

**EVALUATION OF DROUGHT TOLERANCE POTENTIAL
IN EARLY GENERATION PROGENIES OF SUGARCANE
(*Saccharum* Sp. COMPLEX)**

T h e s i s

SUBMITTED TO THE



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C E R T I F I C A T E

This is to certify that the thesis entitled “**EVALUATION OF DROUGHT TOLERANCE POTENTIAL IN EARLY GENERATION PROGENIES OF SUGARCANE (*Saccharum* Sp. COMPLEX)**” submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Genetics and Plant Breeding** and minor in **Molecular Biology and Biotechnology** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona-fide* research carried out by **Mr. Deepak Koujalagi, Id. No. 49622** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

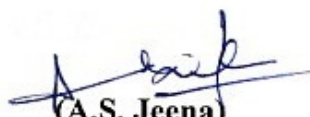
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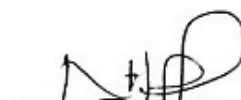
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LIST OF ABBREVIATIONS

Abbreviations	Description	Abbreviations	Description
ANOVA	Analysis of variance	d.f	Degree of freedom
<i>et al.</i> ,	and co workers	⁰	Degree
<i>viz.</i>	namely	%	Per cent
ha	Hectare	/	per
cm	Centimetre	<i>i.e.</i> ,	That is
PC	Pantnagar clone	Cov.	Covariance
m	Meter	Var.	Variance
No.	Number	q/ha	Quintals per hectare
NS	Non significant	'000/ha	Thousand per hectare
*	Significant	spp.	species
**	Highly significant	σ^2_g	Genotypic variance
PCV	Phenotypic coefficient of variation	σ^2_p	Phenotypic variance
GCV	Genotypic coefficient of variation	σ^2_e	Environmental variance
ECV	Environmental coefficient of variation	GA	Genetic advance
t/ha	Tonnes per hectare	CD	Critical difference
H ² b	Broad sense heritability	SD	Standard deviation
GAM	Genetic advance as percent of mean	SE	Standard error
r	Correlation coefficient	Avg.	Average
r _{xy(p)}	Phenotypic correlation coefficient	r _{xy(g)}	Genotypic correlation coefficient
Fig.	Figure	DAS	Days after sowing
DG	Days to germination	G %	Germination percent
CTD	Canopy temperature depression	RWC %	Relative water content percent
S	Sensitive	T	Tolerant
Chl	Chlorophyll	DTC	Drought tolerance capacity
bp	Base pairs		



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**). Sugarcane is an important industrial crop, ranking among the ten most planted crops in the world. Sugarcane is a unique crop with the ability to accumulate sucrose that can reach levels up to 50% of dry weight in its stalks (**Botha and Black, 2000**).

It is generally used to produce sugar and has lately gained increased attention because of ethanol which is derived from cane. Sugarcane bagasse (the chief waste produce generated after extraction of the sucrose from cane juice) is mostly used for energy cogeneration at the mill or for the creation of animal feed increasing by and large efficiency of the crop system. Recently, there has been increased interest in using bagasse for processes such as paper production, as a dietary fibre in bread, as a wood substitute in the production of wood composite and in the synthesis of carbon fibres (**Sangnark and Noomhorm, 2004**). The enzymatic and hydrolytic processes that allow the bagasse carbon units from cellulose and hemicelluloses to be fermented, is scaling it up for ethanol production, turning sugarcane into an efficient crop for energy production as well (**Paiva et al., 2004; Han and Wu, 2004**).

Sugarcane (*Saccharum* ssp.) belongs to the family *Poaceae*, sub- family *Panicoideae* and tribe *Andropogoneae*. The family contains 13 subfamilies (**Sanchez-Ken et al., 2007**), 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**). 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**). 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. Sugarcane belongs to the genus *Saccharum* L.

consisted of hybrids (**Price, 1965**) derived from *Saccharum officinarum* (Noble cane), *S. sinense* (Chinese cane), *S. barberi* (North Indian cane), *S. robustum* and *S. spontaneum*.

Sugarcane has one of the most complex genomes of all cultivated plant species, with a high chromosome number and high degree of aneuploidy (**D'Hont et al., 1996**). The modern hybrids have between 100 and 120 chromosomes, 70–80% of the chromosomes belong to *S. officinarum*, 10–23% of the chromosomes originated from *S. spontaneum* and 8–13% of chromosomes are derived from recombination between the species (**Piperidis and D'Hont, 2001**). The number of chromosomes can vary in commercial cultivars, the basic genome size ranges from 760 to 926 Mb, which is twice the size of the rice genome (389 Mb) and similar to *Sorghum's* (760 Mb) (**D'Hont, 2005; D'Hont and Glaszman, 2001**).

Products like sugar, sweeteners, khandsari and gur are produced to the tune of 25 million tonnes raw value makes India a leading country followed by Brazil in the second place (**Indian Sugar, 2017**). Brazil stands out as the main producer of sugarcane, with a cultivated area estimated to be approximately 8.8 million hectares (as of the 2013/2014 harvest), producing more than 600 million tons, of which 46% was used for sugar production and 54% was used for ethanol production (**Conab, 2013**).

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. Uttar Pradesh has the largest area (2160 thousand ha) among different states cultivating sugarcane whereas Maharashtra tops the list of highest sugarcane (49689 thousand tons) producer among different states and Tamil Nadu being the state with highest productivity (105 tons/ha). In Uttarakhand state, the total area under sugarcane cultivation was 1.0 lakh hectare with a production of about 5.98 million tonnes in year 2017. Haridwar, U.S. Nagar, Dehradun and Nainital are some major sugarcane producing districts in state (**Indian Sugar, 2017**).

Sugarcane cultivation requires a tropical and sub-tropical climate, with a minimum of 60 centimetres (24 inch) of annual moisture. It is one of the most efficient photo-synthesizers in the plant kingdom. Being a C₄ plant with long life cycle, it utilizes high amount of water, nutrients, CO₂ and light energy to produce a considerably high biomass (**Carr and Knox, 2011**). In major cultivating regions, such as India, Pakistan, Peru, Brazil, Bolivia, Australia, Ecuador, Cuba, Philippines, Hawaii and Colombia, sugarcane can produce 20 lb (9 kg) for each square meter exposed to the sun. Though sugarcane produces seeds, present commercial sugarcane cultivation depends on vegetative propagation through stem cuttings which has turn out to be the universal reproduction means.

In recent years, there has been a great demand for renewable energy sources to replace fossil fuels, one of the ‘villains’ of global warming. In this situation, sugarcane ethanol production has been thought as a potential substitute (**Goldemberg, 2007**). However, in order to meet this growing demand there has been an expansion of sugarcane cultivation to marginal areas, where water availability is limited or highly variable (**Azevedo et al., 2011**). The competition for land use for bio-fuels or food production in this context of water efficiency is also an important aspect to be taken into consideration (**Walker, 2010**).

Limitations such as narrow gene pool, complex genome, reduced fertility and the extended breeding/selection cycle make it tricky to carry out improvement programmes. Adding up, current varieties have inconsistent chromosome number ($2n = 100-120$) and which flowers infrequently. Tolerance to various biotic and abiotic stresses, nutrient management and enhanced sugar recovery are few of the challenges to achieve sustainable sugarcane production and productivity (**Suprasanna et al., 2011**).

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**). 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**). 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 in Nigeria (**Olaoye, 1999**). Limitation of irrigation water led to minimizing the cultivated area of sugarcane, so there is a very limited opportunity to increase it horizontally. In the sugarcane industry alone, water stress is estimated to cost an average of \$260 million per annum in lost production. These losses occur despite nearly 60% of the industry receiving supplementary irrigation (**Inman-Bamber, 2007**).

The sugarcane crop cycle usually takes from 12 to 18 months. Once the above ground plant part is harvested, it will re-grow, giving rise to a new plant that will be harvest again. This cycle usually has one plant crop and 3 to 4 ratoon (re-growth) crops. Typically, farmers have about 20% of their area with “new” plants (i.e., cane that will be harvest for the first time). Therefore, unlike other crops with shorter life cycle, such as maize, soybean and wheat, sugarcane farmers do not have any flexibility to avoid dry seasons, because sugarcane will face climate ups and downs that take places along the entire year (**Gentile *et al.*, 2015**).

Sugarcane crop growth can be divided into four stages: germination, tillering, grand growth and maturity (**Gascho and Shih, 1983**). Each of these stages is affected in different ways by water stress. Because a reduction in growth rates is one of the conserved evolutionary responses that plants activate in response to drought, the tillering and grand growth phases are known to be critically affected by water scarcity. Unfortunately, up to 80% of sugarcane yield is produced during these two phases (**Ramesh, 2000; Inman- Bamber and Smith, 2005**).

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 (**Assmann and Grantz, 1990**). 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**). Stalk yield and the content of soluble solids in the stalk juice usually are the key parameters used by breeders to classify the degree of tolerance to drought in sugarcane genotypes. This is because sugarcane productivity is based in these two indexes. Therefore, although most cultivars show decreased yields under drought, some are more affected than others (**Ribeiro *et al.*, 2013**).

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**). 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**).

The two useful parameters: genetic variability and heritability can help the breeder during different stages of crop improvement. Therefore, the knowledge of variability is desirable for a breeder before making any selection program. Genetic diversity that arises due to geographical separation or due to genetic barriers to cross ability or due to different patterns of evolution can be measured (**Adenberg, 1973**).

Development of stress resistant genotypes is a durable, eco-friendly and less expensive solution (**Moore, 1987**). 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 deficits stress, this study is designed towards identification and development of drought tolerant clones based on physiological and biochemical traits which are considered to be concerned with drought tolerance mechanism. As well as Estimation of correlation, Path coefficients and genetic diversity among C₁ clones. The present investigation involves following objectives:

1. To estimate the Genetic parameters, Correlation and Path coefficients for different Physio-biochemical drought tolerance traits among early generation sugarcane clones.
2. Estimation of genetic diversity among early generation sugarcane clones using Mahalanobis D² analysis.
3. Evaluation of sugarcane clones for their response to water stress based on Physiological and Biochemical traits.
4. Validation of candidate gene specific molecular markers for drought tolerance in C₂ clones of different Sugarcane crosses.

*Review
of
Literature*

Sugarcane is tall perennial grass of the genus *Saccharum*; with originally soft, watery culm sugarcane acquired through human selection a distinctive feature of partitioning carbon into sucrose in the stem. Sugarcane is a unique crop regarding the ability to accumulate sucrose that can reach levels up to 50% of dry weight in its stalks **(Botha and Black, 2000; Moore, 1995)**. In most countries where sugarcane is cultivated, there are several foods, drinks and popular dishes derived directly from it under different local names such as syrup, ganne ka ras, guarab, cachaca, rum, falernum, jaggery, panela, molasses, rapadura, rock candy etc.

It originated in the tropics near New Guinea in the South Pacific about 8000 years ago and spread to Soloman Island, the New Hebrides and then to New Caledonia **(Brandes, 1956)**. Its migration spread along human migration routes to South Asia, India and Polynesia. By 327 B.C. it had become an important crop of India. Eastward, it migrated with Pacific Islanders and reached in Hawaii between 600 and 1100 A.D. Sugarcane was introduced in China from India around 800 B.C. It was commercially exploited by Indian Sub-continent in 700 A.D. The Egyptians developed clarification, crystallization and refining processes **(Raza, 2013)**.

It is widely cultivated in tropical and subtropical countries for sugar and biomass production. It ranks sixth in the total production of crops i.e. wheat, corn, rice barley, soybean, sugarcane, oats, other food crops. Worldwide, it is grown on an area of 17 million hectares in over 110 countries with an estimated production of 1, 683 million metric tons which is 22.4% of the total agriculture production by weight **(Moore and Fitch, 1990)**.

Abiotic stresses such as drought, salinity, extreme temperature, chemical toxicity and oxidative stress cause major crop losses worldwide, reducing potential yield up to 70% and average yields of most major crop plants by more than 50% **(Bray et al., 2000)**. The potential yield losses by individual abiotic stresses are estimated at 17% by drought, 20% by salinity, 40% by high temperature, 15% by low temperature and 8% by other factors.

Water is very essential for plant growth and makes up 75 to 95 percent of plant tissues. A vast amount of water moves through-out the plant daily. Plant uses water and carbon dioxide to form sugars and complex carbohydrates. Water acts as carrier of nutrients and also a cooling agent. It also provides an element of support through turgor and as an intercellular reaction medium (**Ashraf and Harris, 2005**).

Knight and Knight (2001) described that water stress imparted by drought and temperature severity is the most prevalent abiotic stress that limits plant growth and productivity. The drought badly affects agriculture, hydropower, water supply and industrial sectors. Repeated drought and unfavourable human activities might cause the desertification of vulnerable arid, semi-arid and dry sub-humid areas (**Kundzewis, 1997**). Worse impact of drought was reported in India which was further provoked by the epidemics followed by famines. The drought with other linked famines affected the Indus Basin during the 19th and the first half of the 20th century, famines averagely came about after 7 to 8 year (**Indian Famine Commission, 1880**). It has been estimated that drought cause an average annual yield loss of 17% in the tropics but losses can be much more severe and total crop failure is possible (**Edmeades *et al.*, 1999**).

The world population is expected to reach from the current 6.7 billion to over 9 billion by 2050 (**Eckardt, *et al.*, 2009**). About 80 million people are being added to the population total each year and 97% of the predicted population growth will take place in developing countries. It is predicted that there is need to produce double food in order to feed over 9.0 billion people by 2050. Increased food production implies increased demand for and consumption of water. This will certainly place more pressure on the environment. The developing countries in particular, are confronted with severe food security challenges (**Raza, 2013**). To face these challenges, there is utter requirement of stress tolerant crops especially against drought to meet the future world food needs. That's why drought tolerance has come to the forefront of agronomic research in recent years due to dwindling irrigation reserves and increased costs associated with irrigation application (**Gowda *et al.*, 2007**).

Studies related to drought tolerance in sugarcane have focused on agronomic, physiological and biochemical traits, which may help in developing and adapting new cultivars to adverse conditions (**Silva *et al.*, 2014**). These responses may be complex due to the genetic variation between cultivars, plants developmental stage and stress duration and severity (**Hemaprabha *et al.*, 2013**).

Keeping all these above points in view, the following review of literature appertained to the objectives of this investigation has been done under the following sub heads:

1. Effect of drought stress on sugarcane crop.
2. Variability, Genetic Parameters, Correlation and Path Coefficients
3. Genetic diversity estimation among sugarcane clones
4. Physio-Biochemical parameters based evaluation for response of sugarcane clones towards moisture stress.
5. Use of molecular markers in breeding for drought tolerance in sugarcane.

2.1 Effect of drought stress on sugarcane crop

Drought usually implies a stress condition that includes a water deficit in the soil, increased temperature during the day, reduced nutrient availability and eventually, increased soil salinity (**Oliver *et al.*, 2011**). Increased effects of drought are expected with climate change and increased water scarcity. Thus, studies aimed at selecting drought-tolerant cultivars to increase yields are necessary for the future of sustainable agriculture (**dos Santos *et al.*, 2014**).

For sugarcane, early development is considered the most sensitive period to water stress, in which 70–80% of its yield is produced during this phase. Therefore, any biotic or abiotic stress throughout the growing phase, especially in this period of rapid growth, can drastically reduce the yield of this crop as well as affect the potential for re-growth and longevity of sugarcane crops (**Silva *et al.*, 2012**). Limited water is one of the most important environmental stress factors that limit the sugarcane production worldwide and yield loss of more than 50% has been recorded due to drought. Moisture stress associated with high temperature is more damaging (**Venkataramana, 2003**).

The reduction in plant growth is the first response of sugarcane to water deficit; however, enhanced production of organic osmolytes (such as glycine betaine and proline), reactive oxygen species (ROS), and reduced photosynthetic capacity have been reported as physiological and biochemical responses of sugarcane to osmotic stress conditions (**Azevedo *et al.*, 2011; Silva *et al.*, 2012**).

Severe water stress, affects the entire plant. Morphological and physiological responses of sugarcane plants vary according to the genotype, the duration (rapid or gradual) and the intensity (severe or mild) of stress and also the type of the tissue affected. Water stress also affects both cane and sugar yield substantially. However, large potentially exploitable genetic variation for cane and sugar yield under water stress has been reported (**Basnayake *et al.*, 2012**). The most common water stress responses in sugarcane are leaf rolling, stomatal closure, inhibition of stalk and leaf growth, leaf senescence and reduced leaf area. Moreover, under water stress, both cell division and cell elongation are interrupted and stem and leaf elongations are the most seriously affected growth processes. Root development is also influenced by water deficit, but relatively less than the above-ground biomass (**Tsunada *et al.*, 2017**).

Water deficit can trigger a negative impact upon growth and development of the crop, compromising plant productivity especially if the drought duration exceeds the capacity of drought tolerance of the plant species (**Smit and Singles, 2006**). Regardless of the temporary nature of the stress, the set of properties and vital functions of the plant gradually decrease according to its duration (**Zingaretti *et al.*, 2012**).

Ramesh and Mahadevaswamy (1999) reported that moderate and severe drought stress decreased mean cane yields by 18.1 and 29.2%, respectively. Drought stress caused severe losses to sugarcane productivity and sucrose recovery in Indian states Maharashtra and Gujarat. It reduced cane yield 20 to 40% where as the sucrose formation and recovery reduces to 5%. It also favours the attack of certain insects like wooly aphids, stalk borers, pink mealy bug, termites and diseases like wilt and smut in sugarcane

Due to glycophytic nature of sugarcane, drought conditions interfere with sugar production by affecting growth rate, yield of the cane, juices of lower sucrose contents, purity, higher acidity and the sucrose content of the stalk. Thus drought may reduce sugarcane yield up to 50% or even more (**Raza, 2013**).

The effect of water stress on the translocation of the products of photosynthesis by sugarcane leaves was investigated by (**Hartt, 1967**) using $^{14}\text{CO}_2$. Water stress depressed translocation more than it did photosynthesis and the commonly observed increase in sucrose content during dry periods could not be reconciled with the results.

Decrease in water potential is directly related to the reduction in plant photosynthesis rates, where during water stress less carbon assimilation occurs, resulting in insufficient dissipation of electrons generated by the electron transfer chain and subsequently an overproduction of reactive oxygen species (ROS) (**Zingaretti *et al.*, 2012**).

Drought stress imposes changes on plant metabolism, but plants also actively respond to stress conditions through alterations in photosynthate partitioning between organs, metabolic flux, and the production of osmo-protectants such as carbohydrates, amino acids, and other osmolytes including proline, glycine betaine, putrescine, and γ -amino butyric acid (**Vital *et al.*, 2017**)

The deficiency of soil moisture lead to imbalance of water potential in plant tissues which results in the reduced crop growth, cane yield and sugar yield (**Srivastava *et al.*, 1997**). Cane yield was reduced by 2-35% in the parents under moderate drought stress and by 6-21% under prolonged drought stress and reduction of 5% under moderate drought stress and 21% under prolonged drought stress were recorded in the progenies of different crosses (**Ishaq and Olaoye, 2009**).

Faced with the reduction of water availability in arid and semi-arid regions, more research is increasingly needed to understand clearly the response mechanisms of sugarcane to water stress, since the search for alternatives to reduce the problems caused by this environmental stress is considered one of the great challenges of agribusiness in Brazil (**Amaral *et al.*, 2003**).

The increasing prevalence, period and intensity of severe water deficit, has encouraged many big sugarcane crop improvement programs to devote attention in water use-efficient and water stress tolerant varieties and water use-efficient crop productions systems (**Tsunada *et al.*, 2017**). The mounting knowledge of stress biology coming from genetic, agronomic and molecular biology studies in different crops, including sugarcane is providing a major thrust to develop strategies for producing water stress tolerant and commercially valuable sugarcane varieties (**Ramiro *et al.*, 2016**).

Water is necessary for several metabolic reactions and its restriction can affect all the aspects of growth and development in plants (**Larcher, 2006**). The negative

effects of water shortage over biochemical, physiological, and morphological processes can be verified in all parts of the plant. In situations of water restriction, common physiological alterations include reduced leaf water potential and the relative water content (Medeiros *et al.*, 2013).

Hemaprabha and Alarmelu (2004) Studied cane formation in 55 clones showing the impact of drought on cane growth and susceptibility of high sugared clones to water deficit stress. Mean values of all the traits were lower and CV were higher (except for cane diameter) under drought. Drastic reduction in single cane weight (64.16%) mainly through reduction in cane height (48.79%) affected cane yield under drought conditions, while juice quality traits were less affected in the high sugared clones studied. A wide range in sucrose content in the high sugared clones under drought indicated the need to identify stable high sucrose types among the high sugared clones.

2.2 Variability, Genetic Parameters, Correlation and Path Coefficients

Kamat and Singh (2001) evaluated fifteen sugarcane varieties of early and mid-late maturity group to study variability for quantitative, biochemical and juice quality characters under rain-fed condition. Quantitative characters like germination percentage, number of shoots, leaf area index in June, cane height, number of millable canes, single cane weight and cane yield recorded moderate to high heritability and genetic advance. Among biochemical parameters proline content also showed high values of heritability. Hence, while selecting varieties tolerant to rain-fed condition, these quantitative characters as well as proline content in leaf may be considered.

Patil *et al.* (2014) evaluated thirty sugarcane genotypes for cane yield, sugar yield and related morphological characters. The characters germination % at 45 days followed by tillers at 120 days and shoots at 240 days showed high GCV and PCV. High estimates of heritability along with high genetic advance (% of mean) were observed for tillers at 120 days (000/ha) followed by germination % at 45 days, internodes/stalk at 360 days, shoots at 240 days (000/ha) and stalk height at 360 days.

Priya *et al.* (2015) evaluated seventy seven clones including four checks during 2011-12 in second clonal stage for study on genetic parameters revealed that the characters viz., shoot population at 240 DAP, stalk length, number of millable canes, fibre content, brix, sucrose, CCS per cent, pol per cent cane, total sugars per cent,

biomass per cane, fibre yield, CCS yield, theoretical yield of alcohol and cane yield showed high heritability coupled with high genetic advance as per cent of mean indicating that these characters were under the influence of additive gene effects and selection would be effective for the improvement of these characters.

Wright (1921) proposed Track or pathway analysis which allows for obtaining information regarding the direct and indirect effects through the breakdown of correlations of a group of characteristics in relation to a certain characteristic that is considered more important and that should be chosen based on the researcher's experience with the species under study.

Silva *et al.* (2012) used path analysis to evaluate the physiological components of sugarcane under two water conditions, with photosynthesis as the basic variable, in order to obtain subsidies that can help the genetic breeding of this crop by selection of superior clones with drought tolerance. Data from nine physiological variables were considered, with six of these being used in the model after exclusion of those that were contributing to multi-collinearity. Variables used in path analysis were enough to explain the variation found in photosynthesis under both water conditions. Stomatal conductance, transpiration and the SPAD index should be considered as a priority in breeding programs for sugarcane aiming to get more productive and tolerant genotypes to water stress, and selection indexes should be used with due consideration of these variables to promote gains in photosynthesis.

Severino *et al.* (2002) emphasized that for breeding purposes, it is important to identify among characteristics of high correlation with the basic variable, those with the most direct effect favorable to selection, so that the correlated response through indirect selection is efficient. In addition, path analysis becomes more effective when based on genotypic values than when applied to phenotypic values, which in many cases may have little applicability and even induce the breeder to make errors.

2.3 Genetic diversity estimation among sugarcane clones

Genetic diversity arises due to geographical isolation or due to genetic barriers to crossability or due to different patterns of evolution and is important for sustainable production in crop species since greater losses of characteristics in any population may

limit its chances of survival and requires greater human efforts for successful production (Trethowan and Kazi, 2008; Bisht *et al.*, 2017).

In sugarcane, crop improvement is impeded by its narrow gene pool, complex genome, and poor fertility, caused by genetic recombination as well as long breeding selection cycle. The success of sugarcane breeding program therefore lies in the proper choice of rich and genetically diverse parents. The genetically diverse parents may be selected on the basis of diverse geographical distribution of the genotypes and information on agronomic characters (Melchinger, 1998).

Normally, the choice of parental lines in sugarcane breeding programs has been defined on the basis of agronomic characters and pedigree records, using bi-parental crosses or polycrosses between elite genotypes. The lack of genealogy data and the inadequate identification of some genotypes may impair an accurate estimation of the genetic diversity among sugarcane accessions. In addition, the continuous selection for the same traits such as sucrose content in breeding programs has caused a reduction in genetic diversity, limiting further advances in sugarcane breeding (Creste *et al.*, 2010).

The estimates of genotypic and phenotypic correlations among yield components has paved the basis for selection of superior genotypes from the diverse breeding populations, thereby establishing appropriate plant attributes for selection to improve the yield and quality status of sugarcane varieties (Shangera *et al.*, 2015)

Divergence analysis is a powerful tool in quantifying the degree of divergence at genotypic level. It quantifies the degree of divergence based on phenotypic observations in different crops. These studies have shown that accessions from the same geographical region may differ genetically as well as phenotypically and also in adaptability. The studies of correlations provide the information that how strongly traits are genetically associated with one another. Thus through the estimates of genotypic and phenotypic correlations among yield components and as a result it paved the basis for selection of superior genotypes from the diverse breeding populations (Kang *et al.*, 2013).

Singh *et al.* (2004) evaluated 51 *Sachharum* genotypes belonging to *Sachharum officinarum*, *S. barberi* and *S. sinense*, grown in Lucknow, Uttar Pradesh, India, during 2000-2002, and genetic diversity was determined using Mahalanobis D² statistics. The

genotypes were grouped into eight clusters and most of the genotypes (32) were in cluster I. Genotypes belonging to the 3 *Saccharum* spp. were randomly distributed into the clusters. The highest inter cluster distance was observed between clusters VI and VII, followed by V and VII. Single cane weight had the highest contribution in the genetic divergence of the material examined.

Mali *et al.* (2009) determined genetic divergence among 21 genotypes and found that generalized distance (D) between two populations varied from 14.043 to 43.592 demonstrating adequate diversity in the experimental material for 15 characters under study also clustering pattern indicated no association between geographical diversity and genetic divergence. Cluster V may be used for cane yield, stalk length, cane diameter, single cane weight whereas, cluster III for quality parameters *viz.*, juice brix, sucrose per cent juice, juice purity per cent and CCS per cent.

Tahir *et al.* (2013) evaluated 25 sugarcane genotypes to determine genetic divergence among the 14 parameters majority of the characters exhibited significant differences while some showed non-significant mean squares. Principal Component Analysis (PCA) exhibited two principal components accounting for 88% of the total variation in the tested breeding material and new components named “Vigour”, and “Quality”. Cluster analysis using Ward’s method on the newly created variables using principal components revealed that there are 3 clusters at a linkage distance of 4.5. Cluster I and III had 11 genotypes each and cluster II had 3 genotypes. Cluster I showed high mean values for Vigour Component while Cluster II for Quality Component and Cluster III showed genotypes with high mean yield. There was no association of the clustering with the geographic location of the genotypes.

Kumar and Kumar (2012) worked with a group of 339 accessions of sugarcane which were collected from different geographical sources to understand the nature of genetic divergence. Accession having genetic similarity were grouped in 14 non-overlapping clusters, appropriateness of the cluster arrangement was tested by sequential F-test. Entries falling into the same cluster presumably diverse very little genetically from one another with respect to aggregate of the traits studied. The maximum intercluster distance was observed between cluster VI and VII followed by IV and XI, I and IV, II and IV and VI. Cluster VI and VII was much diverse than others. Accessions belonging to different clusters having maximum genetic distance

were promising and recommended for hybridization programme to improve yield and quality and can be utilized in generating new commercial cultivar / clones.

The success of the development of new varieties of sugarcane is associated with the ability to correctly choose the best individuals to be the progenitors of the new varieties, a better understanding of the genetic diversity between the clones used as progenitors is essential in defining the new crosses (**Santos *et al.*, 2012; Brasileiro *et al.*, 2014**).

Pocovian and Mariotti (2015) deciphered genetic diversity in sixty three sugarcane (*Saccharum officinarum* L.) accessions from INTA germplasm bank (Argentina) which, will be of great importance for germplasm collection and breeding improvement as the results of diversity analysis will identify diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and clustering analysis used for this purpose.

Shehzad *et al.* (2013) evaluated eleven varieties of sugarcane (*S. officinarum* L.) for divergence analysis through various economic important character like plant height, cane height, number of tillers /plant, number of leaves/plant, leaf area, cane diameter, cane weight, dry matter contents, juice contents, sucrose value and brix value and obtained results pertaining to analysis of variance, highly significant differences among the accessions for all the traits except number of leaves/plant and considerable range of variability was observed. Brix value had the maximum contribution to genetic divergence followed by cane height and cane weight owing to genetic dissimilarity among the genotypes for these characters. The cane diameter and sucrose value had zero contribution towards the total genetic divergence owing to genetic similarity amongst the genotypes.

Bisht *et al.* (2017) in their divergence studies through Mahalanobis D² statistics grouped the 36 genotypes into eleven clusters. The maximum numbers of genotypes (21) were grouped in cluster I and the lowest (1) in cluster VI, VII, VIII, IX, X and XI. Members of cluster VII and XI were found to be genetically most diverse on the basis of their inter cluster difference as opposite to clusters I and II (10.77) which are closely related. Cane height contributed maximum (15.397%) to genetic divergence followed by Single cane weight (14.762%) and no. of millable cane (13.016%). These characters

were considered to be most vital for the genetic diversity. Lowest contribution was from juice purity percent (4.286%) followed by Cane thickness (7.301%) and Juice extraction percent (7.619%).

Tena *et al.* (2016) undertook a study to determine genetic diversity of sugarcane germplasm in Ethiopia using 400 sugarcane genotypes (174 local and 226 introduced) in partial balanced lattice design with two replications. 21 quantitative characters were observed which included cane yield and its components, sugar yield and sugar quality traits. ANOVA deciphered highly significant differences ($P < 0.01$) among the genotypes for 21 quantitative traits. Cluster analysis revealed intra cluster D^2 values ranging from 2.16 - 10.60 and inter cluster from 7.24 – 58.64. Six principal components accounting for 79.26% of the total variation in the tested materials were found. Millable stalk count, single cane weight, stalk diameter, cane yield, sugar yield and sugar quality traits showed high positive loading on the first two PCs and accounted for most of the variation observed among the genotypes.

Sajjad and Khan (2009) grouped forty accession of sugarcane into different clusters using Mahalanobis D^2 statistic technique. The Randomized Complete Block Design (RCBD) was used with three replications and block size of 10m x 5m. The characters measured were number of tillers, cane length (cm), cane weight (g), cane diameter (cm), leaf area (cm²), juice content (%), brix value (%), pol value (%), purity %, fibre % and commercial cane sugar (CCS). The contribution of each character in genetic diversity was also calculated. The most contributing character was fibre % followed by cane weight, pol %, brix value, CCS and juice contents. The cane length contributed zero. According to Tocher' method the forty genotypes were grouped into sixteen cluster.

2.4 Physio- Biochemical parameter based evaluation for response of sugarcane clones towards moisture stress

The crop has been cultivated in a region between the 35° parallels north and south of the equator, which includes both tropical and subtropical regions (**Silva *et al.*, 2010**). In many of these areas, especially in the arid and semi-arid regions, rainfall does not provide the amount of water required by the sugarcane. Therefore, water deficit is one of the most important limiting factors for obtaining high production indices of sugarcane.

Studies related to drought tolerance in sugarcane crops have focused on agronomic and physiological characteristics that may be able to assist in the development and adaptation of new genotypes that are able to withstand adverse conditions (**Jangpromma *et al.*, 2010**). Out of the four phenological stages in sugarcane life cycle, the tillering and grand growth phases have been recognized as the critical periods of water demand (**Ramesh and Mahadevaswamy, 2000**). Therefore, during this time period, the damage caused by water deficit is most detrimental to crop production.

Silva *et al.* (2007) reported that physiological characteristics such as PSII maximum quantum yield (ratio between the variable and the maximum chlorophyll-a fluorescence's Fv/Fm), leaf chlorophyll content (SPAD index) and leaf temperature can be used as indirect and reliable tools for selection of drought-tolerant sugarcane genotypes. Other variables related to gas exchange such as stomatal conductance (gs) and transpiration (E) as well as leaf water potential, by being very sensitive to water stress and having a good correlation to photosynthesis, have also been identified as promising ways for discriminating genotypes with characteristics of drought tolerance.

Chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids (Cx+c), in the salt stressed leaves of all genotypes decreased significantly, but the extent of decrease was variable among different genotypes. In contrast, proline content in salt stressed plantlets of all sugarcane genotypes increased markedly, except in genotypes K95, K92-2 and LK92-4. Maximum quantum yield of PSII (Fv/Fm), photon yield of PSII (UPSII), quantum efficiency of PSII (qP) and net photosynthetic rate (Pn) in salt stressed plantlets of all genotypes were significantly dropped (**Cha-um *et al.*, 2012**).

Plants have developed physiological mechanisms that result in saving water for later periods. Stomatal closing is the first line of defense against dehydration. Plants that possess better control of stomatal function are more drought tolerant. In fact, stomata can be regulated based on the level of water deficit by only partially closing, leading to some carbon fixation during drought conditions and an increase in the efficiency of water use (**Lawlor and Tezara, 2009**).

Silva *et al.* (2011) The physiological response of four commercial sugarcane genotypes to water stress was evaluated by measuring the photochemical efficiency of

the photosystem II (chlorophyll a fluorescence ratio, Fv/Fm), estimated chlorophyll content (SPAD unit), leaf temperature (LT) and leaf relative water content (RWC). The analysis of variance showed that the interaction of genotype x irrigation x evaluation date had a significant effect for three physiological markers tested, Fv/Fm, SPAD unit and RWC. Under non-stressed conditions, all genotypes showed similar responses for the four markers. Under water deficiency stress, two drought- tolerant genotypes, HOCP01-523 and TCP89-3505 displayed higher values for Fv/Fm, SPAD unit and RWC, and lower values for LT, and could be classified as tolerant.

Cha-um *et al.* (2012) reported positive correlation between Fv/ Fm and Φ PSII, proline content and non-photochemical quenching (NPQ), Φ PSII and Pn, and Pq and plant dry weight in the sugarcane cultivars. The results were obtained by studying Seven sugarcane (*Saccharum* sp.) commercial cultivars, viz., UT-94-2-483, LK92-11, K84-200, K97-32, K95-84, K88-92 and K 92-80, originally derived from meristem cuttings were subjected to simulated osmotic stress (as 200 mM mannitol) under controlled environmental conditions. Based upon Ward's multivariate cluster analyses of data for proline content, photosynthetic capacity, chlorophyll fluorescence, and growth inhibition, three cultivars (K88-92, K92-80 and UT-94-2-483) were identified as water deficit sensitive, whereas four (K84-200, K95-84, K97-32 and LK92-11) as water deficit tolerant.

Studies on sugarcane crops dealing with aspects of the relationship between physiological characteristics are limited, such that studies are required to better understand the association phenomena among variables. Results obtained by the simple correlation coefficients in the study conducted by (**Silva *et al.*, 2012**) highlights that the SPAD index showed high favorable correlations with net photosynthesis under both water conditions. However, a simple correlation measures only the mutual association between two variables, where causes and effects responsible for such correlation remain embedded.

Silva *et al.* (2013) found that the genotype HoCP93-776 was more sensitive to drought treatment as indicated by the significantly lower values of SPAD index, photosynthetic pigments, water potential (Ψ_w) and relative water content (RWC) variables. The genotype TCP02-4587 had higher water potential, stomatal control efficiency, water use efficiency (WUE), intrinsic instantaneous water use efficiency

(WUE intr), instantaneous carboxylation efficiency and photosynthetic capacity. The highest air vapor pressure deficit during the drought conditions could be due to the stomatal closing in the HoCP93-776, which contributed to its lower photosynthetic capacity.

Deviation of temperature of plant canopies in comparison to ambient temperature, also known as CTD (canopy temperature depression = air temperature – canopy temperature), has been recognized as indicator of overall plant water status (**Idso, 1982**) and used in such practical applications as evaluation of plant response to environmental stress, irrigation scheduling, cultivar comparison for water use and tolerance to heat and drought. High CTD has been used as a selection criterion to improve tolerance to drought and heat and has been associated with yield increase among wheat (*Triticum aestivum* L.) cultivars at CIMMYT (**Karimizadeh and Mohammadi, 2011**).

Varieties differ in their response to drought stress, and it is possible to identify more tolerant ones. This identification is essential, particularly in areas that has long period of water deficit. **Rong-hua et al. (2006)** and **Silva et al. (2011)** showed that indirect and faster methods of measuring photosynthetic activity, such as the chlorophyll a fluorescence technique, and in particular, the maximum photochemical efficiency of photo-system II (PSII) (which can be assessed via the variable-to-maximum chlorophyll a fluorescence ratio, Fv/Fm) and estimated chlorophyll content (SPAD unit), can be just as effective as the more time-consuming gas exchange techniques in revealing differences among drought-tolerant and susceptible genotypes of barley.

Silva et al. (2007) reported that the tolerance-susceptibility classification of a genotype is not associated to the average chlorophyll content, but rather, to the degree of the chlorophyll degradation under the stressful condition. Under water deficit conditions, the average SPAD unit value was significantly lower than for the well-watered conditions. There was a steady reduction in SPAD unit since stress increased with values lower at 90 Days after transplanting (DAT) than those at 0 and 45 DAT.

The relative water content (RWC) or ‘relative turgidity’ of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water

holding capacity at full turgidity. RWC provides a measurement of the ‘water deficit’ of the leaf and may indicate a degree of stress expressed under drought and heat stress. RWC integrates leaf water potential with the effect of osmotic adjustment as a measurement of plant water status. A genotype with the capability to minimize stress by retaining turgid leaves in stressed environments will have physiological reward (Mullan and Pietragalla, 2012).

The use of physiological markers provides a relatively more precise way of assessing drought tolerance, especially in field trials. Physiological parameters such as leaf temperature (LT) and relative water content (RWC) are also very responsive to water stress, and have been shown to correlate well with drought tolerance in sunflower, barley, wheat and weeping love grass. Physiological parameters such as LT and relative water content (RWC) are also very responsive to drought stress and have been shown to correlate well with drought tolerance (Colom and Vazzana, 2003).

Silva *et al.* (2007) demonstrated that water stress by and large resulted in a higher increase in LT in susceptible genotypes. This is based on the theory that increasing plant water deficits led to stomatal closure, decreased leaf transpiration cooling, and consequently, increased LT relative to well-watered plants.

RWC was the physiological attribute that best differentiated both the tolerant and the susceptible genotypes. Differences in RWC were also found on the interaction of irrigation level by evaluation date. Genotypes with no water deficit treatment in the first evaluation date showed high RWC values, but that were decreased significantly with a concurrent increase in days under water deficit treatment. According to Schlemmer *et al.* (2005), RWC in barley, wheat and corn with adequate water was approximately 90%. On the other hand, under water stress conditions, RWC ranged from 45 to 58% for barley, 56 to 72% for wheat and around 65% for corn. The smaller reduction found for sugarcane may be an indication that this species is more tolerant to drought (Silva *et al.* 2011).

Medeiros *et al.* (2013) observed a significant reduction on stomatal conductance, transpiration, and net photosynthesis upon irrigation suspension until total stomata closure, and then re-watered. RB867515 showed a faster stomatal closure while RB962962 slowed the effects of drought on the gas exchanges parameters with a faster

recovering after re-watering. Accumulation of carbohydrates, amino acids, proline, and protein in the leaves and roots of the stressed plants occurred in both varieties, substantially linked to reduction of the leaf water potential. Due to the severity of stress, this accumulation was not enough to maintain the cell turgor pressure, so relative water content was diminished. Water stress affected the contents of chlorophyll (a, b, and total) in both varieties, but not the levels of carotenoids.

Plants response to drought seems to be complex and has different mechanisms. One of the mechanisms might be accumulation of proline that is not only used for osmotic adjustment but also for scavenging and detoxifying oxidants and for preventing membrane damage and protein denaturation during severe drought stress. Generally, drought induces alterations in protein synthesis. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress (**Munawarti and Semiarti, 2014**).

Drought induced osmotic stress triggers a wide range of perturbations ranging from growth and water status disruption to the alteration of ion transport and uptake systems (**Bajji et al., 2000**). Generally, the plants accumulate some kind of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake. Among these solutes, proline is the most widely studied. It has been suggested that the increase of free proline levels is an indicator of injury that results from imbalances in other pathways (**Abbas et al., 2014**).

Accumulation of the amino acid proline has been widely used as a physiological–biochemical indicator of water stress so that an increase in concentration can be correlated with higher drought tolerance (**Molinari et al., 2007**). Diverse functions have been proposed for such proline accumulation: osmotic adjustment, storage of carbon and nitrogen to be used during recovery after the stress period, stabilising of proteins and membranes and reactive oxygen species (ROS) (**Cia et al., 2012**).

Increased proline accumulation was reported in water stressed sorghum. Increased proline in the stressed plants may be an adaptation to overcome the stress condition which constantly supplies energy for growth and survival and thereby helping the plants to tolerate stress (**Abdul et al., 2008**).

Nitrate reductase activity showed reduction in the genotypes (39.11 %) and progenies (44.95 %), while proline accumulation and activities of superoxide dismutase and peroxidase almost doubled in the genotypes and progenies. The tolerant genotypes viz. Co 740, ISH 100, NS 83/247, Co 85019, Co 997 and Co 99008 could be utilized in crop improvement, while Co 419, Co 8021, Co 775 and Co 8368 were susceptible (**Hemaprabha et al., 2013**).

Water stress increases the production of ROS, which causes a redox imbalance in the cells, disrupting the electron transport chain and triggering oxidative stress due to the toxic action of ROS. Lipid peroxidation is commonly used as an indicator of oxidative stress. It is characterised by ROS attack on the polyunsaturated fatty acids of cellular membranes, which may severely affect the integrity and functionality of cells and can cause irreversible damage (**Gratao et al., 2005**).

Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide and hydroxyl radical can cause lipid peroxidation and consequently membrane injury which leads to leakage of cellular content, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands and thus cell death (**Scandalios, 1993**). Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxides damage of cell was controlled by the potency of peroxidase enzyme system (**Sairam and Tyagi, 2004**).

Glycine betaine contents mostly increased when PEG concentration is increased. Drought stress has profound effect on the glycine betaine accumulation in sugarcane genotypes. Glycine betaine provides protection from oxidative stress, which would have otherwise cause damage of cell membrane, protein, DNA structure and inhibition of the photosynthesis will occur under water stress condition (**Sairam and Saxena, 2000**).

A decline in photosynthetic capacity leads to conditions of excess light energy and increased generation and accumulation of reactive oxygen species (ROS) becomes inevitable. ROS can cause extensive damages to photosynthetic machineries and other cellular components that may result in death of plant cells and tissues (**Takahashi and Badger, 2011**). However, plants have evolved elaborate systems of enzymatic antioxidants as Superoxide dismutase (SOD), Catalase (CAT), Peroxidase

(POX), Ascorbate peroxidase (APX) and Glutathione reductase that allow scavenging of ROS and protection of plant cells from oxidative damages during environmental stresses (**Gill and Tuteja, 2010**). Water stress enhanced activities of POX, CAT and SOD in leaves of bread wheat (*Triticum aestivum* L), and greater activities of POX and CAT were found in drought- tolerant variety (**Ngamhui et al., 2015**).

Christy et al. (2012) Sugarcane genotypes such as tolerant (Co 99004), and sensitive (Co 97010) were subjected to NaCl (170 and 250mM) and Poly ethylene glycol (-0.8Mpa and -1.7Mpa) treatments and evaluated through biochemical analysis to assess the commonness between salinity and drought stress. Among the biochemical characters analyzed total chlorophyll decreased and proline, lipid peroxidase, xanthophylls increased with severe stress (both salinity and drought). Peroxidase and superoxide dismutase activity increased with isozymes showing over expression under stress condition. The results showed that the tolerant genotype (Co 99004) expressed better stability under the saline and water stress conditions where as the sensitive genotype (97010) failed to perform under the stress conditions.

Abbas et al. (2013) evaluated antioxidant activity by different assays, antioxidant, 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical, lipid peroxidation (MDA), proline, glycine betaine and total phenolic contents. The genotype S-2003-US-114 exhibited strong antioxidant activity in the DPPH (IC₅₀, 35.08±1.25µg/ml) assay. Drought stress imposed at various stages of crop growth resulted in an increase in lipid peroxidation and decrease in membrane stability, CPHS-35 and S-2003-US-694 had the lowest lipid peroxidation (malondialdehyde content) at control and showed highest membrane stability at PEG 12.5%. There was a significant enhancement in the Proline contents and the reduction in Proline oxidase activities under PEG treatment. Drought stress causes an increase in the amino acid and glycine betaine (GB) content. The results indicate that sugarcane possesses potential antioxidants against drought tolerant.

Wagih (2005) found that varieties L6 and 26 (Ragnar) exhibited significantly ($P < 0.0$) higher DTC values as compared to other three varieties. Based on the quantitative fall in pH, L6, 22 and 26 were consistently be grouped as drought tolerant varieties since the DTC values $> 3\%$, while L14 as drought sensitive with DTC $< 1.70\%$, and L10 as medium variety. The obtained results indicated that a reduction in PH $> 3\%$ for the varieties higher Q77N1232, Co6519 and Ragnar DTC values as compared to other three varieties.

2.5 Use of molecular markers in breeding for drought tolerance in sugarcane

Hybrid genotypes or clones possessing tolerance to drought at a specific level are used in hybridization to develop tolerant varieties. Screening of genotypes for a particular trait by traditional methods is a time-consuming and relatively laborious process. The use of molecular markers in breeding programs, during recent years, has been proved as an efficient tool in this regard (**Srivastava *et al.*, 2012**).

Molecular genetic markers are a valuable tool in the studies of complex genomes such as sugarcane (**Daugrois *et al.*, 1996**). Their incorporation in the selection of economic traits during the early stages of a breeding program, as well as in the choice of the best parents in a cross, may significantly reduce the time for the development of new varieties. These goals can be achieved with the availability of robust polymorphic markers, which co-segregate with economically important traits in sugarcane (**Sharma, 2009**).

With the development of the random amplified polymorphic DNA (RAPD) method (**Williams *et al.*, 1990**), random segments of a genome can be sampled and thereby, genetic traits which differ between different genotypes can be compared. SCAR marker linked to smut resistance gene has been identified and utilized in the sugarcane breeding program (**Que *et al.*, 2008**). SCAR markers, by being both accurate and cost efficient, offer the most practical method for screening numerous samples in a time and labor-saving manner (**Kasai *et al.*, 2000**).

SSRs are stretches of DNA consisting of repetitive sequences of 2 to 6 base pairs, which are evenly distributed across the genome. They are generally present in non-coding regions of the genome. They are highly polymorphic and flanked by sequences that are sufficiently conserved. Therefore, they are considered as a suitable source for generating primers for PCR amplifications (**Sharma, 2009**). SSR primers are developed based on sequence information, which makes them more reliable and consistent compared to the primers generated at random such as RAPD.

The cost of running microsatellite marker analyses is relatively low, but the cost of developing the markers is high, limiting their application to larger commercial crops such as sugarcane. The high cost of developing plant microsatellite libraries, coupled with the low level of enrichment peculiar to sugarcane libraries justify the use of expressed sequence tags (EST)-SSR markers in sugarcane (**Silva, 2001**). The target

region amplification polymorphism (TRAP) technique allows the identification of polymorphisms in coding regions using one fixed primer designed from a target expressed sequence tag (EST) sequence and a second random primer of arbitrary sequence except for AT- or GC-rich cores that anneal with introns and exons, respectively (Creste *et al.*, 2010).

Using 55 microsatellite markers, scored among 80 modern sugarcane genotypes and 21 EST-SSR markers and 11 TRAP markers related to drought-defining physiological parameters were considered as genuine associations which can further be utilized for screening of sugarcane clones for drought tolerance (Sharma, 2009).

A sequence characterized amplified region (SCAR) marker was used for screening drought tolerant genotypes in sugarcane. Of 150 RAPD decamer primers, used to identify polymorphism amongst five sugarcane genotypes (3 drought tolerant and 2 drought sensitive), 12 were found to be polymorphic. A 724 bp DNA fragment was amplified through PCR with primer OPAK 12 in drought tolerant genotypes ROC22 and GT 18, while it was absent in both sensitive genotypes. High effectiveness in the detection of drought tolerant genotypes, as well as wild species of sugarcane, showed that this marker is appropriate in assisting selection of genotypes with drought tolerance (Srivastava *et al.*, 2012).

McIntyre *et al.* (2005) 1000 single-dose markers, including microsatellite (SSR), amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers, were scored across the population and maps containing approximately 400 markers were constructed for each parent. At $p < 0.01$, two genomic regions, one from the female Q117 map and a different region from the 74C42 male map, plus an unlinked bi-parental simplex marker (single-dose marker present in both parents) were identified as associated with PRR over both years of data collection. These regions explained between 6 and 16% of the phenotypic variation. An additional region was identified in the female map as associated with PRR at $p < 0.01$ in one year and $p < 0.05$ in the second year. To validate these markers and determine whether they would be useful in alternative germplasm, markers from each genomic region associated with PRR or brown rust were screened across a set of 154 elite sugarcane clones; PRR and brown rust ratings were available for 131 and 72 of the clones, respectively. For PRR, three of the 6 markers tested remained significantly associated ($p < 0.01$) with resistance ratings in the elite clone set.

Ukoskit *et al.* (2018) used Quantitative trait loci (QTL) mapping validated with association mapping to identify expressed sequence tag-simple sequence repeats (EST-SSRs) associated with sugar-related traits. For linkage mapping, 524 EST-SSRs, 241 amplified fragment length polymorphisms, and 10 genomic SSR markers were mapped using 283 F1 progenies derived from an interspecific cross. Six regions were identified using multiple QTL mapping, and 14 unlinked markers using single marker analysis. Association analysis was performed on a set of 200 accessions, based on the mixed linear model. Validation of the EST-SSR markers using association mapping within the target QTL genomic regions identified two EST-SSR markers showing a putative relationship with uridine diphosphate (UDP) glycosyltransferase, and beta-amylase, which are associated with pol and sugar yield.

Wagh (2009) conducted molecular analyses of sugarcane F1s and some varieties in relation to salt stress tolerance. The genomic DNA of five salt tolerant F1s and seven salt susceptible F1s along with three salt tolerant varieties were subjected to PCR amplification using 40 random decamer primers sequentially, from OPM 01 to OPM 20 and OPL 01 to OPL 20. The primer OPM09 amplified a 1.5 Kb band which has noticed in five salt tolerant varieties and remained absent in salt susceptible F1s. It was concluded that the OPM091.5 marker may be associated with salt tolerant trait in sugarcane and should be converted to sequence characterized amplified region (SCARs) for further studies.

Priji and Hemaprabha (2014) screened 14 putative candidate genes belonging to abscisic acid (ABA) independent pathway on a set of tolerant species clones belonging to *Saccharum spontaneum*, *S. barberi*, *S. sinense*, *S. robustum* and *Erianthus* and the susceptible species clones of *S. officinarum*. Out of these, 11 genes viz., DREB 1A, DRF 1, Hep2, HRD, NAC 2, NIT 1, PIN 3, SHN 1, S1Z 1, Snac 1 and Wrky 38 were present in *Saccharum* and *Erianthus*, highlighting the role of these genes in drought tolerance. All the drought tolerant species clones exhibited eight to eleven genes indicating the role of these genes in imparting drought tolerance in sugarcane. The drought tolerant species clones could serve as potential donors in pre-breeding activities for genetic improvement of drought tolerance in sugarcane and the eleven sugarcane specific drought responsive genes can serve as novel and functional candidate gene markers in marker-assisted introgression breeding programmes.



*Materials
and
Methods*



The present investigation was conducted at Sugarcane Breeding Block, Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar with early generation clones of Sugarcane. These early generation clones were selected from bi-parental progenies of the crosses involving five drought tolerant parents viz; Co 1148, Co 1158, ISH 100, Co 617 and Co 85002 and three drought sensitive parents viz; UP 9530, Bo 91 and Co Pant 97222. Hybridization was performed at which were performed at National Hybridization Garden, ICAR-Sugarcane Breeding Institute (SBI) Coimbatore during Oct - Nov 2015. The important features of the parents involved in the crosses are presented in Table 3.1. The screening for response to drought tolerance was conducted under artificially created stress condition in rain-out shelter (polyhouse) at Pantnagar Centre for Plant Genetic Resources, Pantnagar and the molecular analysis was carried out at Plant Biotechnology Laboratory, Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar. The work flow of present study including the generation of experimental material and the evaluation for drought tolerance is being presented in the Fig 1.

Table 3.1: Salient Features of the Parental Varieties Used in Genration of Experimental Material

S.No	Parental variety	Salient Features
1.	Co 1148	Tolerant to drought and smut, susceptible to red rot, high yield and moderate sucrose content. Co 291 bridges it with <i>Saccharum spontaneum</i> .
2.	Co 1158	Tolerant to drought and wilt, susceptible to red rot, high yield and moderate sucrose content. Co 285 bridges it with <i>Saccharum spontaneum</i> .
3.	ISH 100	Tolerant to drought , moderately resistant to red rot, CoC 671 bridges it with <i>Saccharum robustum</i> .
4.	Co 617	Tolerant to drought , moderate sucrose content. Co 285 bridges it with <i>Saccharum spontaneum</i> .
5.	Co 85002	Tolerant to drought and smut, moderately susceptible to red rot, high yield and high sucrose content
6.	UP 9530	Sensitive to drought, resistant to red rot , moderate yield and low sucrose content
7.	Bo 91	Mid-late maturity, tolerant to flooded condition , good ratooner, resistant to red rot , moderately resistant to smut, high yield and moderate sucrose content
8.	Co Pant 97222	Mid-late maturity, moderately resistant to red rot, high yield and high sucrose content

3.1 Experimental Site:

Pantnagar is located at the foothills of Himalayas (Shiwalik range) and geographically falls in the humid subtropical climate zone. It is situated at 29° N latitude, 79.3°E longitudes and at an altitude of 243.84 m above the mean sea level. The region is popularly known as “Tarai” due to subtropical humid climate.

Activity carried out	Place	Time Period
Crossing of 8 parents to generate F₁ progenies	National Hybridisation Garden (NHG), ICAR-SBI, Coimbatore	Oct –Nov 2015
↓		
Fluff sowing	Sugarcane Poly-house, NEB-CRC, GBPUAT, Pantnagar	June, 2016 (2 nd week)
↓		
Seedling transplantation	Sugarcane breeding block, NEB-CRC, GBPUAT, Pantnagar	August, 2016 (1 st week)
↓		
Evaluation of 65 C₁ clones for Drought response in Control and Stress Environment (Spring crop)	Sugarcane breeding block, CRC for control & PCPGR, GBPUAT, Pantnagar for stress environment	March, 2017
↓		
Evaluation of 65 C₂ clones for Drought response in Control and Stress Environment (Autumn crop)	Sugarcane breeding block, CRC for control & PCPGR for stress environment	Oct, 2017
↓		
Validation of 10 molecular markers for drought tolerance	Plant Biotechnology Lab, Deptt. of Genetics and Plant breeding, GBPUAT, Pantnagar	April–May 2018

Fig. 1: Flow Diagram Representing the Work Flow of the Present Study over the Period

3.2 Experiment1: Evaluation for drought tolerance potential of early generation sugarcane clones through physio-biochemical parameters involved in drought tolerance.

3.2.1 Experimental material:

The experimental material consisted of 65 C₁ and C₂ clones, seven parents and six standard check varieties. The details of the 72 genotypes (parents and clones) is presented in Table 3.2. C₁ and C₂ clones were planted using single budded setts during March (Spring 2017-18) and C₂ clones in October (Autumn 2017-18) using augmented block design with three blocks and six checks in two sets respectively. One set (Control) was planted in field condition with normal course of irrigation at NEB- CRC, Pantnagr and another set (Stress) 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.

3.2.2 Physio- Biochemical Parameters Recorded:

The study aimed at evaluation of drought response of sugarcane genotypes. Therefore, for the purpose of study 16 Physio-Biochemical characters were observed which included parameters reported to be involved in conferring drought tolerance to the plant. Observations were recorded from a sample of five plants per plot. All the characters were recorded at different stage of development especially during the period 60-200 DAS. The details of characters recorded are given below.

3.2.2.1 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.

3.2.2.2 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

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

3.2.2.3 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) in both (Control and Stress condition) sets of planting. During each Leaf Temperature measurement, the natural leaf orientation with respect to the sun was maintained to avoid shade effects.

3.2.2.4 SPAD Readings at 60 DAS:

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

3.2.2.5 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) in both the sets of planting.

3.2.2.6 SPAD Readings at 120 DAS:

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

3.2.2.7 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) in both the sets of planting.

3.2.2.8 SPAD Readings at 180 DAS:

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

3.2.2.9 Relative Water Content (RWC %):

Leaf discs of 2.5 cm in diameter was collected from the top-most fully expanded leaf receiving sunlight, typically the flag leaf and the samples were immediately packed in laminated paper envelopes. The leaf disc fresh weight was determined, and the samples then individually immersed in deionised-distilled water for 6 h under low irradiance. The excess water was gently removed from the discs with absorbent paper prior to the determination of the turgid weight. The disc dry weight was determined after 72 h at 70° C (Smart and Bingham, 1974). The RWC was obtained as follows:

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

Table 3.2. Details of the Sugarcane Genotypes and Checks Used in the Present Study

S. No	Clones	Cross	Cross Type	S. No	Clones	Cross	Cross Type
1	PC (2015-16)-1	UP 9530 x Co Pant 97222	S x S	37	PC (2015-16)-37	Bo 91 x Co 1148	S x T
2	PC (2015-16)-2	UP 9530 x Co Pant 97222	S x S	38	PC (2015-16)-38	Bo 91 x Co 1148	S x T
3	PC (2015-16)-3	UP 9530 x Co Pant 97222	S x S	39	PC (2015-16)-39	Bo 91 x Co 1148	S x T
4	PC (2015-16)-4	UP 9530 x Co Pant 97222	S x S	40	PC (2015-16)-40	Bo 91 x Co 1148	S x T
5	PC (2015-16)-5	UP 9530 x Co Pant 97222	S x S	41	PC (2015-16)-41	Bo 91 x Co 1148	S x T
6	PC (2015-16)-6	UP 9530 x Co Pant 97222	S x S	42	PC (2015-16)-42	Bo 91 x Co 1148	S x T
7	PC (2015-16)-7	UP 9530 x Co Pant 97222	S x S	43	PC (2015-16)-43	Bo 91 x Co 1148	S x T
8	PC (2015-16)-8	Bo 91 x Co 1158	S x T	44	PC (2015-16)-44	Bo 91 x Co 1148	S x T
9	PC (2015-16)-9	Bo 91 x Co 1158	S x T	45	PC (2015-16)-45	Bo 91 x Co 1148	S x T
10	PC (2015-16)-10	Bo 91 x Co 1158	S x T	46	PC (2015-16)-46	Bo 91 x Co 1148	S x T
11	PC (2015-16)-11	Bo 91 x Co 1158	S x T	47	PC (2015-16)-47	UP 9530 x Co 1148	S x T
12	PC (2015-16)-12	Bo 91 x Co 1158	S x T	48	PC (2015-16)-48	UP 9530 x Co 1148	S x T
13	PC (2015-16)-13	Bo 91 x Co 1158	S x T	49	PC (2015-16)-49	UP 9530 x Co 1148	S x T
14	PC (2015-16)-14	Co 617 x Co 1158	T x T	50	PC (2015-16)-50	UP 9530 x Co 1148	S x T
15	PC (2015-16)-15	Co 617 x Co Pant 97222	T x S	51	PC (2015-16)-51	UP 9530 x Co 1148	S x T
16	PC (2015-16)-16	Co 617 x Co Pant 97222	T x S	52	PC (2015-16)-52	UP 9530 x Co 1148	S x T
17	PC (2015-16)-17	Co 617 x Co Pant 97222	T x S	53	PC (2015-16)-53	UP 9530 x Co 1148	S x T
18	PC (2015-16)-18	Co 617 x Co Pant 97222	T x S	54	PC (2015-16)-54	UP 9530 x Co 1148	S x T
19	PC (2015-16)-19	Co 617 x Co Pant 97222	T x S	55	PC (2015-16)-55	UP 9530 x Co 1148	S x T

20	PC (2015-16)-20	Co 617 x Co Pant 97222	T x S	56	PC (2015-16)-56	UP 9530 x Co 1148	S x T
21	PC (2015-16)-21	Co 617 x Co Pant 97222	T x S	57	PC (2015-16)-57	Co 85002 x Co 1158	T x T
22	PC (2015-16)-22	Bo 91 x Co Pant 97222	S x S	58	PC (2015-16)-58	Co 85002 x Co 1158	T x T
23	PC (2015-16)-23	Bo 91 x Co Pant 97222	S x S	59	PC (2015-16)-59	Co 85002 x Co 1158	T x T
24	PC (2015-16)-24	Bo 91 x Co Pant 97222	S x S	60	PC (2015-16)-60	Co 85002 x Co 1158	T x T
25	PC (2015-16)-25	ISH 100 x Co Pant 97222	T x S	61	PC (2015-16)-61	Co 85002 x Co 1158	T x T
26	PC (2015-16)-26	ISH 100 x Co Pant 97222	T x S	62	PC (2015-16)-62	Co 85002 x Co 1158	T x T
27	PC (2015-16)-27	ISH 100 x Co Pant 97222	T x S	63	PC (2015-16)-63	ISH 100 x Co 1158	T x T
28	PC (2015-16)-28	ISH 100 x Co Pant 97222	T x S	64	PC (2015-16)-64	ISH 100 x Co 1158	T x T
29	PC (2015-16)-29	ISH 100 x Co Pant 97222	T x S	65	PC (2015-16)-65	ISH 100 x Co 1158	T x T
30	PC (2015-16)-30	ISH 100 x Co Pant 97222	T x S	S. No.	Parents	Parentage	
31	PC (2015-16)-31	ISH 100 x Co Pant 97222	T x S	1	BO 91	Bo 55 x Bo 43	-
32	PC (2015-16)-32	ISH 100 x Co Pant 97222	T x S	2	Co 1148	P 4383 x Co 301	-
33	PC (2015-16)-33	ISH 100 x Co Pant 97222	T x S	3	Co 617	PoJ 2878 x Co 285	-
34	PC (2015-16)-34	ISH 100 x Co Pant 97222	T x S	4	Co 85002	Co 62198 x CoC 671	-
35	PC (2015-16)-35	ISH 100 x Co Pant 97222	T x S	5	ISH 100	Co 7201 x CoC 671	-
36	PC (2015-16)-36	ISH 100 x Co Pant 97222	T x S	6	UP 9530	Seo 1084/86 x Seo 122/85	
				7	Co Pant 97222	Co Pant 84212 GC	-

Check varieties:

Co 238, Co Pant 03220 and CoJ 64 are Early and Co Pant 97222, 05224 and CoS 8436 are Mid-late varieties.

3.2.2.10 Proline Content:

The proline content was determined using the method of **Bates *et al.* (1973)**. Leaf samples were homogenised in 3% (w/v) of Sulphosalicylic acid and centrifuged at 6000 rpm for 30 min at 25°C. 2ml of supernatant are added to 2 ml acid Ninhydrin solution (1.25 g Ninhydrin, 30 ml glacial acetic acid, 20 ml phosphoric acid 6M) and 2ml glacial acetic acid and incubated at 100°C for 1 h and transferred to an ice bath. The absorbance was measured at 520 nm and the Proline content was determined using a Proline standard (0–100 µg ml⁻¹) Proline.

3.2.2.11 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)** and **Lichtenthaler (1987)**, respectively. 100 mg of leaf material was collected from the second and third nodes of the shoot tip. The leaf samples are placed in a 25 ml glass vial, along with 10 ml of 95.5% acetone and blended using a homogenizer. The glass vials are sealed with parafilm to prevent evaporation and then stored at 4°C for 48 h. The Chl *a* concentration was measured using a UV-visible spectrophotometer at 662 nm and 644 nm wavelengths. A solution of 95.5% acetone is used as a blank.

The Chl *a*, (µg g⁻¹ FW) in the leaf tissues are calculated according to the following equations:

$$\text{Chl } a = 9.784D_{662} - 0.99D_{644}$$

3.2.2.12 Chlorophyll *b* (Chl *b*) concentrations:

Sample preparation and estimation were carried out using similar methods mentioned in section **3.2.2.11**. Chl *b* concentrations (µg g⁻¹ FW) in the leaf tissues are calculated according to the following equations: $\text{Chl } b = 21.42D_{644} - 4.65D_{662}$

3.2.2.13 Total Chlorophyll concentrations:

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

3.2.2.14 Total Carotenoid (Cx+c) concentrations:

Sample preparation and estimation were carried out using similar methods mentioned in section 3.2.2.11. 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:

$$\text{Cx+c} = 1000D_{470} - 1.90\text{Chl } a - 63.14\text{Chl } b / 214$$

3.2.2.15 Drought Tolerance Capacity (DTC):

Drought tolerance capacity were determined following Na- EDTA method developed by **Dwivedi *et al.* (1988)**, by extracting one gram air dry leaf in 20 ml 0.025 M Na₂ EDTA and boiling it for 25-30 minutes in a water bath. After cooling, pH was measured. The percent fall in pH as compared to the control (field crop) was recorded as DTC. Quantitatively, fall of pH ≥ 3 % indicates as drought tolerant genotype (5) and fall of pH ≤ 1.7 % as sensitive genotypes (1).

3.2.2.16 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**). 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). Leaf firing provides a good assessment of overall drought resistance.

3.2.3 Statistical analysis:

Statistical analysis involves analysis of variances for augmented block-II design, F- test to test homogeneity of variances over season, pooled analysis of variance, estimation of variability and genetic parameters, correlation and path coefficient analysis for 16 physio-biochemical parameters and estimation of diversity through Mahalanobis D² analysis. All these estimations were carried out using MS Excel 2007, SPSS 16.0v, and WINDOSTAT 9.2v.

3.2.3.1 Augmented block II ANOVA

An augmented design-II (**Federer, 1956**), 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. Randomization is done in such a way that all the checks (c) and a part of

test genotypes fall only once in each block. Equal number of test genotypes was planted in each block to facilitate statistical analysis.

The analysis of variance for augmented design was done for all 4 trials conducted in two different environments (Control and Stress) during the crop seasons viz; spring 2017-18 and autumn 2017-18 using the method as described by **Federer and Ragavarao (1975)**.

The structure of ANOVA for Augmented Design-II

Source of variation	d. f.	SS	MSS	F
Blocks	b -1	bSS	bMS	bMS/EMS
Entries	e -1	eSS	eMS	eMS/EMS
Checks	c – 1	cSS	cMS	cMS/EMS
Varieties	v – 1	vSS	vMS	vMS/EMS
Checks vs Varieties	1	cvSS	cvMS	cvMS/EMS
Error	(c-1) (b-1)	ESS	EMS	
Total	N-1	TSS		

Where,

b= No. of blocks, **c**= No. of checks, **v**= No. of test varieties, **e**= c + v, **N**= bc + v

Different components of ANOVA can be calculated as follows. However, since only the checks (c) but not the test varieties (v) are replicated in this design, adjustment of means of 'v' must be done before ANOVA with the help of the following effects.

Block effects (b_j) = $1/c (T b_j - \bar{c} - T v b_j)$ (j= 1 to b) Counter check: $\sum_1^b b_j \approx 0$

Mean effect (m) = $1/e (GT-(b-1) c - \sum_1^b n_j b_j) \sum n_j b_j$

Where,

n_j is the number of varieties occurring in j^{th} block

Check effects ($\bar{c}_i$) = $c_i - m$ (i = 1 to c)

GCF (General correction factor) = GT^2 / N

$$\mathbf{bSS} = \sum_1^b T b_j^2 / (c + n_j) - \text{GCF}$$

$$\mathbf{bMS} = \mathbf{bSS} / (b-1)$$

$$\mathbf{TSS} = \sum_{i=1}^c \sum_{j=1}^b c_{ij}^2 + \sum_1^v v_i - \text{GCF}$$

$$\mathbf{cCF} \text{ (Check correction factor)} = T c^2 / bc$$

$$\mathbf{cSS} = \sum_1^c T c_i / b - cCF$$

$$\mathbf{cMS} = \mathbf{cSS} / (c-1)$$

$$\mathbf{eSS} = (m \times GT) + \sum_1^b b_j(T b_j) + \sum_1^c T c_i(C_i) + \sum_1^v v_i(v_i) - [\sum_1^b T b_j^2 / (c+n_j)]$$

$$\mathbf{eMS} = \mathbf{eSS} / (e-1)$$

$$\mathbf{vCF} = T v^2 / v$$

$$\mathbf{vSS} = \sum_1^v v_i^2 - vCF$$

$$\mathbf{vMS} = \mathbf{vSS} / (v-1)$$

$$\mathbf{cvSS} = \mathbf{cvMS} = \mathbf{eSS} - \mathbf{cSS} - \mathbf{vSS}$$

$$\mathbf{ESS} = \mathbf{TSS} - \mathbf{eSS} - \mathbf{bSS}$$

$$\mathbf{EMS} = \mathbf{ESS} / (c-1) (b-1)$$

If the block effect is significant, following four standard errors are helpful in drawing conclusion.

$$\mathbf{SEd}_1 \text{ (between any two check means)} = \sqrt{\frac{2EMS}{b}}$$

$$\mathbf{SEd}_2 \text{ (between any two means of test varieties)} = \sqrt{2EMS}$$

$$\mathbf{SEd}_3 \text{ (between any two entries of the same block)} = \sqrt{2EMS(1+1/c)}$$

$$\mathbf{SEd}_4 \text{ (between means of a check and a test variety)} = \sqrt{EMS(1+1/b+1/c+1/bc)}$$

CD is then calculated using these $\mathbf{SEd}_s \times 't'$ at 5% or 1% levels.

3.2.3.2 F-test to test homogeneity of variances

In order to perform combined analysis or Pooled analysis of variances the primary requirement is to satisfy homogeneity of error variances over seasons using one of the homogeneity test like F-test of homogeneity {When, error variances (p)=2} and Bartlett's Chi-square test {When, error variances (p)>2} depending upon the nature of data and number of environments. In our study we have to compare error variance from only two environments therefore we used F-test as suggested by **Lal (2018)**.

Here null and alternate hypothesis are $H_0: \sigma_1 = \sigma_2$ and $H_1: \sigma_1 \neq \sigma_2$. Let Se_1 and Se_2 are the mean square errors (MSE) for the two years. Then the value of F statistics will be Se_1 / Se_2 and this value will be tested against the Table F value at n_1 and n_2 degrees of freedom at 5 % level of significance, where n_1 and n_2 are degrees of freedom (df) for error for the two years, respectively. If the calculated value of F is greater than tabulated F value then the null hypothesis of homogeneity of variance is rejected and the data is heterogeneous in different years, otherwise it is homogeneous.

3.2.3.3 Pooled analysis of variance:

Pooled analysis of variance was carried out after satisfying the F-test for homogeneity of variances over seasons. Similar environment (control and stress) over seasons were considered as replications and analysis of variance (ANOVA) was carried for both control and stress environment separately.

3.2.3.3.1 Analysis of variance (ANOVA):

The pooled Analysis of variance (ANOVA) will be done as explained by **Panase and Sukhatme (1967)**. The data were analyzed to test the significance of difference between the genotypic mean for various characteristics.

Source of Variation	df	Mean Square	Expected Mean Square	F value
Replication (r)	r-1	MS_r	$\sigma^2_e + r \sigma^2_g + \sigma^2_r$	MS_r / MS_e
Treatment (t)	t-1	MS_t	$\sigma^2_e + r \sigma^2_g$	MS_t / MS_e
Error (e)	(r-1) (t-1)	MS_e	σ^2_e	
Total	rt- 1			

Where;

MS_r = Mean square due to replications

MS_t = Mean square due to treatment

MS_e = Mean square due to error

Genotypic variance (σ^2_g) = $MS_t - MS_e/r$

Phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

Error variance (σ^2_e) = Mean square due to error

The significance of difference among genotypic mean was tested by F test. If, $F_{cal} > F_{tab}$ then variance is significant. Wherever the F test was found to be significant, Critical Difference (C.D) was calculated for comparing particular genotypes.

$C.D (0.05) = SE_d \times 't'$

Where;

't' = table value of 't' at error degree of freedom and 5% level of probability.

SE_d = Standard error of difference between two treatment means.

Standard error of difference: $SE_d = \sqrt{2 \times EMS / r}$

Where;

EMS = error mean square

r = number of replications

3.2.3.4 Estimation of Variability and Genetic Parameters

3.2.3.4.1 Mean:

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

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

3.2.3.4.3 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)**

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

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

Where, σ_g = genotypic standard deviation.

σ_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 **Sivasubramanian and Menon (1973)**.

>20 %-high

10-20 %-moderate

0-10 %-low

3.2.3.4.4 Heritability (h^2)

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

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

σ_g^2 = Genotypic variances

σ_p^2 = Phenotypic variances

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

>60 %-high

30-60 %-moderate

0-30 %-low

3.2.3.4.5 Genetic advance

Genetic advance (GA) for each character was computed by adopting the formulae given by **Johnson *et al.* (1955)**:

$$GA = h^2 \cdot K \cdot \sigma_p$$

Where,

h^2 = Heritability of the character.

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

σ_p = Phenotypic standard deviation of the character.

3.2.3.4.6 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)**.

>20 %-high

10-20 %-moderate

0-10 %-low

3.2.3.5 Estimation of Correlation and Path Coefficients:

3.2.3.5.1 Correlation Coefficients:

Correlation coefficients were computed to determine the association among all the yield contributing characters.

The general formula for computing the simple correlation coefficient is as follows:

$$r_{xy} = \frac{\text{cov}(xy)}{\sqrt{\text{var}(x) \times \text{var}(y)}}$$

Where,

r_{xy} = correlation coefficient between character X and Y

Cov(xy) = covariance between character X and Y

Var(x) = variance due to character X

Var(y) = variance due to character Y

The phenotypic, genotypic and environmental correlation coefficients were calculated using the following formula:

Genotypic correlation coefficient:

$$G_{xy} = \frac{\text{covGxy}}{\sqrt{\text{var}(G_{xx}) \times \text{var}(G_{yy})}}$$

Where,

Cov Gxy= Genotypic covariance between character X and Y

Var Gxx = Genotypic variance for character X

Var Gyy = Genotypic variance for character Y

Phenotypic correlation coefficient:

$$P_{xy} = \frac{\text{cov}P_{xy}}{\sqrt{\text{var}(P_{xx}) \times \text{var}(P_{yy})}}$$

Where,Cov P_{xy}= Phenotypic covariance between character X and YVar P_{xx} = Phenotypic variance for character XVar P_{yy} = Phenotypic variance for character Y**Environmental correlation coefficient:**

$$E_{xy} = \frac{\text{cov}E_{xy}}{\sqrt{\text{var}(E_{xx}) \times \text{var}(E_{yy})}}$$

Where,Cov E_{xy}= Environmental covariance between character X and YVar E_{xx} = Environmental variance for character XVar E_{yy} = Environmental variance for character Y

The significance of correlation coefficient (r) was tested by comparing the calculated value of t with tabulated 't' value at (n-2) d.f. (**Snedecor and Cochran, 1967**).

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

3.2.3.5.2 Estimation of Path Coefficient

Path coefficient analysis was done according to the procedure given by **Wright (1921)** and as elaborated by **Dewey and Lu (1959)**. Path coefficient analysis was carried out by taking DTC as dependent variable and other drought related traits as independent variables. 15 drought related physio-biochemical characters were considered in path coefficient analysis to estimate their direct and indirect effects.

Path coefficient was estimated by solving the following equation which expresses the basic relationship between correlation and path coefficient. The equation is as follows:

$$ry1 = Py1 + Py1 r12 + Py3 r13 + ...+ Pyn r1n$$

$$ry2 = Py2 + Py1 r21 + Py3 r23 + ...+ Pyn r2n$$

$$ryn = Pyn + Py1 rn1 + Pyn rn2 + ...+ Pyn rn(n-1)$$

Where,

ry1, ry2,.....,ryn are correlation coefficients of dependent variable (y) with various independent variables (1, 2, ..., n)

Py1, Py2,.....,Pyn are the direct path effects of independent variables (1, 2, ..., n) on dependent variable (y)

r12, r13.....r1n.....rn(n-1) are correlation coefficients between various independent Variables (1, 2,....., n)

Path coefficients were obtained by replacing the corresponding elements in A and B matrix by correlation coefficients. B matrix was inverted and the inverted B matrix was multiplied by A matrix to obtain path coefficients. Thus there will be set of simultaneous equations which can be written in the form of matrices as follows:

$$\begin{matrix} A \\ \begin{bmatrix} r1y \\ r2y \\ r3y \\ rn y \end{bmatrix} \end{matrix} = \begin{matrix} B \\ \begin{bmatrix} r11 & r12 & ... & r1n \\ r21 & r22 & ... & r2n \\ r31 & r32 & ... & r3n \\ rn1 & rn2 & ... & rnn \end{bmatrix} \end{matrix} \times \begin{matrix} C \\ \begin{bmatrix} P1y \\ P2y \\ P3y \\ Pny \end{bmatrix} \end{matrix}$$

Where,

r1y = correlation coefficient between character 1 and y

rnny = correlation coefficient between character n and y

P1y = direct effect of character 1 and y

Pny = direct effect of character n and y

The solution for vector C may be obtained as follows: $C = B^{-1} A$

Where,

B^{-1} = Inverse of matrix B

The residual factor which measures the contribution of the rest of the characters of causal scheme was obtained as given below:

$$P_{xy} = \sqrt{1 - r^2}$$

$$R^2 = \sum_{i=1}^r P_{iy}^2 + 2 \sum_{i=j}^r P_{iy} \cdot P_{jy} \cdot R_{ij}$$

Where,

R_{ij} = Denotes all possible combinations of independent character of character y

R^2 = Coefficient of multiple determination

3.2.3.6 Genetic Divergence Analysis:

3.2.3.6.1 Estimation of Genetic Divergence

The estimation of genetic divergence was done with the help of Mahalanobis' "D²" statistic (generalized distance) as suggested by **Rao (1952)**. For this all physio-biochemical characters were considered. Its calculation involved the following steps

- a- A set of uncorrelated linear combinations (Y,s) was obtained by Pivotal condensation of the common dispersion matrix formed by a set of correlated variables (X,s). The common dispersion matrix was obtained with the help of error mean squares and sum of products.
- b- Using the relationship between Y,s & X,s the mean values of different genotypes for different characters were transformed into mean value of a set of uncorrelated linear combinations.
- c- The D² value between 'ith' & 'jth' genotypes for kth character was calculated as:

$$D_{ij}^2 = \sum_{t=1}^k (Y_{it} - Y_{jt})^2$$

- d- The 'k' component, D² values for each combination were ranked in descending order of magnitude.
- e- The ranks were added up for each component D² values over all combinations and rank totals were obtained.

3.2.3.6.2 Group Constellation

All the genotypes were grouped into clusters on the basis of D² values, as suggested by Tocher. In the said method, two genotypes belonging to the same cluster should at least, on the average, show a smaller D² value than those belonging to two

different clusters. The average D^2 values of all possible genotype combinations in one cluster with those in the other were computed and its square root was used to represent the statistical distance between two clusters.

3.2.3.6.3 Intra and Intercluster Distances

For the measure of intracuster D^2 values, the following formula was used:

$$\text{Intracuster } D^2 = \sum D_i^2 / n$$

$$n = P(P-1)/2$$

Where,

$\sum D_i^2$ = is the sum of D^2 values between all possible combinations (n) of the populations (P) included in a cluster.

n = all possible combinations among the populations in a cluster.

P = number of populations included in a cluster.

The square root of intracuster D^2 values ($d = \sqrt{D^2}$) was used to represent intra-cluster distance of a cluster. The average D^2 values of all possible genotype combinations in one cluster with those in the other were computed and its square root used to represent the statistical intercluster distance between the two clusters.

3.2.3.6.4 Contribution of different Characters towards Divergence

The relative contribution of different characters to the total divergence between each pair of genotypes was given a score of 1 to P (P being the number of characters) based on the magnitude of D^2 values due to each character. A rank of 1 represents the highest contribution and P the lowest of character 'X'

Contribution of each character was calculated using the following formula:

$$\text{Percent contribution of a character} = \frac{N(X)}{n(n-1)/2} \times 100$$

Where,

N(X) = Number of genotypic combinations which were ranked first for the character 'X', out of the total genotypic combinations of $n(n-1)/2$

n = Number of genotypes

3.2.3.7: Selection indices for various drought tolerance traits

In crop improvement programmes, several traits with varying degrees of agronomic and economic importance are usually considered at the same time. Because genetic correlations exist between traits, their selection cannot be considered separately. The improvement of one will provoke changes in others, usually in a sense contrary to that desired.

The alternative methodology available to plant breeders to counteract this type of problem comprises selection indices (**Hazel, 1943**). Selection based on indices permits maximizing the response to selection for one or a group of traits. In reality, selection based on indices reflects not only the response with direct selection, but also the correlated response as selection is practised for other traits.

The various steps involved in construction of selection indices are explained below:

1. Estimation of variance and covariance matrix:

Using some type of genetic design and experimental design that permit obtaining estimates of genetic and phenotypic variances and covariance's among the traits and consequently, their coefficients of heritability and genetic and phenotypic correlations.

2. Setting of Simultaneous Equations:

Selection is based on the phenotypic evaluation of several traits that are frequently obtained from the means of several replications. This is carried out to minimize the influence of experimental error and thus increase the precision of those means. That is, one can say that

$$\bar{P}_i = G_i + \bar{E}_i$$

and that, as a result, the phenotypic mean approaches the genotypic value.

When considering several traits at the same time, the genotypic value (G_j) of each family for the various traits should be obtained (**Baker, 1986**):

$$G_j = a_1 G_{1j} + a_2 G_{2j} + \dots + a_m G_{mj} = \sum_i^m a_i G_{ij}$$

Where,

a_i corresponds to the relative weights attributed to the various traits according to their agronomic or economic importance

G_j corresponds to the genotypic value of family $j(j=1,2,...,n)$

The problem is that the values of G_j are unknown; only their approximate values- the phenotypic values ($\bar{P}_j$) are known. Hence, for each individual (or family), a selection index based on phenotypic values is obtained, that is:

$$I_j = b_1 \bar{P}_{1j} + b_2 \bar{P}_{2j} + \dots + b_m \bar{P}_{mj} = \sum_i b_i \bar{P}_{ij}$$

Where,

b_i corresponds to the weights of the phenotypic values (that will be estimated)

$\bar{P}_j$ corresponds to the mean phenotypic value of family $j(j=1,2,...,n)$

Once the initial weights (a_i) are attributed to each trait, the estimates for b_i are obtained so that the correlation between G_j and I_j is maximized. That is, the value $r(G_j, I_j)$ should be the maximum possible. Under such conditions, we have the following matrix representation:

$$GA = PB$$

Where,

G is the matrix of the genetic variances and co-variances

P is the matrix of the phenotypic variances and co-variances

A is the vector of the weights attributed to the genotypic values

B is the vector of the weights of the phenotypic values to be estimated

The solution of this matrix system is given as:

$$\hat{B} = P^{-1}GA$$

Where,

P^{-1} is the inverse matrix of P .

