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HERITABILITY AND GENETIC ADVANCE IN RECOMBINANT INBRED LINES FOR DROUGHT TOLERANCE AND OTHER RELATED TRAITS IN SORGHUM (*Sorghum bicolor*)

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ABSTRACT
Recombinant inbred lines (RILs) of the sorghum cross N13 x E36-1 were evaluated for thirteen quantitative traits at University of Agricultural Sciences, Dharwad, India during 2007/08 post rainy season. The objectives of the experiment were to estimate the genetic coefficient of variation (GCV), heritability and genetic advance expected from selection. Highly significant differences were obtained among the RILs for all traits studied. Grain yield, stay green traits, panicle exertion and number of spikelets per head showed a relatively high GCV and PCV (21–34%). Comparatively high heritability (63-99%) were obtained from all traits except for green leaf area at 15 days after flowering(GLA15), days to 50% flowering and yield, which showed moderate heritability (52-57%) value. High genetic advance as percent of the mean were obtained from green leaf area at 30 and 45 days after flowering (%GLA30 & %GLA45(25-33%), green leaf area at flowering (GLA) (27%), plant height(26%), panicle exertion(17%), head length(35%) and yield(22%) among the RILs at 5% selection intensity, which indicated the possibility of improving these traits. Several RILs were identified that have exceeded the better yielding parent over the study period. Grain yield showed a strong positive association (r =0.34–0.83) with %GLA30, %GLA45, leaves number per plant, stem thickness, number of spiklet per head and green leaf area at flowering. Overall, the present results showed that a) the availability of genetic variance for some drought tolerance and other related useful traits in the RILs for exploitation through selection, and b) the availability of superior inbred lines for further breeding work.

KEYWORDS: Genetic Advance (GA), grain yield, heritability, stagy-green, sorghum.

INTRODUCTION
Sorghum (2n = 2x = 20) is a C4 crop that displays excellent tolerance to high moisture stress (Doggett H, 1998). It has the highest water use efficiency among major crop plants and is unusually tolerant to low soil fertility, traits essential for survival and productivity in arid and semi-arid areas with limited irrigation capability (Zhanguo *et. al.2008*). Global cultivation of sorghum covers an area of 43.73mha with annual production of 64mt (Sasaki and Antonio, 2009). It is the fifth most important cereal crop grown globally after wheat, maize, rice and barley production (FAO, 2006), providing food and fodder for the inhabitants of drought-prone regions. Recently, sorghum has been demonstrated as a viable bio-energy feedstock (Wang, *et.al.2008*). Its remarkable ability to reliably produce grains under adverse conditions makes sorghum important "fail-safe" sources of food, feed and fuel (Addissu, 2011)

Sorghum is one of the most widely grown cereal crops in Ethiopia. It is a staple food crop on which the lives of millions of poor Ethiopians depend. It has tremendous uses for the Ethiopians as no part of this plant is ignored. Sorghum grows in a wide range of agroecologies most importantly in the moisture stressed parts where other crops can least survive and food insecurity is rampant (Asfaw, 2007). Being an indigenous crop, tremendous amount of variability exists in the country. As a result, a large number of accessions have been collected by the Institute of Biodiversity Conservation (IBC) of Ethiopia. Many of these accessions have been evaluated in the country and some were released as commercial cultivars for the highlands. However, in recent years, the variability is becoming less in the lowlands where vulnerability due to recurrent drought and incidence of pests mainly spotted stem borer (*Chilo partellus*) is very high (Gebrekidan,1973). The local cultivars in these areas are late-maturing resulting in failure of the crop as the rain ceases early in the season before or at the time of flowering. For these areas, it is indispensable to find out sorghum inbred line that can tolerant post flowering moisture stress and it needs concerted effort to improve the yield levels of sorghum. Several attempts have been made to understand drought tolerance mechanisms so as to identify and improve sorghum genotypes that could fit to moisture deficit. In sorghum, the best characterized form of drought tolerance during grain filling stage of crop growth is the so called "stay-green" trait which is defined as the ability of the plant to resist premature plant senescence, retain green leaf area, fill grain normally, and resist lodging under conditions of post-flowering drought stress (Rosenow, 1987; Bohnert,1995). Sorghum genotypes with the stay-green trait continue to fill their grains normally and retain good fodder quality even under limited water or moisture stress conditions (Borrell and Hammer, 2000). Delay in the onset and reduced rate of leaf senescence (*i.e*., two distinct components traits of stay-green) offer an effective strategy for increasing grain production, fodder quality and grain crop residues particularly under water limited conditions (Haussmann, 2002). Stay-green sorghum genotypes maintain photosynthetically active leaf area better than genotypes that do not posses this trait under limited soil moisture during grain filling stage (Sanchez *et.al.*2002)

