CROP BREEDING, GENETICS & CYTOLOGY

Diallel Analysis of the Stay-Green Trait and Its Components in Sorghum

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ABSTRACT

Stay-green in sorghum [*Sorghum bicolor* (L.) Moench] is an important component of post-flowering drought tolerance. This research was conducted to describe (i) stay-green as post-flowering green leaf area duration (GLAD) and its components [green leaf area (GLA) at flowering, timing for onset of senescence, and senescence rate] and (ii) the expression of heterosis for stay-green in terms of heterosis for its components. The study was conducted during the 1992 to 1993 and 1993 to 1994 post-rainy season at ICRISAT headquarters near Hyderabad, India. It involved a nine-parent complete diallel, in two experiments differing in soil-water availability after flowering. Weekly estimations of % GLA were made on 36 leaves per plot. Relative (%days) and absolute (m^2 days) GLAD and their components were derived from a fitted logistic function. The variances in both relative and absolute GLAD were each fully ($R^2 > 0.96$) accounted for by their components. In spite of significant genotype × environment interactions for the component traits, the expression of heterosis for non-senescence as related to the stay-green trait was stable across experiments. The inheritance of the onset of senescence was additive, but a slow senescence rate was dominant over a fast rate. Consequently, a large relative GLAD (slow senescence) was partially dominant over a small relative GLAD. Because of the dominance of a large leaf area at flowering, the partial dominance in relative GLAD translated into overdominance for a large absolute GLAD. These results offer an opportunity for improving drought tolerance of sorghum in environments with post-flowering drought stress.

A NUMBER OF ANNUAL CROP SPECIES contain genetic variation for the degree or rate of senescence following seed maturation (Thomas and Smart, 1993). Stay-green, which is a stress induced parameter, is a component of tolerance to post-flowering drought and resistance to charcoal rot [*Macrophomina phaseolina* (Tassi) Goid] (Rosenow et al., 1977; Mughogho and Pande, 1984) in sorghum. It also improves the quality and quantity of the stover for cattle feed (McBee et al., 1983).

Stay-green has been described as a reduced progressive senescence in sorghum (McBee, 1984), resulting in greater functional leaf area during grain filling and in an extension of the photosynthetic capability in the upper canopy leaves after physiological grain maturity. Stay-green thus reduces the need for translocation of stored assimilates from the stem during grain filling, and extends the period of active assimilation past maturity. As a result, non-senescent sorghum accumulates more soluble sugars in the stem than does senescent sorghum, both during and after grain filling (Duncan et al., 1981; McBee et al., 1983). The higher concentration of stem sugars improves the digestible energy content of the stover or, if translated into growth of axial branches (Vietor et al., 1989), increases the amount of total harvestable fodder. The presence of green leaves also increases the market price of the stover as fodder in some areas (M. M. Anders, ICRISAT, 1994, personal communication). Stay-green is thus a particularly valuable trait in dual purpose (grain plus fodder) sorghum in semi-arid environments.

Stay-green can also enhance resistance to stalk rots, as moisture stress predisposes sorghum to infection by soil-borne pathogens that infect senescing or dead tissue (Jordan et al., 1984). Observations on stay-green, done at the end of grain development on plants grown under moisture stress, have been used as a selection criterion for charcoal rot resistance (Rosenow, 1980; Mughogho and Pande, 1984). However, these two traits are not different expressions of a single trait, because of dissimilar segregation patterns (Tenkouano et al., 1993).

Since non-senescent genotypes remain physiologically active during the late stages of grain filling, non-senescence enhances the stress tolerance of the crop by increasing the assimilate supply for grain filling, maintaining the root function and water uptake, or both. Green leaf area (GLA) after flowering has been related to grain yield under moisture stress in wheat (*Triticum aestivum* L.)(Fischer and Kohn, 1966) and maize (*Zea mays* L.) (Tollenaar and Daynard, 1978). Stay-green therefore has been suggested as an indirect selection criterion for post-flowering drought tolerance (Rosenow et al., 1983).

