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Research Note

Stay-green and other physiological traits as efficient selection criteria for grain yield under drought stress condition in sorghum (*Sorghum bicolor* L. Moench)

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Abstract
An experiment was conducted to study the co-efficient of variation, heritability, genetic advance, correlation coefficients and path analysis for yield and other physiological traits of sorghum genotypes under moisture stress condition with the aim of assessing the potential genetic gain. High estimates of heritability and genetic advance as per cent of mean were observed for leaf area index, stay-green score, relative water content, proline, SPAD chlorophyll, plant height and grain yield. Positive and significant correlation of grain yield with leaf area index, stay-green, relative water content, SPAD chlorophyll content and proline were observed. The characters like leaf area index, stay-green, relative water content and SPAD chlorophyll had positive direct effect on single plant yield under drought stress condition at genotypic level. This suggests the possibilities of improvement of these characters through selection for drought tolerance breeding programme.

Key words
Sorghum bicolor, genetic variability, heritability, genetic advance and genotypes

Sorghum is considered an important crop grown widely in the world for food, feed and industrial value. It is estimated that the sorghum cultivated area permanently in the world is affected by 28% due to drought stress (Li *et al.*, 2009). Drought is a major stress factor and complex phenomenon that limits the agriculture production globally. Hence, understanding of various characters (morphological, physiological and biochemical) associated with drought tolerance can be rewarded. Drought stress has been categorized in to pre and post flowering. In which post-flowering drought stress is important, since it is associated with grain filling and yield. Improvement of sorghum cultivars through appropriate screening of genotypes, breeding methods and accurate genotyping and phenotyping can help in greater extent of yield under drought situations.

In this study, one hundred sorghum genotypes were evaluated for their plant water relations under water stress at flowering (anthesis) stage to post-flowering (grain yield) based on the estimation of genetic gain and relationship between yield and other physiological component traits. The ultimate aim of the study was to establish possible selection criteria for sorghum genotypes under moisture stress condition and future hybridization for developing cultivars for combating water stress.

The experiment was carried out at Tamil Nadu Agricultural University, Coimbatore during *kharif* 2016. The experiment was laid out under randomized block design (RBD) with two replications as under post flowering moisture stress imposed by withholding irrigation from flowering to maturity. In this study genotype B-35, was used as the drought resistant check and variety CO 26 (TNAU released grain sorghum) as the drought susceptible check. It was ensured that no rain was recorded during the moisture stress imposition i.e., from anthesis to crop maturity phase along with normal recommended cultural practices were adopted during the crop period. Data were recorded for days to 50 per cent flowering, leaf area index, SPAD chlorophyll, relative water content, plant height, proline, stay-green and grain yield traits with two replications.

Plant physiological traits and statistical analysis: Water stress indicator traits like relative water content (RWC) were calculated using the formula suggested by Barrs and Weatherly (1962) and total leaf chlorophyll contents were measured with a Minolta chlorophyll meter SPAD-502. The stay-green was estimated visually on a plot basis on a scale of 1 to 5 based on the degree of leaf and plant death at physiological maturity in the field both under post-flowering drought stress, by following the visual ratings of stay-green trait suggested by Wanous *et al.* (1991) in sorghum. Proline estimation was done for stress condition based on Bates *et al.* 1973. Differences between genotypes for different characters were tested for significance by using analysis of variance technique (Panse and Sukhatme, 1954). Heritability (broad sense) was calculated as the ratio between genotypic variance to total or phenotypic variance and expressed as percentage (Allard, 1960). The expected genetic advance was obtained as the difference between the mean of the progeny of selected individuals and base populations, computed with the help of formula suggested by Johnson *et al.* (1955). Phenotypic and genotypic correlation were calculated by the formula suggested by Al-Jibouri *et al.* (1958) using the variance and covariance estimates from the analysis of variance and analysis of covariance tables. The phenotypic and

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genotypic correlations coefficients were further partitioned into direct and indirect effects as suggested by Wright (1921).

Variance analysis: Significant differences were observed among the genotypes for SPAD chlorophyll reading, stay green, relative water content, leaf area index, proline and grain yield and days to 50% flowering under drought stress has justified the relative contributions of these traits to the variability, hence are amenable to selection (Table 1). These tested genotypes also significantly differed for drought tolerant indicators such as SPAD chlorophyll reading, stay green, relative water content, leaf area index and proline indicating a substantial influence of environment on these traits.

