit reduced GLAM by 67% in the terminal drought treatment compared with the fully irrigated control. Under terminal water deficit, hybrids possessing the B35 and KS19 sources of stay-green retained more GLAM ($1260 \text{ cm}^2 \text{ plant}^{-1}$) compared with intermediate ($780 \text{ cm}^2 \text{ plant}^{-1}$) and senescent ($670 \text{ cm}^2 \text{ plant}^{-1}$) hybrids. RQL12 hybrids (KS19 source of stay-green) displayed delayed onset and reduced rate of senescence; A35 hybrids displayed only delayed onset. Visual rating of green leaf retention was highly correlated with measured GLAM, although this procedure is constrained by an inability to distinguish among the functional mechanisms determining the phenotype. Linking functional rather than phenotypic differences to molecular markers may improve the efficiency of selecting for traits such as stay-green.

SORGHUM is an important cereal crop in semiarid regions of the world. One of the major challenges for sorghum improvement programs is to develop plants that have an advantage in water-limited environments. Historically, sorghum breeders have used empirical methods to select for yield under drought conditions. However, more recently, some breeders have identified secondary traits that confer a yield advantage under drought and have developed criteria for selecting these traits (Rosenow et al., 1983; Henzell et al., 1992). Results from these programs suggest that advances in crop improvement under water-limited conditions are more likely if drought resistance traits are selected in addition to yield per se.

Stay-green, or delayed foliar senescence, is one such secondary trait. Rapid premature leaf death generally occurs in sorghum when water is limiting during the

grain-filling period (Stout and Simpson, 1978; Rosenow and Clark, 1981). During postanthesis drought, genotypes possessing the stay-green trait maintain more photosynthetically active leaves than genotypes not possessing this trait (Rosenow et al., 1983; McBee, 1984). Expression of stay-green has been reported in some other cereals including maize, *Zea mays* L. (Tollenaar and Daynard, 1978; Crafts-Brandner et al., 1984a, 1984b; Gentinetta et al., 1986; Rajcan and Tollenaar, 1999a, 1999b); rice, *Oryza sativa* L. (Mondal and Choudhuri, 1985; Wada and Wada, 1991); and oat, *Avena sativa* L. (Helsel and Frey, 1978). In addition, a stay-green mutant of the pasture grass *Festuca pratensis* Huds. has been identified and subsequently studied, leading to a better understanding of the biochemistry of senescence (Thomas and Stoddart, 1975; Thomas and Smart, 1993).

Of all the factors contributing to the stay-green phenomenon, N status of the leaf is central to senescence (Thomas and Rogers, 1990). During senescence, protein is degraded and amino acids are transported out of the leaf. The characteristic yellowing of the leaf indicates the loss of chlorophyll from the pigment-protein complexes of the photosynthetic apparatus. In fact, leaf senescence is thought to be triggered by an increased demand for N elsewhere in the plant.

Four classes of stay-green have been identified by Thomas and Smart (1993). The first two classes are functionally stay-green and may occur after alteration of genes involved in the onset of senescence and the regulation of its rate of progress. However, stay-green in the remaining two classes is cosmetic; that is, the plants are green but lack photosynthetic competence. This may be due to a loss in photosynthetic capability that normally accompanies senescence combined with maintenance of leaf chlorophyll, or it may be related to premature death seen in herbarium specimens or frozen foods that retain greenness because they are rapidly killed at harvest.

Green leaf area at physiological maturity has proved to be an excellent indicator of stay-green, and has successfully been used to select drought-resistant sorghums

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Abbreviations: APL, area per leaf; CNPP, culm number per plant; DAE, days after emergence; FTN, fertile tiller number; GLAM, green leaf area at maturity; LAI, leaf area index; LAR, leaf appearance rate; ND, no water deficit treatment; PFD, postflowering water deficit treatment; SPLA, senesced plant leaf area; TD, terminal water deficit treatment; TLN, total leaf number on the main culm; TPLA, total plant leaf area; TPLA_{max}, maximum total plant leaf area; TT, thermal time. *, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

in the USA (Rosenow et al., 1983) and in Australia (Henzell et al., 1992). Key components determining GLAM are: (i) maximum green leaf area ($TPLA_{max}$) at ≈ 4 d before anthesis, (ii) duration of leaf senescence, and (iii) rate of leaf senescence. Duration of leaf senescence is a function of the timing of the onset of senescence and the timing of physiological maturity.

Two factors affecting the components of GLAM are water deficit and genotype. Timing and severity of drought are critical in determining both leaf area development and subsequent senescence. Environmental conditions resulting in high leaf area production at anthesis followed by severe postanthesis water deficit are most conducive to the expression of stay-green. Furthermore, genotypic differences in leaf area production and senescence among six diverse grain sorghum hybrids were observed by Hammer et al. (1987), and genetic variation in the inheritance of ($TPLA_{max}$), onset of leaf senescence, and rate of leaf senescence have been reported by Van Oosterom et al. (1996). The latter study found the inheritance of stay-green to be a function of the inheritance of its components, and concluded that the inheritance of the onset of senescence was additive, whereas the inheritance of the rate of senescence was completely dominant for a slow rate. Therefore, the relative 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.

To improve yield under drought, knowledge of the extent of genotypic variation in the components of GLAM is required. In particular, higher $TPLA_{max}$, delayed onset of leaf senescence, and reduced rate of leaf senescence are all pathways to increased GLAM. Coefficients for these parameters are also required to modify leaf senescence routines in sorghum simulation models (Hammer and Muchow, 1994). Using these coefficients, together with an understanding of the functional basis of physiological responses, would enable simulation of effects of the stay-green drought-resistance trait. The model then could be used to assess the value of stay-green in a range of target environments.

Quantitative measurement of stay-green in plant breeding programs can be time consuming and expensive. Therefore, visual rating of stay-green in the field is very important to plant breeders for screening large segregating populations. Wanous et al. (1991) found that GLAM was correlated with visual ratings of both green leaf retention ($r = 0.91$) and green leaf number ($r = 0.95$) for sorghum grown under drought. A visual score for leaf senescence has already been used to select for drought resistance in maize, although these parameters were poorly correlated (Bolaños and Edmeades, 1996). The lack of association between green leaf retention and grain yield observed in their studies may be due to increased mobilization of N from the leaves of the higher yielding hybrids, thereby inducing senescence, a phenomenon observed by Muchow (1994).

The objectives of this study were twofold. First, we determined the effects of water regime and hybrid on the components of GLAM: $TPLA_{max}$, duration of leaf senescence, and rate of leaf senescence. Second, the

association between visual rating of green leaf retention and measured green leaf area at maturity was examined under water-limited conditions to assess the accuracy of this visual approach. The association between GLAM and grain yield will be discussed in the second paper of this series (Borrell et al., 2000).

MATERIALS AND METHODS

Experiment Site

A field experiment was conducted at Hermitage Research Station (altitude 480 m, $28^{\circ}10'S$, $152^{\circ}02'E$) in the sorghum cropping zone of southern Queensland, Australia, in the 1994-1995 season. The experiment design was a split plot with three replicates. Three irrigation treatments were applied to main plots and nine hybrids varying in rate of leaf senescence were allocated to subplots. Main plots were 6 by 31.5 m, with a 2.8-m buffer zone between them, and subplots were 3.5 (5 rows) by 6 m. Replicates were separated by a 4-m roadway adjoining a 4-m cropped buffer zone.