Knowledge about the inheritance of stay-green is a prerequisite for the successful use of this trait as a selection criterion. Non-senescence in sorghum, measured as GLA retention, was reported to be regulated by both dominant and recessive epistatic interactions (Tenkouano et al., 1993). However, the level of dominant gene action depends on the environment (Walulu et al., 1994). Two different types of functional stay-green can be distinguished, involving either a delayed onset of leaf senescence or a slower rate of senescence (Thomas and Smart, 1993). The onset and rate of leaf senescence, together with the GLA at flowering, determine the total GLAD after flowering, which is the best descriptor of stay-green (Rosenow et al., 1983). An understanding of the contributions of these three components to stay-green, and of their individual modes of inheritance, should provide a better understanding of heterosis and of the contribution of individual parental lines to stay-green expression.

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Abbreviations: DAF, days after flowering; GE, genotype × environment; GLA, green leaf area; GLAD, green leaf area duration; PE, parent × environment.

The aims of this study were (i) to describe stay-green in terms of GLAD and its components (GLA at flowering, timing for onset of leaf senescence, senescence rate) and (ii) to express heterosis of GLAD as a function of heterosis of its components, in a 9 × 9 diallel experiment grown in two contrasting environments.

MATERIALS AND METHODS

Genotypes

The study was based on a complete diallel of nine tropically adapted, but very diverse sorghum cultivars (Table 1). Four parents were known sources of stay-green: E 36-1, a widely-adapted, zera-zera germplasm line from Ethiopia; IS 9377, a landrace from South Africa with a large leaf area and a slow rate of leaf senescence; Q 102 and Q 104, breeding lines from Queensland, Australia, which are derived from KS 19 (Combine Kafir-60 × Short Kaura). Three parents were known to be senescent: R 16, a high-yielding cultivar from Maharashtra, India, which is adapted to the post-rainy season (stored soil moisture) but has a very rapid rate of leaf senescence and is susceptible to charcoal rot; SPV 475 (ICSV 112), a high-yielding, senescent cultivar for the rainy season, bred by ICRISAT; SPV 783, a high-yielding cultivar for the post-rainy season, also bred by ICRISAT. The last two parents were expected to be stay-green, based on characteristics related to stay-green: BJ 111, a charcoal rot-resistant cultivar from Karnakata, India, adapted to the post-rainy season; IS 22380, a drought-tolerant landrace from Dabar-Baladi, Sudan.

The nine parents were crossed (by hand emasculation and pollination) in all combinations, including reciprocals, during the 1991 to 1992 and 1992 to 1993 dry seasons. The resulting 72 hybrids and nine parents were grown in a 9 × 9 diallel experiment under contrasting environmental conditions during the 1992 to 1993 and 1993 to 1994 post-rainy seasons at ICRISAT Center, near Hyderabad, India.

Experiments

Experiment I (post-rainy season conditions) was sown on 23 Sep. 1992 in a medium deep vertisol (very fine montmorillonitic isohyperthermic Typic Pellustert) field, which had been fallowed during the preceding rainy season (the standard practice for post-rainy season sorghum in peninsular India). The field was fertilized with 40 kg N ha^{-1} and 18 kg P ha^{-1}, banded before sowing, and an additional 46 kg N ha^{-1} side-dressed 27 d after sowing (DAS). The field received light sprinkler irrigations at sowing (15 mm), at 5 and 14 DAS (7 and 10 mm) to establish the crop, and at 27 DAS (15 mm), following side-dressing. The moisture stored in the soil plus rainfall of 77 mm (56 DAS) was sufficient to complete the crop season without serious drought stress.

Experiment II (simulated rainy season conditions) was sown on an alfisol (clayey-skeletal mixed isohyperthermic Udic Rhodustalf) field on 24 Sep. 1993. The crop was grown under full irrigation until 2 wk after the mean flowering date, in contrast to the stored soil moisture conditions of Exp. I. The normal day length of 12 h was extended to 18 h until 32 d after emergence (DAE), with 100 watt incandescent bulbs suspended over the field on a 3- by 5-m grid. This simulated the longer pre-floral initiation developmental periods of rainy season crops. The crop was fertilized in the same manner as Exp. I.

Both experiments were grown in a randomized complete block design with three replications. Individual plots were four rows of 4.0 by 0.75 m. Due to the limited quantities of F_1 seed, only the two central rows of each plot were sown to the test genotype (parent or F_1); the other two rows were sown to M 35-1, a genotype derived from a local landrace.