Co-efficient of variation: The present investigation revealed that the percent phenotypic co-efficient of variation (PCV) was higher than the genotypic co-efficient of variation (GCV) for all the traits under drought stress was presented in the table 2. It can be due to the influence of environment on the expression of these traits. The maximum GCV was obtained for proline, followed by leaf area index, stay-green, relative water content, plant height, chlorophyll content and grain yield per plant. It is also suggested that the variability at the phenotypic level includes both genotypic and environmental variability. The values of genotypic and phenotypic co-efficients of variation for these traits under stress justified the relative contributions of the variability of genotypes. Ultimately, the success of the selection depends on the extent of genetic variability present for the traits under consideration. The present finding substantiates the findings of Geleta and Daba (2005) as genotypic and phenotypic co-efficients of variation is higher for plant height and grain yield per plant. The data further indicated that characters like proline, leaf area index, stay-green, relative water content and plant height showed high value for phenotypic and genotypic co-efficient of variation. High values of GCV for these characters suggest better scope of improvement by selection under drought stress condition. Days to 50% flowering showed the lowest co-efficient of variation at phenotypic and genotypic levels. Similar results were reported for theses traits with respect to PCV and GCV (Geleta and Daba, 2005).

Heritability and Genetic advance: The heritability expresses the proportion of total variance that is attributed by genetic cause. Heritability in broad sense indicates the relative success of the selection programme. In this study all the characters studied were showed higher heritability values (Table 2) for relative water content followed by SPAD chlorophyll reading and plant height, leaf area index, proline, stay-green, grain yield and days to 50 per cent flowering. From the above investigation it is evident that most of the traits studied have high heritability estimates, which indicated that most likely the heritability was due to additive gene effects and direct selection may be effective. Kebede *et al*. (2001) reported high heritability for stay-green ranging from 72% to 84% suggesting it as useful selection criteria under drought. Since, stay-green plants can maintain leaf senescence till its maturity.

The expected genetic advances for the traits under consideration were presented in Table 2. Relative comparison of heritability estimates and expected genetic advance as percentage of mean will give an idea about the nature of gene action governing a particular character. High heritability along with high genetic advance as percentage of mean was observed for proline, leaf area index, stay-green score, relative water content, plant height, SPAD chlorophyll and grain yield. In the present investigation, characters like proline, leaf area index, stay-green score, relative water content, plant height, SPAD chlorophyll and grain yield, exhibited high heritability and expected genetic advance. Among the characters studied, high estimates of heritability (>80%) and genetic advance expected (>40%) were obtained for relative water content, stay-green, leaf area index and proline. These characters exhibited high heritability, high genetic advance as percentage of mean with high genotypic co-efficient of variation indicating importance of additive genetic variance for these characters which could be used for selection and improvement of genotypes under moisture stress environment. The character days to 50% flowering recorded the lowest heritability, genetic advance as per cent of mean and genotypic coefficient of variation indicating larger influence of environmental conditions (Vinodhana *et al.,* 2009).

Association analysis for yield and other physiological characters: A perusal of table 3 indicates positive and significant genotypic correlation of grain yield with leaf area index, stay-green, relative water content, SPAD chlorophyll content and proline. It is also indicates that the values of the phenotypic correlation coefficients were in the same order or lower than that of the genotypic correlation coefficients. From the above results it can be seen that characters like leaf area index, stay-green, relative water content, SPAD chlorophyll content and proline showed positive correlation with yield under moisture deficit stress condition. In this study relative water content had highly significant positive correlation with SPAD chlorophyll reading and grain yield under stress. So the genotypes with high leaf relative water content exhibited less reduction in biomass, maintaining chlorophyll and yield due to relative water content as an alternative measure of plant water status, reflecting metabolic activity in tissues

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and used as a most meaningful index for dehydration tolerance. However, relative water content and SPAD chlorophyll reading had highly significant negative association with stay-green score trait, (the scale of stay-green trait lower is advantageous and higher is disadvantageous for drought screening) so stay-green cultivars with more internal plant water status coupled with high chlorophyll content influenced the grain yield positively. Similarly, Xu *et al.* (2000) investigated the relationship between the visual stay-green rating and the leaf chlorophyll concentration in sorghum and reported that the chlorophyll content was significantly negative correlation to stay-green rating ($r = -0.90$). On the other hand, there is a significant correlation between grain yield and RWC, proline and whole of chlorophyll. Therefore, these characters should be given utmost consideration for breeding varieties tolerant to moisture deficit stress condition in order to minimize the losses in grain yield.

Analyses of direct and indirect effects provide an effective means of association between yield and other physiological component traits. It allows recognizing the specific forces acting to produce a given correlation and measures the relative significance of each causal factor. The characters like leaf area index, stay-green, relative water content and SPAD chlorophyll had positive direct effect on single plant yield under drought stress condition (Table 4). Simple direct selection based on these traits would be rewarding. This information would be of greater value in selecting the useful traits and thus optimize the observations on a few related traits in the preliminary trail involving a large number of germplasm accessions.

It could be concluded from this study that analysis of variance, genotypic and phenotypic co-efficient of variation, heritability, genetic advance and association analysis have implications in selecting genotypes by assessing potential variability present among the genotypes under consideration thereby further it can be useful in sorghum drought tolerance improvement program and providing new sources of variation for physiological traits that can enhance genetic potential of sorghum for developing drought tolerant cultivars. Grain yield as dependent variable that selection of the genotypes should be based on the characters having high direct effect as stay green, relative water content, SPAD chlorophyll and leaf area index can have scope of selection for better yield under moisture deficit stress conditions as drought tolerant genotypes, which could be used as the donor parents in hybridization programme for sorghum drought tolerance breeding.