As a result, the estimated vector B generates the relative weights of the phenotypic values ($\hat{b}_1, \hat{b}_2, \dots, \hat{b}_m$) thus permitting us to obtain each family's indices.

3. Expected Genetic Gain:

The expected genetic gain through selection is predicted by the following formula (Singh and Chaudhary, 1977):

$$(Z|v) \sum a_i b_j G_{ij} / (V_P)^{\frac{1}{2}}$$

Where,

(Z|v): Standardized selection differential (s) indicating selection intensity

a_i : Economic weightage

b_j : Regression coefficient

G_{ij} : Genotypic variance-covariance matrix

V_P : Phenotypic variance-covariance matrix

4. Relative efficiency of the index:

The relative efficiency of the index is determined as the percent ratio of the genetic gain by the index to the genetic gain by direct selection. The relative efficiency is determined using the following formula (Charumathi, 2011):

$$\text{Relative efficiency of index} = \frac{\text{Genetic gain by the index}}{\text{Genetic gain by the direct selection}} \times 100$$

3.3 Experiment 2: Validation of candidate gene specific molecular marker for drought tolerance in C₁ clones of different Sugarcane crosses

3.3.1 Experimental material:

The experimental material consisted of 20 selected C₂ clones of sugarcane selected based on performance for Physio-biochemical parameters observed in experiment 1 and five parental varieties. These genotypes comprised of 10 drought tolerant clones, 10 drought sensitive clones and five parental varieties (3 tolerant and 2 sensitive). The details of the clones used for the study are presented in Table 3.3.

Table 3.3: Details of the clones used for validation of candidate gene specific marker

S. No.	Genotype	Parentage	Drought tolerance capacity
1	PC (2015-16)-9	UP 9530 x Co Pant 97222	5
2	PC (2015-16)-16	UP 9530 x Co Pant 97222	1
3	PC (2015-16)-28	ISH 100 x Co Pant 97222	5
4	PC (2015-16)-29	ISH 100 x Co Pant 97222	5
5	PC (2015-16)-31	Bo 91 x Co 1158	5
6	PC (2015-16)-32	Co 617 x Co Pant 97222	1
7	PC (2015-16)-33	ISH 100 x Co Pant 97222	5
8	PC (2015-16)-34	ISH 100 x Co Pant 97222	5
9	PC (2015-16)-35	ISH 100 x Co Pant 97222	1
10	PC (2015-16)-36	ISH 100 x Co Pant 97222	1
11	PC (2015-16)-39	ISH 100 x Co Pant 97222	1
12	PC (2015-16)-43	ISH 100 x Co Pant 97222	1
14	PC (2015-16)-48	UP 9530 x Co 1148	1
15	PC (2015-16)-54	UP 9530 x Co 1148	5
16	PC (2015-16)-56	UP 9530 x Co 1148	1
17	PC (2015-16)-60	Co 85002 x Co 1158	5
18	PC (2015-16)-61	ISH 100 x Co 1158	1
19	PC (2015-16)-64	ISH 100 x Co 1158	5
20	PC (2015-16)-65	ISH 100 x Co 1158	5
21	Co 1148	P 4383 x Co 301	5
22	Co 617	PoJ 2878 x Co 285	5
23	Co Pant 97222	Co Pant 84212 GC	1
24	ISH 100	Co 7201 x CoC 671	5
25	UP 9530	Seo 1084/86 x Seo 122/85	1

***5: Tolerance and 1: Sensitive**

Molecular Marker Analysis:

Ten candidate gene specific molecular markers for drought tolerance are tested for validation in the above mentioned genotypes. The details of the markers used are presented in Table 3.4.

Table 3.4. Details of the molecular markers for molecular screening of sugarcane clones

S. No.	Marker	Forward Primer	Amplicons Size	Reference
1	DREB1A	AGATGTGCGGGATCAAGCAG TCGCGTAGTACAGGTCCCAG	408 bp	Priji and Hemaprabha, 2014
2	NAC 2	GGTTGCTGGCCACCATTCT GCCGTTTGGTACCTTCTGCT	305 bp	Priji and Hemaprabha, 2014
3	SHN 1	ATCCTCAGCGCCAAACTGAG GTGGTCGGAGCAAGAATAGCG	502 bp	Priji and Hemaprabha, 2014
4	SIZ 1	TGTAGCCAACGGCATGGAAC TCTCAGACAGGGAACAAACCAG	300 bp	Priji and Hemaprabha, 2014
5	Snac1	TACAAGTTCGACCCGTGGGA GCGACGAGTAGAAGTCGCC	270 bp	Priji and Hemaprabha, 2014
6	CIPK 14	GTGGAATTTCCGCCGAGAA GGGATGCGAAATTCCCCCTT	200 bp	Priji and Hemaprabha, 2015
7	LEA 3	AACCAGAACCAGGCCAGCTA AAATCAAGCGCGAACGATGC	270 bp	Priji and Hemaprabha, 2015
8	ABF 2	GGCTCAGGCTCAGAGTCAGA GCTCCTGCGACGATGAACTT	280 bp	Priji and Hemaprabha, 2015
9	DRF 1	AGCAAGCTCAAGCAGTCAGT GGGGTTGGCTGTCAAGCTTA	300 bp	Priji and Hemaprabha, 2014
10	HRD	TGGCGGCAATAGCCTATGAC CTATTCATGCAAGCCACACCAC	420 bp	Priji and Hemaprabha, 2014

3.3.2.1 Experimental method:

For the validation studies of 10 candidate gene specific molecular markers for drought tolerance the DNA from 25 genotypes was extracted from the young leaves of sugarcane. The detailed procedure for DNA extraction is given below.

DNA Extraction CTAB Method:

The genomic DNA was extracted for molecular characterization studies by using the CTAB method of **Doyle and Doyle (1990)** with some modifications.

Principle

CTAB is a cationic detergent that has the useful property of precipitating carbohydrates at high temperature and high salt concentration and it precipitates the nucleic acids at low salt concentration and low temperature.

Reagents for DNA extraction:

(1) 1 M Tris Cl Buffer (100ml)

12.114g of Tris base was dissolved in 80 ml of de-ionized water. pH was adjusted to 8.0 with 6N HCl and volume was made finally to 100 ml with de-ionized water. The entire solution was autoclaved and stored at room temperature.

(2) 0.5 M EDTA (50ml)

9.3 g EDTA was dissolved in 30 ml of de-ionized water containing few pellets of NaOH (EDTA dissolves at pH 8.0). pH was adjusted to 8.0 and final volume was made to 50 ml with de-ionized water. It was then autoclaved and kept at room temperature.

(3) 5 M NaCl (100ml)

Dissolve 29.22 g NaCl in 80 ml double distilled water. Heat water for 2-3 min and dissolve NaCl. Add double distilled water and make up the volume to 100 ml.

(4) 10 per cent CTAB (100ml)

Dissolve 10 g of CTAB in 70 ml of double distilled water, then heat in microwave oven then stir. Make up the volume to 100 ml.

(5) 0.2 percent beta mercaptaethanol: Added fresh.

(6) DNA Extraction buffer (100 ml)

Dissolve 20 ml of 10% CTAB, 10 ml of 1M Tris chloride, 28 ml of 5M NaCl and 4ml of 0.5M EDTA in double distilled water. Adjust the pH to 8.0 with 6N HCl. Make up the volume to 100 ml. Autoclave for 20 min. and store at 4°C.

(7) 70% Ethanol (100ml)

Dissolve 70 ml of Absolute ethyl alcohol in 30 ml of De-ionized water. Stored at 4°C.

(8) 5 M Sodium acetate (10ml)

4.1g of Sodium acetate was dissolved in 8 ml of de-ionized water with constant stirring. pH was adjusted to **7.5** with glacial acetic acid. Final volume was made up to 10 ml, autoclaved and stored at 4°C.

(9) Chloroform: Isoamyl alcohol 24:1 (100 ml)

Dissolve 4ml of Isoamyl alcohol to 96 ml of Chloroform. Mixed thoroughly and stored at 4°C.

(10) Isopropanol

A ready to use reagent is used and which has to be stored at -20°C.

(11) TE Buffer (100ml)

1ml of 1M Tris chloride buffer and 0.2 ml of 0.5M EDTA were added to 80 ml of de-ionized water and pH was adjusted to 8.0 with 6 N HCl. Final volume was made up to 100 ml, autoclaved and stored at room temperature.

3.3.2.2 Selection of experimental tissues for DNA extraction:

Young fresh leaves from ten randomly selected plants of each sugarcane clone were harvested using a sharp sterilized blade. Leaves were washed with autoclaved double distilled water and then sterilized with absolute alcohol.

DNA Isolation procedure

The method described by **Doyle and Doyle (1990)** was followed for extraction of genomic DNA.

- Using electronic balance 5.0 g of sterilized leaf material was weighed for DNA extraction.
- Pre-heat CTAB buffer at 65°C for 30' min a water bath. Take 50ml centrifuge tube and mark them from 1 to 25.
- In each centrifuge tube add 20 ml of extraction buffer (10% CTAB).
- Grind the sample to a fine powder in a pre-cooled mortar and pestle using liquid nitrogen.
- Mix extraction buffer and powdered sample thoroughly.

- Incubate at 65° for one hour (cap should be slightly loose). Mix it intermittently at almost 10 minutes interval.
- After taking tubes out from the water bath, allow it to come to room temperature and add 10 ml of chloroform and isoamyl alcohol (24:1) mixture. Swirl the tube gently by moving wrist in the shape of 8 for approximately 10 minutes.
- Centrifuge at 8500 rpm for 20 minutes.
- Transfer the aqueous phase to a fresh centrifuge tube. Add double amount of isopropanol. Mix the contents very gently by inverting the tube 10-15 times.
- Keep the tubes overnight at -20°C.
- Spool out the DNA thread using a wide-bore tip into a micro-centrifuge tube.
- The samples were centrifuged for 10 minutes at 5,000 rpm, 4°C. The solution was poured without disturbing the DNA pellet at the bottom of the tubes.
- The DNA pellet was washed in 70% ethanol for 20 min.
- The pellet was dried and dissolved in 500 µl TE buffer. It was stored at -20°C.

3.3.2.3 Determination of Quality and Quantity of Isolated DNA:

3.3.2.3.1 Qualitative analysis “agarose gel electrophoresis”:

Electrophoresis through Agarose is the standard method used to check the quality of DNA fragments. The technique is simple, rapid to perform and capable of resolving fragments of DNA. Submerged gel electrophoresis unit was used for fractionating genomic DNA on agarose gel.

3.3.2.3.2 Reagents required:

- DNA loading dye (Double dye): Bromophenol blue (0.25 w/v), Xylene cyanol, Sucrose (40% w/v).
- Electrophoresis buffer (50X TAE): 24.2g of Tris base, 5.7 ml of acetic acid and 10 ml of EDTA are dissolved in 100 ml of autoclaved distilled water and adjust the pH to 8.0.
- Ethidium bromide (10,000 X): 100mg of ethidium bromide was dissolved in 10ml of sterilized water and stored at 4°C. Ethidium bromide is highly carcinogenic and mutagenic. Working solution for staining the gel is made by dissolving 60µl EtBr stock (10mg/ml) in 300ml of water.

3.3.2.3.3 Procedure:

1. The open end of a clean, dry plastic tray supplied with the electrophoresis apparatus was sealed with tape so as to form mould.
2. 0.8% Agarose gel was prepared by dissolving (boiling) 1X TAE buffer. Cool it down to approximately 50⁰C. 8µl of ethidium bromide was added in this agarose solution and poured it in gel casting plate with already adjusted gel comb.
3. The agarose solution was left to solidify at room temperature for 30-40 minutes.
4. After complete setting of gel the comb was removed carefully with the tape and the gel was mounted in an electrophoresis tank filled with 1X TAE buffer.
5. The DNA loading dye was mixed with DNA sample in 1:6 ratio and loaded in gel along with one sample of known quantity of DNA.
6. Electrophoresis was done by running the gel at 75volts for 45 minutes.
7. The gel was then visualized on an UV transilluminator.
8. The photograph of gel was taken in a gel documentation unit.

3.3.2.4 DNA purification: RNase A treatment

Ribonuclease A is an endonuclease enzyme, having the capacity to digest RNA. DNase free RNase, supplied by Genei Pvt. Ltd., Bangalore was used. This RNase was supplied as a powder and dissolves easily in water.

3.3.2.4.1 Preparation:

One ml sterile de-ionized water (zero conductivity) was added to the vial containing RNase powder. Aliquots of 50 µl were made in sterile Eppendorf tubes. The tubes were kept in boiling water at 100⁰C for 15 min. Eppendorf tubes were covered with a Paraffin film and stored at -20⁰C.

3.3.2.4.2 RNase treatment procedure:

1. 5µl RNase A (10 mg/ml) was added to the Eppendorf tube containing 100 µl of extracted DNA and then incubated for 1 h at 37⁰C in a water bath.
2. Equal volume of Tris saturated phenol: chloroform: iso amylalcohol (25:24:1) was added and mixed gently by inverting the tubes. Centrifuged at 8000 rpm for 5 min.

3. Aqueous layer (i.e., upper layer) was collected and equal amount of chloroform + isoamyl alcohol (24:1) was added. The tubes were spun at 8,500 rpm for 5 min and top layer of DNA was removed.
4. Supernatant was taken into a fresh Eppendorf tube. To this, 5 M sodium acetate (1/10 vol, pH = 7.5) and chilled absolute ethanol was added. The contents were mixed and kept at -20°C for 30 min. Centrifuge the content to obtain the pellet.
5. Finally the pellet was washed with 70 per cent ethanol, dried and dissolved in 100 µl TE buffer. The genomic DNA dissolved in TE was quantified by UV absorbance by using Genesys UV spectrophotometer.

3.3.2.4.3 Quantification of genomic DNA:

The purified genomic DNA dissolved in TE buffer was taken for quantification by UV absorbance at 260nm in a UV spectrophotometer.

To measure the concentration by using UV spectrophotometer, the blank as set against TE buffer and then after proper rinsing of Quartz cuvette, the sample with appropriate dilution was loaded in cuvette. The optical density (O.D.) was measured at 260nm for estimating the concentration at DNA. The O.D. was also recorded at 280nm to determine protein contamination. The concentration of DNA was calculated by using following equation:

$$\text{Concentration of DNA } (\mu\text{g}/\mu\text{l}) = \text{O.D.}_{260} \times 50 \times \text{dilution factor} / 1000$$

The ratio of $\text{O.D.}_{260/280}$ gives the amount of RNA or protein contamination in the preparation. A value of 1.8 is optimum for best DNA preparation. A value of ratio below 1.8 indicated presence of protein in the preparation and a value above 1.8 indicates RNA contamination in the isolated DNA.

3.3.2.5. PCR Amplification

The Polymerase Chain Reaction (PCR) is a powerful, extremely sensitive technique based on the enzymatic amplification of DNA fragments that is flanked by oligo-nucleotide primers hybridizing to opposite strands of the target sequence. The PCR involves three basic steps which constitute a single cycle:

- (i) Denaturation of the target DNA at 92-94°C
- (ii) Annealing of the primers to the template DNA.

- (iii) Primer extension by addition of nucleotides to the 3' end of the primers by the enzyme DNA polymerase.

PCR amplifications were performed in a volume of 20 µl reaction setup as detailed in Table 3.5:

Table 3.5: Details of the PCR Reaction set up used in the study

Components	Conc.	Final Conc.	Single tube (µl)
1. DNA template (50ng/µl)		50ng	1.0
2. dNTPs mix (100 mM)		10 mM	0.4
3. Taq DNA polymerase (5U/ µl)		1U	0.2
4. Reaction buffer (10X)		1X	2.5
5. Primer forward(20 ng/ µl)		20 ng	1.0
6. Primer reverse (20 ng/ µl)		20 ng	1.0
7. De-ionized water		15.0 µl	13.9
Total volume			20.0

The PCR amplification was carried out in Himedia Prima™ Thermocycler as per the protocol presented in the Table 3.6. As per requirement of individual molecular marker the annealing temperature with slight modification were used.

3.3.2.6. Agarose gel electrophoresis

Horizontal gel electrophoresis unit was used for fractionating the PCR products on agarose gel. Agarose gel (3%) was prepared by dissolving 3g of agarose in 100 ml of 1X TAE buffer. For each well, DNA loading dye and DNA samples were mixed in 1:6 ratios and loaded with a micropipette. Electrophoresis was done at 75 V for 2 h in 1X TAE electrophoresis buffer. The gel was then stained in ethidium bromide solution. After de-staining in de-ionized water, the gel image was viewed in U.V. transilluminator and stored in gel documentation system.

Table 3.6: PCR Amplification Protocol used for 10 candidate gene specific markers

	Denaturation		Annealing		Polymerization	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94°C	5 min	-	-	-	-
36 Cycles	94°C	1 min	50-63°C	1 min	72°C	1 min
Last cycle	-	-	-	-	72°C	7 min

The amplified products were electrophoresed on agarose gel.

3.3.2.7. Validation of markers in the Elite Clones

10 candidate gene specific molecular markers identified and reported by various workers as potential markers for Carrying out MAS for drought tolerance, were selected for their validation in the present experimental material. All the 10 molecular markers were scored as present or absent for each of the 25 samples in the C₂ clones. For each marker, C₂ clones were grouped into two classes: those with the marker and those without. The phenotypic mean of the two classes was then statistically compared by using t- test (McIntyre *et al.*, 2005). t- Test was conducted using the following formula:

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$



Results and Discussion



Sugarcane (*Saccharum* spp.) is a water loving crop and suffers from water stress. The crop consumes about one tonne of water to produce 454 g of sugar. Sugarcane in rain-fed condition requires a minimum of rainfall of 1000-1200 mm per annum. Under irrigated condition sugarcane normally receives enough water however the process creates pressure on water resources and aggravates water scarcity. Low output of cane and subsequent reduction in sugar per unit area, as well as low sugar recovery from net weight of cane has been attributed to severe drought stress (**Moore, 1987**). However, studies have also shown that the capacity to tolerate drought is genotype dependent and so can be bred. Breeding for high yield in drought stress environments can be achieved either by selecting for characters that increase yield under moisture deficit or by incorporating drought tolerance trait into an existing breeding programme (**Ishaq and Olaoye, 2009**).

With the objective to develop clones exhibiting good tolerance ability towards water stress, the present study was carried out in two experiments. The experimental results are presented in the following heads:

Experiment 1:

Sixteen different physio-biochemical characters were recorded among all 65 Bi-parental clones, seven parents and six standard checks planted in two seasons (spring and autumn) with two different environments (control and stress) in each of the season during 2017-18. The entire data set were subjected to statistical analysis and the experimental results are presented below under following heading.

- 4.1 Analysis of variance
- 4.2 Mean performance of clones
- 4.3 Estimates of Selection parameter
- 4.4 Estimates of Correlation and Path coefficient
- 4.5 Assessment of genetic diversity
- 4.6 Selection indices for various drought tolerance traits
- 4.7 Evaluation of 65 bi-parental progenies along-with parents for their response to drought stress

4.1. Analysis of variance:

For each of four sets of data recorded from two seasons each with two environments which comprises sixteen different physiological and biochemical parameters, analysis of variance (ANOVA) was carried out individually following the standard procedure for augmented block design- II (**Federer, 1956**), and the results are summarized individually in following sections.

4.1.1 Analysis of Variance for Spring planted Control Environment

The results of ANOVA are represented in Table 4.1. Analysis of variance for spring planted control environment during 2017-18 revealed that there were significant differences among all 65 bi-parental C₁ clones and their parents for 14 characters except two traits namely proline content (11.245) and leaf firing (0.187) for which non-significant differences found. Traits like SPAD 60 (52.004), SPAD 120 (40.506), CTD 180 (3.763), RWC% (8.406), Total Chlorophyll (0.153) and Total carotenoids (0.002) recorded highly significant differences. No significant differences were recorded between the 6 standards used for traits like days to germination, germination percent, SPAD 120 (7.623), SPAD 180 (13.743), CTD 60 (1.862), CTD 120 (1.451), CTD 180 (0.876), Chl b (0.003), proline content (8.256) and leaf firing (0.00).

4.1.2 Analysis of Variance for Spring planted Stress Environment

The results of ANOVA are summarized in Table 4.2. Analysis of variance for 16 characters under stress environment in spring 2017-18 indicated significant differences among all 65 bi-parental C₁ 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.

4.1.3 Analysis of Variance for Autumn planted Control Environment

The results of ANOVA are summarized in Table 4.3. Similar to spring planted crop, analysis of variance under control environment during autumn 2017-18 also indicated significant differences among all 65 bi-parental C₂ clones and their parents for 14 characters out of 16 characters and non significant difference were observed for two characters namely proline and leaf firing. No significant differences were observed between the 6 standards for traits like days to germination, germination percent, SPAD 60 (9.818), SPAD 120 (9.818), SPAD 180 (13.743), CTD 60 (4.357), CTD 120 (1.143), CTD 180 (1.129), Chl b (0.004), proline content (12.262) and leaf firing (0.00).

Table 4.1: Analysis of Variance for Various Drought Tolerance Traits in Sugarcane Under Spring Planted Control Environment

Source of Variation	DF	Days to Germination	Germination%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
Block	2	120.278 **	7.947	68.442***	1.716	65.013**	1.180	43.904	3.965**
Entries	77	63.222**	58.756**	52.004***	4.277**	40.506***	4.338**	42.205*	3.763***
Checks	5	12.500	25.14	18.102*	1.862	7.623	1.451	13.743	0.876
Varieties	71	57.903**	57.011**	55.041***	4.325**	41.968***	4.425**	43.490*	4.019***
Checks vs. Varieties	1	694.445***	36.152**	5.919	12.935**	101.113**	12.578**	93.300*	0.015
Error	10	12.500	1.945	4.481	0.971	4.964	0.715	13.548	0.296

Source of Variation	DF	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	DTC
Block	2	2.439	0.663**	0.005	0.592***	0.001***	18.417*	0.000	0.122***
Entries	77	8.406***	0.154**	0.011**	0.153***	0.002***	11.245	0.187	0.062***
Checks	5	5.111**	0.064**	0.003	0.085***	0.000*	8.256	0.000	0.071***
Varieties	71	6.894***	0.156**	0.009**	0.159***	0.002***	11.856	0.190	0.060***
Checks vs. Varieties	1	132.181**	0.440**	0.167***	0.065**	0.001***	10.297	0.000	0.138***
Error	10	0.818	0.004	0.002	0.006	0.000	4.562	0.956	0.004

*, **, ***, 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 4.2: 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 4.3: Analysis of Variance for Various Drought Tolerance Traits in Sugarcane Under Autumn Planted Control Environment

Source of Variation	DF	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
Block	2	5.833	6.512	8.207	5.232	11.641	16.353**	10.381	6.609
Entries	77	45.747**	52.756**	72.219**	5.884*	72.291**	5.943*	75.812**	6.305*
Checks	5	12.500	15.14	9.818	4.357	9.818	1.143	13.743	1.129
Varieties	71	48.415*	54.251**	63.447**	5.694*	63.527**	6.313*	69.293**	6.572*
Checks vs. Varieties	1	22.500	30.152**	1007.012****	27.027**	1006.845****	3.654	848.978****	13.187*
Error	10	12.500	2.495	14.146	1.771	14.146	1.937	13.548	2.018

Source of Variation	DF	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing	DTC
Block	2	1.874	0.656***	0.005*	0.583***	0.001***	14.859*	0.000	0.114***
Entries	77	7.573***	0.153***	0.010***	0.152***	0.002***	10.631	0.787	0.064***
Checks	5	4.664**	0.052***	0.004	0.077***	0.000**	12.262	0.000	0.084***
Varieties	71	6.894***	0.156***	0.009***	0.159***	0.002***	13.355	0.990	0.060***
Checks vs. Varieties	1	70.313***	0.378***	0.113***	0.077**	0.001***	15.027*	2.900	0.247***
Error	10	0.771	0.004	0.001	0.004	0.000	5.450	0.856	0.006

*, **, ***, 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 4.4: Analysis of Variance for Various Drought Tolerance Traits in Sugarcane Under Autumn 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	54.444**	19.647**	60.232**	3.073*	14.898	15.917***	55.950***	29.493***
Entries	77	36.079**	91.458***	73.431***	3.255**	45.446**	5.876***	73.754***	16.045***
Checks	5	48.889**	56.23***	27.531**	2.569*	16.730	7.029***	36.143***	1.732
Varieties	71	34.620**	92.56***	71.806***	3.209**	45.662**	5.742***	73.676***	16.670***
Checks vs. Varieties	1	75.625**	156.254***	418.372***	9.973**	173.736***	9.591***	267.289***	43.222**
Error	10	5.556	1.926**	4.866	0.682	6.530	0.447	2.311	0.606

Source of Variation	DF	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	leaf Firing	DTC
Block	2	25.486*	0.776***	0.015***	0.999***	0.000**	13280.150***	0.344***	0.082**
Entries	77	82.561***	0.002	0.000	0.319***	0.000**	3177.846***	0.255***	0.087***
Checks	5	24.767**	0.227***	0.011***	0.042***	0.000**	7.585	0.000	0.101***
Varieties	71	68.604***	0.030***	0.001*	0.326***	0.000**	2749.892***	0.244***	0.080***
Checks vs. Varieties	1	1362.433***	0.233***	0.011***	1.217***	0.000**	49413.870***	2.336***	0.492***
Error	10	3.479	0.002	0.000	0.002	0.000	2.738	0.000	0.009

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

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

4.1.4 Analysis of Variance for Autumn planted Stress Environment

The results of ANOVA are summarized in Table 4.4. Analysis of variance for 16 characters under stress environment during autumn 2017-18 indicated that there were significant differences among all 65 bi-parental C₂ clones and their parents for all the 16 characters recorded. No significant differences were recorded between the 6 standards for traits like SPAD 120 (16.730), CTD 180 (1.732), proline content (7.585) and leaf firing (0.00).

Comparing analysis of variance for all the four environments (Two season + Two growing condition) we found that few of the traits like proline content and leaf firing exhibited no significant differences in control environment but highly significant difference under stress condition, indicating that these traits are of very high utility in selection for drought tolerance.

4.1.5 Test for Homogeneity of Error Variances between Two Seasons

In order to perform combined analysis or Pooled analysis of variances the primary requirement is to satisfy homogeneity of error variances over seasons using one of the homogeneity test like F-test of homogeneity {When, error variances (p)=2} and Bartlett's Chi-square test {When, error variances (p)>2} depending upon the nature of data and number of environments. In our study we have to compare error variance from only two environments therefore we used F-test as suggested by Lal, (2018). The F-test was carried out to test homogeneity of variances between two similar environments over season i.e. control environment in spring and autumn planted crops. Similarly stress environment in spring and autumn planted crops were tested for homogeneity. The results for both the test are presented in following sections.

4.1.5.1 F-Test to Test Homogeneity of Variances between Two Control Environments

Homogeneity of variances test was performed for all the 16 characters in both the seasons (spring and autumn) for control environment. The results of the F-test are presented in the Table 4.5. The results obtained indicated that error variances for all the traits are homogeneous at 1% level of significance except for CTD 180 (6.818) and SPAD 60 (3.157). For these characters error variances were found heterogeneous at 5% level of significance.

4.1.5.2 F-Test to Test Homogeneity of Variances between Two Stress Environments

The results of the F-test are presented in the Table 4.6. revealed homogeneous error variances at 1% level of significance for all the 16 characters in both the seasons (spring and autumn). Traits like SPAD 60 (3.365) and RWC% (3.681) were found to exhibit heterogeneity of error variances at 5% level of significance.

Table 4.5: F-Test to test Homogeneity of Variances between Two Control Environments

S. No.	Traits	Control Spring	Control Autumn	F test	5%= 2.9	1%= 4.8
1	Days to Germination	12.5	12.5	1.000	NS	NS
2	Germination%	1.945	2.495	1.282	NS	NS
3	SPAD 60	4.481	14.146	3.157	S	NS
4	CTD 60	0.971	1.771	1.824	NS	NS
5	SPAD 120	4.964	14.146	2.850	NS	NS
6	CTD 120	0.715	1.937	2.709	NS	NS
7	SPAD 180	13.548	13.548	1.000	NS	NS
8	CTD 180	0.296	2.018	6.818	S	S
9	RWC%	0.818	0.771	1.061	NS	NS
10	Chl a	0.004	0.004	1.000	NS	NS
11	Chl b	0.002	0.001	2.000	NS	NS
12	Total Chl	0.006	0.004	1.500	NS	NS
13	Total Carotenoids	0.000	0.000	0.000	NS	NS
14	Proline	0.903	1.26	1.395	NS	NS
15	Leaf firing	0.854	0.652	1.310	NS	NS
16	D T C	0.004	0.006	0.667	NS	NS

Table 4.6: F-Test to test Homogeneity of Variances Between Two Stress Seasons

S. No.	Traits	Stress Spring	Stress Autumn	F test	5%= 2.9	1%= 4.8
1	Days to Germination	4.566	5.556	1.216	NS	NS
2	Germination%	2.526	1.926	1.311	NS	NS
3	SPAD 60	1.446	4.866	3.365	S	NS
4	CTD 60	0.402	0.682	1.696	NS	NS
5	SPAD 120	4.33	6.53	1.508	NS	NS
6	CTD 120	0.66	0.447	1.476	NS	NS
7	SPAD 180	1.773	2.311	1.303	NS	NS
8	CTD 180	1.37	0.606	2.260	NS	NS
9	RWC%	0.945	3.479	3.681	S	NS
10	Chl a	0.003	0.002	1.500	NS	NS
11	Chl b	0.000	0.000	0.000	NS	NS
12	Total Chl	0.004	0.002	2.000	NS	NS
13	Total Carotenoids	0.000	0.000	0.000	NS	NS
14	Proline	3.126	2.738	1.141	NS	NS
15	Leaf firing	0.000	0.000	0.000	NS	NS
16	D T C	0.017	0.009	1.888	NS	NS

4.1.6 Pooled Analysis of Variance for Control and Stress Environment

Pooled or combined analysis of variances was performed after satisfying the requirement of F-test of homogeneity. Pooled analysis for control and stress environment was performed separately considering each season as replication for all the 65 bi-parental clones, their parents and the 6 standards and the results have been summarized in the following sections.

4.1.6.1 Pooled Analysis of Variance for Control Environment

Pooled analysis of variances was carried out for control environment with 65 bi-parental progenies, their parents and 6 standards in two replications where each of the seasons (spring and autumn) was considered as a single replicate. The results of the pooled analysis are presented in Table 4.7. The results revealed that all the genotypes (bi-parental clones, parents and standards) differ significantly for 14 characters, whereas for two characters like proline content (15.788) and leaf firing (4.781) no significant differences were recorded.

4.1.6.2 Pooled Analysis of Variance for Stress Environment

Pooled analysis of variances was performed for stress environment comprising of 65 bi-parental progenies, parental genotypes and 6 standards. The results of the pooled analysis are presented in Table 4.8. The results showed that all the bi-parental clones differ significantly from each other for all the 16 characters.

The existence of significant differences for almost all the traits indicates that sufficient variability exist among the clones evaluated in the study. Coefficient of variability (CV) for control environment and stress environment ranged from 1.001 to 17.34 and 0.863 to 16.216 respectively. The wide range of coefficient of variability was due to higher CV values for days to germination (17.34 and 16.216 respectively) in both environmental conditions. CV for the remaining traits was recorded to be less than the 10 which indicates consistency in the execution of the experiments (**Bisht *et al.*, 2014**).

Comparing the results from two different environment (control and stress), it's found that the traits like proline content and leaf firing varied between both the environment. Significant difference for proline content and leaf firing in stress environment unlike control environment signifies that these two traits have significant role in water stress environment.

Table 4.7: Pooled Analysis of Variance for Various Drought Tolerance Traits in Sugarcane under Control Environment

Source of Variation	d.f.	Mean Squares							
		Days to Germination	Germination%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
Replications	1	2417.36	7.947	2641.103	0.0007	1451.483	3.428	948.486	4.601
Treatments	71	70.64**	57.011**	71.925*	6.806**	71.000**	6.256**	78.141**	7.842**
Error	71	35.67	1.495	46.562	1.066	34.494	1.7186	34.641	0.904
CV%		17.3410	3.1138	16.4726	15.3994	14.1245	16.5030	15.0439	15.209

Source of Variation	d.f.	Mean Squares							
		RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing	D T C
Replications	1	1.488	0.005	0.044	0.019	0.0001	163.840	0.000	0.000
Treatments	71	15.669**	0.310**	0.016**	0.309**	0.0004**	15.788	4.781	5.101**
Error	71	0.768	0.0009	0.0005	0.001	0.000	0.773	0.000	0.000
CV%		1.001	1.435	4.593	1.360	3.336	1.840	0.000	0.000

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

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

Table 4.8: Pooled Analysis of Variance for Various Drought Tolerance Traits in Sugarcane Under Stress Environment

Source of variation	d.f.	Mean Squares							
		Days to Germination	Germination%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
Replications	1	289.00	19.647	3.546	0.448	34.310	0.022	91.808	2.921
Treatments	71	49.624**	91.458**	96.348**	5.755**	92.889**	8.496**	109.002**	8.593**
Error	71	21.394	1.926	4.326	0.205	4.280	0.337	10.757	0.463
CV%		16.216	3.804	4.789	9.070	4.886	11.272	8.198	11.246

Source of variation	d.f.	Mean Squares							
		RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing	D T C
Replications	1	3.712	0.279	0.006	0.367	0.0002	910.128	0.173	0.173
Treatments	71	135.989**	0.473**	0.019**	0.648**	0.0005**	5474.911**	5.119**	5.119**
Error	71	0.364	0.005	0.0003	0.005	0.000	232.320	0.117	0.117
CV%		0.823	4.176	6.377	3.683	3.399	9.769	10.125	10.125

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

Pooled analysis of variances allows further reduction in error mean sum of squares as one or more source of variability is taken out from experimental error thus reducing the experimental error. This may further lead to reduction in coefficient of variation (Lal, 2018).

4.2 Mean performance of clones

The performance of 65 bi-parental clones along with their parents the standards viz., Co 0238, Co Pant 03220, Co J 64 (early) and Co Pant 97222, Co Pant 05224, Co S 8436 (Mid late) were evaluated in both the environments (control and stress). The data was recorded for 16 different physiological and biochemical parameters such as days to germination, germination percent, SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC%, Chl a, Chl b, Total Chl, Total carotenoids, proline content, leaf firing and drought tolerance capacity (DTC). Mean performance of all genotypes in both the environments (control and stress) is summarized in the Table 4.9.

4.2.1 Days to Germination

Mean number of days to germination in control and stress environments was recorded to be 28.9 ± 4.19 and 24.47 ± 3.24 , respectively which ranged from 18 for PC (2015-16)-1 to 45 for PC (2015-16)-34 in control and 15 for PC (2015-16)-1 and 30 for PC (2015-16)-11 under stress environment, respectively. All the checks recorded a mean number of days to germination to be 30 days in both the environments. Critical difference of 8.36 and 6.47 at 5% was recorded in control and stress environment, respectively. Among 65 bi-parental clones 11 clone were found to be on par with the best parent ISH 100.

4.2.2 Germination Percent at 45 DAS

Mean germination percent in control and stress environments was recorded to be 38.80 ± 0.85 and 36.20 ± 0.97 , respectively with a range from 26 for PC (2015-16)-23 to 47.60 for PC (2015-16)-37 and 19.75 for PC (2015-16)-23 and 45.97 for PC (2015-16)-29 in control and stress environments, respectively. Critical difference of 1.71 and 1.94 at 5% was recorded in control and stress environments, respectively. For germination percent seven clones were found to be significantly superior than the best parent Co Pant 97222.

4.2.3 SPAD Reading at 60 DAS

Mean SPAD Readings in control and stress environments was recorded to be 41.05 ± 4.79 and 42.98 ± 1.46 , ranged from 30.50 for PC (2015-16)-53 to 56.30 for PC (2015-16)-20 and 28.20 for PC (2015-16)-53 and 54.81 for PC (2015-16)-20 respectively. Critical difference of 9.55 and 2.91 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones three clones were found to be on par with the best parent ISH 100 for SPAD 60.

4.2.4 CTD at 60 DAS

Mean CTD in control and stress environments was recorded to be 4.30 ± 0.72 and 4.93 ± 0.31 , respectively which ranged from 0.40 for PC (2015-16)-35 to 8.00 for PC (2015-16)-40 and 4.56 for PC (2015-16)-13 and 7.88 for PC (2015-16)-9, respectively in control and stress environments. Critical difference of 1.44 and 0.63 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones PC (2015-16)-9 was the only clone found to be significantly superior than the best parent ISH 100.

4.2.5 SPAD Reading at 120 DAS

Mean SPAD Readings in control and stress environments was recorded to be 41.17 ± 4.12 and 42.12 ± 1.45 which ranged from 29.90 for PC (2015-16)-53 to 55.80 for PC (2015-16)-20 and 28.95 for PC (2015-16)-48 and 54.32 for PC (2015-16)-31 respectively. Critical difference of 8.22 and 2.89 at 5% was recorded in control and stress environment respectively. For SPAD 120 readings four clones were found to be significantly superior than the best parent ISH 100.

4.2.6 CTD at 120 DAS

Mean CTD in control and stress environments was recorded to be 5.27 ± 0.92 and 5.09 ± 0.40 which ranged from 0.70 for PC (2015-16)-35 to 9.53 for PC (2015-16)-15 and 1.15 for PC (2015-16)-32 and 9.15 for PC (2015-16)-31 respectively. Critical difference of 1.83 and 0.81 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones 12 clones were found to be significantly superior than the best parent Co 617.

4.2.7 SPAD Reading at 180 DAS

Mean SPAD Readings in control and stress environments was recorded to be 38.75 ± 4.13 and 39.63 ± 2.30 which ranged from 28.60 for PC (2015-16)-53 to 52.40 for PC (2015-16)-55 and 23.48 for PC (2015-16)-61 and 53.44 for PC (2015-16)-31 respectively. Critical difference of 8.24 and 4.59 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones PC (2015-16)-31 was the only clone found to be significantly superior than the best parent ISH 100.

4.2.8 CTD at 180 DAS

Mean CTD in control and stress environments was recorded to be 4.67 ± 0.66 and 5.95 ± 0.47 which ranged from 0.30 for PC (2015-16)-13 to 9.23 for PC (2015-16)-64 and 2.33 for PC (2015-16)-25 and 10.62 for PC (2015-16)-33 respectively. Critical difference of 1.33 and 0.95 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones two clones were found to be significantly superior than the best parent Co 617 for CTD 180.

4.2.9 Relative Water Content Percent (RWC%)

Mean RWC in control and stress environments was recorded to be 87.35 ± 0.61 and 72.52 ± 0.42 which ranged from 80.66 for PC (2015-16)-62 to 91.50 for ISH 100 and 55.01 for PC (2015-16)-35 and 86.60 for PC (2015-16)-33 respectively. Critical difference of 1.22 and 0.84 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones four clones were found to be significantly superior than the best parent ISH 100.

4.2.10 Chlorophyll a (Chl a)

Mean Chl a in control and stress environments was recorded to be 2.08 ± 0.02 and 1.79 ± 0.05 which ranged from 1.54 for PC (2015-16)-11 to 3.33 for ISH 100 and 1.12 for PC (2015-16)-61 and 3.28 for ISH 100 respectively. Critical difference of 0.04 and 0.10 at 5% was recorded in control and stress environment respectively. None of the clones were found to be significantly superior than the best parent ISH 100.

4.2.11 Chlorophyll b (Chl b)

Mean Chl b in control and stress environments was recorded to be 0.51 ± 0.01 and 0.28 ± 0.01 which ranged from 0.20 for PC (2015-16)-50 to 0.70 for PC (2015-16)-7 and 0.10 for PC (2015-16)-62 and 0.63 for PC (2015-16)-58 respectively. Critical

difference of 0.03 and 0.02 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones PC (2015-16)-58 was the only clone found to be significantly superior than the best parent ISH 100.

4.2.12 Total Chlorophyll (Total Chl)

Mean Total Chl in control and stress environments was recorded to be 2.59 ± 0.02 and 2.07 ± 0.05 which ranged from 1.99 for PC (2015-16)-61 to 3.89 for ISH 100 and 1.25 for PC (2015-16)-61 and 3.80 for ISH 100 respectively. Critical difference of 0.04 and 0.10 at 5% was recorded in control and stress environment respectively. None of the clones were found to be significantly superior than the best parent ISH 100.

4.2.13 Total Carotenoids

Mean Total Carotenoids in control and stress environments was recorded to be 0.05 ± 0.00 and 0.05 ± 0.00 which ranged from 0.03 for PC (2015-16)-52 to 0.09 for ISH 100 and 0.03 for PC (2015-16)-1 and 0.09 for ISH 100 respectively. Critical difference of 0.00 and 0.00 at 5% was recorded in control and stress environment respectively. For total carotenoids none of the clones were found to be significantly superior than the best parent Co Pant 97222.

4.2.14 Proline Content

Mean proline content in control and stress environments was recorded to be 47.43 ± 0.61 and 151.44 ± 10.70 which ranged from 41.18 for PC (2015-16)-23 to 54.98 for PC (2015-16)-14 and 92.77 for PC (2015-16)-15 and 233.58 for PC (2015-16)-64 respectively. Critical difference of 11.32 and 21.34 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones 10 clones were found to be on par with the best parent ISH 100 for proline content.

4.2.15 Leaf Firing

Mean leaf firing in control and stress environments was recorded to be 4.08 ± 0.00 and 3.21 ± 0.24 which ranged from 1.00 for PC (2015-16)-5 to 5.00 for ISH 100 and 1.00 for PC (2015-16)-4 and 5.00 for ISH 100 respectively. Critical difference of 0.00 and 0.47 at 5% was recorded in control and stress environment respectively. Among 65 bi-parental clones 41 clones were found to be on par with the best parent ISH 100. Few of the genotypes exhibiting leaf firing symptoms are presented in Plate 1.