Treatments

The water regime treatments were no deficit (ND), post-flowering deficit (PFD) and terminal deficit (TD). The two contrasting water-limited environments (PFD and TD) were based on the classifications of Ludlow and Muchow (1990) for crop production in the semiarid tropics. Intermittent stress typifies the wet season in the monsoonal semiarid tropics, with stress occurring at any time and with varying intensities between emergence and maturity. Postflowering deficit represents one pattern of intermittent stress. Terminal stress typifies the dry season of the semiarid tropics, where crops are usually grown solely on stored soil moisture in heavy soils. The crop matures on a progressively depleted soil moisture profile. The TD treatment was designed to mimic this pattern of water stress.

The experiment block was furrow irrigated between 3 and 11 Nov. 1994 (≈ 38 d before sowing) and ≈ 300 mm of water was available for the crop at sowing. An analysis of initial soil water content found no significant differences ($P > 0.05$) among plots in this parameter at sowing. All treatments were covered with black plastic prior to sowing to exclude rainfall and prevent evaporation losses. The ND treatment was irrigated regularly on a 30-mm deficit (pan evaporation minus rainfall). Irrigation scheduling was based on crop factors of 0.3, 0.7, 1.0, and 0.7 for each 30-d increment of crop growth. The PFD treatment was irrigated at the same frequency and magnitude as the ND treatment until 28 d before flowering, at which time irrigation ceased. No irrigation was applied to TD plots. Plants in this treatment should have relied solely on stored soil water; however, an additional 80 mm of water entered the profile through the plastic in a series of rainfall events near anthesis. The magnitude of water entry under the plastic was determined by the neutron scattering technique using a neutron probe (Model 503R, CPN Corp., Martinez, CA). An analysis of the magnitude of water entry found no significant differences ($P > 0.05$) among plots in this parameter.

Nine hybrids were examined from crosses of three females varying in the B35 source of stay-green (AQL39, senescent; AQL41, intermediate; A35, stay-green) and three males in the KS19 source of stay-green (R69264, senescent; RQL36, intermediate; RQL12, stay-green). A35 is the male-sterile version of B35. The B35 and KS19 sources of stay-green are derived from sorghum lines native to Ethiopia and Nigeria,

respectively. B35 is derived from IS12555, a durra landrace in Ethiopia, while RQL12 is derived from KS19, which in turn was derived from the cross between Combine Kafir 60 and Short Kaura, the latter being from Nigeria. Hybrid parents were initially characterized for stay-green by visually rating these lines for GLAM in a range of multi-environment trials across a number of years within the Australian sorghum breeding program.

Agronomy

Soil type was a cracking and self-mulching gray clay with abundant CaCO_3 concretions (Elphinstone depositional, McKeown, 1978; Ug 5.16, Northcote, 1974). The degree of swelling on wetting indicates a high montmorillonite clay content and the soil is a linear gilgai complex (McKeown, 1978). The experiment site has a slope of $\approx 2\%$ and the profile is moderately well drained. At the surface (0–0.1 m) pH, electrical conductivity, and Cl were 7.9, 0.125 mS cm^{-1} , and 15 mg kg^{-1} , respectively, and increased to 9.1, 0.366 mS cm^{-1} , and 74 mg kg^{-1} , respectively, at depth (0.8–0.9 m). Organic C was 13 g kg^{-1} at the surface (0–0.1 m), decreasing to 6 g kg^{-1} at depth (0.8–0.9 m).

The experiment site was fertilized on 19 Oct. 1994 (57 d before sowing) with 300 kg N ha^{-1} as urea. Two days later a mixture containing 40, 30, and 10 kg ha^{-1} P, K, and Zn, respectively, was applied. The site was rotary-hoed immediately after fertilizer application to break down larger clods for ridge construction and seedbed preparation. Ridges of height 0.2 m were established 0.7 m apart parallel to the direction of slope. Trickle irrigation tape was laid near the crest of each ridge in the ND and PFD treatments and connected to a main irrigation line. Water was applied to each irrigation treatment block via an in-line flowmeter in the main line. After establishing the irrigation infrastructure, each replicate was covered in a single black polyethylene sheet (Garden Nursery Products, Beenleigh, Queensland, Australia) (8 by 150 m by 200 μm). Sheets were secured by placing sand over the plastic in the furrows. Entry of water under the plastic was prevented by burying the sheets to a depth of 1 m upslope of each replicate, and by constructing wide and shallow drainage channels below each replicate. No weeds grew under the plastic and therefore no weed control was required.

Cross-cuts (55-mm diameter) were made in the plastic with a metal spear at 100-mm intervals in a line along the crest of each ridge. After making depressions in the soil below each cut, about five seeds were dropped through each of 12 holes with a hand-held planting device, then covered and compressed (15 Dec. 1994). Five rows of each hybrid were planted in each treatment block and three guard rows of sorghum (cv. Buster) were planted at the ends of all treatment blocks. All plots were watered by hand after sowing, and following emergence (18 Dec. 1994) and establishment, seedlings were thinned to one per hole (3 Jan. 1995), giving a population density of ≈ 14 plants m^{-2} .

Anthesis was defined as the time when 50% of the anthers had extruded from 50% of 10 tagged panicles in each plot. Physiological maturity was determined by assessing black layer twice weekly in 10 tagged plants, beginning with the grain in the uppermost quartile of the panicle and finishing with the grain in the basal quartile. Physiological maturity was defined as the time at which basal grains in 50% of the tagged panicles attained black layer. The plots were hand harvested on 10 to 12 Apr. 1995.

Leaf Observations

Early in crop growth, 10 representative plants were tagged from the center of Row 1 of each plot. Production of leaves

on both main and tiller culms was measured on four tagged plants by identifying and marking a known leaf number early in crop growth and then recording the number of fully expanded and senesced leaves at weekly intervals (Hammer et al., 1993). A leaf was considered fully expanded when its ligule became visible above the enclosing sheath of the previous leaf. A leaf was considered senesced when more than 50% of its area had senesced. In addition, the green area of all fully expanded leaves on both main and tiller culms was measured (Delta-T DIAS image analysis system, Cambridge, UK) on two tagged plants at each of three harvest times corresponding with the expansion of the 6th, 12th, and flag leaves. The number of leaves senesced on each tagged plant, together with the known area of those leaves, was used to calculate senesced plant leaf area (SPLA).

A single row of length 1 m was also cut from one of three center rows of each plot at 30, 46, 59 (anthesis + 3d), 87, and 114 d after emergence (DAE). At least 0.5-m intervals of crop were left between sampling areas within a row and no adjacent areas were sampled. Harvests at 30, 59, and 114 DAE corresponded with the phenological stages of panicle differentiation, anthesis, and physiological maturity. Green leaf area was determined for each plot at all harvest times with an electronic planimeter (Delta-T DIAS image analysis system). For partly senesced leaves, the senesced portion was cut away from the leaf prior to measurement so that only green leaf area was determined.