Sorghum improvement done so far through germplasm selection was limited totally to the available gene pool with in the population. Therefore, in germplasm collection and selection, creation of new genetically different from the original population is hardly possible. This issue can be addressed through gene recombination which occurs at early phases of meiosis and assures the transfer of required genes to the progeny.

According to Teklu, 1998, tef varieties developed through trait recombination have shown a 9% yield advantage as compared to those developed through direct selection from germplasm materials. To observe similar effect, there were limited information on the level of genetic variability, heritability and genetic advance of drought tolerance and related traits in recombinant inbred lines of sorghum. This study was, therefore, aimed to estimate the level of genetic coefficient of variation, heritability and genetic advance in recombinant inbred lines of sorghum.

MATERIAL AND METHODS

Cultivars N13 and E36-1 were crossed to produce the F_8 recombinant inbred lines (RILs) used for this study. The line E36-1 is a donor for stay-green traits and lodging resistance and high yielding breeding line assigned to guinea-caudatum hybrid race with Ethiopian origin. N13 is sensitive to moisture deficit, susceptible to charcoal rot and non-stay-green type but resistance to *striga heromontica* weed (Haussmann, 2004). A total of two hundred twenty six F_8 generation of Recombinant Inbred lines (RILs) were used for this experiment which was developed through single seed descent method. The experiment was conducted during the post-rainy seasons (October to March) of 2007/08 at Dharwad, University of Agricultural science, India. This season is ideal for evaluating the expression of adaptive traits for terminal moisture-deficit conditions, as the crop is dependent almost entirely on stored soil moisture and undergoes a long progressive stress under moderate evaporative-demand conditions. The recombinant inbred lines seed materials were sown together with the two respective parental lines in a 19 x□12 lattice design with four replications. The experimental units were 3-row plots, with each row being 5m long and spaced 0.50m apart. A basal application of 54 kg urea ha^{-1} and 227kg DAP ha^{-1} was banded before sowing. The field was irrigated with overhead sprinklers to ensure germination. To eliminate the border effect three rows of M35-1 and SPV86 sorghum cultivars were sown on all sides of the field. The plant population was successfully thinned 10 days after emergence to about 100,000 plants ha^{-1} when the seedlings were at 4 leaf stage. Twenty days after emergence, an additional 86 kg urea ha^{-1} was side-dressed and the field given a light (15-mm) - sprinkler irrigation. Appropriate pesticides were used were protected from both leaf feeding insect pests and stem borers.

During the course of study, quantitative traits assessed in the trial included the plant height at full maturity, days to 50% flowering, head length, panicle exertion, stem thickness, number of spikelet per head, 1000 seed weight, number of leaves per plant at full maturity stage, yield per plant, and the three stay-green traits (percentage of green leaf area at 15, 30 and 45 days after flowering) are recorded and described as below.