4.2.16 Drought Tolerance Capacity (DTC)

Mean DTC in control and stress environments was recorded to be 3.28 ± 0.00 and 3.31 ± 0.24 which ranged from 1.00 for PC (2015-16)-16 to 5.00 for ISH 100 and 1.00 for PC (2015-16)-4 and 5.00 for ISH 100 respectively. Critical difference of 0.00 and 0.47 at 5% was recorded in control and stress environment respectively. For DTC 29 clones were found to be on par with the best parent ISH 100.

Critical perusal of mean performance of all the 72 genotypes for 16 physiological and biochemical traits in both control and stress environment, it was found that there was a enormous effect of water stress on the mean performance of all the genotypes for all the characters. The effect of water stress was clearly reflected by the reduced values for almost all the traits except for proline content under stress. Increased proline content was also reported earlier due to up-regulation of proline coding gene in response to water stress conditions (**Wagih, 2005**).

Traits like SPAD readings at various time intervals exhibited a gradual decrease under stress condition. Similar trend of gradual decrease for SPAD readings reported in sugarcane **Silva et al. (2011)** and in wheat **Karimizadeh and Mohammadi (2011)**. The gradual decrease in SPAD readings are presented as Fig. 2.

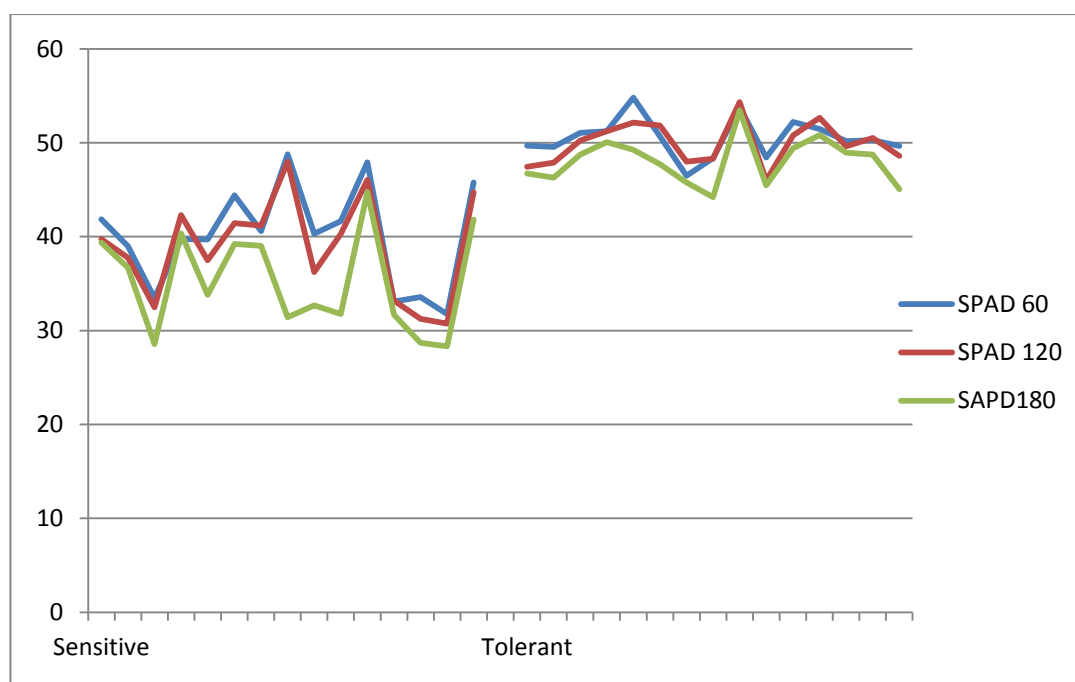


Fig.2: SPAD Readings at Various Intervals Under Stress Environment



PC (2015-16)-5



PC (2015-16)-23



PC (2015-16)-48

Plate 1: Leaf Firing symptoms due to water stress in Sugarcane

Table 4.9: Mean Performance of 72 Sugarcane Genotypes for various Drought Tolerance Traits in Control and Stress Environment

S. No.	Genotype	Days to Germination		Germination%		SPAD 60		CTD 60		SPAD 120	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1	PC (2015-16)-1	18.00	15.00	32.85	31.70	45.05	41.84	5.38	5.15	45.71	39.74
2	PC (2015-16)-2	23.00	18.00	35.80	32.50	40.00	38.99	5.48	4.19	39.61	37.77
3	PC (2015-16)-3	23.00	18.00	32.70	27.80	31.50	33.48	4.03	2.69	31.86	32.48
4	PC (2015-16)-4	18.00	15.00	30.70	24.80	43.80	39.73	5.01	5.24	44.48	42.28
5	PC (2015-16)-5	38.00	30.00	35.05	30.50	39.25	39.72	5.15	4.69	38.16	37.49
6	PC (2015-16)-6	23.00	23.00	32.65	31.00	38.70	44.40	5.17	5.11	38.22	41.45
7	PC (2015-16)-7	23.00	23.00	34.65	30.50	38.90	40.60	5.33	5.17	39.18	41.18
8	PC (2015-16)-8	33.00	25.00	44.85	43.70	41.45	48.77	6.70	7.31	42.07	47.98
9	PC (2015-16)-9	33.00	25.00	42.85	42.20	40.20	49.68	7.08	7.88	40.71	47.43
10	PC (2015-16)-10	20.00	15.00	36.36	31.38	41.60	40.31	1.70	2.71	40.30	36.23
11	PC (2015-16)-11	30.00	15.00	29.00	26.45	38.80	41.65	1.00	2.88	37.50	40.28
12	PC (2015-16)-12	25.00	25.00	41.78	45.85	41.10	47.93	5.20	6.13	40.71	46.06
13	PC (2015-16)-13	20.00	15.00	35.35	30.85	36.30	33.10	2.10	1.56	35.00	33.19
14	PC (2015-16)-14	30.00	30.00	41.25	44.55	52.10	49.56	6.00	6.18	48.90	47.89
15	PC (2015-16)-15	33.00	25.00	37.45	33.95	33.05	33.56	7.03	4.62	32.10	31.24
16	PC (2015-16)-16	23.00	30.00	37.50	35.70	32.80	31.75	2.48	1.92	32.98	30.75
17	PC (2015-16)-17	30.00	15.00	36.65	33.70	43.95	45.78	1.33	4.25	43.84	44.72
18	PC (2015-16)-18	25.00	20.00	41.25	44.40	43.40	51.05	6.21	6.98	45.75	50.25
19	PC (2015-16)-19	30.00	30.00	39.10	36.85	34.25	42.40	2.11	4.55	35.88	41.55
20	PC (2015-16)-20	30.00	30.00	44.90	45.65	56.30	51.22	7.00	7.37	55.80	51.21
21	PC (2015-16)-21	30.00	15.00	27.15	24.80	43.50	42.52	3.40	3.61	43.00	39.71
22	PC (2015-16)-22	23.00	23.00	26.85	22.60	40.90	44.20	3.65	4.26	41.68	42.73
23	PC (2015-16)-23	33.00	25.00	26.00	19.75	37.20	44.50	4.87	4.84	37.01	43.10
24	PC (2015-16)-24	23.00	18.00	30.25	25.82	34.35	37.87	4.43	3.19	34.48	35.23
25	PC (2015-16)-25	25.00	23.00	35.85	30.25	37.75	35.69	2.99	3.61	37.66	37.25
26	PC (2015-16)-26	28.00	30.00	42.75	42.20	44.00	54.81	5.45	7.52	46.82	52.15

Table 4.9: Contd....

S. No.	Genotype	Days to Germination		Germination%		SPAD 60		CTD 60		SPAD 120	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
27	PC (2015-16)-27	33.00	25.00	40.20	35.95	37.35	42.68	5.90	4.23	39.23	41.79
28	PC (2015-16)-28	25.00	20.00	43.45	42.23	46.95	50.68	5.80	7.20	45.93	51.85
29	PC (2015-16)-29	25.00	25.00	47.25	45.97	40.30	46.49	5.65	7.05	42.27	47.98
30	PC (2015-16)-30	25.00	25.00	43.75	43.10	42.05	48.35	5.76	6.75	45.48	48.30
31	PC (2015-16)-31	30.00	25.00	43.20	40.38	45.10	53.81	5.73	5.61	48.03	54.32
32	PC (2015-16)-32	30.00	20.00	40.35	30.47	35.70	30.72	1.10	2.45	36.18	34.44
33	PC (2015-16)-33	25.00	25.00	41.95	41.20	42.85	49.33	4.66	6.15	43.28	42.40
34	PC (2015-16)-34	35.00	30.00	41.20	41.01	54.20	50.88	0.70	7.33	53.70	49.93
35	PC (2015-16)-35	45.00	20.00	37.15	33.45	37.70	48.36	0.40	6.82	37.20	46.23
36	PC (2015-16)-36	30.00	30.00	36.20	32.85	50.90	45.11	2.70	2.90	50.40	43.27
37	PC (2015-16)-37	23.00	23.00	47.60	43.85	42.10	48.43	5.40	6.38	42.13	45.99
38	PC (2015-16)-38	20.00	18.00	45.70	44.89	33.35	52.21	5.65	6.68	34.18	50.77
39	PC (2015-16)-39	25.00	30.00	35.75	29.00	33.80	32.23	6.17	2.46	33.07	33.91
40	PC (2015-16)-40	25.00	20.00	41.95	40.55	42.95	51.45	8.00	6.80	46.96	52.67
41	PC (2015-16)-41	25.00	25.00	40.55	41.20	41.45	48.83	5.81	6.11	41.81	46.43
42	PC (2015-16)-42	20.00	20.00	31.20	27.25	40.30	37.57	3.93	4.04	40.13	42.13
43	PC (2015-16)-43	30.00	23.00	30.50	23.80	41.70	39.03	5.35	3.28	41.92	40.88
44	PC (2015-16)-44	33.00	20.00	37.70	30.86	38.60	39.30	1.53	4.72	39.28	35.72
45	PC (2015-16)-45	30.00	30.00	46.25	41.49	51.40	48.39	5.50	6.81	50.80	47.18
46	PC (2015-16)-46	30.00	30.00	30.40	25.20	39.10	36.31	6.30	5.33	38.50	36.13
47	PC (2015-16)-47	25.00	20.00	40.75	40.20	35.20	47.29	5.30	6.31	35.58	47.62
48	PC (2015-16)-48	33.00	30.00	40.35	41.75	31.25	28.20	4.15	3.86	31.98	28.95
49	PC (2015-16)-49	30.00	30.00	42.90	42.05	41.20	46.29	6.38	5.80	40.83	46.73
50	PC (2015-16)-50	23.00	23.00	40.40	43.20	41.65	54.65	5.80	6.91	47.74	53.40
51	PC (2015-16)-51	25.00	30.00	40.74	42.70	47.10	49.87	5.78	6.84	48.18	50.28
52	PC (2015-16)-52	30.00	30.00	40.25	33.60	39.35	42.48	4.98	3.98	39.33	39.67

Table 4.9: Contd....

S. No.	Genotype	Days to Germination		Germination%		SPAD 60		CTD 60		SPAD 120	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
53	PC (2015-16)-53	30.00	20.00	40.45	33.85	30.50	31.21	2.80	2.50	29.90	29.15
54	PC (2015-16)-54	30.00	20.00	47.50	45.75	52.20	50.18	2.10	6.98	51.10	49.62
55	PC (2015-16)-55	45.00	30.00	41.81	42.20	54.30	50.15	7.20	7.09	53.20	49.55
56	PC (2015-16)-56	30.00	30.00	39.92	37.30	45.50	41.73	3.80	3.86	44.40	39.91
57	PC (2015-16)-57	25.00	20.00	44.75	43.30	49.60	50.26	6.44	6.43	49.23	50.52
58	PC (2015-16)-58	25.00	25.00	42.30	41.75	44.65	50.65	6.36	5.76	44.10	49.93
59	PC (2015-16)-59	25.00	25.00	41.70	36.70	33.90	43.45	3.10	2.78	35.88	37.13
60	PC (2015-16)-60	38.00	30.00	35.90	30.90	33.20	31.25	5.15	3.15	33.38	30.73
61	PC (2015-16)-61	45.00	30.00	40.25	36.90	33.20	30.72	2.00	2.35	32.10	29.52
62	PC (2015-16)-62	30.00	30.00	40.75	37.45	47.10	35.39	3.00	3.82	46.00	33.90
63	PC (2015-16)-63	28.00	25.00	41.30	39.29	36.85	36.88	3.68	2.74	37.38	37.85
64	PC (2015-16)-64	28.00	28.00	46.10	41.80	44.65	49.65	4.75	6.16	48.63	48.60
65	PC (2015-16)-65	23.00	23.00	47.15	43.30	50.23	47.32	2.53	6.98	48.13	46.72
66	BO 91	30.00	30.00	40.25	35.65	36.30	32.77	3.00	3.62	35.20	30.75
67	Co 1148	30.00	30.00	46.10	42.20	47.80	47.10	5.90	6.71	45.25	45.42
68	Co 617	30.00	25.00	45.70	43.65	50.85	48.70	4.00	5.35	50.38	47.38
69	Co 85002	30.00	25.00	43.80	40.25	46.50	45.71	5.10	6.29	45.55	40.67
70	ISH 100	25.00	20.00	44.30	42.85	43.65	52.82	1.36	6.97	43.18	49.14
71	UP 9530	25.00	25.00	40.75	35.65	39.00	38.40	2.99	3.77	38.53	38.95
72	Co Pant 97222	25.00	25.00	37.70	33.65	45.40	43.05	1.94	4.09	44.93	39.90
Mean		28.29	24.47	38.80	36.20	41.05	42.98	4.30	4.93	41.17	42.12
Minimum		18.00	15.00	26.00	19.75	30.50	28.20	0.40	1.56	29.90	28.95
Maximum		45.00	30.00	47.60	45.97	56.30	54.81	8.00	7.88	55.80	54.32
SE (Mean)		4.19	3.24	0.85	0.97	4.79	1.46	0.72	0.31	4.12	1.45
CD at 5%		8.36	6.47	1.71	1.94	9.55	2.91	1.44	0.63	8.22	2.89

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

Table 4.9: Contd....

S. No.	Genotype	CTD 120		SPAD 180		CTD 180		RWC%		Chl a	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1	PC (2015-16)-1	6.32	4.47	45.26	39.36	5.62	5.15	90.24	78.16	1.87	1.54
2	PC (2015-16)-2	6.07	4.16	40.16	36.70	5.62	5.01	90.94	77.77	1.77	1.44
3	PC (2015-16)-3	7.97	3.19	29.93	28.56	6.72	5.04	85.84	71.75	1.65	1.33
4	PC (2015-16)-4	6.90	5.33	42.41	40.34	5.14	3.72	85.04	70.17	1.55	1.17
5	PC (2015-16)-5	6.33	4.31	36.16	33.81	5.07	4.78	87.86	77.33	1.66	1.26
6	PC (2015-16)-6	6.25	5.40	37.27	39.21	5.40	5.70	90.74	78.52	1.73	1.36
7	PC (2015-16)-7	5.50	4.87	38.70	39.02	4.40	4.78	89.99	70.22	1.85	1.26
8	PC (2015-16)-8	7.20	8.69	41.57	31.41	6.45	5.18	90.48	82.63	2.56	2.37
9	PC (2015-16)-9	8.85	7.36	39.71	46.73	8.25	4.83	89.04	81.82	2.22	2.08
10	PC (2015-16)-10	3.00	1.35	36.70	32.68	1.50	4.23	86.93	65.72	1.65	1.36
11	PC (2015-16)-11	2.81	1.33	32.20	31.75	1.90	4.67	88.68	72.72	1.54	1.28
12	PC (2015-16)-12	6.56	7.10	40.56	44.76	5.81	6.04	90.29	80.51	2.46	2.27
13	PC (2015-16)-13	0.80	1.23	30.20	31.69	0.30	2.57	88.99	70.65	1.86	1.37
14	PC (2015-16)-14	5.20	6.33	42.30	46.30	5.60	6.16	88.74	81.93	2.22	2.11
15	PC (2015-16)-15	9.53	4.75	30.15	28.69	8.28	6.04	85.36	68.08	1.97	1.60
16	PC (2015-16)-16	3.23	2.57	31.58	28.30	1.95	2.49	86.99	69.11	1.96	1.65
17	PC (2015-16)-17	2.53	4.06	42.14	41.82	3.05	5.25	85.89	70.07	1.94	1.45
18	PC (2015-16)-18	6.12	7.90	42.98	48.74	3.83	9.05	89.62	81.72	2.18	2.13
19	PC (2015-16)-19	2.96	3.95	35.23	37.21	1.56	3.43	85.27	70.68	1.70	1.27
20	PC (2015-16)-20	4.12	6.69	52.30	50.06	7.00	9.23	85.97	80.60	2.12	2.03
21	PC (2015-16)-21	3.60	1.27	40.60	39.20	2.90	5.28	82.04	64.11	1.70	1.47
22	PC (2015-16)-22	4.26	3.03	41.33	41.29	4.06	3.98	85.83	67.74	1.70	1.29
23	PC (2015-16)-23	6.04	4.39	34.16	38.71	4.14	4.81	85.73	65.60	1.87	1.38
24	PC (2015-16)-24	3.66	3.62	30.88	32.30	3.26	4.87	84.81	61.69	1.70	1.26
25	PC (2015-16)-25	4.04	4.32	35.16	34.41	3.00	2.33	86.02	64.85	1.70	1.32
26	PC (2015-16)-26	6.31	7.81	44.67	49.24	7.61	8.27	90.84	82.53	2.55	2.34
27	PC (2015-16)-27	7.03	5.06	37.03	38.71	5.67	4.87	83.79	60.65	1.81	1.31

Table 4.9: Contd....

S. No.	Genotype	CTD 120		SPAD 180		CTD 180		RWC%		Chl a	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
28	PC (2015-16)-28	5.16	6.25	43.28	47.73	3.81	8.73	91.09	85.57	2.31	2.18
29	PC (2015-16)-29	6.01	8.02	39.30	45.76	6.81	7.75	88.93	79.47	2.51	2.38
30	PC (2015-16)-30	5.91	7.46	42.93	44.20	6.88	8.98	90.69	81.79	2.29	2.07
31	PC (2015-16)-31	4.40	9.15	46.81	53.44	4.78	8.88	89.79	81.43	2.40	2.28
32	PC (2015-16)-32	4.87	1.15	31.13	30.23	2.84	2.41	85.79	71.22	1.88	1.39
33	PC (2015-16)-33	6.18	6.87	40.17	42.81	7.02	10.62	90.84	86.80	2.52	2.35
34	PC (2015-16)-34	4.60	6.99	51.30	48.81	5.20	7.46	88.96	82.35	2.35	2.16
35	PC (2015-16)-35	0.70	1.34	30.20	40.62	0.40	2.66	84.99	55.01	1.83	1.29
36	PC (2015-16)-36	7.70	3.40	49.50	41.70	7.50	5.34	84.04	71.25	1.86	1.48
37	PC (2015-16)-37	6.52	5.02	39.66	45.46	5.72	8.35	90.79	84.74	2.71	2.47
38	PC (2015-16)-38	5.91	8.02	30.33	49.41	4.61	7.55	88.89	80.73	2.42	2.46
39	PC (2015-16)-39	6.13	4.18	31.44	30.25	5.73	5.44	84.84	70.70	2.12	1.66
40	PC (2015-16)-40	9.15	7.93	44.74	50.81	8.10	9.05	90.89	81.84	2.65	2.58
41	PC (2015-16)-41	6.07	6.24	37.78	44.61	3.42	8.87	90.78	80.88	2.96	2.81
42	PC (2015-16)-42	5.21	4.66	37.28	39.30	3.96	4.54	85.82	69.75	1.70	1.37
43	PC (2015-16)-43	5.61	4.05	38.43	39.11	5.76	5.62	83.83	67.03	2.23	1.79
44	PC (2015-16)-44	2.54	4.34	37.25	33.79	4.98	5.97	86.36	66.10	2.33	1.79
45	PC (2015-16)-45	5.60	6.28	49.50	47.11	1.70	6.18	90.08	82.40	2.83	2.61
46	PC (2015-16)-46	6.70	4.31	35.00	30.78	4.10	4.49	88.97	68.23	1.94	1.60
47	PC (2015-16)-47	5.91	6.80	31.54	44.79	3.51	5.97	85.04	75.83	1.88	1.72
48	PC (2015-16)-48	4.56	4.53	29.93	28.53	3.01	2.62	83.79	70.62	1.89	1.39
49	PC (2015-16)-49	5.54	5.64	38.33	43.95	3.59	7.88	89.78	80.67	1.94	1.80
50	PC (2015-16)-50	7.73	7.96	43.28	50.77	6.63	9.53	91.14	81.55	2.27	1.90
51	PC (2015-16)-51	7.36	9.10	44.32	49.20	5.91	6.13	88.96	78.94	2.37	2.43
52	PC (2015-16)-52	5.66	5.07	36.18	40.55	4.21	5.05	85.73	64.64	1.77	1.70
53	PC (2015-16)-53	3.70	3.63	28.60	27.56	4.10	6.00	86.72	72.34	2.24	1.72
54	PC (2015-16)-54	5.40	6.74	49.10	48.94	2.70	8.45	90.78	82.79	2.43	2.32

Table 4.9: Contd....

S. No.	Genotype	CTD 120		SPAD 180		CTD 180		RWC%		Chl a	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
55	PC (2015-16)-55	7.90	8.39	52.40	50.05	7.80	7.10	90.93	84.31	2.53	2.36
56	PC (2015-16)-56	3.80	3.45	40.60	38.47	4.10	5.24	85.82	70.64	1.66	1.58
57	PC (2015-16)-57	6.21	8.07	46.40	48.73	6.83	6.00	89.89	78.04	2.26	2.08
58	PC (2015-16)-58	4.59	5.75	42.38	48.89	5.14	9.86	90.00	80.10	2.78	2.52
59	PC (2015-16)-59	2.76	3.63	32.85	36.55	1.83	5.55	82.94	56.12	1.64	1.58
60	PC (2015-16)-60	4.92	3.93	30.73	29.72	2.81	4.09	83.74	68.69	1.94	1.47
61	PC (2015-16)-61	4.50	2.20	30.50	23.48	3.70	4.39	80.89	69.75	1.55	1.12
62	PC (2015-16)-62	6.00	3.84	45.20	31.31	2.50	8.52	80.66	65.69	1.71	1.37
63	PC (2015-16)-63	3.73	4.85	32.35	34.36	4.88	4.62	86.59	59.11	1.72	1.49
64	PC (2015-16)-64	8.05	7.85	46.73	45.04	9.23	8.68	89.02	78.07	2.17	1.82
65	PC (2015-16)-65	2.81	6.97	46.69	46.05	3.29	9.16	87.26	82.11	2.84	2.70
66	BO 91	4.30	3.64	30.20	26.58	3.70	6.60	83.34	58.43	2.26	1.75
67	Co 1148	5.70	6.60	45.36	44.69	6.10	6.57	89.32	80.13	2.98	2.82
68	Co 617	5.75	6.68	44.70	46.74	5.50	8.61	90.14	80.76	2.51	2.43
69	Co 85002	5.20	4.77	40.20	40.54	7.10	6.55	90.84	80.76	1.71	1.46
70	ISH 100	6.00	6.63	45.25	48.44	6.69	8.03	91.50	82.00	3.33	3.28
71	UP 9530	4.20	4.61	38.10	38.65	3.65	4.60	86.78	60.61	2.14	1.75
72	Co Pant 97222	4.90	3.60	42.70	39.23	3.03	3.45	86.34	61.17	2.15	1.77
Mean		5.27	5.09	38.75	39.63	4.67	5.95	87.31	72.52	2.08	1.79
Minimum		0.70	1.15	28.60	23.48	0.30	2.33	80.66	55.01	1.54	1.12
Maximum		9.53	9.15	52.40	53.44	9.23	10.62	91.50	86.80	3.33	3.28
SE (Mean)		0.92	0.40	4.13	2.30	0.66	0.47	0.61	0.42	0.02	0.05
CD at 5%		1.83	0.81	8.24	4.59	1.33	0.95	1.22	0.84	0.04	0.10

Table 4.9: Contd....

S. No.	Genotype	Chl b		Total Chl		Total Carotenoids		Proline		Leaf Firing		DTC	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1	PC (2015-16)-1	0.65	0.25	2.52	1.79	0.04	0.03	45.96	112.24	5.00	5.00	3.00	3.00
2	PC (2015-16)-2	0.53	0.21	2.30	1.65	0.04	0.04	43.39	103.76	5.00	5.00	3.00	3.00
3	PC (2015-16)-3	0.57	0.23	2.22	1.56	0.04	0.04	43.08	97.77	5.00	5.00	3.00	3.00
4	PC (2015-16)-4	0.50	0.18	2.05	1.35	0.04	0.03	49.52	97.77	5.00	5.00	1.00	1.00
5	PC (2015-16)-5	0.50	0.22	2.16	1.48	0.04	0.03	50.84	99.27	3.00	3.00	1.00	1.00
6	PC (2015-16)-6	0.48	0.22	2.21	1.58	0.04	0.03	43.80	97.70	5.00	5.00	3.00	3.00
7	PC (2015-16)-7	0.70	0.25	2.55	1.50	0.04	0.03	47.51	99.22	3.00	3.00	1.00	1.00
8	PC (2015-16)-8	0.46	0.38	3.01	2.74	0.07	0.06	52.25	204.19	5.00	5.00	5.00	5.00
9	PC (2015-16)-9	0.48	0.37	2.69	2.45	0.06	0.06	53.10	212.60	5.00	5.00	5.00	5.00
10	PC (2015-16)-10	0.51	0.23	2.16	1.58	0.04	0.03	43.63	95.41	3.00	3.00	1.00	3.00
11	PC (2015-16)-11	0.59	0.24	2.13	1.52	0.04	0.04	47.06	113.17	5.00	5.00	3.00	3.00
12	PC (2015-16)-12	0.43	0.37	2.89	2.63	0.07	0.07	53.94	200.77	5.00	5.00	5.00	5.00
13	PC (2015-16)-13	0.54	0.25	2.40	1.61	0.05	0.04	53.63	112.18	3.00	3.00	3.00	3.00
14	PC (2015-16)-14	0.46	0.36	2.68	2.46	0.06	0.06	54.98	206.30	5.00	5.00	5.00	5.00
15	PC (2015-16)-15	0.63	0.26	2.60	1.86	0.05	0.04	42.48	92.77	3.00	3.00	3.00	3.00
16	PC (2015-16)-16	0.39	0.16	2.35	1.80	0.05	0.05	42.63	98.21	3.00	3.00	1.00	1.00
17	PC (2015-16)-17	0.49	0.17	2.43	1.61	0.04	0.04	49.20	101.22	3.00	3.00	3.00	3.00
18	PC (2015-16)-18	0.46	0.35	2.64	2.48	0.07	0.06	54.48	222.72	5.00	5.00	5.00	5.00
19	PC (2015-16)-19	0.45	0.20	2.15	1.47	0.06	0.05	50.31	113.73	5.00	5.00	3.00	3.00
20	PC (2015-16)-20	0.41	0.33	2.53	2.35	0.06	0.06	54.95	208.80	5.00	5.00	5.00	5.00
21	PC (2015-16)-21	0.43	0.19	2.13	1.66	0.05	0.05	50.52	100.42	5.00	5.00	3.00	3.00
22	PC (2015-16)-22	0.64	0.25	2.34	1.54	0.04	0.03	50.80	101.92	5.00	5.00	3.00	3.00
23	PC (2015-16)-23	0.55	0.22	2.41	1.59	0.04	0.04	41.18	99.49	3.00	3.00	1.00	1.00
24	PC (2015-16)-24	0.59	0.26	2.28	1.52	0.05	0.04	51.48	99.33	3.00	3.00	3.00	3.00
25	PC (2015-16)-25	0.56	0.25	2.25	1.56	0.06	0.05	53.10	98.92	5.00	5.00	3.00	3.00

Table 4.9: Contd....

S. No.	Genotype	Chl b		Total Chl		Total Carotenoids		Proline		Leaf Firing		DTC	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
26	PC (2015-16)-26	0.50	0.36	3.05	2.70	0.06	0.06	53.59	210.26	5.00	5.00	5.00	5.00
27	PC (2015-16)-27	0.58	0.24	2.39	1.54	0.04	0.03	49.50	113.70	3.00	3.00	1.00	1.00
28	PC (2015-16)-28	0.46	0.39	2.77	2.57	0.07	0.07	48.57	227.68	5.00	5.00	5.00	5.00
29	PC (2015-16)-29	0.48	0.40	2.98	2.78	0.07	0.06	47.22	223.22	5.00	5.00	5.00	5.00
30	PC (2015-16)-30	0.39	0.36	2.68	2.42	0.06	0.06	48.95	223.65	5.00	5.00	5.00	5.00
31	PC (2015-16)-31	0.46	0.36	2.86	2.64	0.07	0.07	53.62	200.25	5.00	5.00	5.00	5.00
32	PC (2015-16)-32	0.62	0.28	2.50	1.67	0.04	0.03	49.30	113.12	5.00	5.00	3.00	3.00
33	PC (2015-16)-33	0.45	0.34	2.97	2.69	0.07	0.06	51.67	112.95	5.00	5.00	5.00	5.00
34	PC (2015-16)-34	0.53	0.42	2.88	2.58	0.04	0.04	48.73	210.36	5.00	5.00	5.00	5.00
35	PC (2015-16)-35	0.58	0.25	2.41	1.54	0.04	0.04	51.03	101.42	3.00	3.00	1.00	1.00
36	PC (2015-16)-36	0.59	0.27	2.44	1.74	0.04	0.03	49.48	100.18	1.00	1.00	1.00	1.00
37	PC (2015-16)-37	0.45	0.35	3.16	2.81	0.07	0.07	49.86	226.03	5.00	5.00	5.00	5.00
38	PC (2015-16)-38	0.45	0.41	2.87	2.87	0.07	0.07	49.17	200.23	5.00	5.00	5.00	5.00
39	PC (2015-16)-39	0.46	0.16	2.58	1.82	0.04	0.04	49.74	96.68	3.00	3.00	1.00	1.00
40	PC (2015-16)-40	0.46	0.43	3.10	3.01	0.07	0.07	47.46	223.75	5.00	5.00	5.00	5.00
41	PC (2015-16)-41	0.53	0.45	3.48	3.26	0.08	0.08	44.67	216.91	5.00	5.00	5.00	5.00
42	PC (2015-16)-42	0.55	0.19	2.25	1.56	0.04	0.04	44.52	112.70	3.00	3.00	1.00	1.00
43	PC (2015-16)-43	0.45	0.17	2.67	1.95	0.04	0.03	42.26	122.58	3.00	3.00	1.00	1.00
44	PC (2015-16)-44	0.62	0.24	2.95	2.02	0.05	0.04	50.91	117.25	5.00	5.00	3.00	3.00
45	PC (2015-16)-45	0.47	0.35	3.30	2.96	0.06	0.06	44.01	203.74	5.00	5.00	5.00	5.00
46	PC (2015-16)-46	0.44	0.22	2.38	1.82	0.05	0.04	46.88	100.64	3.00	3.00	3.00	3.00
47	PC (2015-16)-47	0.50	0.34	2.37	2.06	0.07	0.07	42.35	208.68	5.00	5.00	5.00	5.00
48	PC (2015-16)-48	0.45	0.23	2.34	1.62	0.04	0.04	42.86	170.69	3.00	3.00	3.00	3.00
49	PC (2015-16)-49	0.33	0.26	2.27	2.06	0.05	0.05	44.39	212.20	5.00	5.00	5.00	5.00
50	PC (2015-16)-50	0.20	0.15	2.46	2.04	0.06	0.06	45.45	208.56	5.00	5.00	5.00	5.00

Table 4.9: Contd....

S. No.	Genotype	Chl b		Total Chl		Total Carotenoids		Proline		Leaf Firing		DTC	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
51	PC (2015-16)-51	0.45	0.42	2.82	2.85	0.07	0.06	43.25	213.24	5.00	5.00	5.00	5.00
52	PC (2015-16)-52	0.44	0.15	2.21	1.85	0.03	0.03	49.84	191.70	5.00	5.00	5.00	5.00
53	PC (2015-16)-53	0.64	0.25	2.87	1.97	0.04	0.03	47.80	122.75	3.00	3.00	3.00	3.00
54	PC (2015-16)-54	0.42	0.35	2.85	2.66	0.06	0.06	51.00	224.89	5.00	5.00	5.00	5.00
55	PC (2015-16)-55	0.45	0.34	2.98	2.69	0.07	0.07	46.95	223.71	5.00	5.00	5.00	5.00
56	PC (2015-16)-56	0.64	0.26	2.30	1.84	0.05	0.04	43.95	158.86	3.00	3.00	1.00	1.00
57	PC (2015-16)-57	0.41	0.33	2.67	2.41	0.06	0.06	42.95	207.41	5.00	5.00	5.00	5.00
58	PC (2015-16)-58	0.66	0.63	3.44	3.14	0.09	0.09	43.62	205.68	5.00	5.00	5.00	5.00
59	PC (2015-16)-59	0.49	0.17	2.13	1.75	0.04	0.03	42.18	167.71	3.00	3.00	3.00	3.00
60	PC (2015-16)-60	0.43	0.14	2.37	1.61	0.03	0.03	45.13	157.02	3.00	3.00	1.00	1.00
61	PC (2015-16)-61	0.44	0.13	1.99	1.25	0.04	0.03	49.49	170.51	3.00	3.00	3.00	3.00
62	PC (2015-16)-62	0.40	0.10	2.11	1.47	0.04	0.03	50.44	162.93	3.00	3.00	3.00	3.00
63	PC (2015-16)-63	0.54	0.24	2.26	1.73	0.04	0.04	48.86	160.38	3.00	3.00	3.00	3.00
64	PC (2015-16)-64	0.40	0.32	2.57	2.14	0.06	0.06	50.29	233.58	5.00	5.00	5.00	5.00
65	PC (2015-16)-65	0.53	0.41	2.66	3.11	0.04	0.04	41.63	205.69	5.00	5.00	5.00	5.00
66	BO 91	0.65	0.34	2.90	2.08	0.07	0.06	42.68	97.78	3.00	3.00	1.00	1.00
67	Co 1148	0.51	0.44	3.49	3.26	0.09	0.08	47.06	208.71	5.00	5.00	5.00	5.00
68	Co 617	0.46	0.38	2.97	2.80	0.07	0.06	49.90	204.47	5.00	5.00	5.00	5.00
69	Co 85002	0.33	0.25	2.04	1.70	0.05	0.04	48.06	211.76	5.00	5.00	5.00	5.00
70	ISH 100	0.56	0.52	3.89	3.80	0.09	0.09	49.89	213.27	5.00	5.00	5.00	5.00
71	UP 9530	0.57	0.27	2.71	2.02	0.06	0.05	42.40	95.18	3.00	3.00	1.00	1.00
72	Co Pant 97222	0.69	0.44	2.84	2.20	0.06	0.05	43.08	94.80	3.00	3.00	1.00	1.00
Mean		0.51	0.28	2.59	2.07	0.05	0.05	47.43	151.44	4.08	3.00	3.00	3.31
Minimum		0.20	0.10	1.99	1.25	0.03	0.03	41.18	92.77	1.00	1.00	1.00	1.00
Maximum		0.70	0.63	3.89	3.80	0.09	0.09	54.98	233.58	5.00	5.00	5.00	5.00
SE (Mean)		0.01	0.01	0.02	0.05	0.00	0.00	0.61	10.70	0.00	0.24	0.00	0.24
CD at 5%		0.03	0.02	0.04	0.10	0.00	0.00	11.32	21.34	0.00	0.47	0.00	0.47

Mean Chlorophyll a (1.79), chlorophyll b (0.28) and total chlorophyll (2.07) were found to decrease in stress environment when compared to control environment and genotypes differed among themselves for extent of reduction. Genotypes with lower levels of reduction were considered to be tolerant to water stress and genotypes showing higher levels of reductions indicate their sensitivity towards water stress. In contrast, the proline content increased enormously nearly 4-5 times (233.58) in certain genotypes distinguishing them from remaining genotypes which recorded a meager increase of 1.5-2 times (92.77). In response to water stress, plants produce proline, which plays a crucial role during stress conditions. **Abbas *et al.* (2014)** reported that plants accumulate some kind of organic and inorganic solutes like proline in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake. **Cha-um *et al.* (2012)** reported marked increase in proline content of stressed sugarcane genotypes.

Mean days to germination was found to be at lower side (15) in stress environment than control (18) which can be attributed to the enhanced metabolic processes due to higher temperatures in poly-house as compared to the control environment (open air temperatures), which initiated germination a little bit earlier than open fields.

RWC provides a measurement of the 'water deficit' in the leaf and indicate the extent of stress articulated under drought. The mean performance of all genotypes in control environment was similar to each other. However, genotypes differed under water stress conditions. Mean RWC in control (87.31%) which was a way above RWC in stress (72.52%). Certain genotypes recorded lesser changes in RWC% than the remaining. **Mullan and Pietragalla (2012)** reported similar observation for RWC% which supports our findings.

Deficiency of water leads to death of tissues, which appear in the form of leaf firing. All the genotypes responded almost similar for leaf firing under control environment and showed moderate to tolerant behaviour but under stress environment the genotypes differed in their response to stress and the leaf firing observations exhibited in a range from sensitive to tolerant nature. **Riaz *et al.* (2013)** also reported the similar findings for leaf firing in marigold.

4.3 Estimates of Selection parameter

The presence of genetic variability is a key factor for improving the specific traits. Variability can be created by hybridization and so created variability needs to be assessed for revealing the scope of improvement under selection. The C_1 and C_2 clonal population generated by hybridization between 8 different varieties consisting of 65 progenies was evaluated for 16 physiological and biochemical traits. The data obtained from both the environments (control and stress) were subjected to statistical analysis to find out genotypic and phenotypic coefficient of variation, heritability (h^2), genetic advance (GA) and genetic advance over mean (GAM) and the results are summarized in the following sections.

4.3.1 Estimates of Selection parameters in Control environment

The estimates of variability and genetic parameters for all the 16 characters under control environment are presented in the Table 4.10 and summarized below.

4.3.1.1 Days to Germination

High Phenotypic (PCV) coefficient variation (26.052) and moderate Genotypic (GCV) coefficient of variation (14.943) for days to germination were recorded. Moderate heritability (32.9%) and moderate genetic advance over mean (17.656%) were observed and with mean days to germination of (28.290), the expected mean in next generation is (23.959)..

4.3.1.2 Germination Percent at 45 DAS

Moderate PCV (13.772) and moderate GCV (13.415) values were recorded for germination percent. High heritability (94.9%) and high genetic advance over mean (26.920%) were observed and with mean germination percent of (38.800), the expected mean in next generation is (49.846).

4.3.1.3 SPAD Readings at 60 DAS

Moderate PCV (18.581) and moderate GCV (10.597) values were recorded for SPAD 60. Low heritability (21.4%) and low genetic advance over mean (8.194%) were observed and with mean of (41.050), the expected mean in next generation is (44.818).

Table 4.10: Selection Parameters for Drought Tolerance Traits in Sugarcane Genotypes under Control Environment

	Days to Germination	Germination%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180
GCV	14.943	13.415	10.597	38.384	10.275	28.153	11.920	39.579
PCV	26.052	13.772	18.581	44.954	17.466	37.323	19.194	44.440
h²(BroadSense)	0.329	0.949	0.214	0.729	0.346	0.569	0.386	0.793
GeneticAdvance	4.941	10.572	3.394	2.980	5.177	2.341	5.967	3.417
Gen. Advas%ofMean	17.656	26.920	8.194	67.516	12.451	43.746	15.251	72.614
ExpMeanin nextGeneration	23.959	49.846	44.818	7.393	46.759	7.691	45.090	8.123

	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	D T C
GCV	3.117	18.800	17.949	15.138	27.688	7.971	10.236	47.234
PCV	3.274	18.855	18.528	15.199	27.889	8.181	10.563	48.234
h²(BroadSense)	0.906	0.994	0.939	0.992	0.986	0.949	0.992	0.994
GeneticAdvance	5.353	0.808	0.179	0.805	0.030	7.645	3.290	3.340
Gen. Advas%ofMean	6.114	38.616	35.820	31.059	27.688	16.000	97.085	99.363
ExpMeanin nextGeneration	92.911	2.901	0.679	3.397	0.083	55.428	5.236	6.701

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

4.3.1.4 Canopy Temperature Depression at 60 DAS

High PCV (44.954) and high GCV (38.384) values were recorded for CTD 60. High heritability (72.9%) and high genetic advance over mean (67.516%) were observed and with mean of (4.300), the expected mean in next generation is (7.393).

4.3.1.5 SPAD Readings at 120 DAS

Moderate PCV (17.466) and moderate GCV (10.275) for SPAD 120, coupled with moderate heritability (34.6%) and moderate genetic advance over mean (12.451%) were observed. With mean of (41.170), the expected mean in next generation is (46.759).

4.3.1.6 Canopy Temperature Depression at 120 DAS

High PCV (37.323) and high GCV (28.153) for CTD 120, coupled with moderate heritability (56.9%) and high genetic advance over mean (43.746%) were observed. With mean of (5.27), the expected mean in next generation is (7.691).

4.3.1.7 SPAD Readings at 180 DAS

Moderate PCV (19.194) and moderate GCV (11.920) values were recorded for SPAD 180. Moderate heritability (38.6%) and moderate genetic advance over mean (15.251%) were observed and with mean of (38.75), the expected mean in next generation is (45.090).

