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**Koujalagi D**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

**Jeena AS**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

**Singh SP**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

**Tabassum A**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

**Khan KA**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

**Correspondence****Koujalagi D**

Department of Genetics and  
Plant Breeding, GB Pant  
University of Agriculture and  
Technology, Pantnagar,  
Uttarakhand, India

## Study of selection parameters for different drought tolerance imparting Physio-biochemical traits in sugarcane (*Saccharum Sp. complex*)

**Koujalagi D, Jeena AS, Singh SP, Tabassum A and Khan KA**

**Abstract**

The aim of the present investigation was to estimate the selection parameters for Different Drought Tolerance Imparting Physio-Biochemical Traits in Sugarcane (*Saccharum Sp. Complex*). The Physio-Biochemical traits involved in study were days to germination, germination %, SPAD readings, Canopy temperature depression, relative water content, Chlorophyll pigments, proline content, leaf firing and drought tolerance capacity and the selection parameters estimated are mean, range, Phenotypic coefficient of variation (PCV), Genotypic coefficient of variation (GCV), heritability and genetic advance over mean. The results obtained from the study reveals higher mean and wider range for all the traits especially for germination percent, SPAD 60, CTD 120, RWC percent, leaf firing, proline content and drought tolerance capacity. High degree of PCV with moderate GCV was observed for the traits like CTD 60, CTD 120, CTD 180, Total carotenoids, DTC, Chl a, Chl b, Total chlorophyll, Proline content, RWC % and Leaf firing. Heritability and genetic advance was recorded to high for CTD 60 (93.1 % & 66.240 %), CTD 120 (92.4 % & 77.616 %), CTD 180 (89.8 % & 65.020 %), Total carotenoids (68.755 %), Leaf firing (94.15 %), DTC (94.15 %), Chl a (54.564%), Chl b (70.529%), Total chlorophyll (31.059 %) and Proline content 64.790 %). On the basis of an overall consideration of the selection parameters it may be concluded that C<sub>1</sub> clonal population have the potential source for improving the drought tolerance and its associated traits.

**Keywords:** Coefficient of variation, heritability, genetic advance, selection parameters and variability

**Introduction**

Sugarcane (*Saccharum officinarum* L.) a perennial monocotyledonous crop and of a great worldwide economic importance, contributes for approximately 75% of the global sugar production and is gaining relevance in the generation of renewable energy (Commodity Research Bureau, 2015) [6]. Sugarcane is an important industrial crop, ranking among the ten most planted crops in the world. In recent years, sugarcane has become established as a source for the production of ethanol and the co-generation of electricity. Currently new technological opportunities emerging to produce new products (polymers, farnesene, bio-butanol and bio-kerosene) by using sugarcane crop as the raw material (Koujalagi *et al.*, 2017a) [16].

Sugarcane (*Saccharum* spp.) belongs to the family *Poaceae*, sub-family *Panicoideae* and tribe *Andropogoneae*. The family contains 13 subfamilies (Sanchez-Ken *et al.*, 2007) [29], comprising a monophyletic clade that shows some peculiarities, such as the presence of a caryopsis fruit and a well differentiated lateral embryo, a unique combination among monocotyledonous plants (GPWG, 2001) [12]. The tribe *Andropogoneae* contains species that are mostly polyploid and perennial and have a C<sub>4</sub> photosynthetic mechanism. It is one of the biggest tribes of the *Poaceae* family and is extensively distributed in tropical and subtropical regions of the world (Clayton and Renvoize, 1982; Sanchez Ken and Clark, 2010) [5, 28]. The genus name *Saccharum*, is derived from the sanskrit word "sarkara" which means white sugar, which reminds that the plant travelled to the Mediterranean region from India.

In India the sugarcane production recorded 348.44 million tons from an area of 4927 thousand hectares with the productivity of 70.7 tons/ha during 2016-17. There are 526 factories operating with an average duration of 117 days producing 25.12 million tons of sugar and 10885 thousand tons of molasses with an average sugar recovery of 10.62 percent. India exports sugar worth of 8639.83 crore to various countries. There are nearly 4 million growers engaged in cultivating sugarcane occupying 2.5 percent of the total cultivated area. (Indian Sugar, 2017) [13].

