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RESEACH ARTICLE

Evaluation of *Saccharum spontaneum* Clones for Water Deficit Stress Tolerance

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Abstract

Forty *Saccharum spontaneum* accessions were evaluated for water deficit stress tolerance in augmented design along with three standards. After 90 days of the planting water stress was imposed by withholding the irrigation for a period of eight weeks. Morphological traits *viz.,* plant height and number of tillers per clump and physiological traits *viz.,* relative water content, membrane injury index and chlorophyll and carotenoid contents were recorded periodically. Total fresh biomass and dry biomass were recorded after harvesting the crop at the 11th month of the crop growth. Nine genotypes *viz.,* IND 08 – 1491, IND 99 – 848, IND 99 – 847, IND 99 – 863, IND 99 – 849, IND 99 – 882, IND 02 – 1186, SES 121A and IND 99- 850 were found to be superior for maximum no. of drought tolerance related traits. From the correlation analysis among the traits recorded during stress it is found that apart from the plant height and number of tillers per clump, chlorophyll 'a' (0.343*), total chlorophyll (0.326*) and carotenoid (0.320*) contents during stress had a significant positive correlation with total dry biomass. Relative water content had significant positive correlation (0.320*) with the plant height during stress. The correlated traits can be used to identify the drought tolerant genotypes. The identified tolerant genotypes can be evaluated further in replicated trial and can be utilized in sugarcane improvement programmes.

Key words: *Saccharum spontaneum*, Water deficit stress, Morphological traits, Physiological traits, Correlation.

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Introduction

Sugarcane is an important source of sugar and bio energy in the world. Drought stress is becoming a major threat to sugarcane production worldwide in view of climate change. Water deficit stress affects the growth and development of the plant by altering different physiological, biochemical and molecular processes. The dry matter production in plants is directly associated with the water used by them (Tollenaar and Aguilera, 1992; Qing *et al*. 2001). The formative phase (tillering and grand growth stage) of sugarcane is the critical period for water use and water deficit stress during this stage can lead to severe yield loss. Breeding of crop varieties with high yield under unfavorable environmental conditions is a major challenge in view of growing population and changing climate scenario. Among the wild species of sugarcane, *Saccharum spontaneum* is well adapted to unfavourable natural environments and constitutes the primary source of genes for tolerance to biotic and abiotic stresses. However, the utilization of this genetic resources in developing commercial varieties with stress tolerance is hindered by the lack of knowledge about their genetic and phenotypic properties.

S. spontaneum is a perennial grass that can grow well in marginal soils and highly adaptable to various stress conditions. It is also characterised by its high vigor, ratooning ability, high biomass producing ability, resistance to pests and diseases. It is a highly diverse species with a reported 30 cytotypes from Indian subcontinent with a varying chromosome number from 2n = 40 to 128 (Sreenivasan *et al*. 1987) and has wide distribution in India from the sub-Himalayan regions to the peninsular region. Co 205, the first interspecific hybrid variety of sugarcane which leads to a revolution in sugarcane agriculture worldwide was derived from the cross between *S. officinarum* clone Vellai and *S. spontaneum* clone Coimbatore local. Modern sugarcane cultivars are complex aneupolyploids derived from the interspecific crosses involving *S. officinarum* and *S. spontaneum*. By understanding the importance of *S. spontaneum* in sugarcane improvement programs, explorations for *S. spontaneum* in India was started in the year 1933 (Amalraj and Balasundaram, 2006) and still it is continuing. Presently ICAR- Sugarcane Breeding Institute, Coimbatore is the custodian of world's largest field gene bank of *S. spontaneum* with 1963 accessions. This *S. spontaneum* collection is attributed with wide range of phenotypic variations (Kandasami *et al*. 1983; Rao and Vijayalakshmi 1963; Sreenivasan *et al*. 2001; Govindaraj *et al*. 2014).