4.3.1.8 Canopy Temperature Depression at 180 DAS

High PCV (44.440) and high GCV (39.579) values were recorded for CTD 180. High heritability (79.3%) and high genetic advance over mean (72.614%) were observed and with mean of (4.67), the expected mean in next generation is (8.123).

4.3.1.9 Relative Water Content (RWC%)

Very low PCV (3.274) and very low GCV (3.117) values coupled with high heritability (79.3%) and high genetic advance over mean (90.6%) were observed for RWC percent. With mean of (87.31), the expected mean in next generation is (92.911).

4.3.1.10 Chlorophyll a (Chl a)

Moderate PCV (18.855) and moderate GCV (18.800) values coupled with high heritability (94.9%) and high genetic advance over mean (38.616%) were observed for Chl a. With mean of (2.080), the expected mean in next generation is (2.901).

Table 4.11: 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
h² (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

	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
h² (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

4.3.1.11 Chlorophyll b (Chl b)

Moderate PCV (18.528) and moderate GCV (17.949) values coupled with high heritability (93.9%) and high genetic advance over mean (35.820%) were observed for Chl b. With mean of (0.510), the expected mean in next generation is (0.679).

4.3.1.12 Total Chlorophyll

Moderate PCV (15.199) and moderate GCV (15.138) values coupled with high heritability (99.2%) and high genetic advance over mean (31.059%) were observed for total chlorophyll. With mean of (2.59), the expected mean in next generation is (3.397).

4.3.1.13 Total Carotenoids

High PCV (27.889) and high GCV (27.688) values coupled with high heritability (98.6%) and high genetic advance over mean (27.688%) were observed for total carotenoids. With mean of (0.05), the expected mean in next generation is (0.083).

4.3.1.14 Proline Content:

Low PCV (8.181) and low GCV (7.971) values coupled with high heritability (94.9%) and moderate genetic advance over mean (16.000%) were observed for proline content. With mean of (47.43), the expected mean in next generation is (55.428).

4.3.1.15 Leaf firing

Moderate PCV (10.236) and moderate GCV (10.563) values coupled with very high heritability (99.23%) and very high genetic advance over mean (97.085%) were observed for leaf firing. With mean of (4.08), the expected mean in next generation is (5.236).

4.3.1.16 Drought Tolerance Capacity

High PCV (48.234) and high GCV (47.234) values coupled with high heritability (99.4%) and high genetic advance over mean (99.363%) were observed for DTC. With mean of (3.00), the expected mean in next generation is (6.701).

4.3.2 Estimates of Selection parameters in Stress Environment

The estimates of variability and genetic parameters for all the 16 characters under stress environment are presented in the Table 4.11 and summarized below.

4.3.2.1 Days to Germination

High PCV (24.757) and moderate GCV (15.609) values coupled with moderate heritability (39.7%) and high genetic advance over mean (20.273%) were observed for days to germination. With mean of (24.470), the expected mean in next generation is (19.591).

4.3.2.2 Germination Percent at 45 DAS

Moderate PCV (18.733) and moderate GCV (18.343) values coupled with high heritability (95.9%) and high genetic advance over mean (36.998%) were observed for germination percent. With mean of (36.200), the expected mean in next generation is (49.972).

4.3.2.3 SPAD Readings at 60 DAS

Moderate PCV (16.335) and moderate GCV (15.617) values coupled with high heritability (91.4%) and high genetic advance over mean (30.757%) were observed for SPAD 60. With mean of (42.98), the expected mean in next generation is (56.794).

4.3.2.4 Canopy Temperature Depression at 60 DAS

High PCV (34.537) and high GCV (33.325) values coupled with high heritability (93.1%) and high genetic advance over mean (66.240%) were observed for CTD 60. With mean of (4.930), the expected mean in next generation is (8.310).

4.3.2.5 SPAD Readings at 120 DAS

Moderate PCV (16.463) and moderate GCV (15.721) values coupled with high heritability (91.2%) and high genetic advance over mean (30.925%) were observed for SPAD 120. With mean of (42.120), the expected mean in next generation is (55.434).

4.3.2.6 Canopy Temperature Depression at 120 DAS

High PCV (40.793) and high GCV (39.204) values coupled with moderate heritability (92.4%) and high genetic advance over mean (77.616%) were observed for CTD 120. With mean of (5.090), the expected mean in next generation is (9.151).

4.3.2.7 SPAD Readings at 180 DAS

Moderate PCV (19.342) and moderate GCV (17.518) values coupled with high heritability (82.0%) and high genetic advance over mean (32.686%) were observed for SPAD 180. With mean of (39.630), the expected mean in next generation is (53.085).

4.3.2.8 Canopy Temperature Depression at 180 DAS

High PCV (35.161) and high GCV (33.313) values coupled with high heritability (89.8%) and high genetic advance over mean (65.020%) were observed for CTD 180. With mean of (5.95), the expected mean in next generation is (9.988).

4.3.2.9 Relative Water Content (RWC%)

Moderate PCV (11.260) and moderate GCV (11.230) values coupled with high heritability (99.5%) and high genetic advance over mean (23.072%) were observed for RWC percent. With mean of (72.52), the expected mean in next generation is (90.246).

4.3.2.10 Chlorophyll a (Chl a)

High PCV (27.131) and moderate GCV (26.807) values coupled with high heritability (97.6%) and high genetic advance over mean (54.564%) were observed for Chl a. With mean of (1.790), the expected mean in next generation is (2.789).

4.3.2.11 Chlorophyll b (Chl b)

High PCV (35.387) and high GCV (34.807) values coupled with high heritability (96.8%) and high genetic advance over mean (70.529%) were observed for Chl b. With mean of (0.280), the expected mean in next generation is (0.481).

4.3.2.12 Total Chlorophyll

High PCV (27.418) and high GCV (27.169) values coupled with high heritability (98.2%) and high genetic advance over mean (31.059%) were observed for total chlorophyll. With mean of (2.070), the expected mean in next generation is (3.243).

4.3.2.13 Total Carotenoids

High PCV (33.719) and high GCV (33.547) values coupled with high heritability (99.0%) and high genetic advance over mean (68.755%) were recorded for total carotenoids. With mean of (0.050), the expected mean in next generation is (0.083).

4.3.2.14 Proline Content

High (34.239) and high GCV (32.815) values coupled with high heritability (91.9%) and high genetic advance over mean (64.790%) were recorded for proline content. With mean of (151.44), the expected mean in next generation is (257.105).

4.3.2.15 Leaf firing

High PCV (47.847) and high GCV (46.763) values coupled with very high heritability (95.5%) and very high genetic advance over mean (94.151%) were recorded for leaf firing. With mean of (3.00), the expected mean in next generation is (6.566).

4.3.2.16 Drought Tolerance Capacity

High PCV (47.847) and high GCV (46.763) values coupled with high heritability (95.5%) and moderate genetic advance over mean (94.151%) were observed for DTC. With mean of (3.31), the expected mean in next generation is (6.566).

Estimates of selection parameters for all the 16 characters under both control and stress environment recorded high PCV and high GCV for traits like CTD 60, CTD 120, CTD 180, Total carotenoids and DTC under both the environments. Few more traits like Chl a, Chl b, Total chlorophyll, Proline content, RWC% and Leaf firing recorded high PCV and high GCV only under stress environment. 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)**. Higher PCV and GCV estimates only in stress environment depicts that those traits are playing an important role towards drought tolerance.

A very high broad sense heritability estimates were recorded for traits like Germination%, Chl a, Chl b, Total chlorophyll, Proline content, RWC%, Leaf firing and DTC in both the environments. Few of the traits like SPAD 60 (91.4%), SPAD 120 (91.2%), SPAD 180 (82.0%), CTD 60 (93.1%), CTD 120 (92.4%), CTD 180 (89.8%) recorded high heritability under stress environment only. 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**) or degree of genetic determination. It gives an indication of repeatability of performance if selection is practiced for the particular character (**Falconer, 1960**).

Genetic advance as percent of mean (GAM) was recorded to be high for CTD 60, CTD 120, CTD 180, Total carotenoids, Leaf firing and DTC under both the environments and Chl a (54.564%), Chl b (70.529%), Total chlorophyll (31.059%) and

Proline content 64.790%) were found to have higher GAM only under stress environment. 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). 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).**

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.

PCV and GCV for Relative water content (3.117 & 3.274) and proline content (7.971 & 8.181) was recorded to be lower respectively under control environment. Similarly lower heritability (21.4%) for SPAD 60 and lower genetic advance as percent of mean also recorded low for SPAD 60 (8.194) and relative water content% (6.114) under control environment only. None of the trait recorded lower levels for any of the selection parameters under stress environment.

4.4 Estimates of Correlation and Path coefficient

Correlation and path coefficients were estimated for all the 16 characters in both the environments independently. The corresponding results are presented in the following sections for correlation and path coefficients.

4.4.1 Estimates of Correlation Coefficients between Various Drought Tolerance Traits.

Phenotypic and genotypic correlation coefficients were estimated for all the 16 traits under control as well stress environments. The estimations are presented separately for both the environments.

4.4.1.1 Estimates of Correlation Coefficients between Various Drought Tolerance Traits under control environment

Phenotypic and genotypic correlation coefficients for all the 16 traits are presented in the Table 4.12 and Fig.3 (a & b).

Days to germination recorded significant positive correlation with SPAD 60 (0.1921) and RWC percent (-0.2187). With all the remaining traits non-significant negative correlation coefficients at both phenotypic and genotypic levels were recorded.

Germination percent recorded significant positive association with SPAD 60 (0.267), CTD 60 (0.178), SPA 120 (0.302), SPAD 180 (0.304), CTD 180 (0.258), RWC percent (0.355), Chl a (0.560), total chlorophyll (0.5108) and DTC (0.614). Whereas, it recorded significant negative correlations with Chl b (-0.345), and leaf firing (-0.268). Non-significant correlation were observed with CTD 120 (0.130), total carotenoids (0.079) and proline (0.159).

SPAD 60 recorded significant positive association with SPAD 120 (0.963), SPAD 180 (0.92), CTD 180 (0.256), RWC percent (0.230), Chl a (0.285), total chlorophyll (0.242) and DTC (0.267). whereas, it recorded negative significant associations with Chl b (-0.169) and non-significant associations with SPAD 120 (1.00), total carotenoids (0.051) proline (0.007) and leaf firing (-0.05).

CTD 60 recorded significant positive correlations with SPAD 120 (0.178), CTD 120 (0.639), SPAD 180 (0.184), CTD 180 (0.534), RWC percent (0.376), Chl a (0.298), total chlorophyll (0.222), total carotenoids (0.169) and DTC (0.430). significant negative associations were recorded with Chl b (-0.303) and non significant associations with proline and leaf firing.

SPAD 120 recorded significant positive correlation with CTD 120 (0.168), SPAD 180 (0.961), CTD 180 (0.318), RWC percent (0.35), Chl a (0.329), total chlorophyll (0.262), proline content (0.176) and DTC (0.333). whereas, recorded significant negative correlations with Chl b (-0.405) and leaf firing (-0.270) only at genotypic level and non significant association with total carotenoids.

CTD 120 recorded significant positive correlation with SPAD 180 (0.209), CTD 180 (0.7301), RWC percent (0.319), Chl a (0.258), total chlorophyll (0.204) and DTC (0.284). whereas, it recorded significant negative correlations with Chl b (-0.216) and non significant correlations with total carotenoids, proline and leaf firing.

SPAD 180 recorded significant positive correlation with CTD 180 (0.354), RWC percent (0.364), Chl a (0.357), total chlorophyll (0.303) and DTC (0.321). whereas, it recorded significant negative correlation with Chl b. It recorded significant positive association with proline (0.259) and significant negative correlations with leaf firing (-0.214) only at genotypic level.

CTD 180 recorded significant positive correlation with RWC percent (0.380), Chl a (0.334), total chlorophyll (0.289) and DTC (0.361). whereas, it recorded non-significant correlations with Chl b, total carotenoids, proline content and leaf firing.

RWC percent recorded significant positive associations with Chl a (0.566), total chlorophyll (0.520), total carotenoids (0.282) and DTC (0.581). whereas, it recorded significant negative with Chl b (-0.175) and leaf firing (0.414). non significant association were recorded with total carotenoids.

Chl a recorded significant positive associations with total Chlorophyll (0.971), total carotenoids (0.197) and DTC (0.583). whereas, it recorded significant negative association with leaf firing (-0.33) and non significant associations with Chl b and proline.

Chl b recorded significant positive associations with total carotenoid (Chl a recorded significant positive associations with total Chlorophyll (0.183) and leaf firing (0.233). whereas, it recorded significant negative association with proline content (-0.167) and DTC (-0.46). Non significant correlation with total chlorophyll.

Total Chlorophyll recorded significant positive associations with total carotenoid (0.240) and DTC (0.465). whereas, it recorded significant negative associations with leaf firing (-0.278) and non significant associations with proline content.

Table 4.12: Correlation Coefficients between Various Drought Tolerance Characters in Sugarcane under Control Environment

	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing	D T C
Days to Germination	1.000															
Germination%	0.089	1.000														
	0.130															
SPAD 60	0.192*	0.267**	1.000													
	-.138	0.603														
CTD 60	-0.155	0.178*	0.130	1.000												
	-.130	0.213	0.285													
SPAD 120	0.139	0.302***	0.963***	0.178*	1.000											
	-.143	0.553	1.000	0.346												
CTD 120	-0.088	0.130	0.122	0.639***	0.168*	1.000										
	.022	0.196	0.379	0.761	0.471											
SPAD 180	0.092	0.304***	0.920***	0.184*	0.961***	0.209*	1.000									
	-.119	0.514	1.000	0.364	0.969	0.558										
CTD 180	0.006	0.258**	0.256**	0.564***	0.318***	0.730***	0.354***	1.000								
	-.011	0.290	0.478	0.639	0.535	0.844	0.581									
RWC%	-0.219**	0.355***	0.230***	0.376***	0.35***	0.319***	0.364***	0.380***	1.000							
	-0.381	0.365	0.648	0.440	0.605	0.423	0.585	0.426								
Chl a	-0.029	0.560***	0.285**	0.298**	0.329***	0.258**	0.357***	0.334***	0.566***	1.000						
	-0.051	0.615	0.615	0.349	0.547	0.342	0.575	0.376	0.566							
Chl b	-0.042	-0.345***	-0.169*	-0.303**	-0.238**	-0.216**	-0.211*	-0.178	-0.175 *	-0.091	1.000					
	-0.074	-0.354	-0.364	-0.355	-0.405	-0.286	-0.340	-0.200	-0.175	-0.091						
Total Chl	-0.039	0.5108***	0.242**	0.222**	0.262**	0.204*	0.303***	0.289***	0.520***	0.971***	0.152	1.000				
	-0.069	0.524	0.522	0.260	0.445	0.270	0.489	0.324	0.520	0.971	0.152					
Total Carotenoids	-0.124	0.077	0.051	0.169*	0.064	0.090	0.098	0.079	0.282***	0.197*	0.183*	0.240**	1.000			
	-0.217	0.079	0.110	0.198	0.109	0.120	0.158	0.089	0.282	0.197	0.183	0.240				
Proline	0.123	0.155	0.148	0.007	0.176*	-0.040	0.161	0.143	0.096	0.069	-0.167*	0.028	0.039	1.000		
	0.217	0.159	0.320	0.008	0.299	-0.053	0.259	0.160	0.096	0.069	-0.167	0.028	0.039			
Leaf firing	0.006	-0.268**	-0.138	-0.055	-0.159	-0.029	-0.133	-0.143	-0.414**	-0.33***	0.233**	-.278**	0.067	-0.18*	1.000	
	0.010	-0.275	-0.297	-0.064	-0.270	-0.038	-0.214	-0.160	-0.414	-0.337	0.233	-0.278	0.067	-0.188		
D T C	-0.087	0.614***	0.267*	0.430**	0.333*	0.284*	0.321*	0.361*	0.581**	0.583**	-0.46**	0.465**	0.054**	0.309*	-0.32*	1.000
	-0.151	0.630	0.577	0.504	0.566	0.376	0.518	0.405	0.581	0.583	-0.469	0.465	0.054	0.309	-0.38	

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

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

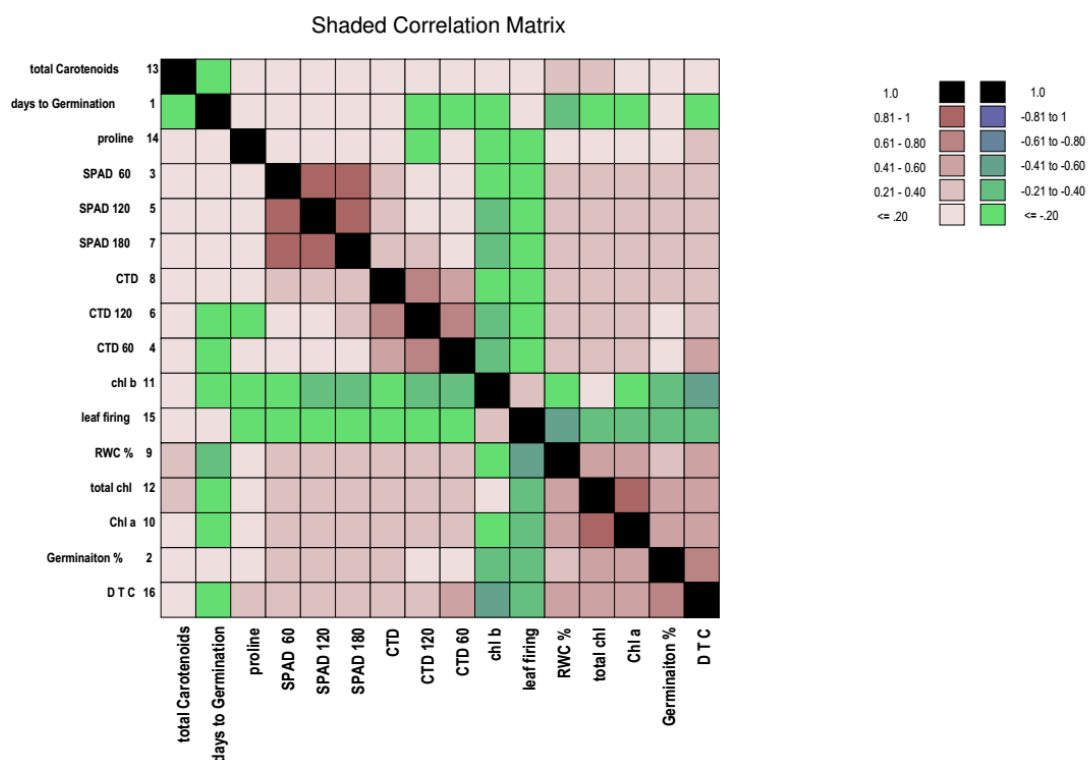


Fig. 3 (a): Phenotypic correlations between various drought tolerance traits under control environment

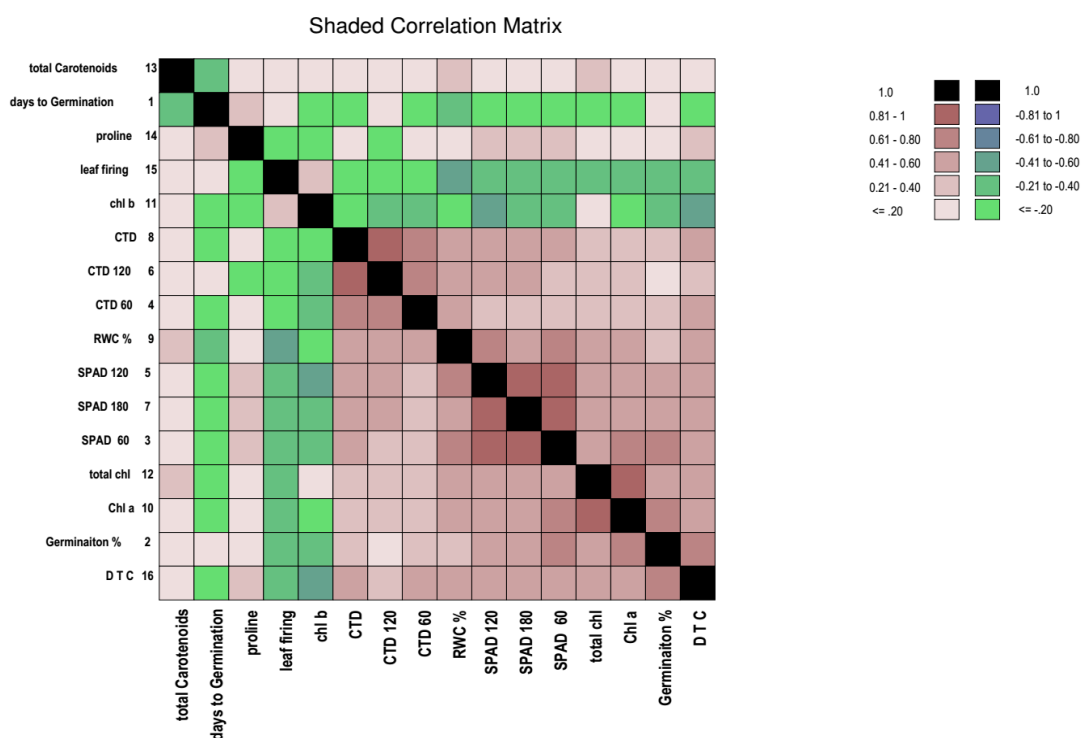


Fig. 3 (b): Genotypic correlations between various drought tolerance traits under control environment

Total carotenoids recorded non significant association with proline content, leaf firing and DTC. Proline content recorded significant positive association with DTC (0.309) and significant negative with leaf firing (-0.18). Leaf firing recorded significant negative correlation with DTC (-0.32).

4.4.1.2 Estimates of Correlation Coefficients between Various Drought Tolerance Traits under stress environment

Phenotypic and genotypic correlation coefficients for all the 16 traits are presented in the Table 4.13 and Fig 4 (a & b).

Days to germination was found to be significantly associated with germination percent (0.246), CTD 120 (0.189) and proline content (0.192). Non-significant negative phenotypic correlation with SPAD 60 (-0.018), SPAD 120 (-0.017) and SPAD 180 (-0.032) were observed whereas, corresponding genotypic correlation were found to be non-significant positive. Similarly days to germination was observed to be associated non-significantly with the all other traits

Leaf firing was found to exhibit significantly negative associations with all the traits except non-significant negative association with days to germination (-0.150). Significant positive correlations were recorded between all the remaining traits such as germination percent, SPAD 60, CTD 60, SPAD 120, CTD 120, SPAD 180, CTD 180, RWC percent, Chl a, Chl b, total chlorophyll, total carotenoids, proline content and DTC.

Estimates of correlation coefficients for all the 16 traits under both environments (control & stress) revealed that traits like proline content, total carotenoids and leaf firing are not correlated with most of the other traits unlike their highly significant association in stress environment. These traits might get triggered due to water stress and expressed at higher levels which make their presence counted only in stress environment.

A characteristic pattern was observed under control environment where most of the physiological traits were found to be associated with other physiological traits but not with the biochemical estimations and vice versa. This also depicts about a kind of interaction or interdependence between physiological and biochemical traits. Significant selection for drought tolerance. The high correlation between two characteristics can be the result of a third effect on them, or a group of characteristics. Thus, it is not possible to observe if the estimate is established by real cause and effect relationships (Silva *et al.*, 2012).

Table 4.13: Correlations between Various Drought Tolerance Characters in Sugarcane under Stress Environment

	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing	D T C
Days to Germination	1.000															
Germination %	0.246** 0.401	1.000														
SPAD 60	-0.019 0.027	0.575*** 0.612	1.000													
CTD 60	0.093 0.196	0.605*** 0.648	0.817*** 0.863	1.000												
SPAD 120	-0.018 -0.007	0.550*** 0.592	0.942*** 0.979	0.810*** 0.873	1.000											
CTD 120	0.189* 0.326	0.678*** 0.714	0.696*** 0.745	0.781*** 0.835	0.741*** 0.781	1.000										
SPAD 180	-0.033 0.067	0.547*** 0.611	0.884*** 0.980	0.744*** 0.847	0.913** 0.987	0.703*** 0.776	1.000									
CTD 180	0.107 0.125	0.606*** 0.650	0.617*** 0.719	0.560*** 0.620	0.591*** 0.653	0.630*** 0.668	0.602*** 0.693	1.000								
RWC %	0.044 0.069	0.591*** 0.603	0.592*** 0.622	0.680*** 0.705	0.619*** 0.648	0.685*** 0.713	0.584*** 0.645	0.563*** 0.594	1.000							
Chl a	0.122 0.194	0.695*** 0.710	0.634*** 0.666	0.642*** 0.665	0.617*** 0.646	0.691*** 0.719	0.617*** 0.681	0.654*** 0.690	0.670*** 0.669	1.000						
Chl b	0.038 0.061	0.581*** 0.593	0.602*** 0.632	0.622*** 0.645	0.603*** 0.631	0.576*** 0.599	0.578*** 0.638	0.466*** 0.492	0.576*** 0.576	0.813*** 0.813	1.000					
Total Chl	0.110 0.175	0.694*** 0.708	0.645*** 0.678	0.656*** 0.680	0.631*** 0.661	0.689*** 0.717	0.627*** 0.692	0.638*** 0.673	0.670*** 0.670	0.994*** 0.994	0.870*** 0.870	1.000				
Total Carotenoids	0.065 0.106	0.676*** 0.691	0.652*** 0.685	0.669*** 0.693	0.660*** 0.691	0.663*** 0.6899	0.632*** 0.698	0.579*** 0.611	0.668*** 0.668	0.890*** 0.890	0.873*** 0.873	0.912*** 0.912	1.000			
Proline	0.192* 0.304	0.842*** 0.860	0.647** 0.680	0.670*** 0.694	0.645*** 0.676	0.7365*** 0.7664	0.633*** 0.699	0.681*** 0.719	0.718*** 0.718	0.753*** 0.753	0.594*** 0.594	0.745*** 0.745	0.706*** 0.706	1.000		
Leaf firing	-0.150 -0.267	-0.729*** -0.787	-0.68*** -0.783	-0.739*** -0.814	-0.707*** -0.783	-0.7246*** -0.7982	-0.666*** -0.776	-0.627*** -0.691	-0.795*** -0.841	-0.751*** -0.795	-0.667 -0.706	-0.756*** -0.801	-0.773*** -0.818	-0.821*** -0.869	1.000	
D T C	0.021 0.033	0.715*** 0.731	0.618*** 0.649	0.638*** 0.660	0.600*** 0.628	0.6733*** 0.7006	0.579*** 0.639	0.610*** 0.644	0.751*** 0.751	0.704*** 0.704	0.589*** 0.589	0.702*** 0.702	0.707*** 0.707	0.862*** 0.862	-0.78* -0.82	1.000

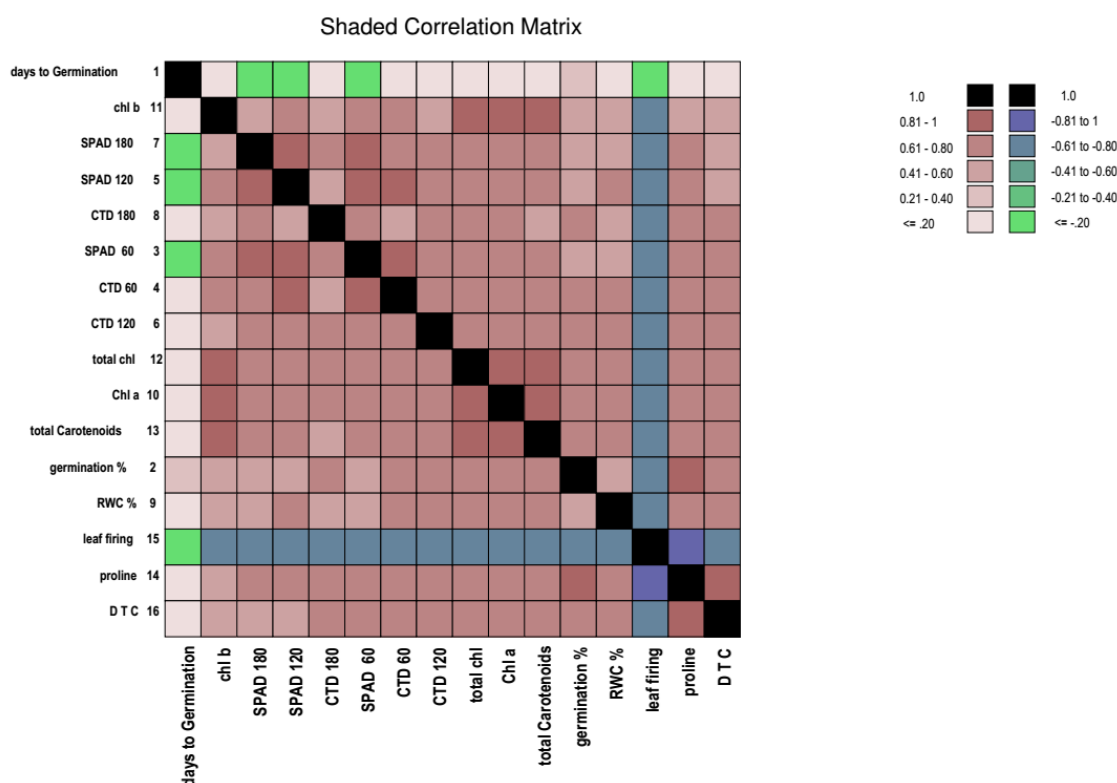


Fig 4 (a): Phenotypic Correlations Between Various Drought Tolerance Traits under Stress Environment

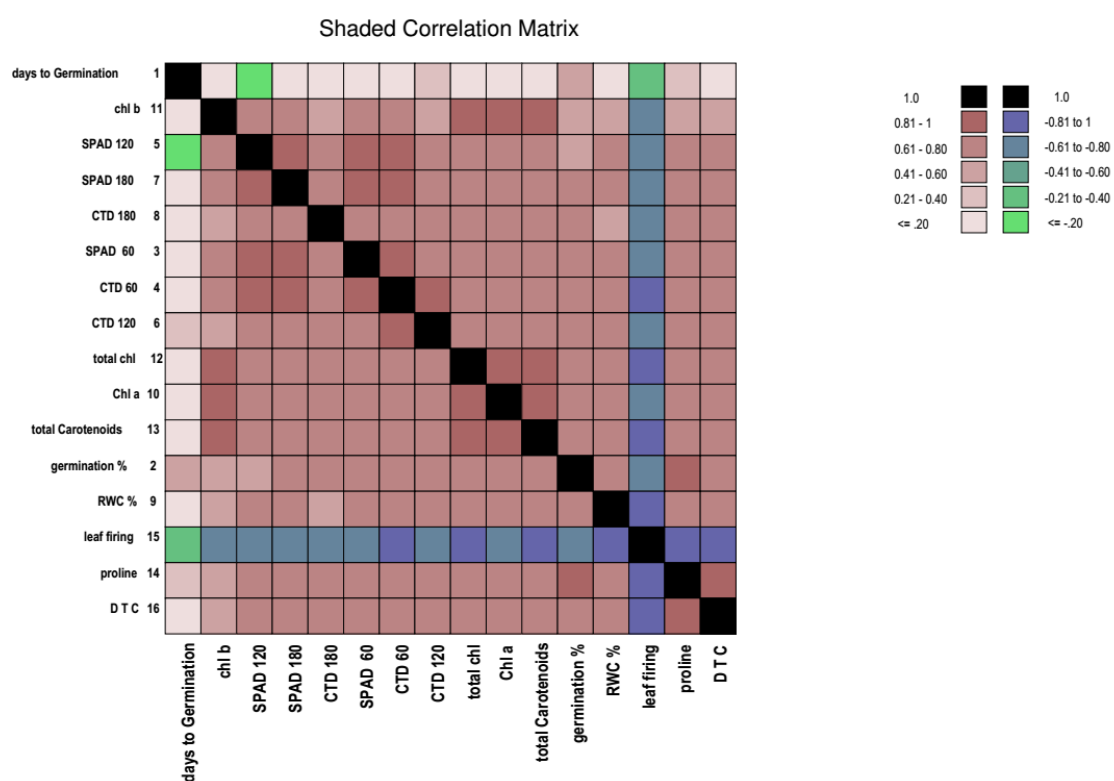


Fig 4 (b): Genotypic Correlations Between Various Drought Tolerance Traits under Stress Environment

4.4.2 Estimates of Path Coefficients for Various Drought Tolerance Traits

The drought tolerance is a complex trait which is influenced by quite a lot of traits from the beginning of germination to the final development of crop. Thus, the drought tolerance is dependent trait and many other characters have an effect on or show association with it. Correlation coefficient provides measure of relationship between any two characters but they do not provide information as to what extent and the manner in which those correlations have established, when one of the characters seems to be dependent over a number of other characters.

Thus, in order to determine the contribution of various characters towards drought tolerance it is essential that the correlations between drought tolerance and the other component characters are partitioned into a series of direct and indirect effects depicting the specific forces which are acting to develop a given correlation. To answer this problem path coefficient analysis was used to partition the correlation coefficients of all the physiological and biochemical traits studied into direct and indirect effects as suggested by **Wright (1921)** and elaborated by **Dewey and Lu (1959)**.

In the present investigation, path coefficient analysis was undertaken considering drought tolerance capacity (DTC) as dependent variable and the rest 15 characters as independent variable. Path coefficient analysis was carried out for both the environments (control & stress). The results of path coefficient analysis for both the environment are presented in the following sections.

4.4.2.1 Estimates of Path Coefficients for Various Drought Tolerance Traits under Control Environment

Path analysis for drought tolerance capacity (DTC) involved estimations of phenotypic and genotypic path coefficients for all the 15 traits as independent variables.

4.4.2.1.1 Phenotypic Path coefficients for DTC:

The results of phenotypic path analysis are presented in table 4.14 and Fig 5.

Direct effects:

Path coefficients for DTC at phenotypic levels depicted that germination percent (0.279) recorded highest positive direct effect on DTC, followed by RWC (0.278), Chl a (0.233), SPAD 120 (0.220), Proline content (0.207) and CTD 60 (0.178) whereas, Chl b (-0.214) conferred highest negative direct effect on DTC, which was

Table 4.14: Phenotypic Path Matrix Exhibiting Direct and Indirect Effects of Drought Tolerance Traits on Drought Tolerance Capacity in Sugarcane under Control Environment

	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing
Days to Germination	-0.067	-0.006	-0.013	0.011	-0.009	0.006	-0.006	0.000	0.015	0.002	0.003	0.003	0.008	-0.008	0.000
Germination%	0.025	0.279	0.074	0.050	0.084	0.036	0.085	0.072	0.099	0.167	-0.096	0.142	0.022	0.043	-0.075
SPAD 60	-0.011	-0.015	-0.057	-0.007	-0.055	-0.007	-0.052	-0.015	-0.017	-0.016	0.010	-0.014	-0.003	-0.008	0.008
CTD 60	-0.028	0.032	0.023	0.178	0.032	0.114	0.033	0.100	0.067	0.053	-0.054	0.040	0.030	0.001	-0.010
SPAD 120	0.031	0.067	0.212	0.039	0.220	0.037	0.212	0.070	0.078	0.071	-0.053	0.058	0.014	0.039	-0.035
CTD 120	0.002	-0.003	-0.003	-0.017	-0.004	-0.027	-0.006	-0.019	-0.009	-0.007	0.006	-0.005	-0.002	0.001	0.001
SPAD 180	-0.017	-0.056	-0.168	-0.034	-0.176	-0.038	-0.183	-0.065	-0.067	-0.065	0.039	-0.056	-0.018	-0.030	0.024
CTD	0.000	-0.005	-0.005	-0.010	-0.006	-0.013	-0.006	-0.018	-0.007	-0.006	0.003	-0.005	-0.001	-0.003	0.003
RWC%	-0.061	0.099	0.084	0.105	0.099	0.089	0.101	0.106	0.278	0.158	-0.049	0.145	0.078	0.027	-0.115
Chl a	-0.007	0.140	0.066	0.069	0.075	0.060	0.083	0.078	0.132	0.233	-0.021	0.226	0.046	0.016	-0.079
Chl b	0.009	0.074	0.036	0.065	0.051	0.046	0.045	0.038	0.038	0.020	-0.214	-0.033	-0.039	0.036	-0.050
Total chl	0.000	-0.004	-0.002	-0.002	-0.002	-0.002	-0.002	-0.002	-0.004	-0.007	-0.001	-0.008	-0.002	0.000	0.002
Total Carotenoids	0.011	-0.007	-0.005	-0.015	-0.006	-0.008	-0.009	-0.007	-0.025	-0.018	-0.016	-0.022	-0.090	0.004	0.006
Proline	0.025	0.032	0.031	0.002	0.036	-0.008	0.033	0.030	0.020	0.014	-0.035	0.006	0.008	0.207	-0.038
Leaf firing	0.000	-0.011	-0.006	-0.002	-0.007	-0.001	-0.006	-0.006	-0.017	-0.014	0.010	-0.012	0.003	-0.008	0.041
D T C	-0.087	0.614	0.267	0.430	0.333	0.284	0.321	0.361	0.581	0.583	-0.469	0.046	0.054	0.310	-0.329
Partial R²	0.006	0.171	-0.015	0.076	0.073	-0.008	-0.059	-0.007	0.162	0.136	0.101	-0.004	-0.005	0.064	-0.014

R SQUARE = 0.6786

RESIDUAL EFFECT = 0.5669

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

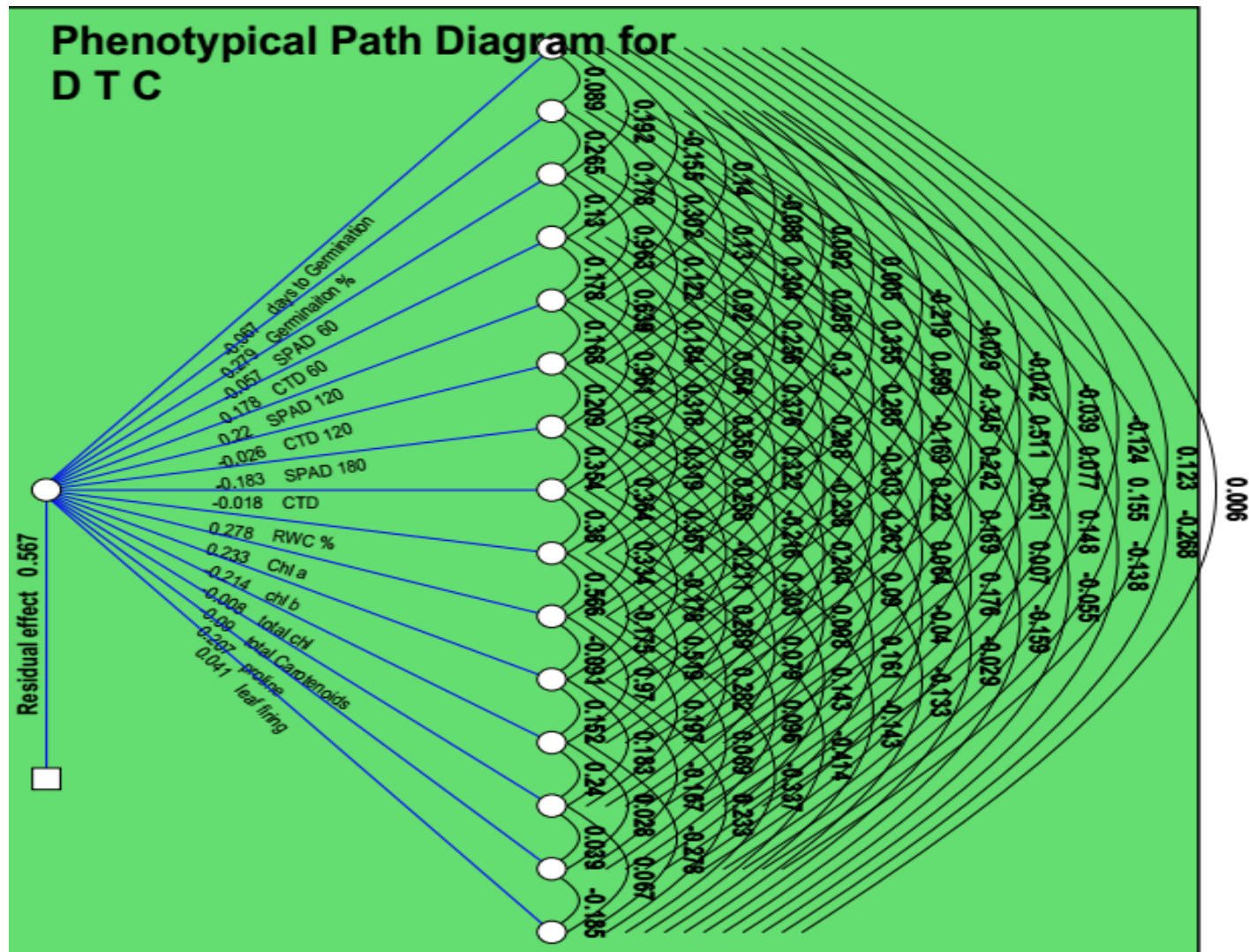


Fig 5: Phenotypic Path Diagram for DTC Depicting Direct and Indirect Effects of Various Drought Tolerance Traits in Sugarcane under Control Environment

followed by CTD 180 (-0.183). Direct effects of the remaining traits on DTC were found to be very low.