Table 1. Mean values of days to flowering, components of stay-green, and stay-green (relative and absolute green leaf area duration), averaged for each parent in each experiment. Values are the means of the upper six leaves of six plants per plot by three replications. Numbers in parentheses are rankings.

Parent	Time from sowing to flowering	Green leaf area at flowering	Time to 95% green leaf area	Maximum rate of leaf senescence	Green leaf area duration†	
					Relative	Absolute
	d	cm^2 $plant^{-1}$	d	% d^{-1}	% d $plant^{-1}$	m^2 d pl^{-1}
Experiment I						
E 36-1	59.3 (4)	2025 (2)	27.7 (3)	2.19 (2)	5537 (3)	11.22 (1)
IS 9377	67.6 (9)	2359 (1)	20.5 (6)	3.59 (5)	3957 (7)	9.37 (2)
Q 102	59.0 (3)	1301 (9)	31.4 (2)	2.00 (1)	5858 (1)	7.66 (4)
Q 104	58.0 (2)	1423 (7)	33.3 (1)	2.67 (4)	5696 (2)	8.12 (3)
BJ 111	61.7 (5)	1526 (5)	22.3 (5)	3.61 (6)	4203 (6)	6.42 (6)
IS 22380	51.7 (1)	1466 (6)	13.3 (9)	2.24 (3)	4204 (5)	6.17 (7)
R 16	63.3 (7)	1607 (4)	20.5 (6)	5.35 (9)	3509 (9)	5.63 (8)
SPV 475	66.7 (8)	1955 (3)	19.4 (8)	4.18 (7)	3726 (8)	7.30 (5)
SPV 783	61.7 (5)	1362 (8)	27.6 (4)	5.24 (8)	4251 (4)	5.46 (9)
Means						
Parents	61.0	1669	24.0	3.45	4549	7.48
Crosses	57.7	1924	25.9	2.85	4944	9.47
Experiment II						
E 36-1	73.3 (5)	1647 (7)	36.2 (6)	2.29 (4)	6581 (7)	10.49 (7)
IS 9377	78.0 (8)	2285 (1)	41.7 (4)	1.90 (1)	7405 (3)	16.84 (1)
Q 102	69.7 (2)	1823 (5)	58.3 (2)	2.57 (5)	8435 (2)	15.39 (2)
Q 104	70.7 (3)	1526 (8)	69.6 (1)	4.47 (8)	8577 (1)	13.08 (3)
BJ 111	73.3 (5)	877 (9)	48.7 (3)	3.81 (7)	7027 (5)	6.68 (9)
IS 22380	66.0 (1)	2093 (2)	33.6 (7)	2.92 (6)	5873 (8)	12.26 (6)
R 16	75.7 (7)	1739 (6)	40.9 (5)	2.14 (3)	7199 (4)	12.55 (4)
SPV 475	71.7 (4)	1859 (3)	33.4 (8)	2.07 (2)	6590 (6)	12.39 (5)
SPV 783	84.0 (9)	1842 (4)	29.6 (9)	5.34 (9)	4837 (9)	9.12 (8)
Means						
Parents	73.6	1743	43.5	3.06	6947	12.09
Crosses	68.6	2056	43.5	2.21	7404	15.27

† At 70 d after flowering (DAF) in Exp. I and 100 DAF in Exp. II.

Field Observations

Six representative plants per plot were harvested at 50% flowering, and the area of each of the upper six leaves were measured (LI300 leaf area meter, LI-COR Inc., Lincoln, Nebraska). A second set of six representative plants per plot was tagged for regular visual estimation of the percentage of green area of each of the upper six leaves. These visual estimates were done weekly to the nearest 10% GLA, on all tagged plants in all replications (108 leaves per genotype per week). Observations began at flowering in Exp. I and 2 wk after flowering in Exp. II. They continued until 3 to 4 wk after physiological grain maturity (Exp. I), or until the mean relative GLA of the upper six leaves for each plot had declined to about 30% (Exp. II).

The % GLA for each leaf (flag leaf to Leaf 6) was averaged for the six plants per plot for each weekly observation. The resulting mean % GLA per leaf was then multiplied by the mean measured leaf area at flowering, to obtain the actual GLA for each leaf at each observation. The GLA for each individual leaf was summed over the six leaves to estimate the absolute GLA per plant each week. The relative GLA per plant for each weekly observation was obtained by dividing the absolute GLA by the GLA at flowering and multiplying by 100.