At present, a very few *S. spontaneum* genotypes have been utilized in commercial sugarcane breeding programmes. The trait specific characterization and extensive utilization of germplasm collections are lacking behind, albeit the availability of a large germplasm collection. Incorporating new germplasm lines in the breeding programme not only release huge genetic variation but also lead to broadening of the genetic base. Hence this study was aimed to identify drought tolerant *S. spontaneum* accessions for further exploitation to combat with climate change challenges.

Materials and Methods

A set of 40 *S. spontaneum* accessions were planted in augmented design along with three standards *viz.,* Ponape 1, Pampa and Taiwan 96 during 2017-18 crop season at ICAR-Sugarcane Breeding Institute, Coimbatore. The plant material consisted of genotypes collected from different states of India *viz.,* Kerala, Tamil Nadu, Andhra Pradesh, Telangana, Rajasthan and Karnataka as a part of germplasm exploration and collection. Each genotype was planted in a 3 m row with spacing of 2.4 m in between the rows. Standard package of practices was followed to raise a good and healthy crop. At 90 days of crop growth plant height (cm), no.of tillers per clump, leaf midrib thickness (mm) and leaf lamina width (cm) were recorded. Plant height was measured from the soil surface to the first fully expanded leaf. After 90 days of the crop growth, during the tillering stage, drought was imposed by withholding the irrigation for eight weeks.

Plant height and number of tillers per clump were recorded just before the imposition of drought (at 90 days of crop growth) and four and eight weeks after commencement of the drought. The same observations were recorded after the crop revived from the stress at 210th days of the plant growth. The growth rates for plant height and no. of tillers per clump during the stress period were calculated as follows,

(The observed value at the end of the drought period– The observed value at the start of the drought period)/ total number of days in the drought stress period.

Since the measurements had been recorded on the same experimental units repeatedly over time, data recorded at different time intervals tend to be highly correlated. The analysis of variance for these two traits was done using repeated measures analysis in R software. Different variance-covariance structures such as compound symmetry (CS), autoregressive 1 (AR 1) and the unstructured (UN) were employed for obtaining the best fit. To identify the best model information criteria *viz*., the Akaike (AIC) and Bayesian (BIC) were used. Parameters were estimated by the method of restricted maximum likelihood (REML). Random coefficient models were constructed for both these traits, where measurements were recorded over time periods. In these models, time was considered as fixed effect and genotypes were taken as random. This mixed model equations were analysed in lme4 package (Bates *et al*. 2015) in R. The random slope effects were then used for constructing the biplot, by considering slope effects for plant height and tiller number on X and Y axis, respectively.

Relative water content (RWC) of the excised fresh leaves were estimated during the stress period according to the method of Barrs and Weatherley (1962) based on the following formula:

$$\text{RWC (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid Weight} - \text{Dry Weight})] \times 100.$$

After taking the fresh weight, the leaves were soaked in water for four hours at room temperature for recording the turgid weight. Dry weight was recorded after drying the leaves at 80^{o}C for 48 hours.

The chlorophyll and carotenoid content in the leaves were estimated using dimethyl sulphoxide (DMSO) method (Hiscox and Israelstam,1979) during the drought stress and the revival period. The absorbance of the aliquot obtained after DMSO extraction, was measured at 663, 645 and 470 nm using UV visible spectrophotometer. Chlorophyll 'a' and 'b', and total chlorophyll content were calculated according to Arnon's (1949) and carotenoid content was calculated according to Lichtenthaler and Wellburn (1983) and expressed as mg g^{-1} fresh weight.