Analyses

Thermal Time

Thermal time (TT) each day (δTT , $^{\circ}\text{C d}$) was calculated from a broken-stick function of temperature (T),

$$\delta\text{TT} = 0 \quad T < T_b \text{ or } T > T_{\text{max}} \quad [1]$$

$$\delta\text{TT} = T - T_b \quad T_b < T < T_{\text{opt}} \quad [2]$$

$$\delta\text{TT} = (T_{\text{opt}} - T_b) \left[1 - \frac{(T - T_{\text{opt}})}{(T_{\text{max}} - T_{\text{opt}})} \right] \quad T_{\text{opt}} < T < T_{\text{max}} \quad [3]$$

if the base (T_b), optimum (T_{opt}), and maximum (T_{max}) temperatures are known. This model was developed in controlled environment studies (Ong and Monteith, 1985; Monteith, 1987) and later applied to studies in sorghum on germination response to temperature (Wade et al., 1993) and modeling of leaf area dynamics (Hammer et al., 1993). Thermal time was determined by accumulating δTT after emergence and to minimize error associated with diurnal temperature variation, 3-h averages were used in Eq. [1] to [3], according to the method described by Jones and Kiniry (1986).

Values for T_{max} , T_{opt} , and T_b were determined using the temperature response for rate of appearance of main culm leaves. In studies on leaf appearance rate in sorghum, Alagar-swamy et al. (1986) found T_{max} and T_{opt} of ≈ 42 and 32°C , and Nelson (1986) reported T_b of $\approx 10^{\circ}\text{C}$. In modeling leaf area dynamics for sorghum, Hammer et al. (1993) used base, optimum, and maximum temperatures of 11, 30, and 42°C , respectively, to calculate TT. Since the experiments were undertaken in northern Australia, these values were used in our study.

Onset and Rate of Leaf Senescence

Maximum green leaf area per plant was the asymptote of the TPLA function. A broken-stick function was fitted to the individual plot data for the SPLA regression on TT. Onset of leaf senescence was estimated as the time at which the two linear phases of the SPLA function intersected. Rate of leaf senescence was determined by the slope of the second linear phase of the SPLA function.

Table 1. Means of monthly rainfall, daily pan evaporation, monthly maximum and minimum temperatures, and daily solar radiation recorded at Hermitage Research Station, Queensland, Australia, during the 1994-1995 experiment period.

Month	Rainfall	Mean daily pan evaporation	Mean maximum temperature	Mean minimum temperature	Mean daily solar radiation
	mm		°C		MJ m ⁻² d ⁻¹
December	63.5	7.4	32.9	17.0	27.0
January	72.5	9.2	28.9	16.2	25.7
February	118.5	4.4	27.4	16.7	20.1
March	24.5	4.0	28.5	13.9	21.1
April	0.0	7.4	25.1	7.7	21.0

Green leaf area at maturity can be described mathematically as follows:

$$\text{GLAM} = \text{TPLA} - (\text{Duration}_{\text{sen}} \times \text{Rate}_{\text{sen}}) \quad [4]$$

where TPLA is the total plant leaf area (cm² plant⁻¹, asymptote attained ≈4 d before anthesis), Duration_{sen} is the duration of leaf senescence (°C d), and Rate_{sen} is the rate of leaf senescence (cm² plant⁻¹ °C d).

Duration of leaf senescence is defined as the number of degree days from the onset of senescence to physiological maturity.

In addition, relative rate of leaf senescence was calculated from the slope of the linear decline over time from anthesis to maturity of green leaf area, relative to green leaf area at anthesis, expressed as loss of relative green leaf area (%) per day:

$$\frac{[(1 - \text{GLAM}/\text{GLAA})100]}{\text{days from anthesis to maturity}} \quad [5]$$

where GLAM is the green leaf area at maturity (cm² m⁻²) and GLAA is the green leaf area at anthesis (cm² m⁻²).

Visual Rating of Leaf Senescence

The association between visual rating of green leaf retention and measured GLAM was examined under TD to determine the accuracy of this visual approach. Green leaf area was measured directly with an electronic planimeter (Delta-T DIAS image analysis system) at the maturity harvest (114 DAE). In addition, green leaf retention was assessed visually by Dr. Bob Henzell (sorghum breeder with Queensland Department of Primary Industries, Australia) at 103 and 115 DAE, using a nine-point scale (1 = whole plant green; 9 = whole plant dead).

Statistical Analyses

Data were analyzed by standard analysis of variance, and pairwise comparisons of means were performed using the protected LSD procedure at $P = 0.05$ (Carmer and Swanson, 1973). For all water regimes, correlations were calculated between TPLA and its components: culm number per plant, leaf number per culm, and area per leaf (APL). Furthermore, correlations were calculated between leaf number per culm and both leaf appearance rate and TT to flag leaf appearance. Correlations were also calculated between GLAM and both the visual rating of green leaf retention at maturity and the relative rate of leaf senescence.

Meteorological Data

A portable meteorological station (Easidata Mk 3, Environdata, Warwick, Queensland, Australia) was installed at the eastern end of Replicate 2 to measure temperatures and solar radiation. Rain gauges (Nylex Corp. Ltd., Melbourne, Australia, 250 mm) were positioned at each of the four corners of the experiment site and a Class A pan was installed at the eastern end of Replicate 1 to measure pan evaporation. Totals

of 125 and 131 mm of rain were recorded during the pre- and postanthesis periods, with 38 and 68 mm falling 3 and 8 days after anthesis (Table 1). Mean monthly maximum temperatures decreased from 33°C in December to 25°C in April. Solar radiation declined from ≈26 MJ m⁻² d⁻¹ in December and January to 21 MJ m⁻² d⁻¹ from February to April.

RESULTS AND DISCUSSION

Leaf Growth

Leaf growth is examined in terms of leaf number and leaf size. The number of senesced leaves on the main stem are plotted against TT for the three water regimes in Fig. 1, together with the number of fully expanded leaves for the ND treatment. Leaf growth and senescence can be divided into three stages. Stage 1 is characterized by a high rate of leaf turnover; that is, as new leaves are expanded at the top of the canopy, older leaves senesce at the base. Stage 1 ends ≈4 d before anthesis when TPLA_{max} has been attained. Stage 2 is an equilibrium phase characterized by negligible leaf senescence, ending with the onset of rapid senescence (Stage 3), particularly in the PFD and TD treatments.

Total plant leaf area is an important determinant of GLAM, since it sets the initial benchmark of green leaf area per plant. It is from this benchmark that leaf area declines according to the onset and rate of senescence,

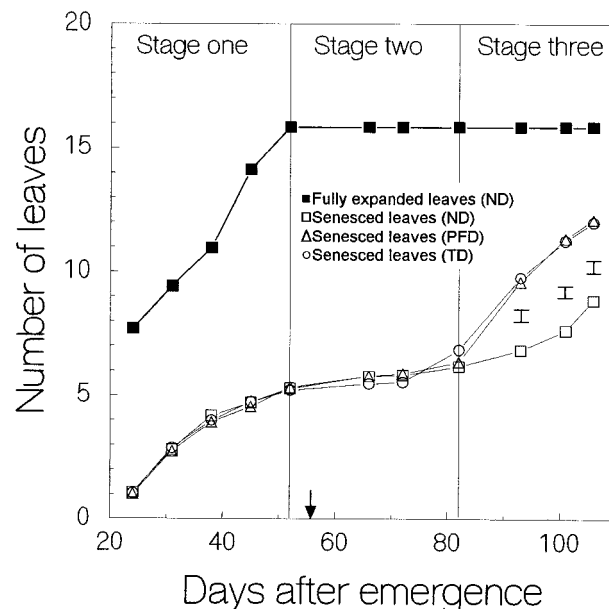


Fig. 1. Temporal pattern of fully expanded leaf number and senesced leaf number for sorghum hybrids grown under three water regimes. Anthesis at Day 56 is marked with an arrow. Vertical bars denote LSD ($P = 0.05$). ND, no water deficit; PFD, postflowering water deficit; TD, terminal water deficit.