Estimation of stay-green trait: At the time of emergence of flag leaf, three representative plants in each plot were tagged; the length and width of the upper six leaves were measured. The area of each leaves estimated as: leaf length x leaf width x 0.7 (The 0.7 factor was determined by measuring the leaf length, breadth and, actual area of 70 randomly selected leaves). At the beginning of flag-leaf emergence, the percentage of each of the upper six leaves of each tagged plant remaining green was visually estimated at weekly intervals according to Mahalakshmi and Bidinger, (2002). The green leaf area (GLA) of each tagged plant was computed by multiplying the percent green-leaf area by measured area of each leaf and summing across the six measured leaves. The percentage of green leaf area (%GLA) for each plant, for each week, was calculated by dividing the estimated GLA for that week by its measured leaf area at flowering. Plot value for %GLA was derived by averaging the three individual plant values for each plot. The weekly % GLA data were used to fit an appropriate equation to describe the pattern of leaf senescence during the period of observations. The fitted equations for each individual plot were used to estimate the %GLA at 15, 30 and 45 days after flowering of the individual entries (%GLA15, %GLA30 and %GLA45) respectively.

The formula employed to calculate stay-green was

$$GLA = \sum_{i=1}^{n=6} (L * W * 0.7) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\%GLA = \sum_{i=1}^{n=6} (L * W * 0.7) - \sum_{i=1}^{n=6} (L * W * 0.7 * VGLA\%) \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where GLA is green leaf area at early stage, L is leaf length and W is width of the leaf, VGLA% is percentage of weekly interval vegetative green leaf area, n is number of measured leaves per plant and %GLA is remaining green leaf area percent at different sorghum growth stage, while 0.7 is correction factor.

Phenotypic and genotypic coefficient of variation: the estimates of phenotypic and genotypic coefficient of variation were obtained as explained by Singh and Chudhary, 1977 as follows

$$PCV\ (\%) = \frac{\sqrt{VP}}{\bar{x}} \times 100$$

$$GCV\ (\%) = \frac{\sqrt{Vg}}{\bar{x}} \times 100$$

Where, PCV is Phenotypic Coefficient of Variation, VP is Phenotypic Variance, GCV is genotypic Coefficient of Variance, Vg is genotypic variance and is Mean of RIL's. GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (20% and above) values as indicated by Sivasubramanian and Menon (1973).

Phenotypic correlation: phenotypic correlation coefficients were estimated by using the following formula (Singh and Choudhary, 1977).

$$rp = \frac{CoVp\ (X,Y)}{\sqrt{Varp\ (x)\ Varp\ (y)}}$$

Where, rp is phenotypic correlation coefficient, CoVp (x, y) is phenotypic covariance between characters x, y, Var_p (x) is phenotypic variance in character x, VarP (y) is phenotypic variance in character y. The observed value of correlation coefficient was compared with the tabulated value for (n-2) degree of freedom for test of significance.

Heritability: it was estimated in RILs for all resistance components as well as the ratio of total genotypic variance to the phenotypic variance according to Falconer (1989).

$$H^2 = \frac{Vg}{Vp} x100,$$

Where, H^2 is % broad sense heritability value, Vg is genotypic variance and Vp is Phenotypic variance. The heritability percentage categorized as low (0-30%), moderate (30-60%) and high ≥ 60%) as given by Robinson *et al.* (1949).

Genetic advance: The extent of genetic advance expected through selection for each of the character was calculated as in Johnson *et al.* (1955).

GA (%) = H x P x K Where, H is Heritability, P is phenotypic standard deviation and K is selection deferential (2.06 at 5%).

Genetic advance as percent of mean (GAM)
GA as per cent mean = (GA/) 100, where GA is genetic advance, is general mean.
Genetic advance as per cent of mean was categorized as low (0-10%), moderate (10-20%) and high (>20%) by following Johnson *et al.* (1995).