Membrane injury index was estimated as per Deshmukh *et al*. (1991); wherein 100 mg fresh leaf samples were collected from the 3rd leaf of the drought induced plants and placed in a test tube containing 10 mL of double distilled deionized water. Tubes were incubated at 45^{o}C in water bath for 30 minutes and electrical conductivity (C1) of the solution was measured with EC meter. The test tubes were subsequently shifted to boiling water bath (100°C) for 10 minutes and cooled at room temperature and conductivity (C2) was measured again. The membrane injury index was calculated as given below:

$$\text{Membrane injury index} = \frac{C1}{C2} \times 100$$

Where,
C = conductivity in millisiemens

Drought scoring of each genotype was done during stress period using a 0 to 9 scale (Table 1) according to Ishaq *et al.* (2008). Besides this, the proportion of dryness was calculated by counting the dried and green leaves in each genotype. The percent dryness was calculated as follows:

Proportion of dryness (%) = Number of dried leaves in the plant / Total no. of leaves in the plant

The total fresh and dry biomasses were recorded after harvesting the crop at the 11th month. Pearson's correlation coefficients between the traits recorded during drought stress were calculated. Analyses of variance for all the observations except plant height and no. of tillers per clump were done by using the SPAD software package for the augmented design. Rest of the analyses were performed in Microsoft excel, unless specified.

Results and Discussion

The analysis of variance for all the observed traits during the stress and revival period showed that the genotypes taken for this study had significant genetic variation. The growth rate during the stress period (8 weeks) was calculated for the plant height and number of tillers per clump. As compared to the plant height growth rate for the tiller count was less and it ranged from -0.19 to 0.16. This can be attributed to tiller mortality during this phase, wherein only certain tillers would mature to form shoots. This tiller mortality is more pronounced in the case of drought. The lowest growth rate for the no. of tillers per clump was recorded in the genotype IND 02-1194 and the highest was in IND 08- 1491. The growth rate for plant height ranged from 0.01 (IND 03-1224) to 1.32 (SES 121A). During the stress period, the plant height and no. of tillers per clump were measured in monthly intervals. Since the measurements had been recorded on the same experimental units repeatedly, the data recorded at different time intervals were highly correlated and the data were subjected to repeated measures analysis. Among the analysed variance – covariance structures, compound symmetry was the ideal one to get best fit for no. of tillers per clump and in case of plant height it was unstructured, as they had corresponding lower AIC and BIC values. The covariance structures and information criteria were presented in Table 2.

Table 1: Scale used for drought scoring in sugarcane according to Ishaq *et al.* 2008

Scale	*Description*	*Rate*
0	No symptoms	Highly tolerant
1	Slight tip drying	Tolerant
3	Tip drying extended to 25% length in most leaves	Moderately tolerant
5	25% to 50% of all leaves fully dried	Moderately susceptible
7	More than 66% of all leaves fully dried	Susceptible
9	All plants apparently dead	Highly susceptible

Table 2: Covariance structures and information criteria for no. of tillers per clump and plant height

Information criteria	*Variance- covariance structures*		
	CS	AR1	UN
	No.of tillers per clump		
AIC	1239.67	1245.22	1238.41
BIC	1616.37	1621.69	1626.28
	Plant Height		
AIC	1262.78	1249.26	1231.62
BIC	1639.25	1625.73	1619.49

The ANOVA (Table 3) showed that the time x treatment interaction was significant for no.of tillers per clump as well as plant height at 1% level of significance. It indicated that there was significant influence of drought on both the traits as the genotypes varied for their height and tiller numbers across the time periods. In case of plant height, there was no or very little improvement in a few genotypes *viz.*, IND 02-1194, IND- 03-1224, IND 03-1226, IND 03-1227, IND 03-1247 and Ponape 1,whereas genotypes like SES 121A, IND 99-847, IND 99-849, IND 99-850, IND 99-863 and IND 02-1166 exhibited a considerable growth in plant height during stress period. There was a general reduction in the number of tillers in all the genotypes during stress period. Severe reduction in the no. of tillers was observed in genotypes like IND 08-1492, IND 99-882, IND 03-1224, IND 02-1214 and IND 02-1186. Genotypes *viz.*, IND 99-863, Taiwan 96, SES 121A, IND 03-1250 and IND 08-1491 reported comparatively less reduction in tiller number during drought stress.

