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 C1 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 biokerosene) by using sugarcane crop as the raw material (Koujalagi et al., 2017a) [16]. Sugarcane (Saccharum sp.) 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 C4 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
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_1, seven parents and six standard check varieties. C_1 clones were planted using single budded sets 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 DAS 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:

\[
\text{RWC} = \left[\left(\text{fresh weight–dry weight}\right) / \left(\text{turgid weight – dry weight}\right)\right] \times 100
\]

#### 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 Shahala et al. (1998) [30] and Lichtenthaler (1987) [17], respectively.
The Chl $a$ (µg g$^{-1}$ FW) in the leaf tissues are calculated according to the following equations: Chl $a = 9.784D662 - 0.99D644$

Chlorophyll $b$ (chl $b$) concentrations
Chl $b$ concentrations (µg g$^{-1}$ FW) in the leaf tissues are calculated according to the following equations: Chl $b = 21.42D644 - 4.65D662$

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

Total Carotenoid (Cx+c) concentrations
The Cx+c concentration was also measured by UV spectrophotometer at 470 nm. Cx+c concentrations (µg g$^{-1}$ FW) in the leaf tissues are calculated according to the following equations: Cx+c = 1000D470 – 1.90Chl $a - 63.14$Chl $b / 214$

Drought Tolerance Capacity (DTC)
Drought tolerance capacity were determined following Na-EDTA method developed by Quantitatively, fall of pH ≥ 3 % indicates as drought tolerant genotype (5) and fall of pH ≤ 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**

\[
\bar{X} = \frac{\sum \text{observations of all the genotypes}}{\text{Number of genotypes}}
\]

**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].

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>Days to Germination</th>
<th>Germination %</th>
<th>SPAD 60</th>
<th>CTD 60</th>
<th>SPAD 120</th>
<th>CTD 120</th>
<th>SPAD 180</th>
<th>CTD 180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>176.011***</td>
<td>21.647**</td>
<td>33.732***</td>
<td>48.181***</td>
<td>69.359***</td>
<td>6.655**</td>
<td>17.165**</td>
<td>2.697**</td>
</tr>
<tr>
<td>Entries</td>
<td>77</td>
<td>42.844***</td>
<td>85.528***</td>
<td>45.856***</td>
<td>6.222**</td>
<td>37.936***</td>
<td>4.967***</td>
<td>60.975***</td>
<td>3.746*</td>
</tr>
<tr>
<td>Checks</td>
<td>5</td>
<td>50.000***</td>
<td>50.233***</td>
<td>30.247***</td>
<td>1.748*</td>
<td>26.747***</td>
<td>4.767**</td>
<td>54.748***</td>
<td>1.487</td>
</tr>
<tr>
<td>Varieties</td>
<td>71</td>
<td>36.399***</td>
<td>88.655***</td>
<td>40.286***</td>
<td>6.624***</td>
<td>35.444***</td>
<td>4.868***</td>
<td>53.250***</td>
<td>3.934*</td>
</tr>
</tbody>
</table>

**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$_4$ 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$_4$ 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$_4$ clonal population are presented in Table 2.
The effect was small for the expression of difference between the values of GCV and PCV indicated that there is less variability and the difficulty of effective selection based on these characters and as their content, RWC % and Leaf firing. Presence of higher PCV and GCV for traits like CTD 60, CTD 120, CTD 180, Total chlorophyll, Total carotenoids, Leaf firing, proline content and drought tolerance capacity recorded all three gradient for water uptake important solute in maintaining both turgor and the driving force of osmotic potential. Proline is considered to be an important solute in maintaining both turgor and the driving force of osmotic potential. Proline content recorded a very wide range over the range of variation. The genetic variability for traits like germination percent, SPAD 60, CTD 60, CTD 120, RWC percent, leaf firing, proline content and drought tolerance capacity recorded all three gradient for water uptake important solute in maintaining both turgor and the driving force of osmotic potential. Proline is considered to be an important solute in maintaining both turgor and the driving force of osmotic potential. Proline content recorded a very wide range

### 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 h² (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 (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 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 genetic 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.

References