Indirect effects:

Germination percent conferred positive indirect effect through Chl a (0.140) and via all the remaining traits indirect effects were negligible. Similarly SPAD 60 recorded positive indirect effect through SPAD 120 (0.212) and negative indirect effect via SPAD 180 (-0.168). CTD 60, SPAD 180, Chl and total chlorophyll recorded positive indirect effect through RWC percent (0.105, 0.101, 0.158 and 0.145 respectively). SPAD 120 conferred negative indirect effect through SPAD 180 (-0.176). Similarly CTD 120 exerted positive indirect effect via CTD 60 (0.114). SPAD 180 conferred positive indirect effect via SPAD 120 (0.212) and RWC percent (0.101). Similarly, CTD 180 exerted positive indirect effect via CTD 60 (0.100) and RWC percent (0.106). RWC percent conferred a positive indirect effect through Chl a (0.132). However, Chl a recorded a positive indirect effect through RWC percent (0.158) and germination percent (0.167). Total chlorophyll conferred positive indirect effect via germination percent (0.142), RWC percent (0.15) and Chl a (0.221). Leaf firing exerted negative indirect effect via Chl a (-0.115).

Coefficient of determination (R^2) was recorded to be 0.6786 and the residual effect was found to be 0.5669.

4.4.2.1.2 Genotypic Path coefficients for DTC:

The results of genotypic path analysis are presented in table 4.15 and Fig 6.

Direct effects:

Genotypic path coefficients revealed that Chl a (0.780) conferred highest positive direct effect on DTC followed by germination percent (0.392), proline content (309), SPAD 120 (0.241), CTD 120 (0.206), RWC (0.191), CTD 60 (0.176). Leaf firing (0.014) conferred least positive direct effect on DTC. However, total chlorophyll (-0.542) recorded highest negative direct effect on DTC followed by SPAD 180 (-0.322), days to germination (-0.220), CTD 180 (-0.141) and total carotenoids (-0.121). Chl b (-0.039) recorded least negative direct effect on DTC.

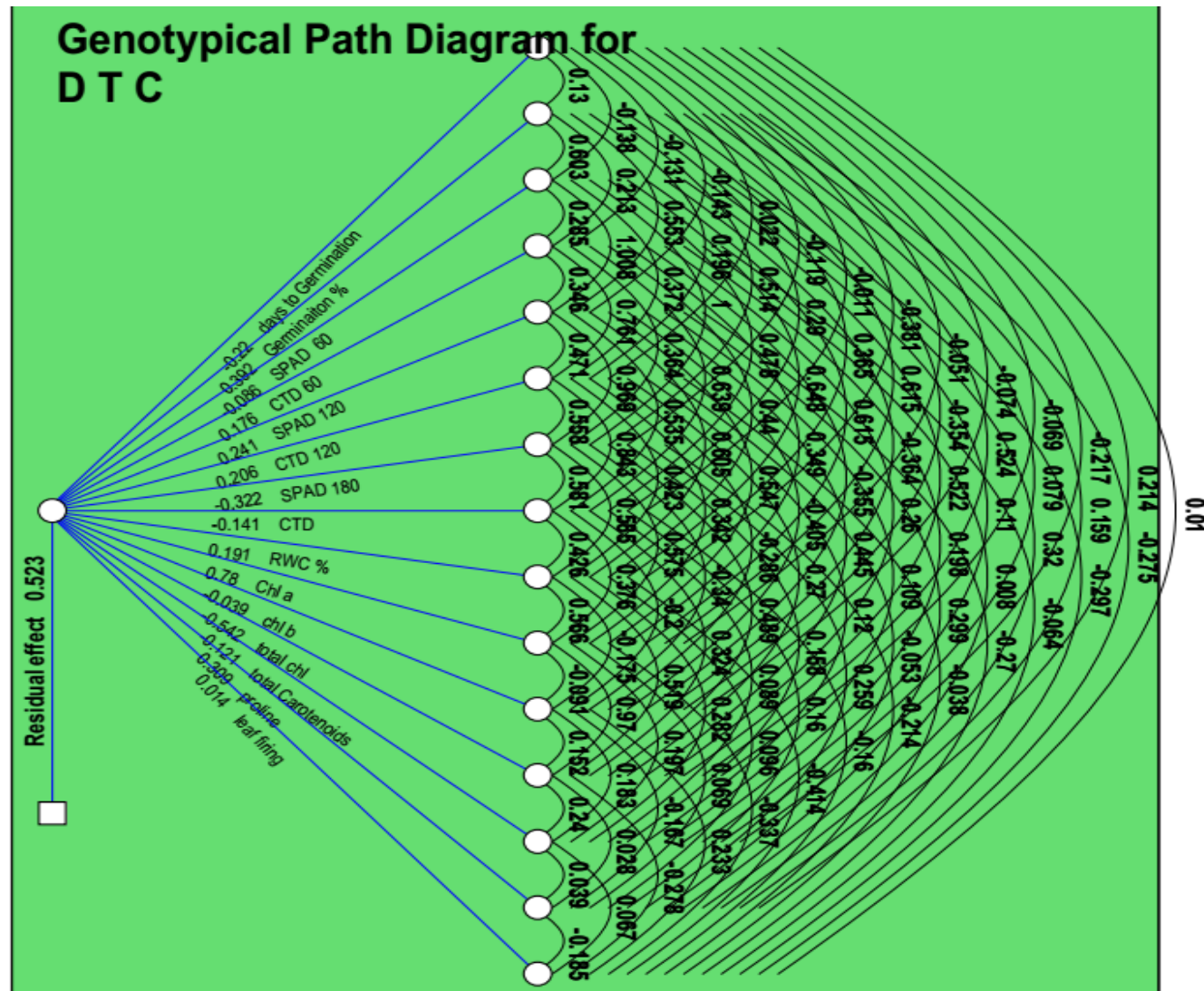


Fig 6: Genotypic Path Diagram for DTC Depicting Direct and Indirect Effects of Various Drought Tolerance Traits in Sugarcane under control environment

Indirect effects:

Germination percent conferred positive indirect effect through Chl a (0.480) and SPAD 120 (0.34) and, negative via SPAD 180 (-0.166) and total chlorophyll (-0.283). Similarly SPAD 60 recorded positive indirect effect through germination percent (0.236) and SPAD 120 (0.243), RWC percent (0.184) and Chl a (0.480). It recorded negative indirect effect via SPAD 180 (-0.323) and total chlorophyll (-0.283). CTD 60, SPAD 120, CTD 120, SPAD180, CTD 180, RWC percent, total chlorophyll and total carotenoids recorded positive indirect effect through Chl a (0.272, 0.427, 0.267, 0.449, 0.293, 0.442, 0.757 and 0.154 respectively). SPAD 120 conferred negative indirect effect through SPAD 180 (-0.312) and via total chlorophyll (0.241). Similarly CTD 120 exerted negative indirect effect via SPAD 180 (-0.180) and CTD 180 (-0.119). SPAD 180 conferred positive indirect effect via SPAD 120 (0.234) and RWC percent (0.112) and, negative indirect effect via (-0.265). Similarly, CTD 180 exerted positive indirect effect via germination percent (0.113), CTD 60 (0.113), SPAD 120 (0.129) and CTD 120 (0.174). RWC percent conferred a positive indirect effect through germination percent (0.143) and SPAD 120 (0.146) and, negative effect via total chlorophyll (-0.282). However, Chl a recorded a positive indirect effect through germination percent (0.241) and, negative effect via total chlorophyll (-0.526). Total chlorophyll conferred positive indirect effect via germination percent (0.205), and Chl a (0.757). Leaf firing exerted negative indirect effect via Chl a (-0.263) and positive via total chlorophyll (0.151).

Coefficient of determination (R^2) was recorded to be 0.726 and the residual effect was found to be 0.523

4.4.2.2 Estimates of Path Coefficients for Various Drought Tolerance Traits under Stress Environment

Path analysis for drought tolerance capacity (DTC) involved estimations of phenotypic and genotypic path coefficients for all the 15 traits as independent variables.

4.4.2.2.1 Phenotypic Path coefficients for DTC

The results of phenotypic path analysis under stress condition are presented in table 4.16 and Fig 7.

Direct effects

Phenotypic path coefficients for DTC revealed that Chl a (3.334) recorded highest positive direct effect on DTC followed by Chl b (0.695), proline content (0.680), total carotenoids (0.239), SPAD 60 (0.259) and RWC (0.201). CTD 120 (0.073) recorded least positive direct effect. However, total chlorophyll (-4.060) conferred highest negative direct effect on DTC followed by SPAD 120 (-0.259), days to germination (-0.128), CTD 120 (-0.073), leaf firing (-0.071), SPAD 180 (-0.064) and germination percent (-0.056). CTD 60 (-0.034) recorded least negative direct effect on DTC.

Indirect effects

Days to germination exerted positive indirect effect via Chl a (0.407) and negative effect via total chlorophyll (-0.448). Germination percent conferred positive indirect effect through Chl a (2.318) and proline content (0.572) and, negative via total chlorophyll (-2.816). A similar trend of positive indirect effects were conferred from traits like SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC percent, Chl a, Chl b, total chlorophyll, total carotenoids and proline via Chl a (>2.00), proline content (>0.400), SPAD 60 (>0.150) and RWC percent (>0.100). Similarly negative indirect effects were conferred from traits like SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC percent, Chl a, Chl b, total carotenoids and proline via total chlorophyll (>-2.500).

Leaf firing conferred a positive indirect effect via total chlorophyll (3.071) and negative indirect effect via Chl a (-2.054), proline (-0.558), Chl b (-0.466), total carotenoids (-0.184), SPAD 60 (-0.177) and RWC percent (-0.159).

Coefficient of determination (R^2) was recorded to be 0.8117 and the residual effect was found to be 0.4339

4.4.2.2.2 Genotypic Path coefficients for DTC

The results of genotypic path analysis are presented in table 4.17 and Fig 8.

Direct effects

Genotypic path coefficients for DTC revealed that total chlorophyll (38.233) recorded highest positive direct effect on DTC followed by SPAD 180 (7.775), CTD 120 (1.358) and germination percent (0.327). Proline content (0.047) recorded least

positive direct effect on DTC. However, Chl a (-33.158) recorded highest negative direct effect on DTC followed by SPAD 120 (-10.216), Chl b (-7.212), leaf firing (-2.678), days to germination (-1.776), RWC (-1.015) and CTD 180 (-0.662). Total carotenoids (-0.062) recorded least negative direct effect on DTC.

Indirect effects

Days to germination recorded a positive indirect effects via CTD 60 (0.230), CTD 120 (0.442), SPAD 180 (0.525), leaf firing (0.715) and total chlorophyll (6.684). whereas, negative effects via Chl a (-6.420) and Chl b (-0.438). A similar trend of positive indirect effects of germination percent, SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC percent, Chl a, Chl b, total chlorophyll, total carotenoids and proline via total chlorophyll (>25.00), SPAD 180 (>0.4.5), CTD 120 (>0.810), CTD 60 (>0.700) and SPAD 60 (>0.350) were recorded. Similarly a trend of negative indirect effects of germination percent, SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC percent, Chl a, Chl b, total chlorophyll, total carotenoids and proline via SPAD 120 (>6.00), CTD 180 (>0.320), RWC percent (>0.550), Chl b (>3.500) were recorded. Traits like germination percent, SPAD 60, SPAD 120, SPAD 180, CTD 60, CTD 120, CTD 180, RWC percent and total carotenoids conferred negative indirect effects via Chl a (>22.00) whereas, traits like Chl b, total chlorophyll, proline content and leaf firing conferred a positive indirect of via Chl a (>24.00). Leaf firing conferred indirect effect via SPAD 120 (8.00), Chl a (26.370) and Chl b (5.09). whereas, it conferred negative indirect effects via total chlorophyll (-30.610), SPAD 180 (6.036) and CTD 120 (-1.084).

Analysing the results for direct and indirect effects conferred by various traits on DTC under both the environments (control and stress), following of the conclusion can be drawn. Under stress conditions total chlorophyll conferred highest positive direct effects and simultaneously total chlorophyll conferred positive indirect effects via Chl a indicating that the indirect effects are the real cause of correlation.

Chl b conferred negative direct effects on DTC under stress environment however, its indirect effects via total chlorophyll and Chl a were recorded to be positive, depicting that the indirect effects via total chlorophyll and Chl a are the real cause of correlation. Similar conclusions can be drawn for SPAD 120 and SPAD 180 where indirect effects via total chlorophyll.

Table 4.16: Phenotypic Path Matrix Exhibiting Direct and Indirect Effects Of Drought Tolerance Traits On Drought Tolerance Capacity in Sugarcane under Stress Environment

	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf firing
Days to Germination	-0.128	-0.031	0.002	-0.012	0.002	-0.024	0.004	-0.013	-0.005	-0.015	-0.004	-0.014	-0.008	-0.024	0.019
Germination%	-0.014	-0.056	-0.032	-0.033	-0.030	-0.037	-0.030	-0.033	-0.032	-0.038	-0.032	-0.038	-0.037	-0.046	0.040
SPAD 60	-0.005	0.149	0.259	0.211	0.244	0.180	0.229	0.159	0.153	0.164	0.156	0.167	0.169	0.167	-0.177
CTD 60	-0.003	-0.022	-0.030	-0.036	-0.029	-0.028	-0.026	-0.020	-0.024	-0.023	-0.022	-0.023	-0.024	-0.024	0.026
SPAD 120	0.005	-0.143	-0.244	-0.210	-0.259	-0.192	-0.236	-0.153	-0.160	-0.159	-0.156	-0.163	-0.171	-0.167	0.183
CTD 120	0.014	0.049	0.051	0.057	0.054	0.073	0.051	0.045	0.049	0.050	0.041	0.050	0.048	0.053	-0.057
SPAD 180	0.002	-0.035	-0.056	-0.047	-0.058	-0.045	-0.064	-0.038	-0.037	-0.039	-0.036	-0.039	-0.040	-0.043	0.042
CTD 180	-0.001	-0.006	-0.006	-0.006	-0.006	-0.006	-0.010	-0.005	-0.006	-0.006	-0.004	-0.006	-0.005	-0.006	0.006
RWC %	0.009	0.119	0.119	0.137	0.124	0.138	0.117	0.113	0.201	0.134	0.115	0.134	0.134	0.144	-0.159
Chl a	0.407	2.318	2.112	2.140	2.056	2.303	2.055	2.181	2.230	3.334	2.710	3.314	2.966	2.510	-2.504
Chl b	0.027	0.404	0.419	0.433	0.419	0.400	0.402	0.324	0.401	0.565	0.695	0.604	0.606	0.413	-0.466
Total Chl	-0.448	-2.816	-2.620	-2.663	-2.563	-2.798	-2.544	-2.590	-2.721	-4.038	-3.532	-4.060	-3.702	-3.027	3.071
Total Carotenoids	0.016	0.161	0.156	0.160	0.157	0.158	0.151	0.138	0.159	0.212	0.208	0.218	0.239	0.168	-0.184
Proline	0.130	0.572	0.440	0.456	0.439	0.501	0.430	0.463	0.488	0.512	0.404	0.506	0.480	0.680	-0.558
Leaf firing	0.011	0.051	0.048	0.052	0.050	0.051	0.047	0.044	0.056	0.053	0.047	0.053	0.055	0.058	-0.071
D T C	0.021	0.715	0.618	0.638	0.600	0.673	0.579	0.610	0.751	0.704	0.589	0.702	0.707	0.862	-0.782
Partial R²	-0.003	-0.040	0.160	-0.023	-0.155	0.049	-0.037	-0.006	0.151	2.347	0.409	-2.851	0.169	0.586	0.055

R SQUARE = 0.8117

RESIDUAL EFFECT = 0.4339

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

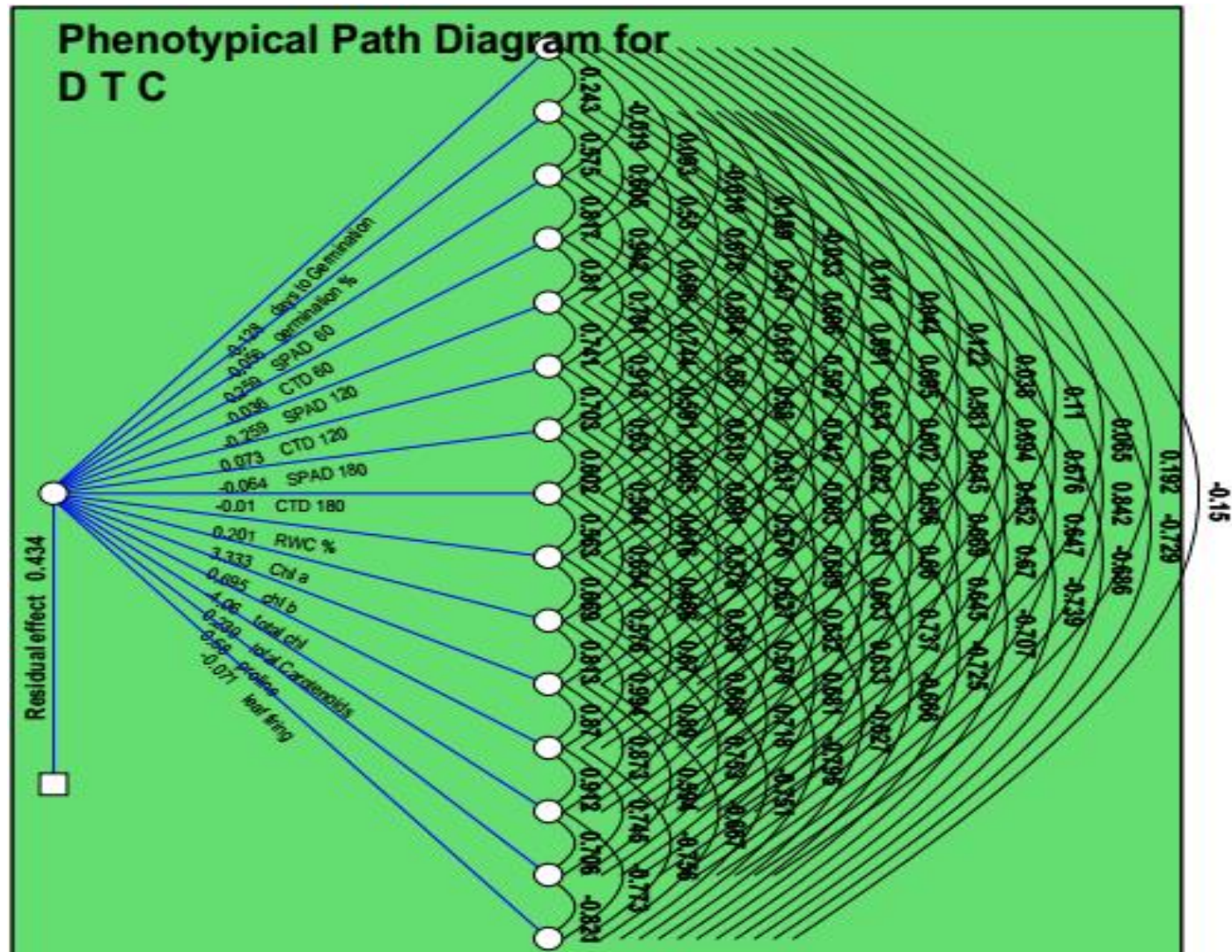


Fig 7: Phenotypic Path Diagram for DTC Depicting Direct and Indirect Effects of Various Drought Tolerance Traits in Sugarcane under Stress Environment

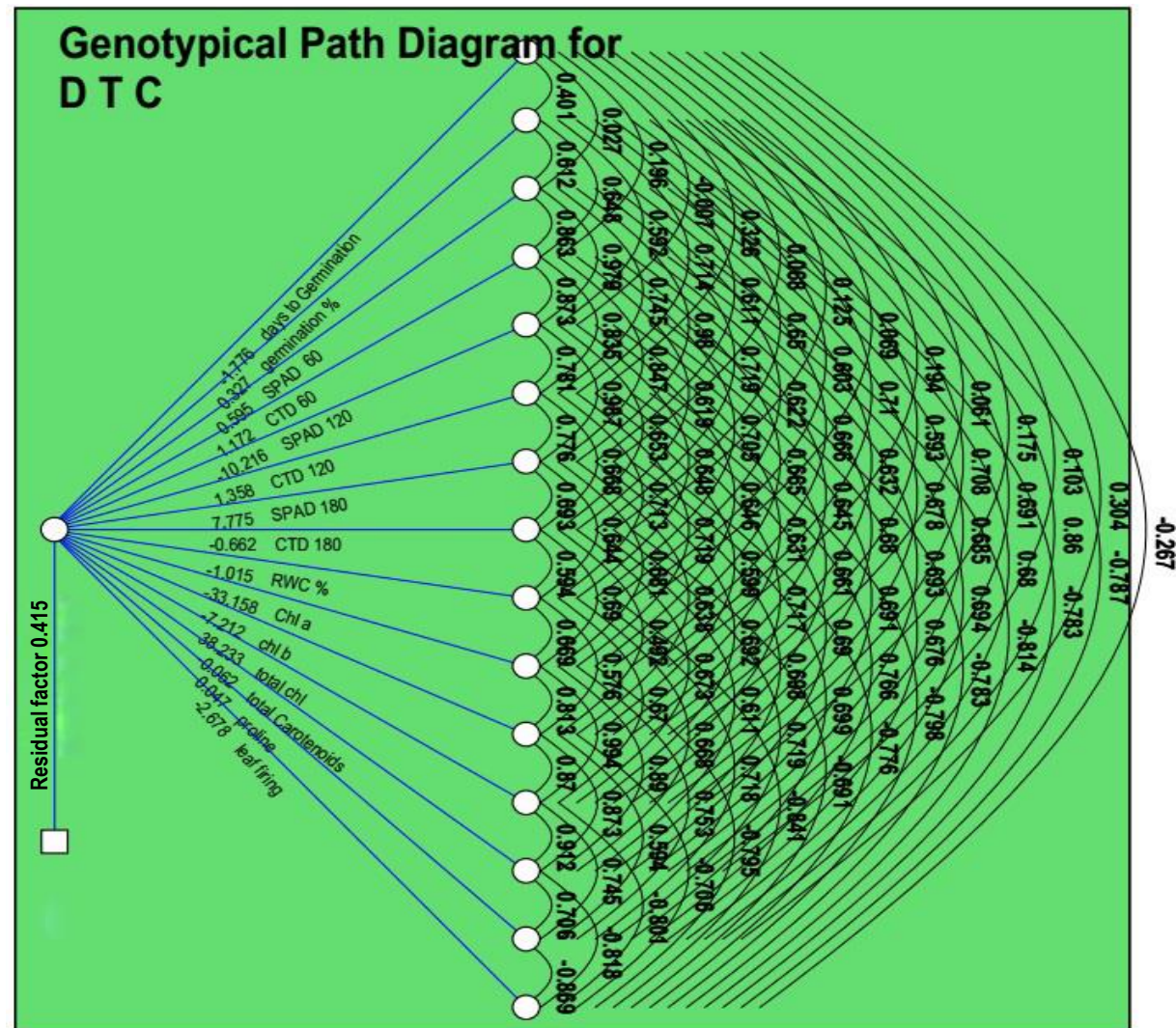


Fig 8: Genotypic Path Diagram for DTC Depicting Direct and Indirect Effects of Various Drought Tolerance Traits in Sugarcane under Stress Environment

Indirect effect via total chlorophyll indicates greater contribution of total chlorophyll towards the correlation. Under such circumstances, indirect causal factors should be considered simultaneously for selection (**Singh and Chaudhary, 1977**).

Under control conditions direct effect of proline content was found to be almost equal to its correlation coefficient which, indicates that the correlation indicates true relationship and direct selection through proline content will be effective. Similarly under stress environment direct effects of Chl b were almost equal to its correlation coefficient.

Unlike negligible effects showed by Chl b under control environment, this trait has to be a determinant of DTC. Since, in addition to its direct effects it conferred higher indirect effects via total chlorophyll under stress (**Silva *et al.*, 2012**).

Traits with higher negative direct and indirect effects under control environment indicate that they are not the determinants for DTC under control environment.

Path coefficients under both the environments and at both phenotypic and genotypic levels explained 100 percent of variations in DTC as revealed by the higher coefficient of determinants. However, the residual effects under control environment was recorded to be > 0.5 which, indicates some more factors need to be included in the analysis to account fully for the variation in DTC.

Traits like Chl a, Chl b, total chlorophyll, SPAD 120, SPAD 180 and proline content were found to confer positive direct as well as indirect effects on DTC which supports their utilization as a reliable tool in selection for drought tolerance in sugarcane.

4.5 Assessment of genetic diversity for drought tolerance traits

Understanding regarding genetic diversity is a necessity for every breeding programme. Origin of genetic diversity is owed to geological division or due to inherent barriers to crossability or due to diverse patterns of evolution. It can be measured following D^2 statistics that determine group distance based on numerous characters (**Mahalanobis, 1928**) and it has turn out to be one of the important methods to determine genetic divergence on the basis of multiple traits. Inclusion of dissimilar parents in hybridization programmes serves the rationale of combining desirable genes

in new combination. Mahalanobis D^2 statistic is a potent tool in estimating the level of divergence at genotypic level. A number of workers have used this method to quantify the degree of divergence based on phenotypic observations in several crops. The genetic divergence present between the clones was estimated by Mahalanobis D^2 statistic as described by **Rao (1952)**. Based on D^2 values, the assemblage of genotypes into clusters was done following Tocher's method **Rao (1952)**.

Genetic diversity using Mahalanobis D^2 statistic among 65 early generation clones of Sugarcane along with parents was estimated for both the environments (control & stress) separately. The results for both the environments are summarized in the following sections.

4.5.1 Estimates of Genetic diversity using Mahalanobis D^2 statistic among 72 sugarcane genotypes under control environment

The estimates of genetic divergence among early generation clones and their parents for drought tolerance under control environment are presented as follows.

4.5.1.1 Clustering of clones:

Based on the Mahalanobis D^2 distances all the 72 genotypes were clustered into 8 clusters and the dendrogram is presented as Fig 9. The compositions of each of the clusters are presented in Table 4.18.

The **cluster I** was found to be the largest cluster with 31 clones falling under this followed by **cluster II** with 22 clones which, comprised of Co 617 a drought tolerant parental variety. The presence of Co 617 in cluster II indicates the tolerance nature of the accompanying clones. **Cluster III** consisted of 10 clones which included UP 9530, Co Pant 97222 and Bo 91 which were used as drought sensitive parents for the present study material. **Cluster IV** comprised of 5 clones including ISH 100 and Co 1148 which were drought tolerant parents. Along with these two parental variety, the clones viz., PC (2015-16)-41, PC (2015-16)-58 and PC (2015-16)-45 also exhibited drought tolerance. **Cluster V** comprised of only 1 clone i.e. PC (2015-16)-21. Similarly cluster **VI, VII and VIII** also consisted of one clone each viz; PC (2015-16)-49, Co 85002 and PC (2015-16)-47. Co 85002 which, was the only genotype in cluster VII also used as tolerant parent.

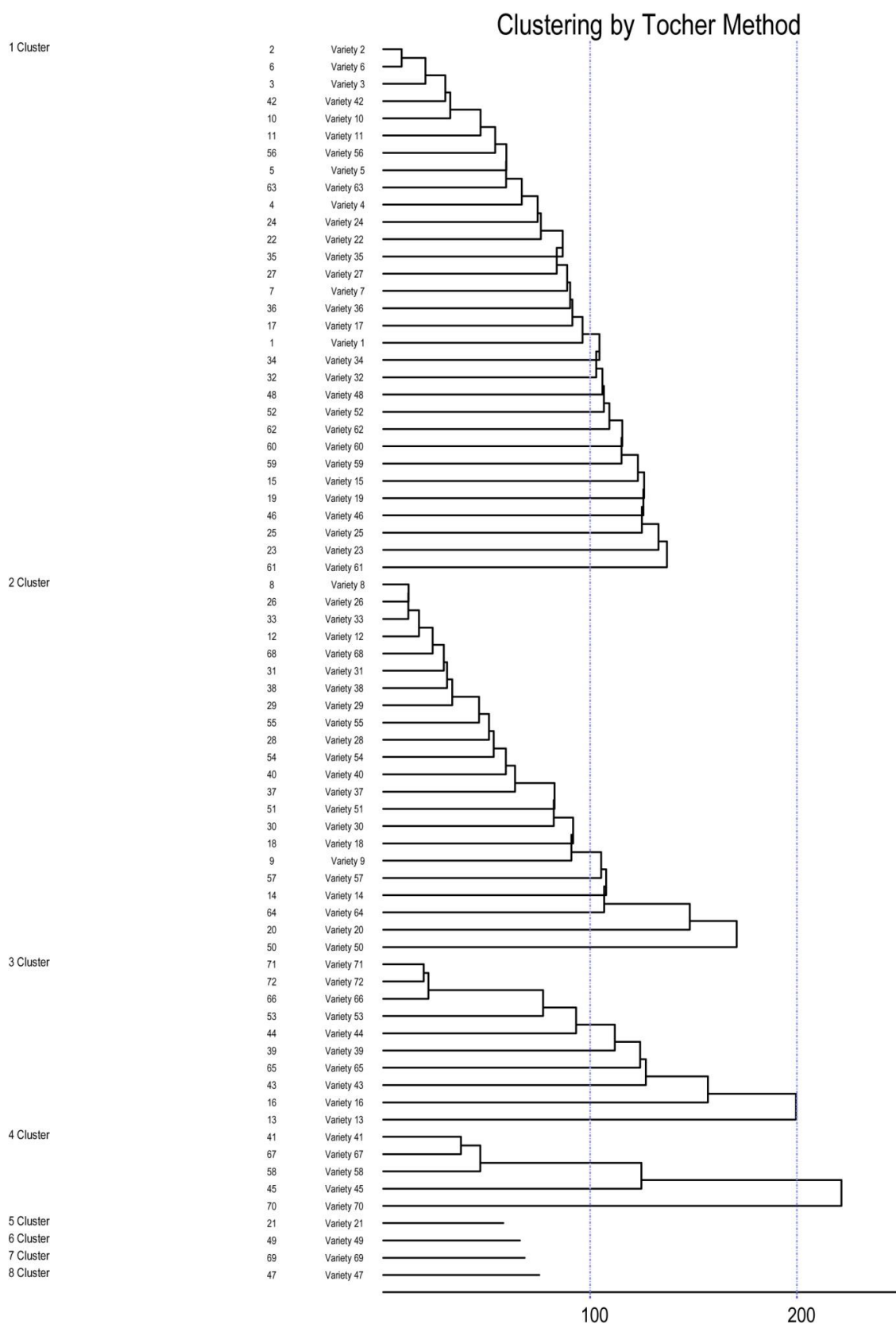


Fig 9: Dendrogram Representing the Clustering Pattern for All 72 Clones in Control Environment.

Table 4.18: Clustering Patterns of 72 Sugarcane Genotypes on the basis of D² Values under Control Environment

Cluster No.	Genotypes included	No. of Genotypes
I	PC (2015-16)-2, PC (2015-16)-6, PC (2015-16)-3, PC (2015-16)-42, PC (2015-16)-10, PC (2015-16)-11, 40-14, PC (2015-16)-5, PC (2015-16)-63, PC (2015-16)-4, PC (2015-16)-24, PC (2015-16)-22, PC (2015-16)-35, PC (2015-16)-27, PC (2015-16)-7, PC (2015-16)-36, PC (2015-16)-17, PC (2015-16)-1, PC (2015-16)-34, PC (2015-16)-32, PC (2015-16)-48, PC (2015-16)-52, PC (2015-16)-62, PC (2015-16)-60, PC (2015-16)-59, PC (2015-16)-15, PC (2015-16)-19, PC (2015-16)-46, PC (2015-16)-25, PC (2015-16)-23, PC (2015-16)-61	31
II	PC (2015-16)-8, PC (2015-16)-26, PC (2015-16)-33, PC (2015-16)-12, Co 617, PC (2015-16)-31, PC (2015-16)-38, PC (2015-16)-29, PC (2015-16)-55, PC (2015-16)-28, PC (2015-16)-54, PC (2015-16)-40, PC (2015-16)-37, PC (2015-16)-51, PC (2015-16)-30, PC (2015-16)-18, PC (2015-16)-9, PC (2015-16)-57, PC (2015-16)-14, PC (2015-16)-58, PC (2015-16)-20, PC (2015-16)-50	22
III	UP 9530, Co Pant 97222 , BO 91, PC (2015-16)-53, PC (2015-16)-44, PC (2015-16)-39, PC (2015-16)-65, PC (2015-16)-43, PC (2015-16)-16, PC (2015-16)-13	10
IV	PC (2015-16)-41, Co 1148, PC (2015-16)-58, PC (2015-16)-45, ISH 100	5
V	PC (2015-16)-21	1
VI	PC (2015-16)-49	1
VII	Co 85002	1
VIII	PC (2015-16)-47	1

4.5.1.2 Average Inter and Intra-Cluster distances:

Average inter and intra-cluster D² values between all the 8 clusters are presented in table 4.19 and depicted as a Fig 10.

The diagonal values in the table indicate the intra-cluster D² values. Intra-cluster D² values ranged from 0.00 to 178.45. Cluster IV (178.45) with 5 clones recorded highest intra-

cluster D² values followed by cluster III (151.66), cluster I (110.94) and cluster II (96.57). All the remaining clusters V, VI, VII and VIII with single clones each

recorded 0.00 intra-cluster D^2 values. All the remaining values apart from diagonal ones in the table indicated the inter cluster D^2 values between all pairs of clusters. Inter cluster D^2 values ranged from 68.41 between cluster VI and VIII to 1451.64 between cluster IV and V.

The relative genetic distance (D), as calculated by taking square root of D^2 values, between different clusters are presented in Table 4.20

The diagonal values in the table indicate the intra-cluster relative genetic distance (D). The maximum intra-cluster genetic distance was recorded in cluster IV (13.36) and minimum in cluster V, VI, VII, VIII (0.00). All the remaining values apart from diagonal ones in the table indicated the inter cluster genetic distances. Maximum inter-cluster distance was recorded between cluster IV and V (38.10) whereas, minimum between cluster VI and VII (8.27).

4.5.1.3 Cluster means for different characters

The cluster means for all the 16 characters were calculated and are presented in Table 4.21. Analysing the cluster means for different characters it was revealed that considerable differences existed between the clusters for all the 16 characters studied.

For trait days to germination, the cluster mean ranged from 25.00 to 30.00. The highest cluster mean was recorded for cluster V, VI and VII (30.00) followed by, cluster I (28.95) and the lowest cluster mean for cluster VIII (25.00). The cluster mean for germination percent ranged from 27.15 to 43.90. The highest cluster mean was for the cluster IV (43.90) followed by, cluster VII (43.80) and the minimum was for cluster V (27.15).

Similarly, cluster mean for SPAD 60 ranged from 35.20 to 45.70. The maximum cluster mean was for cluster IV (45.79) followed by, cluster II (45.21) and minimum for cluster VIII (35.20). The cluster mean for SPAD 120 ranged from 35.58 to 46.25. The highest mean recorded was for cluster II (46.25) followed by, cluster VII (45.55) and the minimum for cluster VIII (35.58). Similarly, the range for SPAD 180 was recorded to be 31.54 to 44.05. Maximum cluster mean was recorded by cluster IV (44.05) followed by, cluster II (43.55) and the minimum cluster mean by cluster VIII (31.54). For CTD 60 the cluster means ranged from cluster III (3.09) to cluster VI (6.38). For CTD 120 the cluster means ranged from 3.60 to 6.45. The maximum mean

Table 4.19: Average Inter and Intra-Cluster (diagonal) D^2 Values between Clusters of Sugarcane Genotypes under Control Environment

Clusters	I	II	III	IV	V	VI	VII	VIII
I	110.94	498.88	226.60	1345.90	173.08	187.02	180.84	221.29
II		96.57	288.16	417.66	536.77	272.52	438.45	318.14
III			151.66	800.57	345.26	231.48	349.63	257.89
IV				178.45	1451.64	1004.93	1405.48	971.16
V					0.00	208.14	184.71	195.86
VI						0.00	68.41	75.45
VII							0.00	165.18
VIII								0.00

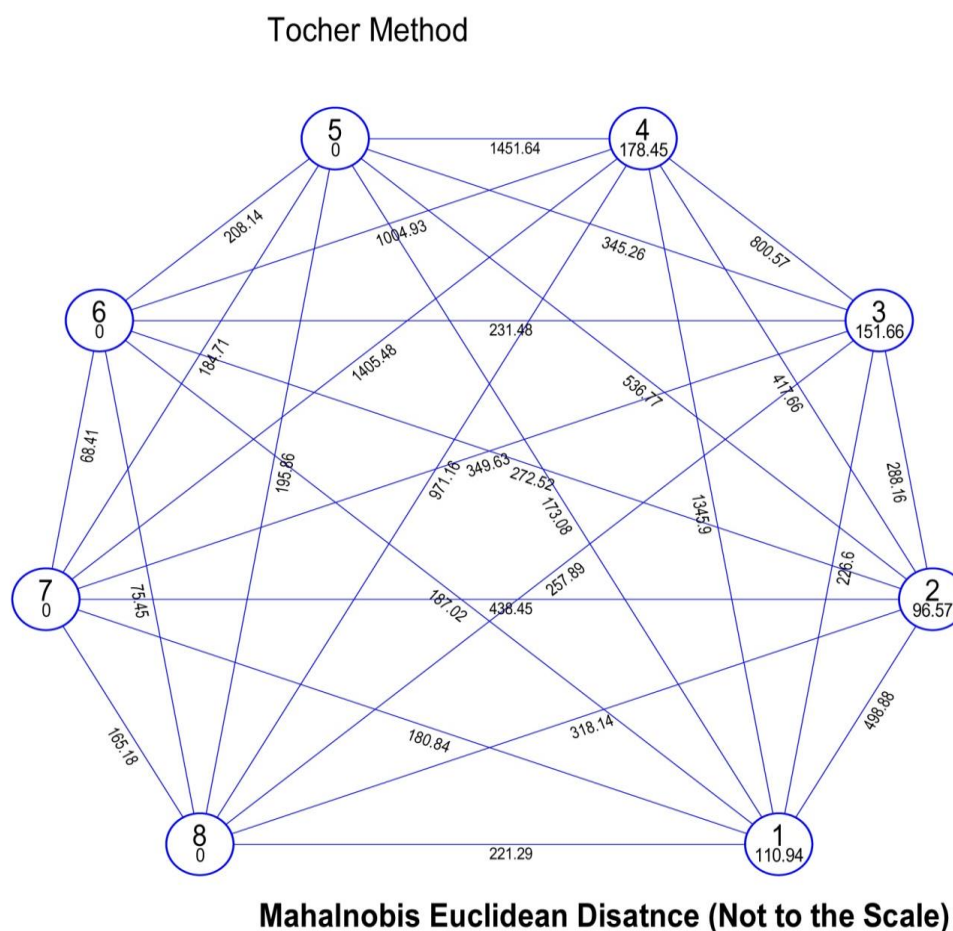


Fig 10: Cluster Diagram based on Average Intra and Inter-Cluster Distances for Sugarcane Genotypes under Control Environment

Table 4.20: Average Inter and Intra-Cluster (diagonal) D Values between Clusters of Sugarcane Genotypes under Control Environment

Clusters	I	II	III	IV	V	VI	VII	VIII
I	10.53	22.34	15.05	36.69	13.16	13.68	13.45	14.88
II		9.83	16.98	20.44	23.17	16.51	20.94	17.84
III			12.32	28.29	18.58	15.21	18.70	16.06
IV				13.36	38.10	31.70	37.49	31.16
V					0.00	14.43	13.59	13.99
VI						0.00	8.27	8.69
VII							0.00	12.85
VIII								0.00

was recorded for cluster II (6.45) followed by, cluster VIII (5.91) and the minimum for cluster V (3.60). For CTD 180 the cluster mean ranged from 2.90 to 7.10. The maximum cluster mean was recorded by cluster VII (7.10) followed by cluster II (6.22) and the minimum by cluster V (2.90).

The range for Chl a ranged from 1.71 to 2.97, with maximum cluster mean for cluster IV (2.97) followed by, cluster II (2.39) and the minimum for cluster V (1.70). For Chl b 0.33 to 0.54 was the range, with maximum cluster mean for cluster I and IV (0.54) and the minimum for cluster VI and VII (0.33). Total carotenoids recorded a range of 0.04 to 0.08, with the maximum mean for cluster IV (0.08) and Minimum for cluster I (0.04).

For RWC percent the cluster mean ranged from 82.04 to 90.84, with maximum cluster mean for cluster VII (90.84) followed by, cluster IV (90.33) and minimum by cluster V (82.04).

For proline cluster mean ranged from 42.35 to 50.52 with maximum mean for cluster V (50.52) followed by, cluster II (50.16) and the minimum for cluster VIII (42.35). For leaf firing cluster mean ranged from 1.80 to 5.00, with maximum mean for cluster II, IV, VI, VII, VIII (5.00) and the minimum for cluster III (1.80). Similarly, for DTC cluster mean ranged from 1.80 to 5.00, with maximum mean for cluster II, IV, VI, VII, VIII (5.00) and the minimum for cluster III (1.80).

Table 4.21: Cluster Mean Values for Different Drought Tolerance Characters in Sugarcane under Control environment

Clusters	Characters															
	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC%	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	DTC
I	28.95	35.77	39.18	3.73	39.14	5.06	36.70	4.04	85.99	1.76	0.54	2.30	0.04	47.27	2.35	2.29
II	27.50	43.70	45.21	5.74	46.25	6.45	43.55	6.22	89.90	2.39	0.43	2.82	0.07	50.16	5.00	5.00
III	26.25	38.31	37.80	3.09	37.29	3.82	35.02	3.65	86.14	2.14	0.55	2.69	0.05	45.67	1.80	1.80
IV	27.00	43.90	45.79	4.98	45.03	5.59	44.05	4.61	90.33	2.97	0.54	3.52	0.08	45.85	5.00	5.00
V	30.00	27.15	43.50	3.40	43.00	3.60	40.60	2.90	82.04	1.70	0.43	2.13	0.05	50.52	3.00	3.00
VI	30.00	42.90	41.20	6.38	40.83	5.54	38.33	3.59	89.78	1.94	0.33	2.27	0.05	44.39	5.00	5.00
VII	30.00	43.80	46.50	5.10	45.55	5.20	40.20	7.10	90.84	1.71	0.33	2.04	0.05	48.06	5.00	5.00
VIII	25.00	40.75	35.20	5.30	35.58	5.91	31.54	3.51	85.04	1.88	0.50	2.37	0.07	42.35	5.00	5.00

4.5.1.4 Contribution of Different Characters towards Divergence:

Percent contribution of different characters towards divergence was measured for all the 16 drought tolerance traits and presented in Table 4.22 and depicted as Fig 11.