Random coefficient models were constructed by considering time period as fixed effect and genotypes as random, assuming both random intercepts and random slopes for plant height and tiller number. Overall slope of fixed effect was positive for plant height, whereas for tiller number it was negative, indicating gradual decrease in tiller number over time, because of tiller mortality. Large variance of random effects for plant height as compared

Table 3: Analysis of variance for number of tillers per clump and plant height using restricted maximum likelihood (REML) method

	DF		*F-value*		*p-value*	
	No. of tillers	*Plant height*	*No. of tillers*	*Plant height*	*No. of tillers*	*Plant height*
Replication	1	1	3.81	1.165	0.0530	0.2824
Time	2	2	107.41	148.69	<0.0001	<0.0001
Genotypes	42	42	3.36	8.667	<0.0001	<0.0001
Time x Treatment	84	84	1.60	2.012	0.0081	0.0002

Table 4: Genotypes with higher growth rate for number of tillers per clump than the best standard

Accessions	*No. of tillers/plant*	*Plant height*
IND 08–1491	0.16	0.12
SES 121A	0.12	1.32
IND 99–864	0.10	0.30
IND 99–850	0.07	0.55
IND 99–849	0.06	0.57
Taiwan 96	0.05	0.14

to tiller number highlighted that the accessions highly differed for increase in plant height (growth rate) before start of the drought period as compared to tiller number. On similar basis, slope variances were larger for plant height indicating varied differential response of genotypes for the trait in contrast to tiller number. Random slope estimate is the measure that relatively evaluates genotypes for their performance during the time period. In this scenario, positive slope estimates indicated that there has been increase in the trait value over time and vice versa. Hence, the genotypes IND 99 – 848, IND 99 – 849, IND 99 – 850, IND 99 – 851, IND 99 – 881, IND 99 – 882 having positive slope estimates for both the traits can be considered superior, by virtue of their increasing height and tillers during drought period (Figure 1). Although SES 121A had highest slope for plant height, there was a marginal reduction in tillers during this period.

From the above data, it is observed that number of tillers per clump was more affected by the drought stress than the plant height. High tillering is one of the peculiarities of this wild relative of sugarcane and the effect of water deficit stress on this trait may have a major impact on the total biomass production.The reduction in plant height and no. of tillers per clump may be due to the reduction in the cell enlargement and increased leaf senescence under water stress as reported by Munawarti *et al*. (2014). Reduction in plant height and number of millable canes in sugarcane due to water stress was reported by Hemaprabha *et al*. (2013); Venkataramana *et al*. (1986); Singh and Reddy (1980) etc. The genotypes with superior growth rate for number of tillers per clump than the best standard during the water stress period were given in Table 4 along with the growth rate for the plant height. Similarly, after the revival from the stress, ten genotypes exhibited significantly high plant height than the best standard Taiwan 96 but in case of no. of tillers per clump only two genotypes had a significant growth than the best standard Ponape1.

Relative water content (RWC) is extensively used in drought experiments to determine the internal water status of the plants. Maintenance of leaf water status is essential for continuation of physiological and metabolic activities. RWC of the genotypes was assessed during the 8thweek of the water stress at 10.81% soil moisture content and it ranged from 53.57 to 80.46%. The highest RWC was recorded by SES 121A and the lowest by IND 02–1194. Only one genotype SES 121A recorded significantly high RWC than the best standard Taiwan 96 (73.58%). The lowest RWC

Table 5: *S. spontaneum* genotypes superior for maximum number of drought tolerance traits

	Percent increase during stress in			*Mean increment rate during stress for*	
Accessions	*Carotenoid*	*Chlorophyll A*	*RWC (%)*	*Plant height*	*No. of tillers/ plant*
IND 08–1491	72.46	56.72	66.71	0.12	0.16
IND 99–848	60.72	51.83	74.29	0.28	-0.05
IND 99–847	57.23	12.69	74.34	0.60	-0.02
IND 99–863	41.88	31.69	76.43	0.58	0.03
IND 99–849	15.17	2.80	71.68	0.57	0.06
IND 99–882	12.87	18.88	66.69	0.34	-0.15
IND 02–1186	6.07	6.28	66.81	0.26	-0.06
SES 121A	-18.01	-9.01	80.46	1.32	0.12
IND 99–850	-18.98	-23.83	74.09	0.55	0.07