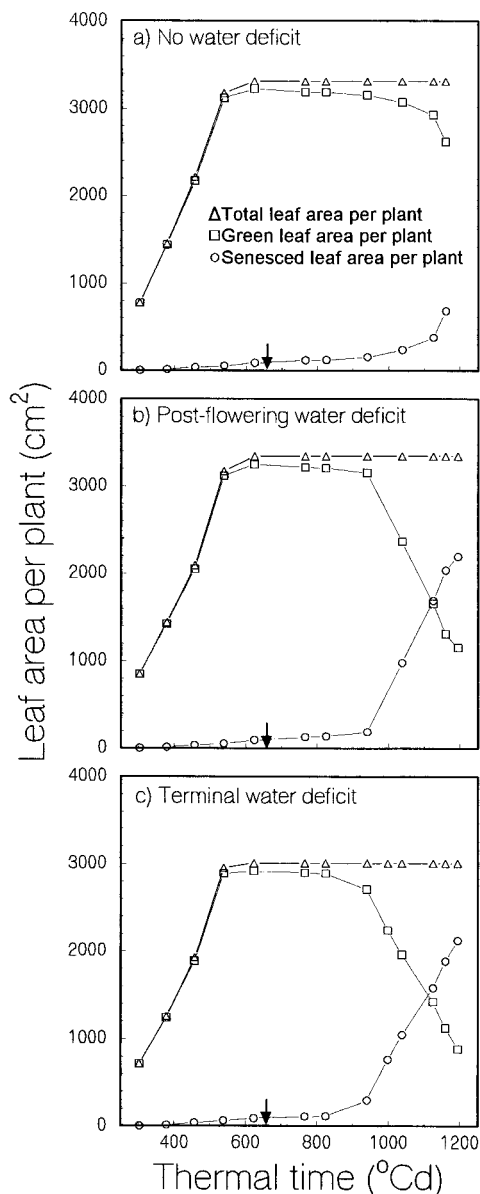


Fig. 2. Changes in leaf area per plant with thermal time from sowing for sorghum hybrids grown under three water regimes: (a) no water deficit, (b) postflowering water deficit, and (c) terminal water deficit. Anthesis at 660 °Cd is marked with arrows.

thus determining the amount of green leaf area maintained throughout grain filling, and ultimately at maturity. No genotype \times water regime interactions were observed for TPLA or any of its components. Water deficit reduced ($P < 0.01$) TPLA by $\approx 12\%$ in TD compared with ND and PFD (Fig. 2). Lower TPLA under TD (Fig. 2c) was primarily due to a reduction ($P < 0.01$) in the size of leaves that emerged after the 9th leaf (≈ 400 °C d), suggesting that the water deficit was sufficiently severe at this stage of crop growth to limit cell expansion in all subsequent emerging leaves (i.e., leaf numbers 10 and above, Fig. 3a). Therefore even if drought is absent after flowering, preanthesis water deficit still has the capacity to reduce green leaf area during grain filling by limiting TPLA before anthesis,

and hence reducing the green leaf benchmark from which senescence will commence. Average APL was also affected ($P < 0.01$) by genotype (Fig. 3b, Table 2), mainly due to larger leaves in RQL36 hybrids.

Genotypic variation ($P < 0.01$) in TPLA was observed, ranging from $3026 \text{ cm}^2 \text{ plant}^{-1}$ (AQL39/RQL12) to $4066 \text{ cm}^2 \text{ plant}^{-1}$ (A35/RQL36) (Table 2). An analysis of the contribution of female parents to hybrid performance found that TPLA was higher ($P < 0.05$) in A35 (stay-green) hybrids compared with AQL39 (senescent) and AQL41 (intermediate) hybrids (Table 2). A similar analysis of TPLA in male parents found RQL36 (intermediate) hybrids were highest ($P < 0.05$), RQL12 (stay-green) hybrids were lowest, and R69264 (senescent) hybrids were intermediate in this parameter (Table 2).

Maximum TPLA can be described in terms of the fertile tiller number per plant (FTN) and the total leaf number on the main culm (TLN) according to the relationship developed by Hammer et al. (1993): $\text{TPLA}_{\text{max}} = (1 + \text{FTN})^{\delta} \text{TLN}^{\gamma}$, where δ and γ are fitted coefficients. They predicted values of TPLA_{max} from TLN and FTN by allowing for a curvilinear increase in TPLA_{max} with TLN and a sequential decrease in total leaf area produced by successive surviving tillers relative to that on the main culm. In our study, culm number per plant ($\text{CNPP} = 1 + \text{FTN}$) was affected ($P < 0.05$) by genotype (Table 2), but not by water regime. Hybrid variation in CNPP ranged from 1.13 (A35/RQL12) to 1.76 (AQL39/R69264). The TLN on the main culm was not affected by water regime, although genotypic variation ($P < 0.01$) was observed (Table 2), ranging from 14.3 leaves (AQL39/RQL12) to 17.1 leaves (A35/RQL36). The TLN correlated significantly with TT to flag leaf appearance and leaf appearance rate (LAR), mainly because of hybrids of A35 (slower maturity and more rapid LAR) and RQL12 (quicker maturity) (Table 2). For the ND treatment, predicted values of TPLA_{max} that were calculated using the relationship of Hammer et al. (1993) with observed values of TLN and FTN for the nine hybrids, correlated highly ($r = 0.81^{**}$, $n = 9$, data not shown) with the observed values of TPLA_{max} .

Differences in leaf size were related to differences in leaf number per culm, evidenced by the positive relationship between averaged TLN and APL values ($r = 0.70^{*}$, $n = 81$) for the nine hybrids (data not shown). The observed relationship between TLN and APL is due largely to the association between area of the largest leaf and TLN, as reported for sorghum by Carberry et al. (1993). This is consistent with findings of Birch et al. (1998) in a study of five maize hybrids varying in maturity and adaptation. It is also consistent with the studies at whole plant level of Hammer et al. (1993) on sorghum, where maximum TPLA was related to TLN and CNPP. Results from this study fitted the relationship for TPLA reported by Hammer et al. (1993), indicating that TPLA on the nine hybrids studied was affected by TLN and CNPP in the same way as found for other sorghum hybrids. It could also be shown that in this study variation in TLN among hybrids contributed more to hybrid differences in TPLA than did variation in CNPP.