Worldwide, water is the major limiting factor to the productivity of rain-fed crops and those with supplementary irrigation. It is likely to further constrain crop production where seasonal rainfall is predicted to be more variable and/or decline and so is the case in sugarcane

(Davies *et al.*, 2011) [17]. Low output of cane, low sugar recovery from net weight of cane and subsequent reduction in sugar per unit area has been attributed to severe drought stress (Moore, 1987) [21]. The ecology is characterized among others by erratic rainfall distribution and/or abrupt cessation of rains during the growing season, thus constituting the greatest hindrance to increase sugarcane production (Olaoye, 1999) [23].

Drought causes several effects in sugarcane. There is evidence that stomata closure, intended to reduce water loss, is triggered by a combination of the water status of adjacent cells, intensity of photon flux and the water deficit sensed by roots. Drought reduces transpiration and photosynthesis and increases leaf temperature. Sugarcane cultivars differ in their responses to drought stress. Usually, the assays to infer the tolerance to drought are done using different cultivars that are ranked according to their yield under drought stress (Rodrigues *et al.*, 2009; 2011) [26, 27].

To counter with water limitation, plants have adopted diverse mechanisms of resistance to drought escape via reduction of the life cycle, drought avoidance and drought tolerance. Plants drought tolerance capacity allows keeping up their metabolism, following a decline in their tissue water potential (Mitra, 2001) [20]. In this process a chain of plant physiological, biochemical and gene regulatory interactions occurs, as well the interactions between plant and microorganisms (Del Pozo *et al.*, 2012) [8].

The two useful parameters: genetic variability and heritability can help the breeder during different stages of crop improvement. High magnitude of variability in a population provides the opportunities for selection to evolve a variety having desirable characters (Arya *et al.*, 2013) [1]. Therefore, it is necessary to estimate and study the genetic variation and mode of inheritance in different physio-biochemical parameters to initiate productive breeding programs. Study of selection parameters from segregating population is useful in understanding the genetic consequences of hybridization. The heritability of a character describes the extent to which it is transmitted from one generation to the next. The genetic advance is the further estimation of expected gain resulting from selection pressure in breeding material (Koujalagi *et al.*, 2017b) [15].

Development of stress resistant genotypes is a durable, eco-friendly and less expensive solution (Moore, 1987) [21]. Hence, there is an instant need to discover sugarcane varieties apt for moisture deficit conditions to set off sustainable sugarcane production. Considering the importance of moisture deficit stress, this study is designed to estimate and study the genetic variation and genetic parameters in early generation clones of sugarcane for physiological and biochemical traits which are considered to be concerned with drought tolerance mechanism.

### Material and methods

The experimental material consisted of 65 C<sub>1</sub>, seven parents and six standard check varieties. C<sub>1</sub> clones were planted using single budded setts during March (Spring 2017-18) using augmented block design with three blocks and six checks in rain out shelter (Polyhouse) at PCPGR to evaluate the response of these clones to drought stress. The drought stress was created by withdrawing the irrigations during the 50 DAS to 200 DAS which overlaps with the grand growth phase and tillering phase of the crop. These two growth stages of crop are considered to be most critical for water requirements during the entire crop duration and are considered to be one of

the major cause for yield loss. The details of characters recorded are given below.

### Days to Germination

Number of days taken from the date of sowing to the day on which most of the buds in each of the entry emerge out of the soil was recorded.

### Germination Percent at 45 DAS

Germination is the process of sprouting and emergence of plant from base cane sets. It was recorded after 45 days of planting. The formula for calculating of germination percentage is given below:

$$\text{Germination \%} = \frac{\text{Number of buds germinated}}{\text{Number of buds planted}} \times 100$$

### Canopy Temperature Depression (CTD) at 60 DAS

Canopy temperature depression of the clones was recorded using hand-held infra-red thermometer (IRT) at 60 Days after sowing (DAS). During each Leaf Temperature measurement, the natural leaf orientation with respect to the sun was maintained to avoid shade effects.

### SPAD Readings at 60 DAS

Chlorophyll content of the leaves was recorded non-destructively using SPAD meter at 60 Days after sowing (DAS).

### Canopy Temperature Depression (CTD) at 120 DAS

Canopy temperature of the clones was recorded using hand-held infra-red thermometer (IRT) at 120 Days after sowing (DAS).

### SPAD Readings at 120 DAS

Chlorophyll content of the leaves was recorded non-destructively using SPAD meter at 120 Days after sowing (DAS).

### Canopy Temperature Depression (CTD) at 180 DAS

Canopy temperature of the clones was recorded using hand-held infra-red thermometer (IRT) at 180 Days after sowing (DAS).