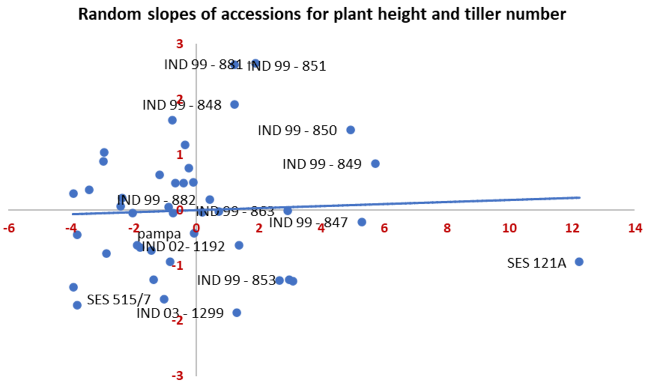

Figure 1: Biplot indicating genotypes with positive slope for both plant height (X axis) and no. of tillers/clump (Y axis)

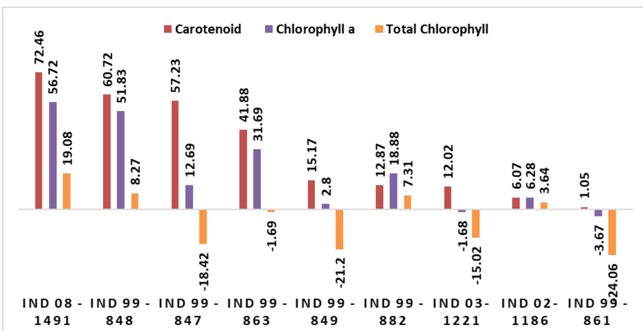

Figure 2: Genotypes with increased carotenoid and chlorophyll content during water deficit stress. Values indicate percent increase in photosynthetic pigments

of IND 02-1194 may have contributed to its lowest growth rate for no. of tillers per clump during stress period. The SES 121A with highest RWC during stress also had highest growth rate for plant height. Augustine *et al.* (2014) reported that the drought tolerant *Erianthus arundinaceus*, wild relative of sugarcane, genotype IK 76-81 recorded high relative water content during drought stress compared to the sugarcane variety Co 86032.

Chlorophyll and carotenoid contents were estimated during the 8th week of the stress (soil moisture content 10.81%) and after the recovery from the stress. The ranges of chlorophyll a, b, total chlorophyll and carotenoid contents during stress were in 0.39 to 1.89 mg/g, 0.22 to 0.62 mg/g, 0.61 to 2.52 mg/g and 0.16 to 0.75 mg/g respectively. A general reduction in chlorophyll and carotenoid contents were observed during stress period as compared to the recovery period from the stress except for few genotypes. The decrease in photosynthetic pigments under drought stress has been considered as an indication of oxidative stress and may be due to pigment photo-oxidation and chlorophyll degradation (Munawarti *et al.* 2014). Few genotypes exhibited an increased chlorophyll a, total chlorophyll and carotenoid contents during the stress period compared to the period after recovery from the stress (Figure 2). These genotypes also exhibited superiority for early growth traits like plant height, tiller number, stalk diameter, no. of leaves on main stem as reported in the previous study (Pathy *et al.* 2022). An increased chlorophyll a/b ratio was observed during the stress compared to the period after recovery. High photosynthetic pigments during stress are an indicative of water stress tolerance. Higher amounts of chlorophyll-a and chlorophyll-b attribute to the accumulation of solutes in the cell sap through passive accumulation resulting from reduced cell size which significantly contributes to increase in osmotic potential thereby resulting in osmotic adjustment ($\Delta\psi\pi$). Energetic status of the chloroplast increases as a consequence of the drought stress which has a direct relationship to that of increased amount of total chlorophyll, chl a and chl b (Ranjbarfordoei *et al.* 2000).