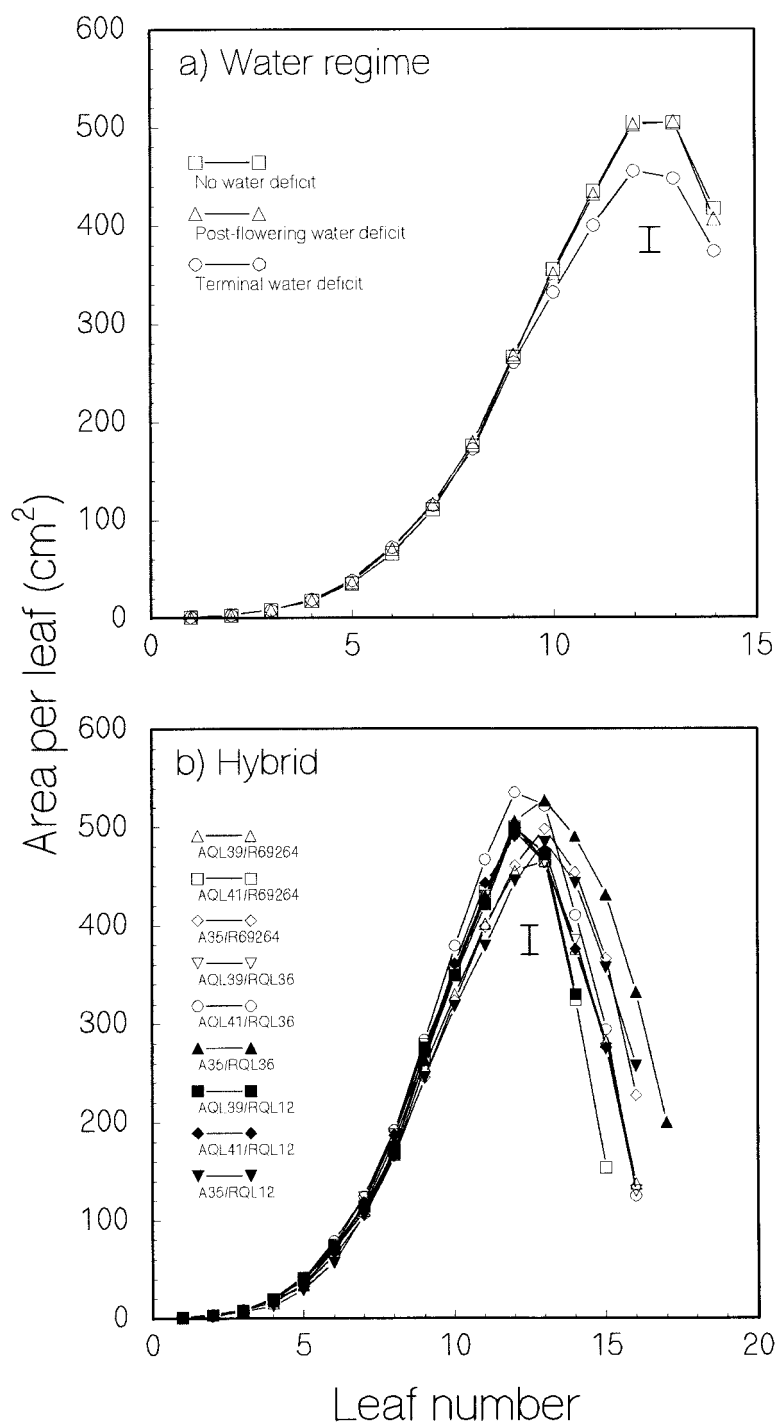


Fig. 3. Leaf-size distribution for the main effects of (a) water regime and (b) hybrid type. Vertical bars denote LSD ($P = 0.05$).

For the KS19 source of stay-green, genotypic variation ($P < 0.01$) in TLN could be explained by phenological differences alone, since variation in LAR was not significant. Genotypic variation ($P < 0.01$) in days to anthesis ranged from 54 d for A35/R69264 and AQL39/RQL12 to 58 d for AQL39/RQL36 and A35/RQL36. Time from emergence to anthesis (56 d) was not affected ($P > 0.05$) by water regime and no genotype \times water regime interaction for days to anthesis was observed. Similar to the current study, Hammer et al. (1993) found no genotypic variation in LAR, hence differences in

TLN were due solely to differences in phenology. However, genotypic differences ($P < 0.01$) in TLN among hybrids varying in the B35 source of stay-green were due to differences in both phenology and LAR (Table 2). Overall, LAR was higher ($P < 0.05$) in A35 hybrids ($0.0264 \text{ leaves}^\circ\text{C d}^{-1}$) compared with AQL39 and AQL41 hybrids ($0.0248 \text{ leaves}^\circ\text{C d}^{-1}$). To our knowledge, this is the first reported variation in LAR among sorghum hybrids. Increased TPLA in A35 hybrids was therefore a result of the combined increases in LAR, TT to flag leaf appearance, and leaf size, and together

Table 2. Total plant leaf area and its components for nine sorghum hybrids and their parents averaged across three water regimes.

Genotype	Total plant leaf area	Culm number per plant	Total leaf number on the main culm	Leaf appearance rate	Thermal time to flag leaf appearance	Area per leaf
	cm ² plant ⁻¹	no. plant ⁻¹	no. culm ⁻¹	leaves °C d ⁻¹	°C d	cm ²
AQL39/R69264	3420	1.76	15.5	0.0252	616	198
AQL41/R69264	3098	1.53	14.9	0.0240	620	200
A35/R69264	3723	1.34	16.4	0.0263	623	210
AQL39/RQL36	3645	1.40	15.5	0.0249	623	210
AQL41/RQL36	3476	1.40	15.5	0.0249	623	223
A35/RQL36	4066	1.42	17.1	0.0274	627	230
AQL39/RQL12	3026	1.66	14.3	0.0245	585	199
AQL41/RQL12	3221	1.38	15.1	0.0250	604	209
A35/RQL12	3344	1.13	15.8	0.0255	618	204
LSD (<i>P</i> = 0.05)	231	0.22	0.7	0.0012	16	13
Female parents						
AQL39	3364	1.61	15.1	0.0249	608	172
AQL41	3265	1.44	15.2	0.0247	616	178
A35	3711	1.30	16.4	0.0264	623	200
LSD (<i>P</i> = 0.05)	134	0.13	0.4	0.0007	9	9
Male parents						
R69264	3414	1.55	15.6	0.0252	620	175
RQL36	3729	1.41	16.1	0.0257	624	199
RQL12	3197	1.39	15.1	0.0250	602	176
LSD (<i>P</i> = 0.05)	134	0.13	0.4	NS	9	9

these adaptations may have enabled the durra landraces in Ethiopia to better survive in a very dry environment (National Academy of Sciences, 1996).

Leaf Senescence

The number of leaves senesced on each plant (Fig. 1), together with the known area of those leaves (Fig. 3), was used to calculate total senesced leaf area per plant (Fig. 2). As there was no genotype × water regime interaction observed for TPLA, onset of leaf senescence or duration of leaf senescence, only main effects are presented. Once the TPLA benchmark has been set, retention of green leaf area during grain filling will be determined by the time at which leaves begin to die

(onset of senescence), and the rate at which death proceeds (rate of senescence).