### SPAD Readings at 180 DAS

Chlorophyll content of the leaves was recorded non-destructively using SPAD meter at 180 Days after sowing (DAS).

### Relative Water Content (RWC %)

Relative water content was estimated using the method describes by (Smart and Bingham, 1974) [31]. The RWC was obtained as follows:

$$\text{RWC} = \frac{[(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \times 100}$$

### Proline Content

The proline content was determined using the method of Bates *et al.* (1973) [2].

### Chlorophyll a (Chl a)

Chlorophyll a (Chl a), Chlorophyll b (Chl b), Total Chlorophyll, and Total Carotenoid (Cx+c) concentrations are analyzed following the methods of Shabala *et al.* (1998) [30] and Lichtenthaler (1987) [17], respectively.

The Chl *a*, ( $\mu\text{g g}^{-1}$  FW) in the leaf tissues are calculated according to the following equations:  $\text{Chl } a = 9.784\text{D}662 - 0.99\text{D}644$

### Chlorophyll *b* (chl *b*) concentrations

Chl *b* concentrations ( $\mu\text{g g}^{-1}$  FW) in the leaf tissues are calculated according to the following equations:  $\text{Chl } b = 21.42\text{D}644 - 4.65\text{D}662$

### Total Chlorophyll concentrations

The Total Chlorophyll (TC) ( $\mu\text{g g}^{-1}$  FW) in the leaf tissues are calculated according to the following equations:  $\text{TC} = \text{Chl } a + \text{Chl } b$

### Total Carotenoid (Cx+c) concentrations

The Cx+c concentration was also measured by UV spectrophotometer at 470 nm. Cx+c concentrations ( $\mu\text{g g}^{-1}$  FW) in the leaf tissues are calculated according to the following equations:  $\text{Cx+c} = 1000\text{D}470 - 1.90\text{Chl } a - 63.14\text{Chl } b / 214$

### Drought Tolerance Capacity (DTC)

Drought tolerance capacity were determined following Na-EDTA method developed by Quantitatively, fall of pH  $\geq 3$  % indicates as drought tolerant genotype (5) and fall of pH  $\leq 1.7$  % as sensitive genotypes (1).

### Leaf firing

Deficiency of water leads to death of tissues, which appear in the form of leaf firing. It is estimated as percent of leaf area showing firing (Riaz *et al.*, 2013) [24]. Leaf firing was classified into three classes *viz*; Sensitive (>50 % of leaf area), Moderate (20-50% of leaf area) and Tolerant (<20% of leaf area).

### Statistical analysis

Statistical analysis involves analysis of variances for augmented block-II design (Federer, 1956) [11], allows evaluation of large breeding materials and incorporates the provision of accommodating single replication of all treatments by spreading it over the blocks (b), while a set of checks (c) are replicated in each block.

### Estimation of Variability and Genetic Parameters Mean

$$\text{General mean } (\bar{X}) = \frac{\text{Sum of observations of all the genotypes}}{\text{Number of genotypes}}$$

**Range** = the minimum and maximum values for each trait within population

### Coefficient of variability

Both genotypic and phenotypic coefficient of variability were computed for each character as per method suggested by Burton and De Vane (1953) [3].

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\sigma_g}{\bar{X}} \times 100$$

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,  $\sigma_g$  = genotypic standard deviation.

$\sigma_p$  = phenotypic standard deviation.

$\bar{X}$  = General mean of the character

GCV and PCV values were categorized as low, moderate and high as indicated by

>20 %-high, 10-20 %-moderate, 0-10 %-low

### Heritability ( $h^2$ )

It was estimated in broad sense by using following formula as suggested by Lush (1940) [18].

$$h^2 (\text{bs}) = (\sigma_g^2 / \sigma_p^2) \times 100$$

**Where**,  $\sigma_g^2$  = Genotypic variances and  $\sigma_p^2$  = Phenotypic variances

The heritability was categorized as low, moderate and high as given by Robinson *et al.* (1949) [25].

>60%-high, 30-60%-moderate, 0-30%-low

### Genetic advance

Genetic advance (GA) for each character was computed by adopting the formulae given by Johnson *et al.* (1955) [14]:  $\text{GA} = h^2 \cdot K \cdot \sigma_p$

Where,  $h^2$  = Heritability of the character.