To describe the patterns of leaf senescence, the differential equation of the logistic function (Causton and Venus, 1981) was fitted to the weekly data of relative GLA for each plot (Fig. 1). The general formula of the equation used was

$$y = a \times e^{(b - c \times t)}/[1 + e^{(b - c \times t)}], \qquad [1]$$

where y is mean relative GLA, t the number of days after flowering, and a, b, and c are constants (a is the upper asymptote, b is related to the onset of senescence, and c to the rate of senescence).

Estimation of Green Leaf Area Duration and its Components

The relative GLAD after flowering for each plot was defined as the area under the logistic curve (Eq. 1), estimated by linear interpolations for 0.2-d intervals. A constant mean relative GLA of 100% was assumed until 10 d after flowering (DAF) in Exp. I and 20 DAF in Exp. II. At those dates, >95% of the field plots in both experiments had an estimated (Eq. 1) mean relative GLA of 100% + 3%. The relative GLAD was calculated from 0 to 70 DAF in Exp. I and from 0 to 100 DAF in Exp. II.

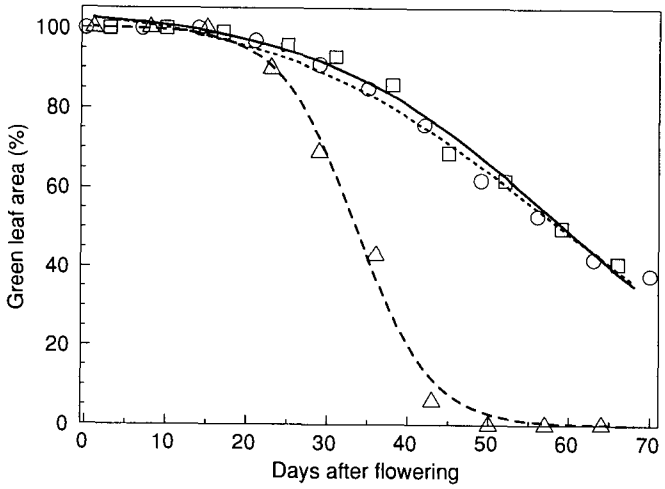

Fig. 1. Percentage of green leaf area after flowering in E36-1 (□——□), R16 (△ – – – △) and the hybrid R16 × E36-1 (○ – – – ○). Data are individual field plots from Exp. I. Curves are fitted according equation 1.

From the fitted functions and the measured GLA at flowering, the following variables were derived for each individual plot:

Onset of senescence—the DAF that the relative GLA reached 95%.

Maximum rate of leaf senescence—the maximum absolute value of the slope of the logistic function.

Green leaf area retention—the relative GLA at the time that the trial mean relative GLA reached about 50% (50 DAF in Exp. I and 75 DAF in Exp. II).

Absolute GLAD—the product of the relative GLAD and the GLA at flowering, divided by 100.

Both relative and absolute GLAD were used to represent stay-green in the analysis. Relative GLAD has the advantage of being independent of GLA at flowering, and thus represents stay-green per se; whereas, absolute GLAD (which includes genetic differences in GLA at flowering) may be agronomically more relevant. Green leaf area at flowering and the onset and rate of leaf senescence were considered as components of stay-green, and GLA retention as an integrated expression of stay-green with practical value in a breeding program. Multiple regression analyses were used to determine the relationship of the components of stay-green to the relative and absolute GLAD. For this purpose, the reciprocal crosses were pooled, leaving 45 genotypes: nine parents and 36 crosses.

Diallel Analysis

For each trait in each season, a diallel analysis according to Griffing's (1956) Model 1, Method 2, was conducted. This model assumes the parents to be fixed and uses both reciprocal crosses in the analysis. However, it does not subdivide heterosis into its components, but calls it specific combining ability (SCA). Since reciprocal effects were either non-significant or weakly significant ($P < 0.05$) for most traits in only one experiment, the two reciprocals in each replication were averaged and the data were re-analyzed using Method 2 of Gardener and Eberhart (1966). This method considers only F_1s and parents and subdivides heterosis into (i) a mean dominance deviation, the difference between the means of the parents and the F_1s, (ii) a parental effect, the difference between the parents in the contribution to heterosis, and (iii) a residual, the difference between individual F_1s in specific heterosis.