Onset of leaf senescence is defined as the time at which the two linear phases of the SPLA function intersected (Fig. 2). Onset of senescence was earlier (*P* < 0.01) in TD (914 °C d) and PFD (943 °C d) plants compared with ND plants (1056 °C d) (Table 3). Irrigation ceased 28 d before flowering in PFD, and although this had no effect on reducing leaf size, and hence TPLA, it did hasten the onset of leaf senescence by 113 °C d compared with ND. Onset of leaf senescence varied (*P* < 0.05) among genotypes (Table 3) and was delayed in AQL41 (intermediate) and A35 (stay-green) hybrids compared with AQL39 (senescent) hybrids, and was also delayed in RQL12 (stay-green) hybrids compared with RQL36 (intermediate) hybrids. Differences in onset of leaf senescence among the nine hybrids were not confounded by phenological differences, since there was no correlation between TT to flag appearance and onset of leaf senescence (data not shown). According to the classification of stay-green by Thomas and Smart (1993), the B35 and KS19 sources of stay-green both displayed Type A behavior; that is, they exhibited delayed onset of senescence, which may have arisen after alteration of genes involved in the timing of the initiation of senescence. Van Oosterom et al. (1996) reported that two QDPI lines derived from KS19 (Q101 and Q102) also displayed delayed onset of leaf senescence. Genes associated with Type A stay-green are likely to be specifically activated at the initiation of senescence. This is the point at which all the various transduction pathways converge, invoking the stay-green syndrome through environmental and internal cues (Thomas and Smart, 1993). Type A stay-green has also been observed in other crop species. For example, soybeans [*Glycine max* (L.) Merr.] exhibiting the delayed leaf senescence (DLS) trait were found to retain chlorophyll, leaf protein, ribulose biphosphate carboxylase (rubisco) activity, and nodule N fixation at higher levels than normal (Abu-Shakra et al., 1978).

Duration of leaf senescence is defined as the number

Table 3. Onset and duration of leaf senescence for nine sorghum hybrids and their parents grown under three water regimes.†

Treatment	Onset of leaf senescence	Duration of leaf senescence
	°C d	
Water regime		
No deficit	1056	146
Postflowering deficit	943	257
Terminal deficit	914	269
LSD (<i>P</i> = 0.05)	35	54
Genotype		
AQL39/R69264	939	252
AQL41/R69264	1001	202
A35/R69264	974	230
AQL39/RQL36	942	249
AQL41/RQL36	958	241
A35/RQL36	963	242
AQL39/RQL12	977	195
AQL41/RQL12	999	202
A35/RQL12	987	204
LSD (<i>P</i> = 0.05)	39	43
Female parents		
AQL39	953	232
AQL41	986	215
A35	975	225
LSD (<i>P</i> = 0.05)	22	NS
Male parents		
R69264	971	228
RQL36	954	244
RQL12	988	200
LSD (<i>P</i> = 0.05)	22	25

† Values were determined using a broken-stick model.

Table 4. Days to physiological maturity in nine sorghum hybrids grown under three levels of water supply.

Female parents	Male parents		
	R69264	RQL36	RQL12
	d		
	No water deficit		
AQL39	113 (55)†	116 (58)	108 (53)
AQL41	114 (55)	115 (58)	113 (56)
A35	115 (56)xf†	114 (59)	113 (57)
	Postflowering water deficit		
AQL39	111 (56)	115 (58)	110 (55)
AQL41	114 (54)	115 (57)	115 (56)
A35	113 (53)	115 (58)	110 (55)
	Terminal water deficit		
AQL39	109 (54)	105 (57)	106 (54)
AQL41	113 (54)	109 (56)	112 (55)
A35	113 (53)	113 (58)	110 (54)
	LSD (0.05) = 4 LSD (0.05) = (2)		

† Days to anthesis are presented in parentheses.

of days from the onset of senescence to physiological maturity, and was longer ($P < 0.01$) for PFD and TD than for ND (Table 3). The contribution of the female parents to hybrid performance was not significant for duration of leaf senescence, despite variation ($P < 0.05$) in onset of senescence. To some extent this is explained by the delay ($P < 0.05$) in physiological maturity in A35 (stay-green) hybrids compared with AQL39 (senescent) hybrids under TD but not under ND or PFD (Table 4). Among the male parents, duration was less ($P < 0.05$) in RQL12 (stay-green) hybrids compared with R69264 (senescent) and RQL36 (intermediate) hybrids. In general, differences in duration of senescence were generated by differences in onset of senescence.

Rate of leaf senescence is defined as the slope of the second linear phase of the SPLA function (Fig. 2), and the response of hybrids varied ($P < 0.05$) with water regime (Table 5). Interestingly, the responses of the B35 and KS19 sources of stay-green were quite different (Table 6). Among the female parents, there was no difference in rate of senescence under TD, yet the rate was higher in A35 (stay-green) than in AQL39 (senescent) hybrid for ND and PFD. On the other hand, among the male parents, rate of senescence was always

Table 5. Rate of leaf senescence for nine hybrids grown under three water regimes.†

Female parents	Male parents		
	R69264	RQL36	RQL12
	cm ² plant ⁻¹ °C d ⁻¹		
	No water deficit		
AQL39	2.86	4.52	4.36
AQL41	6.77	1.16	2.75
A35	6.91	5.20	4.38
	Postflowering water deficit		
AQL39	9.29	8.03	6.53
AQL41	9.89	10.20	9.33
A35	10.17	11.30	8.20
	Terminal water deficit		
AQL39	8.00	8.56	4.96
AQL41	9.23	8.02	6.37
A35	8.36	7.96	6.36
	LSD (0.05) = 2.86 (when comparing means within the same water regime) LSD (0.05) = 2.88 (when comparing means among water regimes)		

† Values were determined using a broken-stick model.

Table 6. Rate of leaf senescence for three female and three male parents grown under three water regimes.†

Female parents	Water regime		
	No deficit	Postflowering deficit	Terminal deficit
	cm ² plant ⁻¹ °C d ⁻¹		
	No water deficit		
AQL39	3.91	7.95	7.17
AQL41	3.56	9.81	7.87
A35	5.50	9.89	7.56
	LSD ($P = 0.05$)		
	0.95	0.95	NS
	Male parents		
R69264	5.51	9.78	8.53
RQL36	3.63	9.84	8.18
RQL12	3.83	8.02	5.90
	LSD ($P = 0.05$)		
	0.95	0.95	0.95

† Values were determined using a broken-stick model.

less ($P < 0.05$) in RQL12 (stay-green) compared with R69264 (senescent) hybrid.

Genotype and water regime also interacted strongly ($P < 0.01$) for relative rate of leaf senescence, expressed as loss of relative green leaf area (%) per day (Table 7). There was little variation in relative rate of leaf senescence among hybrids under ND, yet hybrids ranged from 0.75 to 1.55% loss leaf area index (LAI) d⁻¹ under TD. While there was no difference in relative rate of leaf senescence among the female parents, this parameter was significantly less in RQL12 (stay-green) hybrids than in RQL36 (intermediate) and R69264 (senescent) hybrids (data not shown).

Therefore, hybrids with the KS19 source, but not the B35 source, displayed Type B stay-green; that is, RQL12 hybrids exhibited a reduced rate of senescence that may have arisen after alteration of genes involved in the regulation of its rate of progress. Genes involved in Type B stay-green are more likely to encode for senescence-related activities such as catabolic enzymes or to show increased expression during remobilization (Thomas and Smart, 1993). It appears that some stay-green sorghums contain higher levels of cytokinins in xylem sap than normal (Ambler et al., 1987), and this may be associated with Type B stay-green. Maize varieties have been reported with stay-green of Type B. In the stay-green variety FS854, levels of both chlorophyll and phosphoenolpyruvate carboxylase begin to decline at

Table 7. Relative rate of leaf senescence during the grain filling period in nine sorghum hybrids grown under three levels of water supply.