K = Selection differential which is equal to 2.06 at 5 percent intensity of selection (Lush, 1949) [19].

$\sigma_p$  = Phenotypic standard deviation of the character.

### Genetic advance as percent of mean (GAM)

$$\text{Genetic advance as per cent of mean (\%)} = \frac{\text{Genetic advance}}{\text{General mean of population (Gm)}} \times 100$$

Genetic advance as per cent of mean was categorized as low, moderate and high as given by Johnson *et al.* (1955) [14].

>20%-high, 10-20%-moderate, 0-10%-low

### Result & discussion

The results of ANOVA are summarized in Table 1. Analysis of variance for 16 characters under stress environment in spring 2017-18 indicated significant differences among all 65 bi-parental C<sub>1</sub> clones along-with parents and standards for all the characters recorded. All the characters recorded highly significant differences except for CTD 180 (3.746) which was significant at 5 % level of significance only. The significant difference among genotypes for the traits implies the presence of substantial variation among genotypes C<sub>1</sub> clonal populations which is central to the study of both quantitative and qualitative traits and gives an opportunity to plant breeders for improvement of these characters through breeding. The genetic variability parameters studied in C<sub>1</sub> clonal population are presented in Table 2.

**Table 1:** Analysis of Variance for Various Drought Tolerance Traits in Sugarcane under Spring Planted Stress Environment

Source of Variation	DF	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
Block	2	176.011***	21.647**	33.732***	48.181***	69.359***	6.655**	17.165**	2.697
Entries	77	42.844***	85.528***	45.856***	6.222***	37.936***	4.967***	60.975***	3.746*
Checks	5	50.000***	50.23***	30.247***	1.748*	26.747**	4.767**	54.748***	1.487
Varieties	71	36.399***	88.655***	40.286***	6.624***	35.444***	4.686***	53.250***	3.934*

Checks vs. Varieties	1	464.669***	146.124***	519.409***	0.052	270.781***	25.905***	640.533***	1.691
Error	10	4.566	2.526	1.446	0.402	4.330	0.660	1.773	1.370

Source of Variation	DF	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	D T C
Block	2	29.140***	0.804***	0.016***	1.045***	0.000**	13276.610***	0.078***	0.068*
Entries	77	84.542***	0.224***	0.010***	0.314***	0.000**	3266.970***	0.278***	0.086**
Checks	5	16.652***	0.024**	0.000	0.026**	0.000**	2.565	0.000	0.104**
Varieties	71	68.604***	0.233***	0.011***	0.326***	0.000**	2749.892***	0.253***	0.080**
Checks vs. Varieties	1	1555.591***	0.606***	0.029***	0.901***	0.001	56301.510***	3.403***	0.421***
Error	10	0.945	0.003	0.000	0.004	0.000	3.126	0.000	0.017

\*, \*\*, \*\*\*, Significant at 5%, 1% and 0.5 % level of significance

CTD: Canopy Temperature Depression, RWC %: Relative Water Content %, Chl: Chlorophyll, DTC: Drought tolerance Capacity

**Table 2:** Selection parameters for Drought Tolerance Traits in Sugarcane genotypes under Stress Environment

	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
GCV	15.609	18.343	15.617	33.325	15.721	39.204	17.518	33.313
PCV	24.757	18.733	16.335	34.537	16.463	40.793	19.342	35.161
h <sup>2</sup> (Broad Sense)	0.397	0.959	0.914	0.931	0.912	0.924	0.820	0.898
Genetic Advancement 5%	4.879	13.496	13.359	3.311	13.094	3.999	13.077	3.935
Gen. Adv as % of Mean 5%	20.273	36.998	30.757	66.240	30.925	77.616	32.686	65.020
Exp Mean next Generation	19.591	49.972	56.794	8.310	55.434	9.151	53.085	9.988
Mean	24.47 ± 3.24	36.20 ± 0.97	42.98 ± 1.46	4.93 ± 0.31	42.12 ± 1.45	5.09 ± 0.40	39.63 ± 2.30	5.95 ± 0.47
Range	15-30	19.75-47.95	28.20-54.81	4.56-7.88	28.95-54.32	1.15-9.15	23.48-53.44	2.33-10.62