RESULTS

Environmental and Parental Effects

The environmental effect was highly significant ($P < 0.001$, Table 2) for all traits. Time to flowering was longer in Exp. II, due to the day length extension, than in Exp. I (Table 1). The difference between environments in GLA at flowering was highly significant ($P < 0.001$), but relatively small. Continued irrigation in Exp. II extended the stay-green period due to a delayed onset and reduced rate of senescence. The combined effect of these differences was a significantly longer GLAD in Exp. II, both in relative and absolute terms (Table 1).

The 81 genotypes (72 F_1s and nine parents) differed significantly for all traits (Table 2). Parents that combined a moderate senescence rate with an early onset of senes-

Table 2. Pooled analysis of variance for a 9 × 9 complete diallel over two environments (E). The 81 genotypes have been subdivided into parents (P) and crosses (C).

Source	df	Mean squares					
		Days from sowing to flowering	Green leaf area at flowering ($\times 10^4$)	Days to 95% green leaf area	Maximum rate of senescence	Relative green leaf area duration† ($\times 10^5$)	Absolute green leaf area duration†
Environments	1	14 826.1**	196.1**	38 533**	48.67**	7312.8**	3901.4**
Parents	8	119.9**	58.7**	485**	5.55**	48.4**	25.1**
Crosses	71	60.6**	35.8**	369**	1.36**	30.8**	21.9**
P vs. C	1	828.1**	385.1**	44	24.84**	87.4**	321.5**
P×E	8	31.4**	27.3*	158**	3.81**	17.0**	13.9*
C×E	71	10.9**	15.9*	140**	1.20**	10.6**	12.8**
(P vs. C)×E	1	36.3**	4.3	46	0.85	0.5	17.1
Rep(E)	4	20.8**	39.5**	171**	1.28	31.1**	53.5**
Error‡	321	3.2	11.3	41	0.65	3.8	5.8

*, ** indicates significance at $P < 0.05$ and $P < 0.01$, respectively.
† At 70 d after flowering (DAF) in Exp. I and 100 DAF in Exp. II.
‡ For green leaf area at flowering and absolute green leaf area duration, the error has 319 df.

cence (IS 22380 and SPV 475) had an average relative GLAD that was only 71% of parents with a comparable rate of senescence, but with delayed onset (Q 102 and Q 104, Table 1). Similarly, the parent with an average onset of senescence but a fast rate of leaf senescence (SPV 783), had an average relative GLAD that was 75% that of E 36-1. E 36-1 had a comparable onset but a slower rate of senescence (Table 1). A late onset or slow rate of senescence in general resulted in a high absolute GLAD. However, a high absolute GLAD could also be achieved by a high GLA at flowering, even if the relative GLAD was not particularly high (IS 9377).

The genotype × environment (GE) interaction was significant for all traits, for both the parents, and the crosses (Table 2). However, the size of the interaction and the contribution of each parent differed for individual traits. Among traits, the ratio of the GE variance to the genotype variance was high for the rate of senescence (2.39), but much lower for leaf area at flowering (0.47) and the onset of senescence (0.84). Large differences were found among parents in their contributions to the parent × environment (PE) interactions. The PE interaction for flowering was due to SPV 475 and SPV 783 (Table 3), which reflected their specific adaptation to the two different seasons. SPV 475 flowered relatively early only under the extended day length in Exp. II; SPV 783 flowered relatively early only under the shorter day length of the post-rainy season (Table 1). Three parents contributed little to the PE interaction of both relative and absolute GLAD: IS 22380, Q 104, and SPV 475. E36-1, SPV 783 and R 16 were primarily responsible for the PE interaction for relative GLAD. In the case of R 16, this was due to a large PE interaction for rate of senescence; for E36-1 and SPV 783, it resulted from large PE interactions for the onset of senescence. The PE interaction for absolute GLAD was mainly caused by BJ 111 and E36-1, again due to PE interaction for different components: GLA at flowering in the case of BJ 111 and relative GLAD in the case of E 36-1 (Table 3).