Female parents	Male parents		
	R69264	RQL36	RQL12
	% loss LAI d ⁻¹ †		
	No water deficit		
AQL39	0.28	0.47	0.13
AQL41	0.31	0.18	0.25
A35	0.42	0.32	0.49
	Postflowering water deficit		
AQL39	1.08	1.11	1.05
AQL41	1.35	1.14	1.02
A35	1.08	1.24	1.06
	Terminal water deficit		
AQL39	1.39	1.39	1.12
AQL41	1.47	1.55	0.75
A35	1.38	1.18	0.86
	LSD (0.05) = 0.31 LSD (0.01) = 0.42		

† LAI is leaf area index.

the normal time, but the rate of decrease is reduced compared with other varieties, so that it retains in its leaves more reduced N and NO₃ reductase activity, as well as carboxylating enzymes and chlorophyll varieties (Crafts-Brandner et al., 1984a, 1984b).

In our study, hybrids containing the B35 and KS19 sources of stay-green retained similar leaf greenness at physiological maturity under drought, yet the means of attaining this common end were vastly different. A35 hybrids retained more green leaf area than their intermediate (AQL41) and senescent (AQL39) counterparts by combining high TPLA with delayed onset of leaf senescence, despite having a high rate of senescence. In contrast, RQL12 hybrids (KS19 source of stay-green) maintained more green leaf area than their intermediate (RQL36) and senescent (R69264) counterparts by combining delayed onset and reduced rate of leaf senescence, despite having a low TPLA. Thomas and Smart (1993) emphasized that the stay-green character in one genetic line may have a superficial resemblance to the character in another, yet the common phenotype may arise from very different underlying physiological and biochemical mechanisms. That such differences exist in these mechanisms in the current study should not be surprising, since the B35 and KS19 germplasm is derived from sorghum lines native to Ethiopia and Nigeria, respectively.

Similarly, De Villiers et al. (1993) found that specific aspects of both the timing and rate of senescence differed in three stay-green sorghums (Q101, E36-1 and ICSV745). Variation in the pattern of chlorophyll and protein breakdown during senescence of the three stay-green genotypes indicates that the phenotypic similarity is only superficial. For example, E36-1 displayed normal sequential senescence in both high and low N environments as did Q101, yet in the latter, stability of some of the nitrogen remobilizing enzymes was enhanced, leading to a delay in the onset of leaf senescence (Type A stay-green).

Leaf senescence occurs because of the natural biological process of aging, but it can also be triggered by water deficit (Rosenow et al., 1983; Thomas, 1992), nutrient deficiency (Sinclair and de Wit, 1975; Muchow, 1988; Wolfe et al., 1988ab), shading (Ludwig et al., 1965; Goldsworthly, 1970), insect or disease attack (Thomas and Stoddart, 1980; Waggoner and Berger, 1987), or physical damage (Vanderlip and Reeves, 1972). Genetic variation in senescence has also been observed in sorghum hybrids (Duncan et al., 1981; Hammer et al., 1987). A critical factor in our study is that onset and rate of leaf senescence were triggered by genetic and water regime effects and not by the confounding effects of shading, nutrient deficiencies, insects, or diseases. No foliar diseases were reported in this study, and nonlimiting levels of all essential elements were supplied. The extent to which senescence in lower leaves was induced by shading from the upper canopy was not determined, although low light environments from competitive shading were not considered to be a significant factor in this study. Normal morphological development would also have contributed to the senescence of the lowest leaves, since the sheaths of these leaves are ruptured by culm enlarge-

ment, resulting in their death (Vanderlip and Reeves, 1972).

Green Leaf Area at Maturity

Green leaf area at maturity is defined as the difference between TPLA and SPLA functions at physiological maturity (Fig. 2). Empirically, GLAM is determined by subtracting the product of duration of leaf senescence and rate of leaf senescence from TPLA. Therefore, high TPLA, delayed onset of senescence, and reduced rate of senescence all contribute to increased GLAM. Water deficit reduced GLAM by 67% in TD compared with ND (13 020 vs. 39 220 cm² plant⁻¹). The response of hybrids varied ($P < 0.01$) with water regime such that GLAM declined with increasing water deficit to a greater extent in the five senescent hybrids compared with the four stay-green hybrids (Table 8). Within TD, higher ($P < 0.05$) GLAM in A35 hybrids compared with AQL41 and AQL39 hybrids was due to high TPLA and delayed onset of leaf senescence, and higher ($P < 0.05$) GLAM in RQL12 hybrids compared with RQL36 and R69264 hybrids was due to delayed onset and reduced rate of leaf senescence (Table 9).

Green leaf area was also measured directly at five sampling times during crop growth and is expressed as LAI. At maturity, green LAI declined from about 4 under ND to <1 under TD in the five senescent hybrids (AQL39/R69264, AQL41/R69264, A35/R69264, AQL39/RQL36, AQL41/RQL36). The decline in LAI with increasing water deficit was considerably less in the four stay-green hybrids (A35/RQL36, AQL39/RQL12, AQL41/RQL12, A35/RQL12), decreasing from ≈3.9 under ND to 1.8 under TD. Within TD, genotypic variation in LAI ranged from 0.7 (AQL41/R69264) to 2.3 (AQL41/RQL12), highlighting the importance of the KS19 source of stay-green in maintaining green leaf area in this experiment. These findings show that similar conclusions were reached using the leaf number/leaf size and the LAI approaches to leaf area determination.

In particular, the leaf number/leaf size approach provides a basis to modify the leaf senescence routines in a sorghum simulation model. These coefficients, together with an understanding of the functional basis of physio-

Table 8. Green leaf area at maturity for nine sorghum hybrids grown under three water regimes.

Female parents	Male parents		
	R69264	RQL36	RQL12
	cm ² plant ⁻¹		
	No water deficit		
AQL39	2556	2800	2594
AQL41	2697	2784	2684
A35	2723	3180	2440
	Postflowering water deficit		
AQL39	902	1566	1183
AQL41	464	1210	1079
A35	1517	1392	1262
	Terminal water deficit		
AQL39	590	585	1291
AQL41	350	473	1177
A35	584	1607	1448
LSD (0.05) = 561 (when comparing means within the same water regime)			
LSD (0.05) = 551 (when comparing means among water regimes)			

† Values were determined using a broken-stick model.

logical responses, could be used to simulate the stay-green drought-resistance trait, enabling use of the model to assess the value of stay-green in a wide range of target environments throughout the northern grain belt of Australia. This is the subject of current research.

Visual Rating of Green Leaf Area at Maturity

Visual rating of green leaf retention was highly correlated ($P < 0.01$) with GLAM under terminal water deficit, suggesting that visual rating of stay-green is an adequate means of selecting for variation in this trait. The strength of this correlation was similar for plants visually rated 1 wk before ($r = 0.93^{***}$, $n = 9$) and after ($r = 0.88^{***}$, $n = 9$) physiological maturity, indicating that some flexibility exists in the time at which this parameter can be measured. Similarly, Wanous et al. (1991) reported that the subjective visual rating of green leaf retention was correlated ($r = 0.91$) with measured GLAM in grain sorghum. They also found that visually rating the number of green leaves was highly correlated ($r = 0.96$) with GLAM, supporting the validity of using such visual rating systems in sorghum breeding programs.

In our study, GLAM was also highly correlated ($r = 0.95^{***}$, $n = 27$) with relative rate of leaf senescence (% loss of LAI per day between anthesis and maturity) under TD. Since the determination of GLAM requires only one measurement of leaf area at maturity, additional measurement of leaf area at anthesis should generally not be necessary, except for specific cases requiring direct assessment of the rate of leaf senescence (e.g., the phenotypic characterization of recombinant inbred lines for stay-green).