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Table 1. Analysis of variance (ANOVA) for eight characters under drought stress condition

Sources of variations	d.f.	Days to 50 % flowering	Leaf area index	Plant height	SPAD chlorophyll	Relative water content	Proline	Stay-green score	Grain yield
Replication	1	6.43	0.38	495.88	1.66	40.19	0.77	0.80	57.80
Treatment	99	24.39**	3.53**	4234.49**	134.59**	256.44**	1.11**	2.97**	89.71**
Error	99	3.91	0.20	204.19	4.25	5.8	0.11	0.40	10.34

*, **Significant at 5 and 1 per cent level, respectively

Table 2. Genetic variability parameters of eight quantitative characters of sorghum genotypes under moisture stress condition

Characters	VP	VG	PCV (%)	GCV (%)	h^2 (%)	GAM
Days to 50 % flowering	14.15	10.23	5.84	4.97	72.34	8.71
Leaf area index	1.87	1.66	37.95	35.76	88.79	69.42
Plant height (cm)	2219.34	2015.19	21.16	20.16	90.80	39.58
SPAD chlorophyll reading	69.42	65.16	19.35	18.74	93.86	37.41
Relative water content (%)	131.13	125.31	24.42	23.87	95.56	48.07
Proline (mg/g)	0.61	0.50	55.99	50.42	81.07	93.53
Stay-green score	1.68	1.28	34.92	30.47	76.11	54.76
Grain yield per plant (g)	50.02	39.68	21.68	19.31	79.32	35.43

PV- Phenotypic variance, PCV%- Phenotypic coefficient of variation, GV- Genotypic variance, GCV% - Genotypic coefficient of Variation, h^2 – Heritability broad sense and GAM- Genetic advance as per cent of mean

Table 3. Correlation co-efficient of eight quantitative and physiological characters of 100 sorghum genotypes under drought conditions

Traits		SGR	DFF	LAI	PHT	RWC	PRL	GYP
CHL	G	-0.7572**	0.0070	0.4394**	0.0833	0.4404**	0.3128**	0.5608**
	P	-0.6641**	-0.0029	0.3872**	0.0689	0.4234**	0.2665**	0.4867**
SGR	G		-0.0590	-0.4598**	0.0648	-0.5208**	-0.2553**	-0.6204**
	P		-0.0412	-0.3638**	0.0317	-0.4499**	-0.1877*	-0.5386**
DFF	G			0.0785	-0.2544**	-0.1846	0.1749	-0.1072
	P			0.0683	-0.1907*	-0.1600	0.1208	-0.0561
LAI	G				0.1622	0.4322**	0.2620**	0.6460**
	P				0.1453	0.3952**	0.2191**	0.5240**
PHT	G					0.3158**	0.0094	0.1770
	P					0.3003**	0.0167	0.1609
RWC	G						0.1237	0.6029**
	P						0.1173	0.5542**
PRL	G							0.3612**
	P							0.2925**

*, **Significant at 5 and 1 per cent level, respectively
P-Phenotypic correlation, G-Genotypic correlation, CHL-SPAD chlorophyll reading, SGR- stay-green score, DFF-days to 50% flowering, LAI-leaf area index, PHT-plant height, RWC-relative water content, PRL- proline content, GYP-grain yield/plant

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Table 4. Direct (diagonal) and indirect effects of yield components with single plant yield

Traits	CHL	SGR	DFF	LAI	PHT	RWC	PRL	r_g
CHL	**0.5608**	0.1977	-0.0009	0.1605	0.0017	0.1046	0.0563	0.5608**
SGR	-0310	**-0.6204**	0.0079	-0.1680	0.0013	-0.1237	-0.0459	-0.6204**
DFF	0.0003	0.0154	**-0.1072**	0.0287	-0.0052	-0.0439	0.0315	-0.1072
LAI	0.0180	0.1200	-0.0105	**0.6460**	0.0033	0.1027	0.0471	0.6460**
PHT	0.0034	-0.0169	0.0341	0.0593	**0.1770**	0.0750	0.0017	0.1770
RWC	0.0180	0.1360	0.0247	0.1579	0.0065	**0.6029**	0.0223	0.6029**
PRL	0.0128	0.0666	-0.0234	0.0957	0.0002	0.0294	**0.3612**	0.3612**

Residual effect = 0.5940

r_g = genotypic correlation , *, **significant at 5 and 1 per cent level, respectively

CHL-SPAD chlorophyll reading, SGR- stay-green score, DFF-days to 50% flowering, LAI- leaf area index, PHT-plant height, RWC-relative water content, PRL- proline content