Relationship between Green Leaf Area Duration and Its Components

The onset of senescence in both experiments was not affected by the date of flowering ($r = -0.14$ in Exp. I; $r = -0.28$ in Exp. II; $n = 45$). The rate of senescence increased with a later flowering date in both experiments, however. This effect was more pronounced in Exp. I ($r = 0.75$, $P < 0.001$) than in Exp. II ($r = 0.44$, $P < 0.01$), because stored soil moisture was depleted earlier in Exp. I.

Differences in relative GLAD could be explained by differences in both the initiation and the rate of senescence (Table 4). In both experiments, the multiple correlation of relative GLAD on these two variables explained over 96% of the variation in relative GLAD. The contribution

Table 3. Mean squares for the interaction between environments and the contrast between each parent and the other eight parents for days to flowering, components of stay-green, and stay-green (relative and absolute green leaf area duration).

Parent	Mean squares					
	Days from sowing to flowering	Green leaf area at flowering ($\times 10^4$)	Days to 95% green leaf area	Maximum rate of senescence	Relative green leaf area duration ($\times 10^5$)	Absolute green leaf area duration
E 36-1	3.3	34.5	204.7*	0.4	30.9**	48.0**
IS 9377	8.6	3.7	4.4	2.9*	18.6*	13.8
Q 102	6.3	33.8	90.4	1.6	0.5	16.5
Q 104	0.0	0.1	475.4**	8.2**	3.9	0.2
BJ 111	1.4	88.4**	79.2	0.6	3.1	31.9*
IS 22380	5.1	51.6*	0.9	2.0	9.0	3.8
R 16	0.1	0.6	1.1	13.4**	28.2**	9.0
SPV 475	97.3**	4.9	52.6	5.0**	3.7	0.4
SPV 783	160.1**	27.9	517.7**	0.4	55.4**	1.5
Error	3.2	11.3	41.1	0.6	3.8	5.8

*, ** indicates significance at $P < 0.05$ and $P < 0.01$, respectively.

Table 4. Forward stepwise multiple regressions of relative and absolute green leaf area duration and green leaf area percentage at 50 d (Exp. I) and 75 d (Exp. II) after flowering (DAF) on their component traits, for a half 9 × 9 diallel including the parents (n = 45).

Variable added	Model R^2 Experiment I	Experiment II
	Relative green leaf area duration†	
Onset of senescence	0.590***	0.834***
Senescence rate	0.969***	0.978***
	Absolute green leaf area duration†	
Leaf area at flowering	0.520***	0.595***
Onset of senescence	0.819***	0.945***
Senescence rate	0.968***	0.980***
	Mean relative green leaf area at 50/75 DAF‡	
Senescence rate	0.658***	
Onset of senescence	0.965***	
Onset of senescence		0.867***
Senescence rate		0.962***

*** contribution of added variable (partial R^2) significant at $P < 0.001$.
† Over the period of flowering (FL) to FL + 70 d (Exp. I) or FL + 100 d (Exp. II).
‡ At 50 DAF in Exp. I and 75 DAF in Exp. II.

of both components of senescence was significant at $P < 0.001$ (Table 4), but the initiation of senescence had the highest contribution, especially in Exp. II. The regression of absolute GLAD on total GLA at flowering and the components of relative GLAD also accounted for 96% of the variation in absolute GLAD (Table 4). Leaf area at flowering was the major component of actual GLAD in both experiments, but the contributions of the other two components were also significant.

Mean relative GLA (individual genotype GLA at the point at which the mean experimental GLA averaged 50%) was highly correlated with relative GLAD ($r > 0.99$, n = 45) for both experiments. In addition, the onset and rate of leaf senescence together explained about 96% of the variance in mean relative GLA, although the size of their contributions depended on the experiment (Table 4).

Analysis of Heterosis

The contrast between parents and crosses was highly significant ($P < 0.01$) for most variables (Table 2), suggesting significant non-additive gene effects (heterosis). The only exception was the number of days from flowering to 95% GLA, for which the SCA was non-significant in Exp. II. The heterosis × season interaction was non-significant, except for days to flowering. This was expected because of the extended day length in Exp. II. Thus, despite the significant GE interactions, the expression of heterosis was stable across the two environments. Reciprocal differences between the F_1 hybrids were, according to Griffing's (1956) Model 1 analysis, either non-significant or weakly significant in only one experiment (data not shown), indicating that maternal effects were not important for any of the traits considered.