	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	D T C
GCV	11.230	26.807	34.807	27.169	33.547	32.815	46.763	46.763
PCV	11.260	27.131	35.387	27.418	33.719	34.239	47.847	47.847
h <sup>2</sup> (Broad Sense)	0.995	0.976	0.968	0.982	0.990	0.919	0.955	0.955
Genetic Advancement 5%	16.918	0.985	0.199	1.157	0.034	101.085	3.184	3.184
Gen. Adv as % of Mean 5%	23.072	54.564	70.529	55.461	68.755	64.790	94.151	94.151
Exp Mean in next Generation	90.246	2.789	0.481	3.243	0.083	257.105	6.566	6.566
Mean	72.52 ± 0.42	1.79 ± 0.05	0.28 ± 0.01	2.07 ± 0.05	0.05 ± 0.00	151.44 ± 10.70	3.21 ± 0.24	3.31 ± 0.24
Range	55.01-86.60	1.12-3.28	0.10-0.63	1.85-3.85	0.03-0.09	92.77-233.38	1-5	1-5

### Range of variation

One of the simplest ways in which variability is assessed is by examining the range of variation. The genetic variability for most of the traits such as germination percent, SPAD 60, CTD 120, RWC percent, leaf firing, proline content and drought tolerance capacity indicated higher mean and wider range under evaluation. Proline content recorded a very wide range of 92.77-233.38 which indicates the level of variation present in the clonal population. Proline is considered to be an important solute in maintaining both turgor and the driving gradient for water uptake Cha-um *et al.* (2012) [4]. Leaf firing and drought tolerance capacity recorded all three kinds of clones which recorded sensitive, moderately tolerant and drought tolerant clones under evaluation.

### Genetic variability

Estimates of PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) for all the 16 characters under evaluation recorded high PCV and high GCV for traits like CTD 60, CTD 120, CTD 180, Total carotenoids, DTC, Chl a, Chl b, Total chlorophyll, Proline content, RWC % and Leaf firing. Presence of higher PCV and GCV for different characters allows a breeder to practice effective selection based on these characters and as their phenotypic expression would provide a good indication of the genotypic potential Tadesse *et al.* (2014) [32]. Low GCV indicated that there is less variability and the difficulty of manipulating these traits through plant breeding. Very narrow difference between the values of GCV and PCV indicated that the effect was small for the expression of these characters and these are governed by additive gene action (Koujalagi *et al.*, 2017b) [15].

### Heritability h<sup>2</sup> (Broad Sense)

A very high broad sense heritability estimates were recorded for traits like Germination % (95.9%), Chl a (97.6 %), Chl b (96.8 %), Total chlorophyll (98.2 %), Proline content (91.9 %), RWC % (99.5 %), Leaf firing (95.5 %), DTC (95.5 %), SPAD 60 (91.4%), SPAD 120 (91.2 %), SPAD 180 (82.0 %), CTD 60 (93.1 %), CTD 120 (92.4%), CTD 180 (89.8 %). The heritability (broad sense) plays a vital role in breeding plans for crop improvement. The fraction of total variation which is heritable has been termed as coefficient of heritability in broad sense (Lush, 1940) [18] or degree of genetic determination. It gives an indication of repeatability of performance if selection is practiced for the particular character.

### Genetic advance as percent of mean (GAM)

Genetic advance as percent of mean (GAM) was recorded to be high for CTD 60 (66.240 %), CTD 120 (77.616 %), CTD 180 (65.020 %), Total carotenoids (68.755 %), Leaf firing (94.15 %), DTC (94.15 %), Chl a (54.564%), Chl b (70.529%), Total chlorophyll (31.059 %) and Proline content (64.790 %) were found to have higher GAM. The bigger question of valuable population mass for improvement in character(s) for which selection is followed always was a subject of worry for plant breeders (Negi, 2017) [22]. For answering such questions, the heritability is well thought-out like a measuring rule when articulated in terms of genetic advance. Genetic advance is the handiest estimate as it is the enhancement in the genotypic value in the new population compared to base population Ebid *et al.* (2015) [10]. The estimates of selection parameters for CTD 60, CTD 120, CTD 180, Chl a, Chl b, Total chlorophyll, Total carotenoids

Proline content, RWC % and Leaf firing recorded high PCV and GCV along with high heritability and high genetic advance as percent of mean indicating that selection would be successful for these characters as there is prevalence of additive gene action in expression of these characters. Even with moderate PCV and GCV for SPAD 60, SPAD 120 and SPAD 180 along with high heritability and genetic advance as percent of mean holds promise for their utilization in selection for drought tolerance in sugarcane.

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