RESULTS AND DISCUSSION

For all the studied traits mean squares of RILs showed highly significant variation (Table1). The extent of variability in respect of mean, phenotypic and genotypic coefficients of variation, heritability and genetic advance are shown in Table 2. The present investigation revealed considerable amount of variation for all the characters studied. Such wide variation indicated the scope for improving the population for these characters with respect to drought and other related quantitative traits. The RILs were compared with their parent's value distribution considering the grain yield, stay green and other quantitative traits (Table 2). A good numbers of RILs were identified with superior performance that exceeded the best yielding and stay-green (drought tolerance trait) parent. Such transgressive lines suggest either those favorable additive alleles are brought by both parents, and/or that complementary interactions occur between alleles of different origins.

Table1. Analysis of variance for 13 traits for the 226 F_8 recombinant inbred lines of N13 × E36-1 crosses.

Traits	RILS DF=227	Error DF=448	CV (%)
%GLA15	290.5**	31.4	5.2
%GLA30	540.0**	16.3	8.4
%GLA45	143.6**	25.3	17.1
GLA at flowering(cm^2)	2810.5**	800.4	22.0
Plant height(cm)	29.1**	6.2	12.1
Stem thickness(cm)	82.7**	1.3	4.1
Panicle exertion(cm)	29.8**	6.2	13.0
No. of leaves/plant	290.4**	23.4	12.0
No. of spikelets/head	2.97**	0.6	9.1
100seed weight(g)	0.73**	0.3	14.2
Head length (cm)	109.8**	3.5	7.1
Days to 50% flowering	318.0 **	68.8	3.0
Yield/plant(g)	251461**	48215.0	20.0

** is highly significant at 1% level *is significant at 5% level

Table2. Genetic variability for stay-green, yield and yield component trait in RILs derived from N13 x E36-1(RIP II) cross

Characters	P1	P2	Mean ± S.Em.	GCV (%)	PCV (%)
%GLA15	82.2	95.0	84.3±0.43	9.7	11.8
%GLA30	62.0	84.0	73.0±0.67	14.6	15.5
%GLA45	44.6	65.2	44±1.3	30.2	31.2
GLA at flowering(m^2)	5.7	15.1	12.8.01±1.7	18.3	22.3
Plant height(cm)	178.0	174.0	183±1.9	12.4	13.9
Stem thickness(cm)	45.0	6.7	3.2.05±0.06	13.5	19.5
Panicle exertion(cm)	12.7	7.5	12.2±0.3	36.7	37.9
Number of leaves/plant	6.8	8.5	7.3±0.6	10.5	11.9
Number of spikelets/head	45.0	53.1	41.7±0.5	19.7	22.9
1000 seed weight(g)	3.1	4.1	3.6±0.01	10.4	11.3
Head length (cm)	22.6	25.26	17.8±0.13	30.2	32.3
Days to 50% flowering	68.0	62.0	62.1±0.4	8.2	8.8
Yield/plant(g)	60.0	150	161±0.6	20.7	22.9

P1=N13, P2=E36-1

Frequency distributions of yield and the three stay-green traits in a cross of N13 x E36-1 were unimodal and approximately normal (Fig.1). The two parental lines of the recombinant inbred population (RIP) differed significantly for yield and all other three important stay green traits (% GL15, % GL30 and % GL45) as indicated in fig 1. Reasonable number of RILs were superior in yield and stay green trait than the best parental line.