The variance due to heterosis (h_{ij}) was divided into variance due to average heterosis (h), a parental effect (h_i), and a cross-specific effect (s_{ij}) (Table 5). Significant heterosis was always associated with a significant average heterosis, indicative of significant differences between the means of the parents and the F_1s (Table 5). Parental effects (h_i) were for most traits significant only in the fully irrigated Exp. II. The contribution of individual F_1s to heterosis (s_{ij}) varied with experiment; the contribution of the specific crosses was not consistent across environments, however.

The expression of heterosis (the degree of superiority of the F_1 over the parents) differed for the different measures of stay-green and their component traits. Table 6 contains paired observations (the mean of the two reciprocal F_1s vs. the best parent) for all traits of interest. The F_1s were similar to the best parent for GLA at flowering and for rate of leaf senescence in both experiments. However, for the onset of senescence the F_1 was generally comparable to the mid-parent value (Exp. II and combined experiments). The F_1 exceeded the best parent for days to flowering and absolute GLAD;

Table 5. Mean squares for components of stay green from the diallel analysis according to Method 2 of Gardener and Eberhart (1966) of a half 9 × 9 diallel carried out in two environments.

Source	df	Mean squares: Days from sowing to flowering	Green leaf area at flowering	Days to 95% green leaf area	Maximum rate of senescence	Relative green leaf area duration†	Absolute green leaf area duration†
Experiment I							
Entries	44	16.995**	7.96**	23.04**	0.715**	41.22**	2.567**
Cultivars (c_i)	8	68.250**	21.26**	103.83**	3.152**	191.55**	6.027**
Heterosis (ch_{ij})	36	5.605**	5.00**	5.09*	0.174**	7.82**	1.798**
Average (h)	1	77.828**	46.66**	26.76**	2.558**	113.77**	28.670**
Parents (h_i)	8	7.475**	2.91	5.48	0.138	6.75	0.713
Crosses (s_{ij})	27	2.376**	4.08*	4.17	0.096	4.21	1.124*
Error	88	0.994	2.27	2.84	0.102	3.40	0.583
Experiment II							
Entries	44	16.411**	10.48**	144.01**	0.548**	101.63**	9.410**
Cultivars (c_i)	8	60.031**	34.89**	726.18**	1.002**	465.73**	32.649**
Heterosis (h_{ij})	36	6.717**	5.06**	14.63	0.447*	20.73*	4.246**
Average (h)	1	181.641**	70.54**	0.01	5.234**	150.99**	73.067**
Parent (h_i)	8	2.574**	3.66*	32.62*	0.832**	24.27	1.211
Crosses (s_{ij})	27	1.466**	3.05**	9.85	0.155	14.85	2.596*
Error	88	0.661	1.68	10.87	0.275	11.69	1.445

*, ** inidcates significance at $P < 0.05$ and $P < 0.01$ respectively.
† At 70 d after flowering (DAF) in Exp. I and 100 DAF in Exp. II.

Table 6. Mean values of the F_1, the best parent, and the mean of the parents for relative and absolute green leaf area duration and their components in each experiment separately and in a combined analysis. The type of inheritance reflects the significance of the differences between the means.

Trait	F_1	Best parent	Mean of parents	Type of inheritance
Experiment I				
Days to flowering	57.5 a†	58.2 a	61.0 b	complete dominance
GLA at flowering	1924 a	1876 a	1669 b	complete dominance
Onset of senescence	25.9 b	27.8 a	24.0 c	partial dominance
Senescence rate	2.86 a‡	2.69 a	3.45 b	complete dominance
Relative GLAD	4947 a	5063 a	4549 b	complete dominance
Absolute GLAD	9.48 a	8.58 b	7.48 c	overdominance
Experiment II				
Days to flowering	68.6 a	70.6 b	73.6 c	overdominance
Leaf area at flowering	2056 a	1962 a	1743 b	complete dominance
Onset of senescence	43.5 b	51.1 a	43.5 b	additive
Senescence rate	2.20 a	2.36 a	3.06 b	complete dominance
Relative GLAD	7405 b	7639 a	6947 c	partial dominance
Absolute GLAD	15.3 a	13.9 b	12.1 c	overdominance
Experiment I and II combined				
Days to flowering	63.1 a	64.4 b	67.3 c	overdominance
GLA at flowering	1990 a	1919 a	1706 b	complete dominance
Onset of senescence	34.7 b	39.4 a	33.8 b	additive
Senescence rate	2.53 a	2.53 a	3.26 b	complete dominance
Relative GLAD	6176 b	6351 a	5748 c	partial dominance
Absolute GLAD	12.4 a	11.2 b	9.8 c	overdominance

† Means followed by a different letter are significantly ($P < 0.01$) different according to a t-test with paired observations.
‡ Means followed by the same underlined letter are significantly different at $P < 0.05$ only.

whereas, in the case of the relative GLAD, the F_1 was intermediate between the mid-parent value and the best parent.