However, visual rating of stay-green is limited by an inability to distinguish among the various mechanisms that ultimately determine the phenotype. It is highlighted here that the stay-green character in one source (KS19) displays only a superficial resemblance to the character in the other source (B35), and arises from quite different underlying physiological mechanisms (Borrell and Douglas, 1997). The visual rating approach integrates each of these components in a single phenotypic score. This raises the question as to whether there is any additional benefit in selecting for the determinants of stay-green compared with selecting for the phenotype. Indeed, selection could take place at various

levels. For example, variation in TPLA and onset and rate of leaf senescence could be used as selection criteria, although these determinants are still somewhat empirical. A better approach, perhaps, would be to understand the physiological basis of genetic variation in stay-green, then attempt to link such functional differences to molecular markers. Of course, these alternative strategies would need to be compared with current methodologies to assess their usefulness in plant breeding programs.

CONCLUSIONS

Water deficit reduced TPLA by $\approx 12\%$ in TD compared with ND and PFD, and lower TPLA under TD was mainly due to a reduction in the size of leaves that emerged after the 9th leaf. Hybrids containing the B35 and KS19 sources of stay-green retained significantly more GLAM compared with intermediate (AQL41 and RQL36) and senescent (AQL39 and R69264) hybrids. However, the mechanism of leaf area maintenance varied with the source of stay-green. Using the stay-green classification system of Thomas and Smart (1993), hybrids with the KS19 source of stay-green displayed Types A and B behavior (delayed onset and reduced rate of leaf senescence), while hybrids with the B35 source of stay-green displayed only Type A (delayed onset of leaf senescence). In addition, higher GLAM in A35 (stay-green) hybrids than in AQL39 (senescent) and AQL41 (intermediate) hybrids was due to increased TPLA prior to anthesis, and this advantage was maintained to maturity.

Visual rating of green leaf retention was highly correlated with measured GLAM, further supporting the use of this method to select for the stay-green trait in sorghum breeding programs. However, visual rating of stay-green is constrained by an inability to distinguish among the functional mechanisms determining the phenotype. It is suggested that linking functional rather than phenotypic differences to molecular markers may improve the efficiency of selecting for traits such as stay-green.

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Table 9. Green leaf area at maturity for three female and three male parents grown under three water regimes.

	Water regime		
	No deficit	Postflowering deficit	Terminal deficit
	cm ² plant ⁻¹		
Female parents			
AQL39	2650	1217	822
AQL41	2722	918	667
A35	2781	1390	1213
LSD ($P = 0.05$)	NS	187	187
Male parents			
R69264	2659	961	508
RQL36	2922	1389	888
RQL12	2573	1175	1305
LSD ($P = 0.05$)	187	187	187

† Values were determined using a broken-stick model.

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Does Maintaining Green Leaf Area in Sorghum Improve Yield under Drought? II. Dry Matter Production and Yield

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ABSTRACT

Retention of green leaf area at maturity (GLAM), known as stay-green, is used as an indicator of postanthesis drought resistance in sorghum [*Sorghum bicolor* (L.) Moench] breeding programs in the USA and Australia. The critical issue is whether maintaining green leaves under postanthesis drought increases grain yield in stay-green compared with senescent hybrids. Field studies were undertaken in northeastern Australia on a cracking and self-mulching gray clay. Nine closely related hybrids varying in rate of leaf senescence were grown under two water-limiting regimes, post-flowering water deficit and terminal (pre- and postflowering) water deficit, and a fully irrigated control. Under terminal water deficit, grain yield was correlated positively with GLAM ($r = 0.75^{**}$) and negatively with rate of leaf senescence ($r = -0.74^{**}$). Grain yield also increased by ≈ 0.35 Mg ha⁻¹ for every day that onset of leaf senescence was delayed beyond 76 DAE in the water-limited treatments. Stay-green hybrids produced 47% more postanthesis biomass than their senescent counterparts (920 vs. 624 g m⁻²) under the terminal water deficit regime. No differences in grain yield were found among eight of the nine hybrids under fully irrigated conditions, suggesting that the stay-green trait did not constrain yield in the well-watered control. The results indicate that sorghum hybrids possessing the stay-green trait have a significant yield advantage under postanthesis drought compared with hybrids not possessing this trait.

WATER DEFICIT is the major constraint to rainfed sorghum production worldwide. Drought can occur before and after flowering, and resistance to water deficit at both of these stages has been reported in sorghum (Rosenow et al., 1996). Resistance to postanthesis drought is important in Australia's northern grain belt, since crops generally grow into water deficit (Chapman et al., 2000). Symptoms of susceptibility to postanthesis drought include premature leaf and stem senescence, charcoal rot [*Macrophomina phaseolina* (Tassi) Goidanich], fusarium stalk rot (*Fusarium moniliforme* J. Sheld.), lodging, and reduced seed size. Expression of postanthesis drought symptoms is heightened when crop growth is favorable prior to flowering and is followed by severe water deficit, particularly in the latter half of grain filling. Chapman et al. (2000) reported a

high frequency of this water limitation in Australian sorghum-growing environments.

A mechanism of resistance, known as stay-green (Rosenow, 1977), is indicated by maintenance of green stems and upper leaves when water is limiting during grain filling. Green leaf area at maturity is used as an indicator of postanthesis drought resistance in sorghum breeding programs in the USA (Rosenow et al., 1983) and Australia (Henzell et al., 1992). Green leaf area at maturity and its components have been found to vary with both water regime and genotype (Borrell et al., 2000). The critical issue is whether retention of green leaf area under postanthesis drought actually increases grain yield in stay-green compared with senescent hybrids. Positive associations between green leaf area duration and grain yield have been observed in a range of cereals, including wheat, *Triticum aestivum* L. (Evans et al., 1975); maize, *Zea mays* L. (Tollenaar and Daynard, 1978; Wolfe et al., 1988); oat, *Avena sativa* L. (Helsel and Frey, 1978); and sorghum (Henzell et al., 1992).

There is limited understanding of the physiological processes underlying the stay-green trait, including the basis of genetic variation. According to Bonhert et al. (1995), mechanisms by which plants adapt to abiotic stresses need to be quantified at a physiological, molecular, and genetic level, and future research must be directed at functional characterization and biochemical integration of molecular and genetic data. Sorghum genotypes with the stay-green trait continue to fill their grain normally under drought (Rosenow and Clark, 1981) and exhibit increased resistance to charcoal rot (Rosenow, 1984) and lodging (Henzell et al., 1984; Woodfin et al., 1988). Stay-green genotypes also contain more cytokinins (McBee, 1984) and basal stem sugars (Duncan, 1984) than do senescent genotypes. Increased accumulation of soluble sugars in stay-green types is associated with greater functional leaf area during grain filling, thereby reducing their dependence on stored assimilates from the stem to fill grain (Duncan et al., 1981; McBee et al., 1983). Higher concentration of stem sugars improves the digestible energy content of the straw,

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Abbreviations: AGDM, aboveground dry mass; CGR, crop growth rate; DAE, days after emergence; GLAM, green leaf area at maturity; ND, no water deficit treatment; PFD, postflowering water deficit treatment; TD, terminal water deficit. *, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.