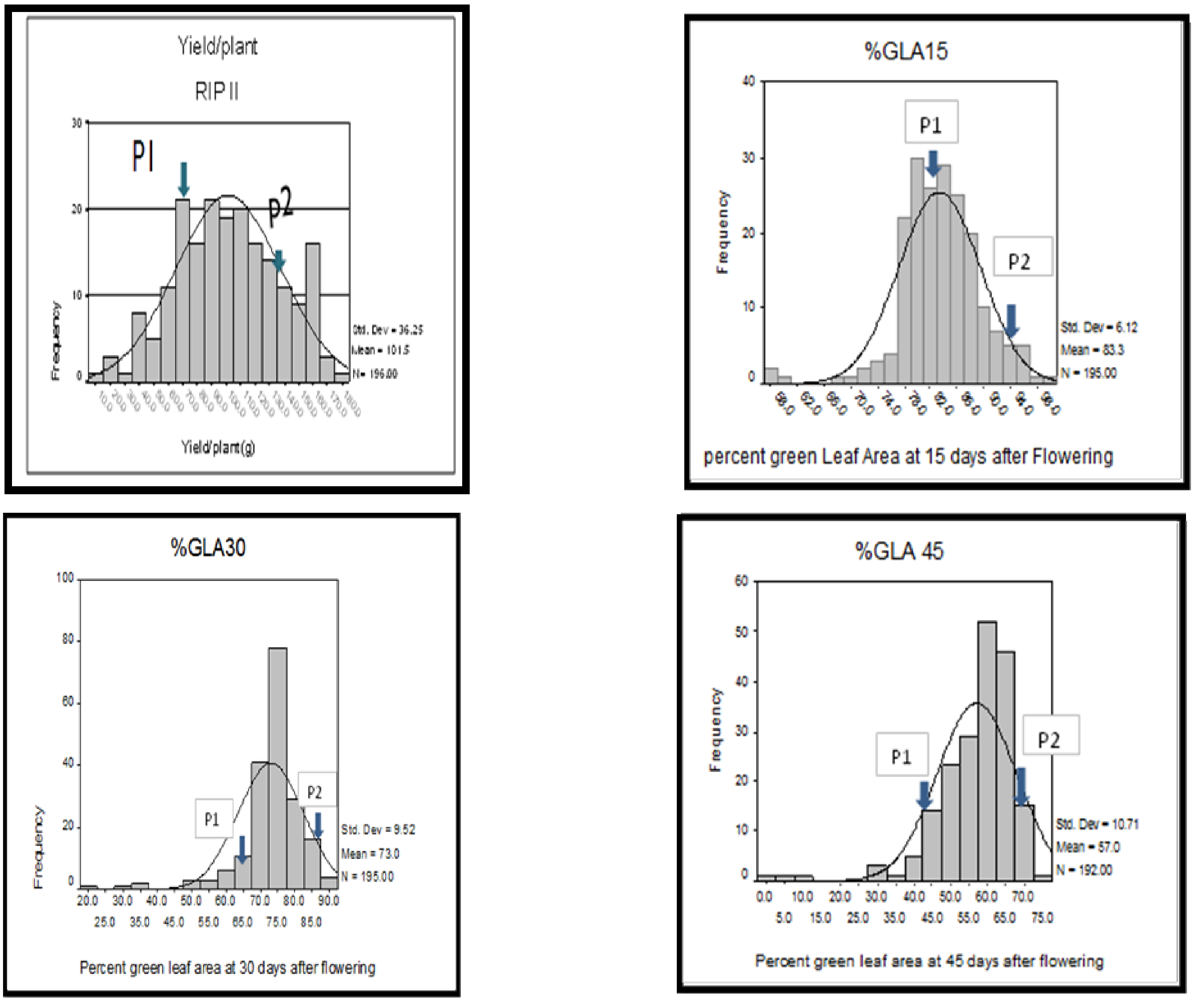

Fig.1. Frequency distribution of the F_8 lines derived from N13 x E36-1 for grain yield and the percentage of green leaf area at A) 15, B) 30 and C) 45 days after flowering (%GLA15, %GLA30, and %GLA45, respectively)