DISCUSSION

Environmental and Parental Effects

The dependence of leaf senescence on soil water, as illustrated by the accelerated senescence under the drier conditions of Exp. I, can result in GE interaction for stay-green if parents differ in their drought tolerance. Both stable (Rosenow, 1984) and unstable (Mughogho and Pande, 1984) expression of stay-green across environments has been reported. The stability of expression for genotypic differences in stay-green (GLAD) will depend on the stability of the particular component that is responsible for the difference between genotypes. This expression is expected to be relatively stable if differences in stay-green are due to differences in component traits with a low GE to G variance ratio, such as time of onset of senescence. It will be unstable if the differences in stay-green result from differences in traits such as the rate of senescence, which has a high GE to G variance ratio. In addition, individual parents varied in the stability of expression of stay-green (Table 3). Therefore, the choice of donor parent(s) in a stay-green breeding program will be critical, both for the traits they contribute to increasing stay-green as well as for their own stability of expression over environments.

Measurement of Stay-Green

Measurement of GLAD to estimate stay-green is too labor intensive to be feasible in any breeding program. Identifying one of its component traits that is highly correlated with GLAD and can be used as an expression of stay-green is, therefore, necessary. GLA retention, estimated at maturity, has been used for this purpose by a number of researchers (Mughogho and Pande, 1984; Tenkouano et al., 1993; Walulu et al., 1994). The results of this study confirm that one single observation on GLA retention can be a reliable substitute for estimating genotypic differences in GLAD, provided the observation is made at the correct time. Genotypic differences in senescence rate are expected to be largest when the senescence rate is highest, i.e., when about 50% of the leaf area has senesced. Since water deficits accelerate senescence, the best timing of this observation depends on the senescence pattern, rather than on the timing of maturity.

Inheritance of Stay-Green and Its Components

The dominance of stay-green over senescence, and the absence of maternal effects in the inheritance of stay-green observed in this experiment have been reported previously (Tenkouano et al., 1993; Walulu et al., 1994). Both findings simplify breeding and selection for the trait, but a better understanding of the basis of the heterosis for stay-green would improve the ability to capitalize on it in breeding.

The non-additive gene effects causing heterosis can occur if the trait under consideration is a complex one, i.e., the sum or product of two or more simpler traits. Subdivision of the complex trait into its components may reveal underlying additive effects in the components (Sparnaaij and Bos, 1993). In this study, relative GLAD could be considered as the sum of (i) the relative GLAD from flowering until the start of senescence and (ii) the relative GLAD during senescence. The first component is largely determined by the time of onset for senescence, and the second by the rate of progress of senescence. The results (Tables 2 and 6) suggested 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. Consequently, the relative GLAD, as the sum of an additively and a dominantly inherited trait, showed partial dominance for a long GLAD. The absolute GLAD was the product of GLA at flowering (complete dominance for high leaf area) and the relative GLAD (partial dominance for long GLAD) and thus showed overdominance: the F_1 on average had an absolute GLAD that exceeded that of the best parent.

The inheritance of stay-green can thus be understood as a function of the inheritance of its components. The dominance of stay-green (relative GLAD) that has been reported (Tenkouano et al., 1993; Walulu et al., 1994), is thus mainly due to dominance for a reduced rate of leaf senescence. The overdominance for absolute GLAD after flowering was encouraging, particularly if this trait enhances grain yield under stress, and therefore tolerance to post-flowering drought stress. Since its relationship with tolerance to pre-flowering drought is unclear (Rosenow, 1984), use as a selection criterion in environments where the crop primarily relies on stored moisture, e.g., the post-rainy season sorghum crop, is worthy of investigation. Its usefulness as a selection criterion needs to be demonstrated through properly controlled selection experiments.

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