The cellular membrane thermostability assay indirectly measures integrity of cellular membrane through quantifying electrolyte leakage after heat treatment. The cell membrane injury at 7.87% soil moisture content ranged from 28.24 to 71.16%. The minimum cell membrane injury was recorded in the standard Ponape 1 while the maximum was recorded by the genotype IND 99–882. Nine genotypes recorded lower membrane injury during stress period and they were on par with the best standard Ponape 1. The genotype SES 121A which exhibited higher growth rate and high RWC during stress also had a lower membrane injury (38.29%). Membrane injury was recorded in these genotypes after recovering from the stress and it is found that all the genotypes recorded a lower membrane injury percent compared to the stress period. The range of membrane injury per cent was 26.76 to 60.07% after the recovery from the stress.

Drought scoring was done in a 0 to 9 scale (Table 1) according to Ishaq *et al.* (2008) during the peak drought stress and the proportion of dryness was also calculated. Seven genotypes *viz*., IND 08-1491, IND 08-1492, IND 99-864, IND 03-1250, IND 03-1301 & SES 121A scored '1' indicating that they were 'tolerant' to drought. All other genotypes were scored as moderately tolerant. Being wild relative of sugarcane, *S. spontaneum* is considered as a hardy crop, which has wider adaptation to different environmental conditions. The proportion of dryness (%) was calculated by counting the number of dried and green leaves in each genotype. The proportion of dryness varied from 14.07-49.08%. The minimum dryness was observed in IND 08-1492 and the maximum was in IND 03–1220. The per cent dryness in the genotype SES 121A was 16.35%.

Total fresh and dry biomasses of the genotypes were recorded after harvesting the crop in the 11th month. The genotypes with highest fresh and dry biomasses wereIND 99–850 (11.895 & 6.380 Kg), IND 99–982 (6.480 & 3.360 Kg) and IND 99–851 (6.475 & 3.365 Kg). The best standard was Taiwan 96 with 2.645 Kg fresh biomass and 1.435 Kg dry biomass. Nine genotypes had significantly high fresh biomass than the best standard Taiwan 96 and four genotypes had significantly high dry biomass than the best standard. Nine S. *spontaneum* genotypes promising under drought condition were identified from this study. These genotypes recorded best values for maximum no. of drought tolerant traits. The details of these genotypes are given in Table 5. This is preliminary evaluation and the genotypes identified need to be evaluated in a replicated trial for multiple years or in different environments.

Pearson's correlation coefficients were worked out between the traits during and after the stress period. Plant height and no. of tillers per clump during the 8^{th} week of the water stress had a significant positive correlation with the total fresh and dry biomass (0.570** & 0.597** and 0.650** & 0.632**, respectively). Chlorophyll 'a' (0.343^{*}), total chlorophyll (0.326^{*}) and carotenoid (0.320^{*}) contents during stress exhibited a low but significant positive correlation with total dry biomass after the harvest. Relative water content had a low significant positive correlation (0.320^{*}) with the plant height during stress. The leaf midrib thickness of the clones showed a positive relationship with plant height during stress (0.432^{**}), fresh biomass (0.417^{**}) and dry biomass (0.418^{**}).The significant positive correlations of morphological traits and photosynthetic pigments with total biomass in this study suggest that these traits can be used to identify the drought tolerant S. *spontaneum* genotypes.

Conclusion

The identified nine drought tolerant genotypes will be evaluated further in replicated trials in multiple seasons/ locations and can be used in the development of drought tolerant sugarcane varieties. In the present scenario of climate change and erratic weather conditions, equipping our breeding programmes with drought/water logging tolerant pre-breeding lines is utmost important in long duration crops like sugarcane. From the correlation analysis it is concluded that photosynthetic pigment contents during drought stress can be used as selection criteria for identifying drought tolerant *S. spontaneum* lines.

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