The genetic and phenotypic coefficient of variation (GCV & PCV) ranged from 8.22% and 8.75% for days to maturity to 36.72% and 37.90% for panicle exertion respectively (Table 2). The highest GCV and PCV were recorded for panicle exertion followed by head length, %GLA45 number of spikelets/head and %GLA30, while moderate GCV and PCV were measured for 1000 seed weight, number of leaves per plant, plant height and stem thickness (cm). The lowest GCV and PCV were recorded for %GLA15 and days to 50% flowering. The high PCV and GCV observed are evident from their high variability that in turn offers good scope for selection. The lowest PCV and GCV indicate limited improvement for the traits through selection and it was conformity with the findings of Sundaresha, (2000). The GCV was near to PCV for most of the characters, indicating a highly significant effect of genotype on phenotypic expression with very little effect of environment heritability estimates observed for most of the characters ranged from 47(stem thickness) to 95 percent(head length). Similar findings were also reported in sorghum (*sorghum bicolor*) by Haussmann *et al.* (2002) for stay-green and yield per plant and Rao and Patil, (1996) for head length panicle exertion and plant height characters.

Simple phenotypic correlation coefficients are shown in Table4. Grain yield showed a strong positive correlations*(r =0*.34–*0.83*) with stay-green traits (%GLA15, %GLA30, (%GLA45), leaves number per plant, stem thickness, number of spiklet per head, head length and green leaf area at flowering(cm2). The two important stay green parameters %GLA30 and %GLA45 revealed strong positive association with grain yield and leave numbers per plant. The correlation coefficient of these two traits is very strong(r= 0.69-0.83) which indicates those recombinant inbred lines possess stay-green (drought tolerance trait) better yield as compared with those lines which do not possess the traits. Contrastingly, highly significant negative associations were observed between yield/plant with days to 50% flowering and panicle exertion which indicates early flowering sorghum lines provide less yield under moisture deficit condition so as to escape from the imposed stress. Similarly strong negative correlation was revealed between panicle exertion and grain yield. As the panicle exertion longer, the grain yield was reduced. This is probably due to sink and source relationship, i.e. the devotion of line for the growth of vegetative part instead of grain filling. The tendency of positive correlation between stay-green trait and grain yield as found in this study has also been reported in various germplasm populations (Haussmann *et al.* 2002; Warkad *et al.* 2008). Other correlation coefficients between pairs of traits that are of some interest to the breeders are shown in Table 4.

Broad sense heritability values for the thirteen traits are presented in Table 3. Traits such as head length, panicle exertion, stay-green, plant height, and green leaf area at flowering showed a relatively high heritability values (>60%). The values estimated for stem thickness (cm), number of leaves/plant, days to 50% flowering and yield per plant were moderate. This result is similar to observation made by Kebede *et al.* 2001. Heritability is a useful quantitative parameter, which considers the role of heredity and environment determining the expression of a character (Allard, 1960; Kukadia *et al.* 1983). Effective selection can be achieved only when additive effects are substantial and environmental effects are small. In the present investigation exceptionally, high estimates of broad sense heritability were noticed for character *viz.*, stay-green (%GLA15, %GLA30 and %GLA45), panicle exertion (cm), stem thickness, head length, number of spikelets per head and 1000 seed weight. Percent green leaf area at 45 days flowering(%GLA45), grain yield and its component traits, head length(cm), GLA at flowering(m^2), plant height(cm), exhibited the highest predicted genetic advance as compared to the other traits (Table 3). The remaining traits showed a moderate to very low amounts of genetic advance. This investigation is in agreement with similar observation made in different crosses by different researchers (Reddy *et al.* 1996; Poornima, 2006; Mahalakshmi and Bridger, 2002).

High GCV along with high heritability and genetic advance provide better information than other parameters alone. On the basis of the present study, stay-green parameters (%GLA15, %GLA30, and % GLA45), yield per plant, panicle exertion, head length and 1000 seed weight are the most important quantitative characters to be taken into consideration for effective selection in sorghum. Opportunities to improve these traits appear to be likely though the degree varies depending on H^2 and GCV values.