Table 4.22: Percent Contribution of Different Drought Tolerance Characters towards Genetic Divergence in Sugarcane under Control Environment

S. No.	Character	Percent Contribution
1	Days to germination	5.47
2	Germination%	10.36
3	SPAD 60	2.23
4	CTD 60	6.76
5	SPAD 120	5.12
6	CTD 120	3.91
7	SPAD 180	3.24
8	CTD 180	6.22
9	RWC%	6.61
10	Chl a	8.80
11	Chl b	7.43
12	Total Chl	0.00
13	Total Carotenoids	8.72
14	Proline	8.80
15	Leaf firing	7.78
16	DTC	8.45

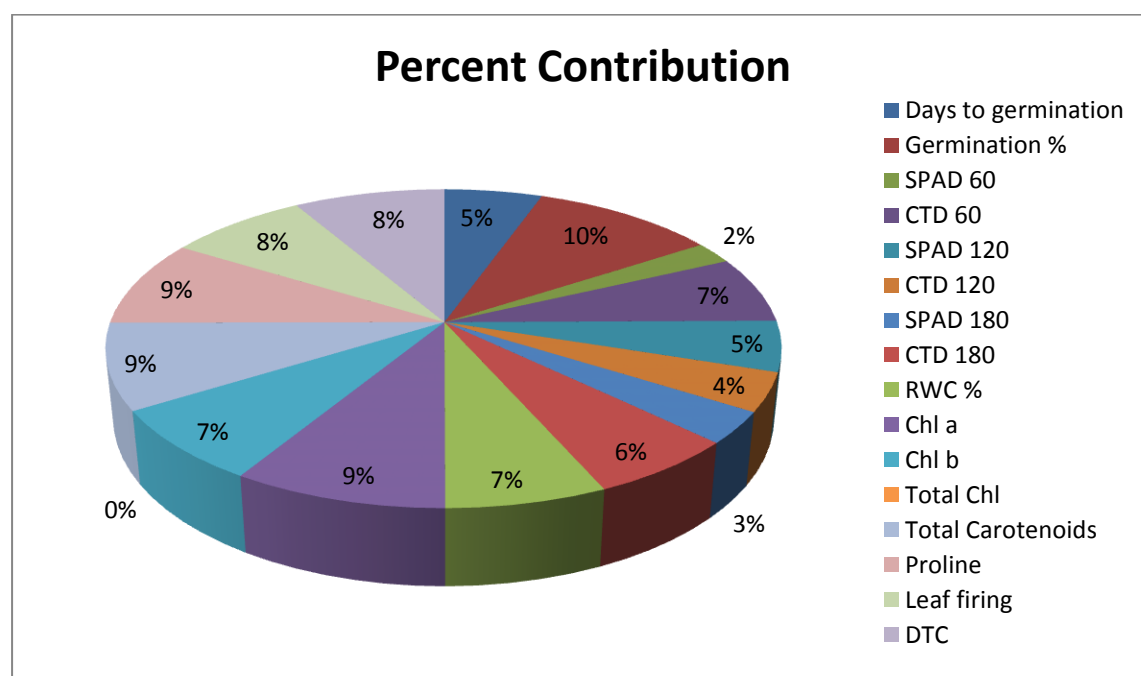


Fig 11: Percent Contribution of Different Drought Tolerance Characters towards Genetic Divergence in Sugarcane under Control environment

The study on individual contribution of characters indicated that the maximum contribution towards divergence was conferred by germination percent (10.36) followed by Chl a and proline content (8.80), total carotenoids (8.72), DTC (8.45), leaf firing (7.78), Chl b (7.43), CTD 60 (6.76), RWC percent (6.61), CTD 180 (6.22), days to germination (5.47), SPAD 120 (5.12), CTD 120 (3.91), SPAD 180 (3.24) and least contribution was recorded by SPAD 60 (2.23), while total chlorophyll (0.00) not contributed towards genetic divergence under control environment.

4.5.2 Estimates of genetic diversity using Mahalanobis D^2 statistic among 72 Sugarcane genotypes under stress environment.

The estimates of genetic divergence among early generation clones for drought tolerance under stress environment are presented as follows.

4.5.2.1 Clustering of clones

Based on the Mahalanobis D^2 distances all the 72 genotypes were clustered into 10 clusters and the dendrogram is presented as Fig 12. The compositions of each of the clusters are presented in Table 4.23.

The **cluster II** was found to be the largest cluster with 24 clones falling under this followed by **cluster I** with 23 clones which, comprised of Co 617 a drought tolerant variety. The presence of Co 617 in cluster I indicate the tolerance nature of the accompanying clones in the cluster. **Cluster VII** comprised of 15 clones which included a sensitive parent UP 9530 used in generation of study material. **Cluster III** consisted of two clones such as PC (2015-16)-41, PC (2015-16)-58 and two tolerant parents viz., Co 1148, ISH 100. **Cluster IV, V, VI, VIII, IX and X** comprised of only single clone / variety such as PC (2015-16)-49, Co 85002, PC (2015-16)-47, BO 91, Co Pant 97222 and PC (2015-16)-35, respectively.

4.5.2.2 Average Inter and Intra-Cluster distances

Average inter and intra-cluster D^2 values between all the 10 clusters are presented in table 4.24 and depicted as a Fig 13.

The diagonal values in the table indicate the intra-cluster D^2 values. Intra-cluster D^2 values ranged from 0.00 to 178.76. Cluster VII (178.76) with 15 clones recorded highest intra-cluster D^2 values followed by cluster II (117.64), cluster III (108.68) and cluster I (74.86). All the remaining clusters IV, V, VI, VIII, IX and X with single clones each recorded 0.00 intra-cluster D^2 values.

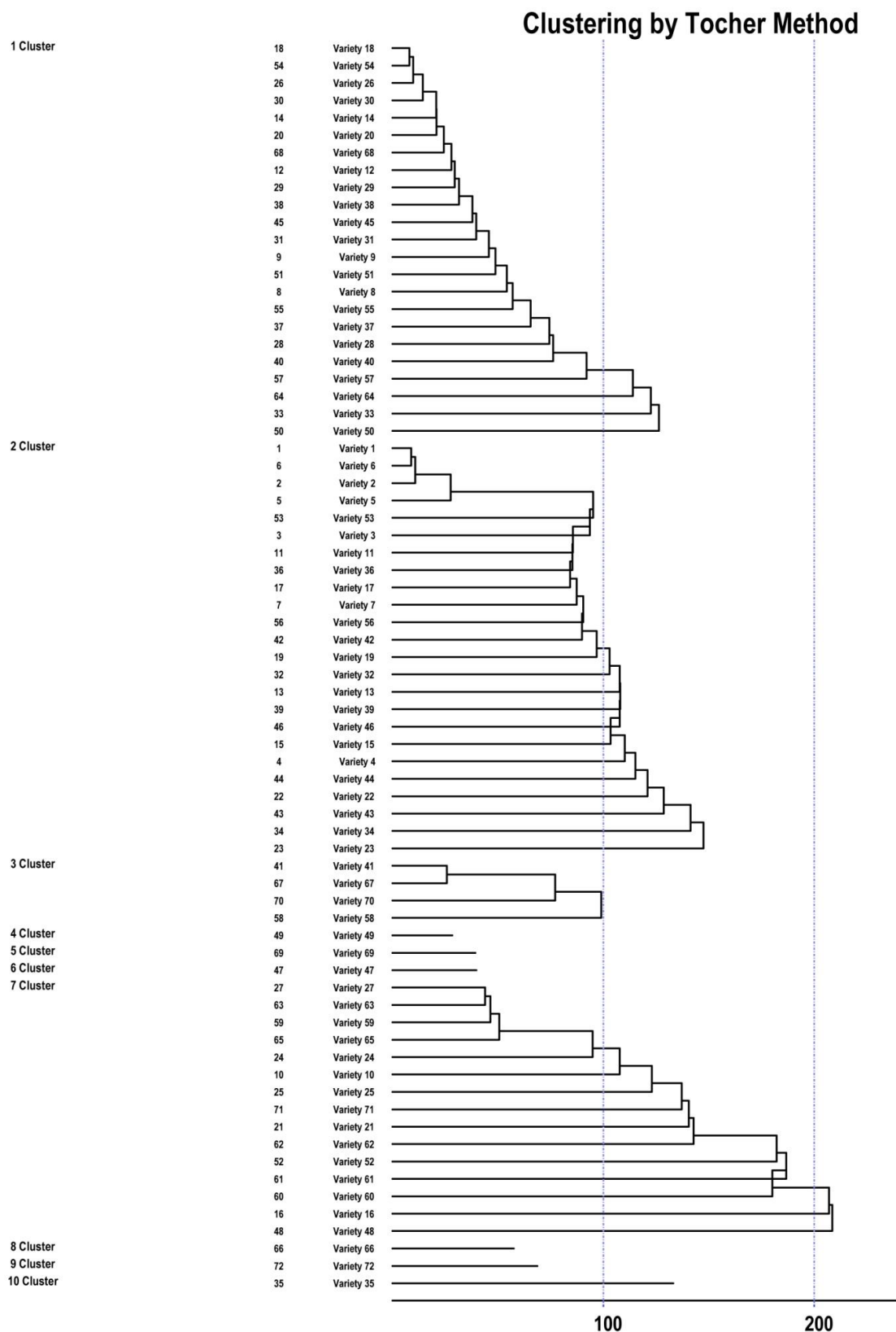


Fig 12: Dendrogram Representing the Clustering Pattern for All 72 Clones in Stress Environment.

Table 4.23: Clustering Patterns of 72 Genotypes on the Basis of D^2 values in Stress Environment

Cluster No.	Genotypes included	No. of Genotypes
I	PC (2015-16)-18, PC (2015-16)-54, PC (2015-16)-26, PC (2015-16)-30, PC (2015-16)-14, PC (2015-16)-20, Co 617, PC (2015-16)-12, PC (2015-16)-29, PC (2015-16)-38, PC (2015-16)-45, PC (2015-16)-31, PC (2015-16)-9, PC (2015-16)-51, PC (2015-16)-8, PC (2015-16)-55, PC (2015-16)-37, PC (2015-16)-28, PC (2015-16)-40, PC (2015-16)-57, PC (2015-16)-64, PC (2015-16)-33, PC (2015-16)-50	23
II	PC (2015-16)-1, PC (2015-16)-6, PC (2015-16)-2, PC (2015-16)-5, PC (2015-16)-53, PC (2015-16)-3, PC (2015-16)-11, PC (2015-16)-36, PC (2015-16)-17, PC (2015-16)-7, PC (2015-16)-56, PC (2015-16)-42, PC (2015-16)-19, PC (2015-16)-32, PC (2015-16)-13, PC (2015-16)-39, PC (2015-16)-46, PC (2015-16)-15, PC (2015-16)-4, PC (2015-16)-44, PC (2015-16)-22, PC (2015-16)-43, PC (2015-16)-34, PC (2015-16)-23	24
III	PC (2015-16)-41, Co 1148, ISH 100, PC (2015-16)-58	4
IV	PC (2015-16)-49	1
V	Co 85002	1
VI	PC (2015-16)-47	1
VII	PC (2015-16)-27, PC (2015-16)-63, PC (2015-16)-59, PC (2015-16)-65, PC (2015-16)-24, PC (2015-16)-10, PC (2015-16)-25, UP 9530, PC (2015-16)-21, PC (2015-16)-62, PC (2015-16)-52, PC (2015-16)-61, PC (2015-16)-60, PC (2015-16)-16, PC (2015-16)-48	15
VIII	BO 91	1
IX	Co Pant 97222	1
X	PC (2015-16)-35	1

All the remaining values apart from diagonal ones in the table indicated the inter cluster D^2 values. Inter cluster D^2 values ranged from 39.35 to 2627.96. Maximum inter cluster D^2 value was recorded between cluster III and X (3667.38) while between cluster IV and V (39.35) was the lowest.

The relative genetic distance (D), as calculated by taking square root of D^2 values, between different clusters are presented in Table 4.25.

Table 4.24: Average Inter and Intra-Cluster (diagonal) D^2 Values in Stress Environment

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	74.86	1162.63	292.65	143.54	280.28	177.38	1745.04	1788.93	1584.82	2751.53
II		117.64	1979.91	659.17	506.83	636.65	252.19	495.59	342.49	640.76
III			108.86	626.31	899.53	517.66	2627.96	2289.53	2147.30	3667.38
IV				0.00	39.35	93.27	1142.58	1371.35	1170.38	2045.04
V					0.00	146.13	950.32	1275.28	1049.59	1781.95
VI						0.00	1026.74	1018.94	875.83	1756.62
VII							178.76	337.50	276.14	362.18
VIII								0.00	86.50	343.80
IX									0.00	279.58
X										0.00

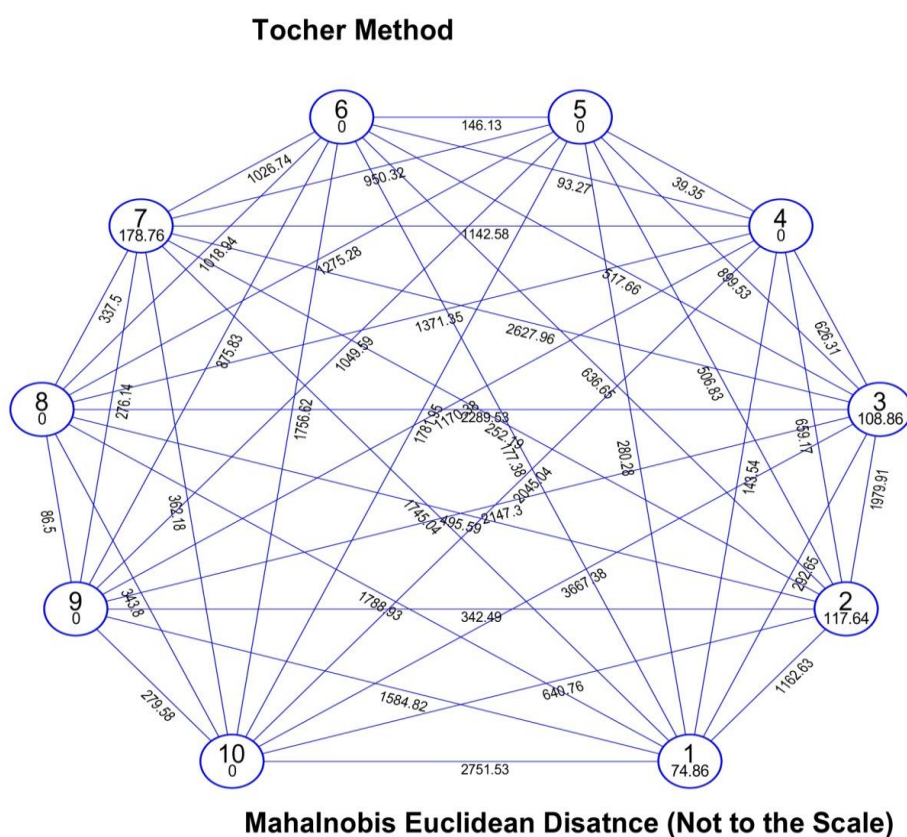


Fig 13: Cluster Diagram based on Average Intra and Inter-Cluster Distances for Sugarcane Clones under Stress Environment

The diagonal values in the table indicate the intra-cluster relative genetic distance (D). The maximum intra-cluster genetic distance was recorded in cluster VII (13.37) followed by cluster II (10.85) and minimum in cluster IV, V, VI, VIII, IX and X(0.00). All the remaining values apart from diagonal ones in the table indicated the inter cluster genetic distances. Maximum inter-cluster distance was recorded between cluster III and X (60.56) whereas, minimum between cluster IV and V (6.27).

Table 4.25: Average Inter and Intra-Cluster (diagonal) D Values in Stress Environment

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	8.65	34.10	17.11	11.98	16.74	13.32	41.77	42.30	39.81	52.46
II		10.85	44.50	25.67	22.51	25.23	15.88	22.26	18.51	25.31
III			10.43	25.03	29.99	22.75	51.26	47.85	46.34	60.56
IV				0.00	6.27	9.66	33.80	37.03	34.21	45.22
V					0.00	12.09	30.83	35.71	32.40	42.21
VI						0.00	32.04	31.92	29.59	41.91
VII							13.37	18.37	16.62	19.03
VIII								0.00	9.30	18.54
IX									0.00	16.72
X										0.00

4.5.2.3 Cluster means for different characters:

The cluster means for all the 16 characters were calculated and are presented in Table 4.26. Analysing the cluster means for different characters it was revealed that considerable differences existed between the clusters for all the 16 characters studied.

For trait days to germination, the cluster mean ranged from 20.00 to 30.00. The highest cluster mean was recorded for cluster IV and VIII (30.00) followed by, cluster III, V, IX (25.00) and the lowest cluster mean for cluster VI and X (20.00). The cluster mean for germination percent ranged from 29.95 to 43.25. The highest cluster mean was for the cluster I (43.25) followed by, cluster IV (42.05) and the minimum was for cluster II (29.95).

Similarly, cluster mean for SPAD 60 ranged from 32.77 to 50.24. The maximum cluster mean was for cluster I (50.24) followed by, cluster III (49.85) and minimum for cluster VIII (32.77). The cluster mean for SPAD 120 ranged from 30.75 to 46.25. The highest mean recorded was for cluster I (49.29) followed by, cluster III (47.73) and the minimum for cluster VIII (30.75). Similarly, the range for SPAD 180 was recorded to be 33.92 to 47.10. Maximum cluster mean was recorded by cluster I (47.10) followed by, cluster III (46.66) and the minimum cluster mean by cluster VII (33.92).

For CTD 60 the cluster means ranged from cluster VII (3.26) to cluster X (6.82). For CTD 120 the cluster means ranged from 1.34 to 7.46. The maximum mean was recorded for cluster I (7.46) followed by, cluster VI (6.80) and the minimum for cluster X (1.34). For CTD 180 the cluster mean ranged from 2.66 to 7.88. The maximum cluster mean was recorded by cluster IV (7.88) followed by cluster I (7.80) and the minimum by cluster X (2.66).

The cluster mean for Chl a ranged from 1.29 to 2.86, with maximum cluster mean for cluster III (2.86) followed by, cluster I (2.26) and the minimum for cluster X (1.29). For Chl b the range was 0.19 to 0.44, with maximum cluster mean for cluster IX (0.44) and the minimum for cluster VII (0.19). Total carotenoids recorded a range of 0.04 to 0.09, with the maximum mean for cluster III (0.09) and Minimum for cluster II, V, VII, X (0.04).

For RWC percent the cluster mean ranged from 55.01 to 81.78, with maximum cluster mean for cluster I (81.78) followed by, cluster III (80.78) and minimum by cluster X (55.01). For proline cluster mean ranged from 94.80 to 212.20 with maximum mean for cluster IV (212.20) followed by, cluster V (211.76) and the minimum for cluster IX (94.80). For leaf firing and DTC cluster mean ranged from 1.00 to 5.00, with maximum mean for cluster I, III, IV, V and VI (5.00) and the minimum for cluster VIII, IX and X (1.00).

Table 4.26: Cluster Means For Different Characters in Stress Environment

Clusters	Characters															
	Days to Germination	Germination %	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC %	Chl a	Chl b	Total Chl	Total Carotenoids	Proline	Leaf Firing	DTC
I	24.78	43.25	50.24	6.72	49.29	7.46	47.10	7.80	81.78	2.26	0.35	2.61	0.06	209.69	5.00	5.00
II	22.52	29.95	39.50	4.08	38.90	3.90	36.09	4.89	71.01	1.45	0.22	1.67	0.04	107.73	2.15	2.15
III	25.00	42.00	49.85	6.39	47.73	6.30	46.66	8.33	80.78	2.86	0.51	3.36	0.09	211.14	5.00	5.00
IV	30.00	42.05	46.29	5.80	46.73	5.64	43.95	7.88	80.67	1.80	0.26	2.06	0.05	212.20	5.00	5.00
V	25.00	40.25	45.71	6.29	40.67	4.77	40.54	6.55	80.76	1.46	0.25	1.70	0.04	211.76	5.00	5.00
VI	20.00	40.20	47.29	6.31	47.62	6.80	44.79	5.97	75.83	1.72	0.34	2.06	0.07	208.68	5.00	5.00
VII	24.83	34.63	37.39	3.26	35.89	3.65	33.92	4.84	63.96	1.46	0.19	1.66	0.04	136.92	2.60	2.60
VIII	30.00	35.65	32.77	3.62	30.75	3.64	26.58	6.60	58.43	1.75	0.34	2.08	0.06	97.78	1.00	1.00
IX	25.00	33.65	43.05	4.09	39.90	3.60	39.23	3.45	61.17	1.77	0.44	2.20	0.05	94.80	1.00	1.00
X	20.00	33.45	48.36	6.82	46.23	1.34	40.62	2.66	55.01	1.29	0.25	1.54	0.04	101.42	1.00	1.00

4.5.2.4 Contribution of Different Characters towards Divergence:

Percent contribution of different characters towards divergence was measured for all the 16 drought tolerance traits and presented in Table 4.27 and depicted as Fig 14.

The study on individual contribution of characters indicated that the maximum contribution towards divergence was conferred by germination percent (10.36) followed by Chl a (8.76), RWC percent (7.62), leaf firing (7.55), total carotenoids (7.35), SPAD 60 (7.23), Chl b (6.69), DTC (6.37), proline content (6.06), CTD 180 (5.98), CTD 60 and CTD 120 (5.71), days to germination (5.35), SPAD 120 (3.95), SPAD 180 (3.20) and total chlorophyll (2.03).

Analysing the results for diversity estimates in both environments, few of the following conclusions can be drawn. In both the environments there were two or three major clusters consisting of approx. 40 and 30 percent genotypes exhibiting a normal distribution of clones. The presence of tolerant and sensitive parents in different clusters along with other clones indicates corresponding response of those clones to water stress. In control environment all the three sensitive parents were clustered in the same cluster revealing their similar nature unlike in stress where all the three parents got distributed into different clusters indicating their differential levels of response to water stress. Normal distribution of clones indicates high degree of divergence among the material tested with respect to traits under study. Maximum inter-cluster distances were observed between cluster III and X under stress and VI and V under control environment which indicates higher genetic diversity between these two groups. Under both the environmental conditions highest inter-cluster distances were found to be between a drought tolerant cluster and a drought sensitive cluster. Thus, hybridization of these two groups could result in transgressive recombinants for drought tolerance as well as for agronomically important traits and exploitation of clones from these two clusters as parents for crossing could produce good sugarcane segregants. Thus, hybridization between genotypes from clusters having maximum inter-cluster distance should result in maximum hybrid vigour and highest number of useful segregants (Shwe *et al.*, 1972; Santos *et al.*, 2012; Brasileiro *et al.*, 2014 and Bisht *et al.*, 2017). The minimum inter-cluster distance was observed between cluster VI and VII under control and IV and V under stress. The lower inter-cluster distance shown that clones

Table 4.27: Contribution of Different Drought Tolerance Characters Towards Genetic Divergence in Sugarcane under Stress Environment

S. No.	Character	Percent Contribution
1	Days to germination	5.35
2	Germination%	10.36
3	SPAD 60	7.23
4	CTD 60	5.71
5	SPAD 120	3.95
6	CTD 120	5.71
7	SPAD 180	3.20
8	CTD 180	5.98
9	RWC%	7.62
10	Chl a	8.76
11	Chl b	6.69
12	Total Chl	2.03
13	Total Carotenoids	7.35
14	Proline	6.06
15	Leaf firing	7.55
16	DTC	6.37

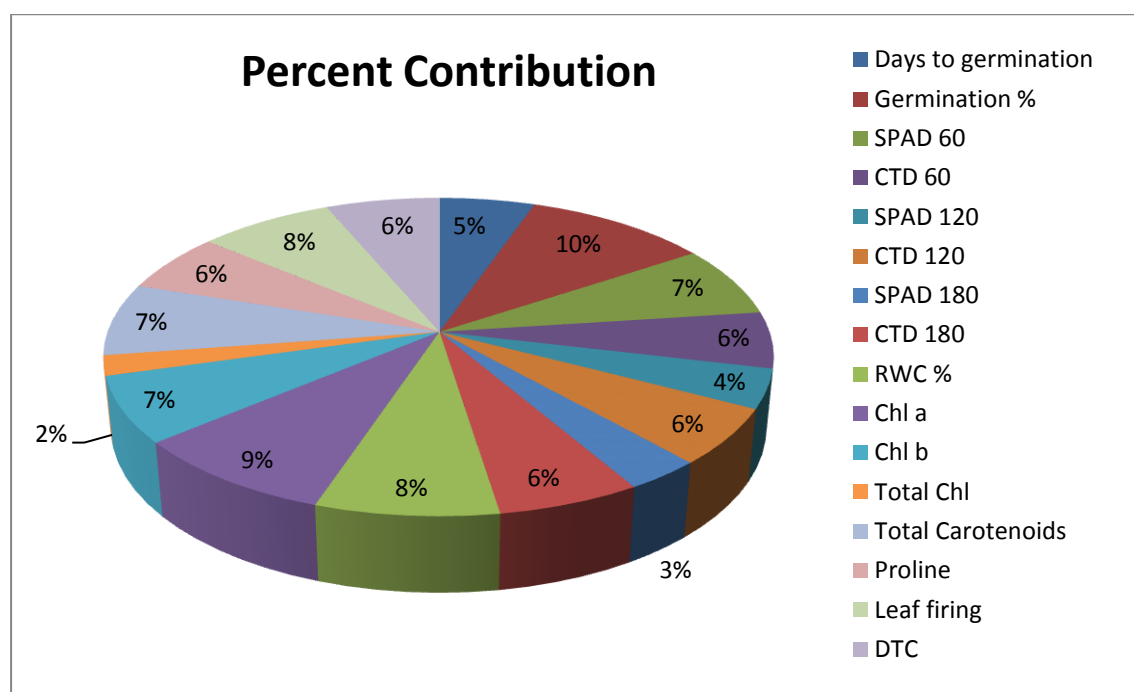


Fig 14: Percent Contribution of Different Drought Tolerance Characters Towards Genetic Divergence in Sugarcane Under Stress Environment

falling in these clusters were strongly interconnected and possibly will not to be used as parents in crossing because resulting hybrids would not be generating desirable segregants for the characters studied.

A critical analysis of cluster means for different character indicated that under stress cluster I had desirable clones for germination percent, SPAD 60, SPAD 120, CTD 120, SPAD 180 and RWC%. Cluster III for CTD 60, Chl a and total carotenoids. Cluster IV for CTD 180 and proline. Cluster IX for Chl b. Cluster VI and X had clones with lowest days to germination which is desirable. Cluster I, III, IV, V and VI had desirable clones for leaf firing and DTC. However, it was suggested that clones with high index for specific character falling into different clusters could be inter-crossed to generate good number of sugarcane progenies having greater potentiality for breeding purpose by virtue of their desirable characters (**Sanghera *et al.* 2015**). The cluster having high mean values for proline, Chl a, leaf firing, DTC, SPAD and CTD could be selected for drought tolerance.

Clusters under control environment were found to consist of desirable clones for almost similar combinations of traits as that of stress environment. Clusters for Proline content, SPAD 180, CTD 180 were found to behaving in a distinct way.

Maximum percent contribution under both the environment was recorded by germination percent depicting the similar pattern for all the traits. Few of the variations observed were unlike in control total carotenoid also contributed towards divergence under stress environment. Under stress environment the percent contribution from SPAD 60 recorded to be higher than in control. In contrast the percent contribution from proline content and DTC got reduced slightly. Under stress all the contributions from various traits was somewhat normally distributed unlike in control environment.

The clustering and genetic distance in addition gives a thought for developing the diverse genetic pool for flourishing breeding programme. Higher the D^2 value, more diverse the genotypes are and these recognized genotypes can be used as parents for widespread hybridisation programme.

4.6 Selection indices for various drought tolerance traits

Selection indices are considered as an aid to the breeder for simultaneous selection of multiple traits. This tool can help the breeder in spotting the desirable

genotype/family of a crop species in a population improvement program. **Smith (1936)** and **Hazel (1943)** illustrated the procedure for constructing a selection index that gives maximum advance from selection. Selection indices provide useful information about which traits to be combined. Selection indices and selection scores were estimated for all 72 genotypes under both environments (control and stress). The results of the selection indices and selection scores estimations for both environments are presented in following sections.

4.6.1 Selection indices for various drought tolerance traits in Sugarcane under control environments

4.6.1.1 Selection indices parameters

Selection indices parameters under control environment are presented in Table 4.28.

Selection indices parameters for all the 16 traits included economic weight, index weights (bi), phenotypical variances, genetic advancement and relative efficiency. Economic weight for all the traits was taken as 1.00 giving equal weights. Index weights (bi) ranged from -8.661 for total chlorophyll to 8.183 for Chl a and Chl b. Total carotenoids recorded index weight of 7.661 followed by leaf firing (2.753), SPAD 60 (-1.990), SPAD 120 (1.798), proline (1.746), CTD 120 (1.645), DTC (-1.567), germination percent (1.516), RWC percent (1.407) and CTD 180 (1.22).

Phenotypical variances ranged from Chl b (-0.930) to germination percent (93.24). SPAD 180 recorded phenotypical variance of 87.828 followed by SPAD 120 (82.534), SPAD 60 (71.625), RWC percent (42.900), proline (42.124), DTC (29.549), leaf firing (29.198) and CTD 180 (27.288).

Genetic advancement ranged from -0.039 to 3.943. The maximum genetic advancement was recorded for germination percent (3.943) followed by SPAD 180 (3.714), SPAD 120 (3.490) and SPAD 60 (3.029)

Relative efficiency of the selection index for improvement of characters under selection ranged from -21.78 to 89.24 percent. The maximum relative efficiency was recorded for SPAD 60 (89.24) followed by SPAD 120 (67.414) and SPAD 180 (62.242).

Table 4.28: Selection Indices Parameters for Various Drought Tolerance Traits in Sugarcane under Control environment

Variables	Economic Weight	bi	Phenotypical Variance	Genetic Advancement	Relative Efficiency
Days to Germination	1.000	0.227	2.374	0.100	2.024
Germination%	1.000	1.516	93.234	3.943	37.297
SPAD 60	1.000	-1.990	71.625	3.029	89.246
CTD 60	1.000	-0.015	19.649	0.831	27.886
SPAD 120	1.000	1.798	82.534	3.490	67.414
CTD 120	1.000	1.645	19.389	0.820	35.028
SPAD 180	1.000	0.798	87.828	3.714	62.242
CTD 180	1.000	1.222	27.288	1.154	33.772
RWC%	1.000	1.407	42.900	1.814	33.888
Chl a	1.000	8.183	6.548	0.277	34.282
Chl b	1.000	8.183	-0.930	-0.039	-21.788
Total Chl	1.000	-8.661	5.618	0.238	29.565
Total Carotenoids	1.000	7.661	0.230	0.010	33.333
Proline	1.000	1.746	42.124	1.781	23.296
Leaf Firing	1.000	2.753	29.198	1.235	37.538
D T C	1.000	-1.567	29.549	1.250	37.425
Selection Intensity 10%: 41.62 5%: 48.71					

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

4.6.1.2 Selection Scores:

The selection scores based on Selection Indices for 72 genotypes under control environment are presented in Table 4.29.

The selection scores ranged from 346.124 to 428.402. The maximum score was recorded by ISH100 (428.402) followed by PC (2015-16)-26 (423.61), PC (2015-16)-8 (415.76), PC (2015-16)-40 (415.12), PC (2015-16)-64 (415.07), PC (2015-16)-9 (414.10), PC (2015-16)-31 (413.57), PC (2015-16)-37 (410.01), Co 1148 (409.83) and PC (2015-16)-55 (409.56). These are the top 10 genotypes having the highest selection scores with respect to drought tolerance characters. Genotypes with least selection

scores with respect to drought tolerance characters are PC (2015-16)-16 (346.12), PC (2015-16)-21 (346.89), PC (2015-16)-23 (347.30), PC (2015-16)-43 (350.84), PC (2015-16)-60 (351.83), PC (2015-16)-10 (352.17), PC (2015-16)-59 (356.17), PC (2015-16)-11 (356.60), PC (2015-16)-48 (357.70), PC (2015-16)-42 (357.89).

Table 4.29: Selection Scores based on Selection Indices for 72 Genotypes of Sugarcane under Control Environment

S. No.	Genotype	Selection Score	S. No.	Genotype	Selection Score	S. No.	Genotype	Selection Score
1	ISH 100	428.40	25	PC (2015-16)-28	395.07	49	BO 91	372.92
2	PC (2015-16)-26	423.61	26	PC (2015-16)-36	394.54	50	PC (2015-16)-4	371.86
3	PC (2015-16)-8	415.76	27	PC (2015-16)-7	394.48	51	PC (2015-16)-19	370.31
4	PC (2015-16)-40	415.12	28	PC (2015-16)-38	392.85	52	PC (2015-16)-39	368.99
5	PC (2015-16)-64	415.07	29	PC (2015-16)-27	392.31	53	PC (2015-16)-13	368.93
6	PC (2015-16)-9	414.10	30	PC (2015-16)-1	392.18	54	PC (2015-16)-62	368.58
7	PC (2015-16)-31	413.57	31	PC (2015-16)-53	388.50	55	PC (2015-16)-49	368.35
8	PC (2015-16)-37	410.01	32	PC (2015-16)-57	388.15	56	PC (2015-16)-35	367.72
9	Co 1148	409.83	33	PC (2015-16)-51	387.40	57	PC (2015-16)-24	366.16
10	PC (2015-16)-55	409.56	34	PC (2015-16)-32	386.80	58	PC (2015-16)-3	366.16
11	PC (2015-16)-58	407.79	35	Co Pant 97222	385.66	59	PC (2015-16)-47	366.12
12	PC (2015-16)-29	406.45	36	PC (2015-16)-15	384.50	60	PC (2015-16)-6	365.39
13	PC (2015-16)-54	406.02	37	PC (2015-16)-63	380.50	61	PC (2015-16)-61	361.59
14	PC (2015-16)-18	405.91	38	PC (2015-16)-25	380.05	62	PC (2015-16)-46	361.31
15	Co 617	405.79	39	PC (2015-16)-65	379.39	63	PC (2015-16)-42	357.89
16	PC (2015-16)-12	405.15	40	PC (2015-16)-50	378.19	64	PC (2015-16)-48	357.70
17	PC (2015-16)-33	404.76	41	PC (2015-16)-56	378.07	65	PC (2015-16)-11	356.60
18	PC (2015-16)-20	403.85	42	PC (2015-16)-5	377.75	66	PC (2015-16)-59	356.17
19	PC (2015-16)-30	401.35	43	Co 85002	377.59	67	PC (2015-16)-10	352.17
20	PC (2015-16)-45	399.59	44	PC (2015-16)-2	377.38	68	PC (2015-16)-60	351.83
21	PC (2015-16)-14	395.96	45	PC (2015-16)-22	377.21	69	PC (2015-16)-43	350.84
22	PC (2015-16)-44	395.64	46	PC (2015-16)-52	376.68	70	PC (2015-16)-23	347.30
23	PC (2015-16)-34	395.56	47	UP 9530	376.38	71	PC (2015-16)-21	346.89
24	PC (2015-16)-41	395.39	48	PC (2015-16)-17	372.95	72	PC (2015-16)-16	346.12

4.6.2 Selection indices for various drought tolerance traits in Stress Environments

4.6.2.1 Selection indices parameters:

Selection indices parameters under stress environment are presented in Table 4.30.

Selection indices parameters for all the 16 traits included economic weight, index weights (bi), phenotypical variances, genetic advancement and relative efficiency. Economic weight for all the traits was taken as 1.00 giving equal weights.

Index weights (bi) ranged from -910.423 for total chlorophyll to 918.922 for Chl a. Leaf firing recorded index weight of 120.529 followed by DTC (-114.745), Total carotenoids (43.595), CTD 120 (3.733), CTD 60 (1.772), germination percent (1.751), CTD 180 (1.155), SPAD 60 1.054), RWC percent (0.990), days to germination (0.785) SPAD 180 (0.723) and proline content (0.675).

Table 4.30: Selection Indices Parameters for Various Drought Tolerance Traits in Stress Environment

Variables	Economic Weight	bi	Variance Phenotypical	Genetic Advancement	Relative Efficiency
Days to Germination	1.000	0.785	91.454	1.050	21.521
Germination%	1.000	1.751	507.709	5.830	43.198
SPAD 60	1.000	1.054	476.562	5.472	40.961
CTD 60	1.000	1.772	119.047	1.367	41.287
SPAD 120	1.000	0.675	469.717	5.394	41.194
CTD 120	1.000	3.733	152.274	1.749	43.736
SPAD 180	1.000	0.723	503.252	5.779	44.192
CTD 180	1.000	1.155	132.320	1.519	38.602
RWC%	1.000	0.990	559.315	6.422	37.960
Chl a	1.000	918.922	34.463	0.396	40.203
Chl b	1.000	894.477	5.635	0.065	32.663
Total Chl	1.000	-910.423	40.087	0.460	39.758
Total Carotenoids	1.000	43.595	1.119	0.013	38.235
Proline	1.000	0.675	4251.379	48.816	48.292
Leaf Firing	1.000	120.529	120.093	1.379	43.310
D T C	1.000	-114.745	120.093	1.379	43.310
Selection Intensity 10%: 153.28 5%: 179.40					

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

Phenotypical variances ranged from 1.119 to 4251.379. The maximum phenotypic variance was recorded by proline content (4251.379) followed by RWC percent (559.315), germination percent (507.709), SPAD 180 (503.252), SPAD 60 (476.562), SPAD 120 (469.717), CTD 120 (152.274), CTD 180 (132.320), leaf firing and DTC (120.093), CTD 60 (119.047), days to germination (91.454), total chlorophyll (40.087), Chl a (34.463), Chl b (5.635) and least phenotypic variance was recorded by total carotenoids (1.119).

Genetic advancement ranged from 0.013 to 48.816. The maximum genetic advancement was recorded for proline (48.816) followed by RWC percent (6.422), germination percent (5.830), SPAD 180 (5.779), SPAD 60 (5.472) and SPAD 120 (5.394).

Relative efficiency of the selection index for improvement in drought tolerance characters ranged from 22.521 to 48.292. The maximum relative efficiency was recorded for proline 48.292 followed by SPAD 180 (44.192), CTD 120 (43.736).

4.6.2.2 Selection Scores:

The selection scores based on Selection Indices for 72 Genotypes under stress environment are presented in Table 4.31.

The selection scores ranged from 324.566 to 553.040. The maximum score was recorded by PC (2015-16)-55 (553.04) followed by PC (2015-16)-26 (547.42), PC (2015-16)-40 (544.67), PC (2015-16)-54 (544.40), PC (2015-16)-18 (544.12), PC (2015-16)-50 (543.56), PC (2015-16)-51 (541.38), PC (2015-16)-20 (541.33), PC (2015-16)-28 (541.29) and PC (2015-16)-29 (540.95). These are the top 10 genotypes having the highest selection scores with respect to drought tolerance. The tolerant parent ISH 100 (538.78) recorded 13th rank based on selection scores. Genotypes with least selection scores are PC (2015-16)-24 (324.56), PC (2015-16)-3 (326.30), PC (2015-16)-10 (328.19), PC (2015-16)-13 (328.95), PC (2015-16)-66 (329.86), PC (2015-16)-16 (330.62), PC (2015-16)-32 (331.09), PC (2015-16)-21 (332.51), PC (2015-16)-39 (333.49) and PC (2015-16)-23 (336.49).

Under both the environments selection scores are estimated and all the 72 clones are ranked from 1 to 72 based on selection scores. Looking at the selection indices under both the environments higher levels of weights were given to traits like Chl a, Chl b and total chlorophyll indicating their larger contribution to the selection index and the score.

Under stress the index weights for chl a, chl b, total chl, total carotenoids, leaf firing and DTC were at higher side in comparison to the control environments revealing their enhanced levels of influence on the index as well as the phenotype under water stress. Similarly CTD (3.733) and SPAD 60 (1.054) recorded higher index weights in stress than in control environments.