In this population, a relatively high GA as percent of the mean were obtained for percent GLA45 and GLA30, head length, 1000 seed weight, grain yield, panicle exertion, plant height and green leaf area at flowering. In the present investigation, high heritability accompanied with high genetic advance over mean were observed for the stay-green characters (%GLA30, %GLA45), head length, 1000 seed weight and panicle exertion suggesting the influence of additive genes and provides scope for selection and their amenability for improvement. Also, high value of heritability along with low genetic advance over mean were observed for the characters number of leaves per plant indicating that variability is mainly due to the non-additive gene effects and hence heterosis breeding can be fruitfully exploited in improving such characters. This finding is in agreement with the finding of Warkad *et al.* 2008 of which similar research made on sorghum germplasm line. At the F_8 generation the RILs are homozygous where additive and additive × additive genetic variances are fixed and prevailing. Thus, selection in this population would prove successful once the fixed genetic component is disentangled from environmental effects.

CONCLUSION

The present data indicated that the prevalence of genetic variance for some useful agronomic traits such stay-green in the RILs that can be exploited through selection as the corresponding to high H^2 and GA estimate allows to do so, and the availability of superior inbred lines in the present cross show the potential and opportunity that exists if more intra-specific populations are created for better gain and establish a reliable information. In the present experiment transgressive inbred lines which superior than the two parents can be promoted to variety trials which can be used under moisture stress condition.

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Table 4. Coefficients of phenotypic correlation among the investigated traits in Recombinant Inbred Lines derived from N13 x E36-1 cross.

Traits	%GL15	%GL30	%GL45	PH	SW	LN	PE	ST	NS	HL	DF	GLA	YP
%GL15	1	0.29*	0.630**	0.19	0.01	0.02	-0.07	0.01	0.03	0.05	-0.03	0.50	0.71**
%GL30		1	0.22*	0.02	0.02	0.46**	-0.01	-0.03	0.01	0.00	-0.02	0.01	0.69**
%GL45			1	0.06	0.03	0.67	-0.12	0.02	0.14	-0.08	-0.21	0.02	0.83**
PH				1	0.01	0.13	0.33**	-0.04	0.03	0.13	-0.06	0.08	0.48**
SW					1	-0.02	-0.06	0.01	-0.05	-0.05	-0.04	0.01	0.02
LN						1	-0.05	0.10	0.07	-0.09	0.03	0.53	0.34*
PE							1	-0.12	-0.08	0.23*	0.03	-0.05	-0.29*
ST								1	0.01	0.04	0.00	0.18	0.51**
NS									1	0.07	0.04	0.06	0.49**
HL										1	0.08	0.01	0.49**
DF											1	-0.02	-0.37
GLA												1	0.42**

%GL15: Per cent of green leaf are at 15 days after flowering, %GL30: Per cent of green leaf are at 30 days after flowering, ST: Stem thickness (cm), NS: Number of spike lets per head %GL45: Per cent of green leaf are at 45 days after flowering, HL: Head length (cm), PH: Plant height (cm), DF: Days to 50% flowering, SW: 1000 seed weight (g) GLA: Green leaf area at flowering (cm^2), LN: Leaves number per plant, YP: Yield/ plant (g), PE: Panicle exertion (cm), ** is highly significant at 1% level *is significant at 5% level

Table3. Estimates of broad sense heritability (H^2) in percent and genetic advance (GA) as percent of the mean for thirteen traits in F_8 recombinant inbred lines of sorghum

Sl.No.	Characters	Heritability (%)	GA (%)
1	%GLA15	67.4	10.2
2	%GLA30	88.9	25.2
3	%GLA45	93.3	33.0
4	GLA at flowering(m^2)	67.2	26.7
5	Plant height(cm)	61.6	26.0
6	Stem thickness(cm)	48.9	17.0
7	Panicle exertion(cm)	93.9	20.7
8	Number of leaves/plant	47.0	8.8
9	Number of spikelets/head	74.1	9.21
10	1000 seed weight(g)	76.2	23.0
11	Head length (cm)	95.0	35.0
12	Days to 50% flowering	58.3	16.5
13	Yield/plant(g)	47.7	22.0

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