Table 4.31: Selection Scores Based on Selection Indices for 72 Sugarcane Genotypes under Stress Environment

S. No.	Genotype	Selection Score	S. No.	Genotype	Selection Score	S. No.	Genotype	Selection Score
1	PC (2015-16)-55	553.04	25	PC (2015-16)-8	519.37	49	PC (2015-16)-5	356.51
2	PC (2015-16)-26	547.42	26	PC (2015-16)-12	518.64	50	PC (2015-16)-53	356.34
3	PC (2015-16)-40	544.67	27	PC (2015-16)-49	515.05	51	PC (2015-16)-7	353.21
4	PC (2015-16)-54	544.40	28	PC (2015-16)-47	500.84	52	PC (2015-16)-43	352.62
5	PC (2015-16)-18	544.12	29	Co 85002	498.28	53	PC (2015-16)-42	350.69
6	PC (2015-16)-50	543.56	30	PC (2015-16)-33	460.46	54	PC (2015-16)-46	348.10
7	PC (2015-16)-51	541.38	31	PC (2015-16)-52	450.65	55	PC (2015-16)-15	347.88
8	PC (2015-16)-20	541.33	32	PC (2015-16)-65	430.53	56	PC (2015-16)-22	347.51
9	PC (2015-16)-28	541.29	33	PC (2015-16)-56	407.31	57	Co Pant 97222	344.56
10	PC (2015-16)-29	540.95	34	PC (2015-16)-62	406.76	58	UP 9530	344.28
11	PC (2015-16)-30	540.69	35	PC (2015-16)-48	405.56	59	PC (2015-16)-35	343.21
12	PC (2015-16)-64	539.50	36	PC (2015-16)-59	404.19	60	PC (2015-16)-25	341.14
13	ISH 100	538.78	37	PC (2015-16)-63	400.51	61	PC (2015-16)-11	338.97
14	PC (2015-16)-31	538.10	38	PC (2015-16)-34	399.03	62	PC (2015-16)-4	338.02
15	PC (2015-16)-37	532.77	39	PC (2015-16)-19	388.21	63	PC (2015-16)-23	336.34
16	PC (2015-16)-38	529.39	40	PC (2015-16)-61	384.96	64	PC (2015-16)-39	333.49
17	PC (2015-16)-9	526.49	41	PC (2015-16)-6	381.39	65	PC (2015-16)-21	332.51
18	PC (2015-16)-14	525.57	42	PC (2015-16)-1	379.36	66	PC (2015-16)-32	331.09
19	Co 1148	523.94	43	PC (2015-16)-17	374.50	67	PC (2015-16)-16	330.62
20	PC (2015-16)-57	523.52	44	PC (2015-16)-60	368.95	68	BO 91	329.85
21	PC (2015-16)-45	522.93	45	PC (2015-16)-2	367.66	69	PC (2015-16)-13	328.95
22	PC (2015-16)-41	522.25	46	PC (2015-16)-27	367.10	70	PC (2015-16)-10	328.19
23	Co 617	521.81	47	PC (2015-16)-44	366.10	71	PC (2015-16)-3	326.30
24	PC (2015-16)-58	520.50	48	PC (2015-16)-36	365.65	72	PC (2015-16)-24	324.56

Genetic advancement under stress environment recorded to be very high for proline, SPAD 60, SPAD 120, SPAD 180, germination percent and RWC percent under stress unlike in control environment. Relative efficiency of selection indices recorded a wide range (-0.22 to 0.89) under control unlike a range of (0.22 to 0.44) under stress environment. Under control environment Chl b recorded negative values for genetic advancement (-0.039) and relative efficiency of selection index (-21.788). These traits can be restricted and restricted selection index can be performed. Similar reports for relative efficiency were reported by **Silva *et al.* (2017)**.

Selection scores based on selection indices ranked all the 72 clones from 1 to 72. Under control environment with highest score ISH 100 was ranked 1st, Co 1148

was 9th and Co 617 was 15th supporting their drought tolerance nature and their utilisation as tolerant parent for generation of the study material.

Similarly under stress environment all these tolerant parents were found to have higher scores and ranked among top 25 clones i.e. ISH 100 was ranked 13th, Co 1148 was 19th and Co 617 was 23rd rank. Based on selection scores under stress, 12 clones were found to be superior than the best parent ISH 100. This clearly indicates that due to recombination's between the parents such transgressive segregants with higher selection scores were observed.

4.7 Evaluation of 72 bi-parental progenies for their response to drought stress

All the 72 genotypes were observed for 16 different drought tolerance physiological and biochemical traits under both the environments (Control and stress). Percent Change in Mean performance of 72 Genotypes for various Drought Tolerance Traits between Control and Stress Environment were estimated. The results for percent change in the mean performance are presented in Table 4.32.

Mean percent change for days to germination was recorded to be 12.64 percent which ranged from -30.43 for PC (2015-16)-16 to 55.56 for PC (2015-16)-35. For germination percent mean percent change was recorded to be 7.64 percent which ranged from -9.74 for PC (2015-16)-12 to 24.49 for PC (2015-16)-32.

Mean percent change for SPAD 60 was recorded to be -5.37 percent which ranged from -56.55 for PC (2015-16)-38 to 24.86 for PC (2015-16)-62. For SPAD 120 mean percent change was recorded to be -2.11 percent which ranged from -48.54 for PC (2015-16)-38 to 26.30 for PC (2015-16)-62. Similarly the recorded mean percent change for SPAD 180 was recorded to be -2.61 percent which ranged from -62.91 for PC (2015-16)-38 to 30.73 for PC (2015-16)-62.

Mean percent change for CTD 60 was recorded to be -38.23 percent which ranged from -947.10.0 for PC (2015-16)-34 to 60.13 for PC (2015-16)-39. For CTD mean percent change 120 was recorded to be -0.64 percent which ranged from -107.95 for PC (2015-16)-31 to 76.39 for PC (2015-16)-32. Similarly the recorded mean percent change for CTD 180 was recorded to be -60.84 percent which ranged from -756.67 for PC (2015-16)-13 to 41.45 for PC (2015-16)-9.

Table 4.32: Percent Change in Mean Performance of 72 Genotypes for various Drought Tolerance Traits between Control and Stress Environment

S. No.	Genotype	D G	G%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC%	Chl a	Chl b	Total Chl	T C	Proline	DTC
1	PC (2015-16)-1	16.67	3.50	7.13	4.28	13.06	29.27	13.04	8.36	13.39	17.65	61.54	28.97	25.00	-144.21	66.67
2	PC (2015-16)-2	21.74	9.22	2.53	23.54	4.65	31.47	8.62	10.85	14.48	18.64	60.38	28.26	0.00	-139.13	66.67
3	PC (2015-16)-3	21.74	14.98	-6.29	33.25	-1.95	59.97	4.58	25.00	16.41	19.39	59.65	29.73	0.00	-126.95	66.67
4	PC (2015-16)-4	16.67	19.22	9.29	-4.59	4.95	22.75	4.88	27.63	17.49	24.52	64.00	34.15	25.00	-97.44	0.00
5	PC (2015-16)-5	21.05	12.98	-1.20	8.93	1.76	31.91	6.50	5.72	11.98	24.10	56.00	31.48	25.00	-95.26	0.00
6	PC (2015-16)-6	0.00	5.05	-14.73	1.16	-8.45	13.60	-5.21	-5.56	13.47	21.39	54.17	28.51	25.00	-123.06	0.00
7	PC (2015-16)-7	0.00	11.98	-4.37	3.00	-5.10	11.45	-0.83	-8.64	21.97	31.89	64.29	41.18	25.00	-108.84	0.00
8	PC (2015-16)-8	24.24	2.56	-17.66	-9.10	-14.05	-20.6	24.44	19.69	8.68	7.42	17.39	8.97	14.29	-290.79	0.00
9	PC (2015-16)-9	24.24	1.52	-23.58	-11.30	-16.51	16.84	-17.6	41.45	8.11	6.31	22.92	8.92	0.00	-300.38	0.00
10	PC (2015-16)-10	25.00	13.70	3.10	-59.41	10.10	55.00	10.95	-182.0	24.40	17.58	54.90	26.85	25.00	-118.68	0.00
11	PC (2015-16)-11	50.00	8.79	-7.35	-188.0	-7.41	52.67	1.40	-145.79	18.00	16.88	59.32	28.64	0.00	-140.48	0.00
12	PC (2015-16)-12	0.00	-9.74	-16.62	-17.88	-13.14	-8.23	-10.36	-3.96	10.83	7.72	13.95	9.00	0.00	-272.21	0.00
13	PC (2015-16)-13	25.00	12.73	8.82	25.71	5.17	-53.75	-4.93	-756.6	20.61	26.34	53.70	32.92	20.00	-109.17	66.67
14	PC (2015-16)-14	0.00	-8.00	4.88	-3.00	2.07	-21.73	-9.46	-10.00	7.67	4.95	21.74	8.21	0.00	-275.23	0.00
15	PC (2015-16)-15	24.24	9.35	-1.54	34.28	2.68	50.16	4.84	27.05	20.24	18.78	58.73	28.46	20.00	-118.39	66.67
16	PC (2015-16)-16	-30.43	4.80	3.20	22.58	6.76	20.43	10.39	-27.69	20.55	15.82	58.97	23.40	0.00	-130.38	0.00
17	PC (2015-16)-17	50.00	8.05	-4.16	-219.5	-2.01	-60.47	0.76	-72.13	18.42	25.26	65.31	33.74	0.00	-105.73	0.00
18	PC (2015-16)-18	20.00	-7.64	-17.63	-12.40	-9.84	-29.08	-13.40	-136.29	8.81	2.29	23.91	6.06	14.29	-308.81	0.00
19	PC (2015-16)-19	0.00	5.75	-23.80	-115.6	-15.80	-33.45	-5.62	-119.87	17.11	25.29	55.56	31.63	16.67	-126.06	66.67
20	PC (2015-16)-20	0.00	-1.67	9.02	-5.29	8.23	-62.38	4.28	-31.86	6.25	4.25	19.51	7.11	0.00	-279.98	0.00
21	PC (2015-16)-21	50.00	8.66	2.25	-6.18	7.65	64.72	3.45	-82.07	21.86	13.53	55.81	22.07	0.00	-98.77	66.67
22	PC (2015-16)-22	0.00	15.83	-8.07	-16.71	-2.52	28.87	0.10	1.97	21.08	24.12	60.94	34.19	25.00	-100.63	66.67
23	PC (2015-16)-23	24.24	24.04	-19.62	0.62	-16.46	27.32	-13.32	-16.18	23.48	26.20	60.00	34.02	0.00	-141.60	0.00
24	PC (2015-16)-24	21.74	14.64	-10.25	27.99	-2.18	1.09	-4.60	-49.39	27.26	25.88	55.93	33.33	20.00	-92.95	66.67
25	PC (2015-16)-25	8.00	15.62	5.46	-20.74	1.09	-6.93	2.13	22.33	24.61	22.35	55.36	30.67	16.67	-86.29	66.67
26	PC (2015-16)-26	-7.14	1.29	-24.57	-37.98	-11.38	-23.77	-10.23	-8.67	9.15	8.24	28.00	11.48	0.00	-292.35	0.00
27	PC (2015-16)-27	24.24	10.57	-14.27	28.31	-6.53	28.02	-4.54	14.11	27.62	27.62	58.62	35.56	25.00	-129.70	0.00

Table 4.32: Contd...

S. No.	Genotype	D G	G%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC%	Chl a	Chl b	Total Chl	T C	Proline	DTC
28	PC (2015-16)-28	20.00	2.81	-7.94	-24.14	-12.89	-21.12	-10.28	-129.13	6.06	5.63	15.22	7.22	0.00	-368.77	0.00
29	PC (2015-16)-29	0.00	2.71	-15.36	-24.78	-13.51	-33.44	-16.44	-13.80	10.64	5.18	16.67	6.71	14.29	-372.72	0.00
30	PC (2015-16)-30	0.00	1.49	-14.98	-17.19	-6.20	-26.23	-2.96	-30.52	9.81	9.61	7.69	9.70	0.00	-356.89	0.00
31	PC (2015-16)-31	16.67	6.53	-19.31	2.09	-13.10	-107.95	-14.16	-85.77	9.31	5.00	21.74	7.69	0.00	-273.46	40.00
32	PC (2015-16)-32	33.33	24.49	13.95	-122.7	4.81	76.39	2.89	15.14	16.98	26.06	54.84	33.20	25.00	-129.45	0.00
33	PC (2015-16)-33	0.00	1.79	-15.12	-31.97	2.03	-11.17	-6.57	-51.28	4.45	6.75	24.44	9.43	14.29	-118.60	0.00
34	PC (2015-16)-34	33.33	14.32	6.13	-947.1	8.88	-30.22	8.75	-43.46	22.27	24.23	50.94	29.96	0.00	-102.46	0.00
35	PC (2015-16)-35	55.56	9.96	-28.28	-16.05	-24.27	-91.43	-34.50	-565.00	35.27	29.51	56.90	36.10	0.00	-98.75	0.00
36	PC (2015-16)-36	0.00	9.25	11.38	-7.41	14.15	55.84	15.76	28.80	15.22	20.43	54.24	28.69	25.00	-102.47	0.00
37	PC (2015-16)-37	0.00	7.88	-15.04	-18.15	-9.16	23.01	-14.62	-45.98	6.66	8.86	22.22	11.08	0.00	-353.33	0.00
38	PC (2015-16)-38	10.00	1.77	-56.55	-18.23	-48.54	-35.70	-62.91	-63.77	9.18	-1.65	8.89	0.00	0.00	-307.22	0.00
39	PC (2015-16)-39	-20.00	18.88	4.64	60.13	-2.54	31.81	3.78	5.06	16.67	21.70	65.22	29.46	0.00	-94.37	0.00
40	PC (2015-16)-40	20.00	3.34	-19.79	15.00	-12.16	13.33	-13.57	-11.73	9.96	2.64	6.52	2.90	0.00	-371.45	0.00
41	PC (2015-16)-41	0.00	-1.60	-17.80	-5.16	-11.05	-2.80	-18.08	-159.36	10.91	5.07	15.09	6.32	0.00	-385.58	0.00
42	PC (2015-16)-42	0.00	12.66	6.77	-2.80	-4.98	10.56	-5.42	-14.65	18.73	19.41	65.45	30.67	0.00	-153.14	66.67
43	PC (2015-16)-43	23.33	21.97	6.40	38.69	2.48	27.81	-1.77	2.43	20.04	19.73	62.22	26.97	25.00	-190.06	0.00
44	PC (2015-16)-44	39.39	18.14	-1.81	-208.50	9.06	-70.87	9.29	-19.88	23.46	23.18	61.29	31.53	20.00	-130.31	0.00
45	PC (2015-16)-45	0.00	10.29	5.86	-23.82	7.13	-12.14	4.83	-263.53	8.53	7.77	25.53	10.30	0.00	-362.94	0.00
46	PC (2015-16)-46	0.00	17.11	7.14	15.40	6.16	35.67	12.06	-9.51	23.31	17.53	50.00	23.53	20.00	-114.68	0.00
47	PC (2015-16)-47	20.00	1.35	-34.35	-19.06	-33.84	-15.06	-42.01	-70.09	10.83	8.51	32.00	13.08	0.00	-392.75	0.00
48	PC (2015-16)-48	9.09	-3.47	9.76	6.99	9.47	0.66	4.68	12.96	15.72	26.46	48.89	30.77	0.00	-298.25	66.67
49	PC (2015-16)-49	0.00	1.98	-12.35	9.09	-14.45	-1.81	-14.66	-119.50	10.15	7.22	21.21	9.25	0.00	-378.04	0.00
50	PC (2015-16)-50	0.00	-6.93	-31.21	-19.14	-11.86	-2.98	-17.31	-43.74	10.52	16.30	25.00	17.07	0.00	-358.88	0.00
51	PC (2015-16)-51	-20.00	-4.81	-5.88	-18.34	-4.36	-23.64	-11.01	-3.72	11.26	-2.53	6.67	-1.06	14.29	-393.04	0.00
52	PC (2015-16)-52	0.00	16.52	-7.95	20.08	-0.86	10.42	-12.08	-19.95	24.60	3.95	65.91	16.29	0.00	-284.63	80.00

Table 4.32: Contd...

S. No.	Genotype	D G	G%	SPAD 60	CTD 60	SPAD 120	CTD 120	SPAD 180	CTD 180	RWC%	Chl a	Chl b	Total Chl	T C	Proline	DTC
53	PC (2015-16)-53	33.33	16.32	-2.33	10.71	2.51	1.89	3.64	-46.34	16.58	23.21	60.94	31.36	25.00	-156.80	66.67
54	PC (2015-16)-54	33.33	3.68	3.87	-232.38	2.90	-24.81	0.33	-212.96	8.80	4.53	16.67	6.67	0.00	-340.96	0.00
55	PC (2015-16)-55	33.33	-0.93	7.64	1.53	6.86	-6.20	4.48	8.97	7.28	6.72	24.44	9.73	0.00	-376.49	0.00
56	PC (2015-16)-56	0.00	6.56	8.29	-1.58	10.11	9.21	5.25	-27.80	17.69	4.82	59.38	20.00	20.00	-261.46	0.00
57	PC (2015-16)-57	20.00	3.24	-1.33	0.16	-2.62	-29.95	-5.02	12.15	13.18	7.96	19.51	9.74	0.00	-382.91	0.00
58	PC (2015-16)-58	0.00	1.30	-13.44	9.43	-13.22	-25.27	-15.36	-91.83	11.00	9.35	4.55	8.72	0.00	-371.53	0.00
59	PC (2015-16)-59	0.00	11.99	-28.17	10.32	-3.48	-31.52	-11.26	-203.28	32.34	3.66	65.31	17.84	25.00	-297.61	0.00
60	PC (2015-16)-60	21.05	13.93	5.87	38.83	7.94	20.12	3.29	-45.55	17.97	24.23	67.44	32.07	0.00	-247.93	0.00
61	PC (2015-16)-61	33.33	8.32	7.47	-17.50	8.04	51.11	23.02	-18.65	13.77	27.74	70.45	37.19	25.00	-244.53	66.67
62	PC (2015-16)-62	0.00	8.10	24.86	-27.33	26.30	36.00	30.73	-240.80	18.56	19.88	75.00	30.33	25.00	-223.02	66.67
63	PC (2015-16)-63	10.71	4.87	-0.08	25.54	-1.26	-30.03	-6.21	5.33	31.74	13.37	55.56	23.45	0.00	-228.24	66.67
64	PC (2015-16)-64	0.00	9.33	-11.20	-29.68	0.06	2.48	3.62	5.96	12.30	16.13	20.00	16.73	0.00	-364.47	0.00
65	PC (2015-16)-65	0.00	8.17	0.64	-28.46	3.35	-41.28	3.93	-178.42	33.41	17.76	62.26	26.69	25.00	-312.42	80.00
66	BO 91	0.00	11.43	9.72	-20.67	12.64	15.35	11.99	-78.38	29.89	22.57	47.69	28.28	14.29	-129.10	0.00
67	Co 1148	0.00	8.46	1.46	-13.73	-0.38	-15.79	1.48	-7.70	10.29	5.37	13.73	6.59	11.11	-343.50	0.00
68	Co 617	16.67	4.49	4.23	-33.75	5.95	-16.17	-4.56	-56.55	10.41	3.19	17.39	5.72	14.29	-309.76	0.00
69	Co 85002	16.67	8.11	1.70	-23.33	10.71	8.27	-0.85	7.75	11.10	14.62	24.24	16.67	20.00	-340.62	0.00
70	ISH 100	20.00	3.27	-21.01	-412.50	-13.80	-10.50	-7.05	-20.03	10.38	1.50	7.14	2.31	0.00	-327.48	0.00
71	UP 9530	0.00	12.52	1.54	-26.09	-1.09	-9.76	-1.44	-26.03	30.16	18.22	52.63	25.46	16.67	-124.48	0.00
72	Co Pant 97222	0.00	10.74	5.18	-110.82	11.20	26.53	8.13	-13.86	29.15	17.67	36.23	22.54	16.67	-120.06	0.00
	Mean	12.64	7.64	-5.37	-60.30	-2.11	-0.64	-2.61	-60.84	16.40	14.77	41.75	20.59	9.97	-227.07	17.59
	Max	55.56	24.49	24.86	60.13	26.30	76.39	30.73	41.45	35.27	31.89	75.00	41.18	25.00	-86.29	80.00
	Min	-30.43	-9.74	-56.55	-1605.0	-48.54	-107.95	-62.91	-756.67	4.45	-2.53	4.55	-1.06	0.00	-393.04	0.00

DG: Days to germination, **G%: Germination%** **CTD:** Canopy Temperature Depression, **RWC%:** Relative Water Content%, **Chl a & b:** Chlorophyll a & b, **TC:** Total carotenoids, **DTC:** Drought tolerance Capacity,

Mean percent change for Chl a was recorded to be 14.77 percent which ranged from -2.53 for PC (2015-16)-51 to 31.89 for PC (2015-16)-7. For Chl b mean percent change was recorded to be 41.75 percent which ranged from 4.55 for PC (2015-16)-58 to 75.00 for PC (2015-16)-62. Total chlorophyll recorded a mean percent change of 20.59 percent which ranged from -1.06 for PC (2015-16)-51 to 41.18 for PC (2015-16)-7.

Similarly the recorded mean percent change for total Carotenoids was recorded to be 9.97 percent which ranged from 0.00 for PC (2015-16)-2 to 25.00 for PC (2015-16)-65.

Mean percent change for RWC% was recorded to be 16.40 percent which ranged from 4.45 for PC (2015-16)-33 to 35.27 for PC (2015-16)-35. For proline content mean percent change content was recorded to be -227.07 percent which ranged from -393.04 for PC (2015-16)-51 to -86.29 for PC (2015-16)-25.

Mean percent change for DTC mean percent change was recorded to be 17.59 percent which ranged from 0.00 for PC (2015-16)-65 to 80.00 for PC (2015-16)-65.

Negative mean values for days to germination indicate that days to germination were found to be earlier under stress than control environment. This change might be because of the higher temperatures under polyhouse that may enhance the metabolic activities and lead to earlier germination. However, earlier germination is desirable so as to capitalise with early vigour. Similarly, positive change in mean value for germination percent indicates that the germination percent under stress was better than the control due to obvious reason of earlier germination in polyhouse. These findings can't be considered as consequence of water stress as the crop was subjected to stress after 60 DAS, therefore the recorded early germination and enhanced germination percent may be owed to the better growing conditions in polyhouse.

Increasing plant water deficits leads to stomatal closure, decreased leaf transpiration cooling, and consequently, increased LT relative to well-watered plants. Genotypes with higher CTD poses better ability to with stand water stress. Therefore, genotypes with higher negative values are more desirable such as PC (2015-16)-54 (-232.38) and ISH 100 (412.50). Present results are in agreement with **Silva *et al.* (2007)** and **Karimizadeh and Mohammadi (2011)** where they demonstrated that water stress by and large resulted in a higher increase in LT in susceptible genotypes.

Negative mean values revealed that SPAD readings at 60 DAS were higher under stress than control. Genotypes with higher percent change towards negative side such as PC (2015-16)-38 (-56.55) will be having higher SPAD readings under stress environment. However, as the stress period advances from 60 DAS to 120 DAS and 180 DAS there was a gradual reduction in the SPAD readings. Therefore, a genotypes with lower reductions in SPAD readings are desirable as lower reduction proves their drought tolerance ability. **Silva et al. (2007)** reported a steady reduction in SPAD unit as the stress increased with values lower at 90 DAT than those at 0 and 45 DAT. Few of the genotypes with lower reductions are PC (2015-16)-51 (-4.363), PC (2015-16)-54 (2.90) and PC (2015-16)-57 (-2.62).

Positive percent mean changes for RWC% indicates that the RWC% has reduced under stress environment. A genotype with the capability to minimize stress by retaining turgid leaves in stressed environments will have physiological reward (**Mullan and Pietragalla, 2012**). The genotypes which are able to maintain the RWC% will be able to tolerate stress. So, the genotypes with lowest reduction in RWC% would be desirable. Few of the genotypes with lower levels of reduction are PC (2015-16)-20 (6.25), PC (2015-16)-28 (6.06) and PC (2015-16)-37 (6.66). **Schlemmer et al. (2005)** and **Silva et al. (2011)** reported similar results in wheat and sugarcane respectively.

Chlorophyll content also recorded a reduction under stress environment than control. The reduction clearly indicates the influence of water stress on plant physiology. Reduction in chlorophyll content will affect the photosynthetic activities and ultimately affecting the overall phenotype of the plant. Genotypes differ for their ability to tolerate water stress and maintain the photosynthetic activity. **Wagih, (2005)** and **Cha-um et al. (2012)** reported that Chlorophyll a (Chla), chlorophyll b (Chl b) and total carotenoids (Cx+c), in the stressed leaves of all genotypes decreased significantly. Genotypes with lower reductions are the desirable ones. Few of such kinds are PC (2015-16)-51 (-2.53), PC (2015-16)-18 (2.29) and ISH 100 (1.53) a tolerant parent used in the study. These results were consistent with that of **Jagtap et al. (1992)**, who reported similar change in chlorophyll contents with decreasing availability of water.

A very high negative percent mean change for proline content indicates that the levels of proline content were very high under stress environment. Genotypes differs for the level of proline contents, some of the genotypes recorded only 1.5-2 times

increase whereas others recorded 5-6 times increase in proline content. A clear differentiation was observed between the genotypes under study for the levels of proline content, which clearly indicates their drought tolerance capacity. **Abbas *et al.* (2014)** reported that plants accumulate some kind of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake and proline is one of major solute involved in drought tolerance. **Hemaprabha *et al.* (2013)** and **Jagtap *et al.* (1988)** also reported similar increase in proline levels.

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 (**Carrow and Duncan, 2003**). Unlike under control all the genotypes under stress exhibited varied response and different levels of leaf firing were observed. Genotypes exhibiting tolerance under stress are the desirable ones such as PC (2015-16)-9, PC (2015-16)-30. The results were consistent with reports of **Riaz *et al.* (2013)** and **Huang, (2004)** where, they observed differential expression for leaf firing under different soil moisture levels in marigold and turf grass respectively.

4.8 Experiment 2: Validation of candidate gene specific molecular marker for drought tolerance in C₁ clones of Sugarcane crosses

Twenty C₁ clones of sugarcane along with five parents were selected for the study based on their performance for 16 physio-biochemical parameters and used for validation of 10 candidate gene specific markers for drought tolerance in sugarcane. The experimental material consisted of 10 drought tolerant clones, 10 drought sensitive clones and five parents (3 Tolerant + 2 sensitive). The results of the molecular analysis for validation are presented under the following sub headings:

4.8.1 Genotyping for 10 candidate gene specific molecular markers

4.8.2 t- Test for Validation of Candidate Gene Specific Molecular Marker

4.8.1 Genotyping for 10 Candidate Gene Specific Molecular Markers

One of the essential steps before utilising any marker for genotyping is confirmation of its presence or absence in the study material. Therefore, all the 10 markers were tested for their presence or absence in the two drought tolerant genotypes

initially. The results of the confirmation studies are presented in Table 4.33 and depicted as Plate 2(a).

Out of 10 candidate gene specific markers only five markers showed their presence in the present study material. The five markers such as DREB 1 A with amplification fragment size (408 bp), NAC 2 (305 bp), Snac 1 (270 bp), LEA 3 (270 bp) and ABF 2 (280 bp) showed their presence with their respective specific fragment size. These markers were consistent with amplification of their respective specific marker fragment size. This confirmed the presence of these five markers in the study material unlike the one which recorded no amplification fragments.

Table 4.33: Confirmation of Markers for Their Presence in the Study Material

S. No.	Markers	Amplicons Size	Presence/Absence	
			PC (2015-16)-28	PC (2015-16)-34
1.	DREB 1 A	408 bp	1	1
2.	NAC 2	305 bp	1	1
3.	SHN 1	502 bp	0	0
4.	SIZ 1	300 bp	0	0
5.	Snac 1	270 bp	1	1
6.	CIPK 14	200 bp	0	0
7.	LEA 3	270 bp	1	1
8.	ABF 2	250 bp	1	1
9.	DRF 1	300 bp	0	0
10.	HRD	420 bp	0	0

*0: Absence and 1: Presence of the marker

The five molecular markers which were confirmed for their presence in the study material were utilised for genotyping all the 25 selected genotypes. The results of genotyping are presented in Table 4.34.

The genotyping of 25 genotypes revealed that 11 genotypes showed varied marker presence. The genotypes PC (2015-16)-28 and PC (2015-16)-34 showed presence of all five markers. PC (2015-16)-9, PC (2015-16)-31, PC (2015-16)-64, PC (2015-16)-65 recorded presence of four marker such as DREB 1A, NAC 2, Snac 1 and LEA 3. PC (2015-16)-29 recorded presence of three markers NAC 1, Snac 1 and ABF 2, ISH 100 recorded DREB 1A, NAC 2 and LEA 3 and PC (2015-16)-54 recorded

DREB 1A, NAC 2 and Snac 1. PC (2015-16)-33 and Co 1148 recorded presence of single marker i.e. DREB 1A. The results of genotyping for DREB 1A, NAC 2, Snac 1, LEA 3 and ABF 2 are depicted as Plate 2 (b-e) respectively.

Table 4.34: Genotyping of 25 Selected Genotypes for Five Drought Related Molecular Markers

S. NO	Genotypes	DREB 1A	NAC 2	Snac 1	LEA 3	ABF2
1	PC (2015-16)-9	1	1	1	1	0
2	PC (2015-16)-16	0	0	0	0	0
3	PC (2015-16)-28	1	1	1	1	1
4	PC (2015-16)-29	0	1	1	0	1
5	PC (2015-16)-31	1	1	1	1	0
6	PC (2015-16)-32	0	0	0	0	0
7	PC (2015-16)-33	1	0	0	0	0
8	PC (2015-16)-34	1	1	1	1	1
9	PC (2015-16)-35	0	0	0	0	0
10	PC (2015-16)-36	0	0	0	0	0
11	PC (2015-16)-39	0	0	0	0	0
12	PC (2015-16)-43	0	0	0	0	0
13	PC (2015-16)-53	0	0	0	0	0
14	PC (2015-16)-48	0	0	0	0	0
15	PC (2015-16)-54	1	1	1	0	0
16	PC (2015-16)-56	0	0	0	0	0
17	PC (2015-16)-60	0	0	0	0	0
18	PC (2015-16)-61	0	0	0	0	0
19	PC (2015-16)-64	1	1	1	1	0
20	PC (2015-16)-65	1	1	1	1	0
21	Co Pant 97222	0	0	0	0	0
22	ISH 100	1	1	0	1	0
23	UP 9530	0	0	0	0	0
24	Co 1148	1	0	0	0	0
25	Co 617	0	0	0	0	0

4.2.2 t- Test for Validation of Candidate Gene Specific Molecular Marker

To test the significance or to validate the association of marker with the drought tolerance t test is applied. The phenotypic mean of the two classes (presence and absence) was statistically compared using t- test. The results of the t-test are presented in Table 4.35.

Table 4.35: t-Test for Validation of Candidate Gene Specific Molecular Marker for Drought Tolerance

S. No.	Marker	N		Mean DTC		Std. Deviation		t_{cal}	d.f.	Significance
		1	0	1	0	1	0			
1	DREB 1A	10	15	4.131	4.676	0.100	0.328	-5.06	23	0.001
2	NAC 2	9	16	4.138	4.637	0.086	0.355	-4.10	23	0.001
3	Snac 1	8	17	4.150	4.602	0.085	0.372	-3.36	23	0.003
4	LEA 3	7	18	4.120	4.589	0.088	0.3617	-3.35	23	0.003
5	ABF 2	3	22	4.160	4.498	0.017	0.382	-3.38	23	0.146

DREB 1A was found to be present in 10 genotypes and absent in 15 genotypes with mean values of 4.131 and 4.676 for group with presence and absence respectively. t_{cal} value was found to be -5.06 which was found to be significant @ 5% level of significance.

NAC 2 showed its presence in nine genotypes and absence in 16 genotypes with mean values of 4.138 and 4.637 for group with presence and absence respectively. t_{cal} value was found to be -4.10 which was found to be significant @ 5% level of significance.

Snac 1 conferred its presence in eight genotypes and absent in 17 genotypes with mean values of 4.150 and 4.602 for group with presence and absence respectively. t_{cal} value was found to be -3.36 which was found to be significant @ 5% level of significance.

LEA 3 conferred its presence in seven genotypes and absent in 18 genotypes with mean values of 4.120 and 4.589 for group with presence and absence respectively.

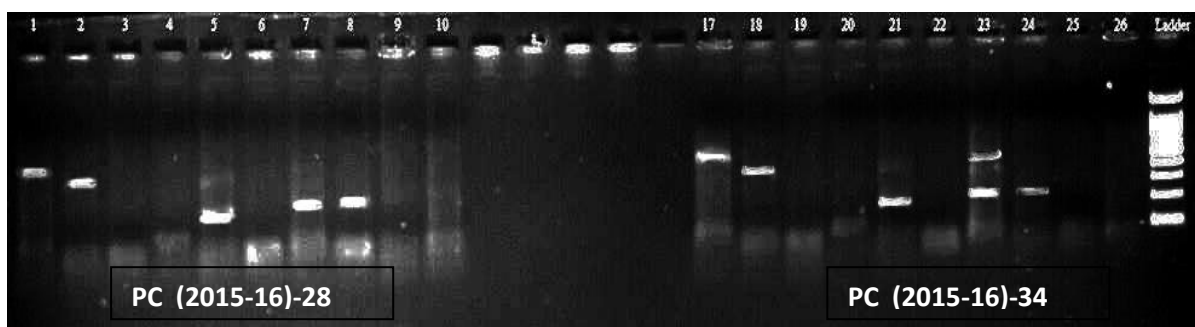


Plate 2(a): Confirmation of Markers for Their Presence in the Study Material

Lane 1, 17: DREB 1A (408 bp). Lane 2, 18: NAC 2 (305 bp). Lane 3, 19: SHN 1. Lane 4, 20: SIZ 1. Lane 5, 21: Snac 1 (270 bp). Lane 6, 22: CIPK 14. Lane 7, 23: LEA (270 bp). Lane 8, 24: ABF2 (280 bp). Lane 9, 25: DRF 1. Lane 10, 25: HRD

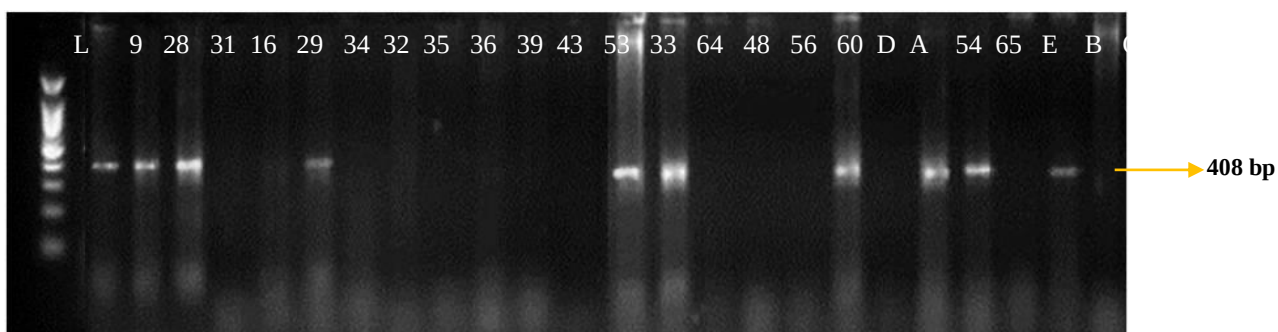


Plate 2(b): Genotyping of 25 Genotypes for DREB 1A (408 bp) Molecular Marker

Lane A: Co Pant 97222, B: ISH 100, C: UP 9530, D: Co 1148, E: Co 617

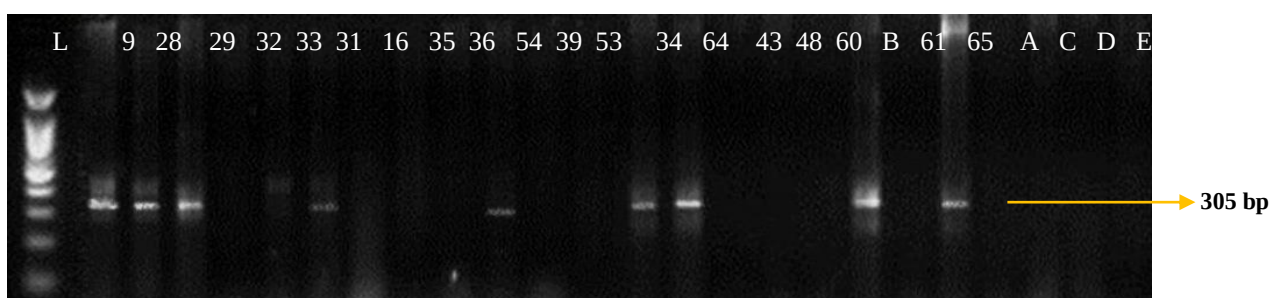


Plate 2(c): Genotyping of 25 Genotypes for NAC 2 (305 bp) Molecular Marker

Lane A: Co Pant 97222, B: ISH 100, C: UP 9530, D: Co 1148, E: Co 617

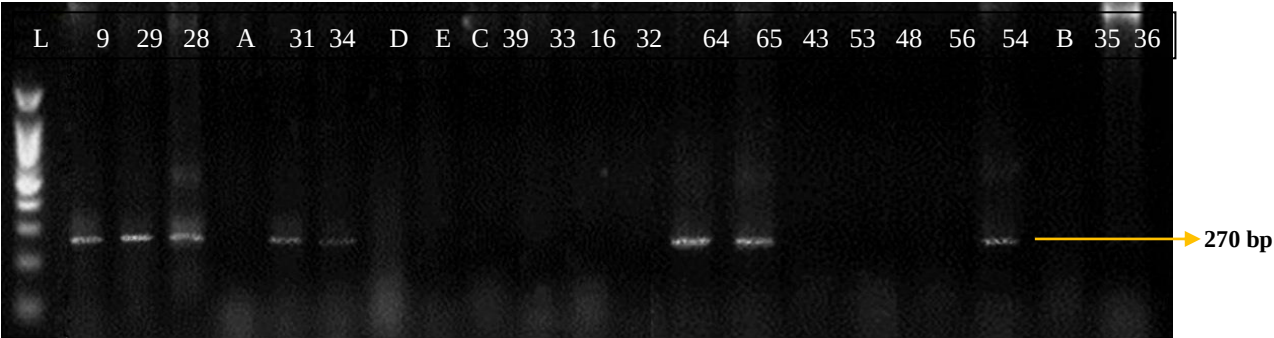


Plate 2(d): Genotyping of 25 Genotypes for Snac 1 (270 bp) Molecular Marker

Lane A: Co Pant 97222, B: ISH 100, C:UP 9530, D:Co 1148, E: Co 617

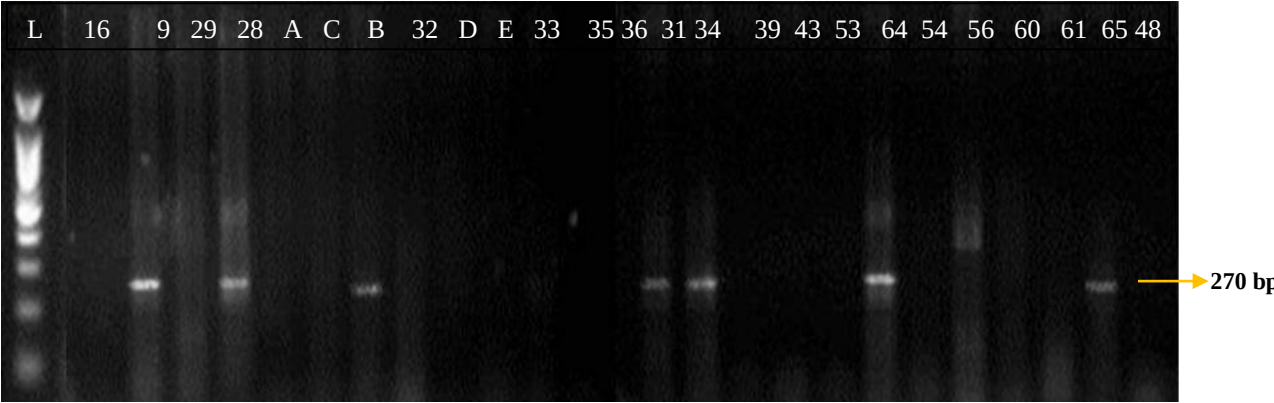


Plate 2(e): Genotyping of 25 Genotypes for LEA 3 (270 bp)Molecular Marker

Lane A: Co Pant 97222, B: ISH 100, C:UP 9530, D:Co 1148, E: Co 617

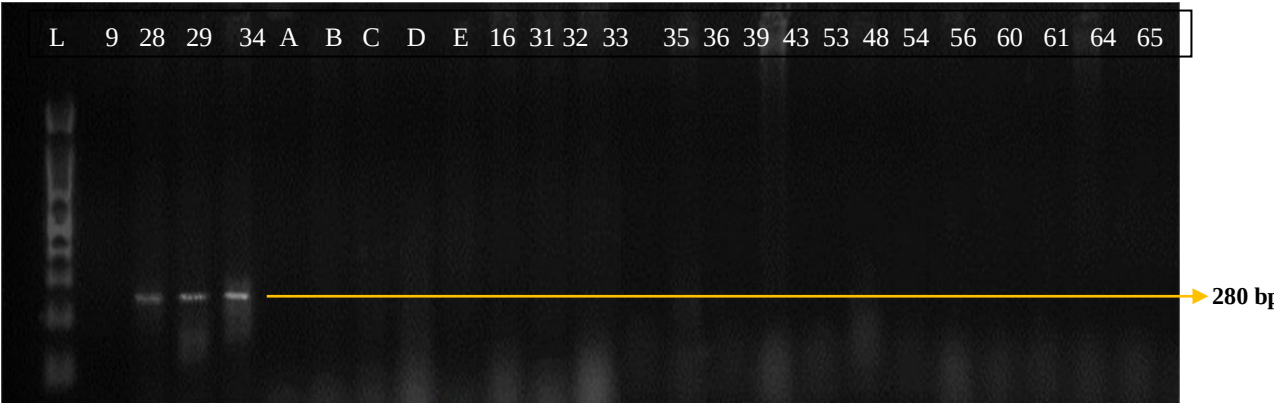


Plate 2(f): Genotyping of 25 Genotypes for ABF 2 (280 bp)Molecular Marker

t_{cal} value was found to be -3.35 which was found to be significant @ 5% level of significance.

ABF 2 conferred its presence in three genotypes and absent in 22 genotypes with mean values of 4.160 and 4.498 for group with presence and absence respectively. t_{cal} value was found to be -3.38 which was found to be non-significant @ 5% level of significance.

t-test used to validate the marker association revealed that four markers DREB 1A, NAC 2, Snac 1 and LEA 3 recorded significant difference between the means of the two groups (marker present and absent). The significant difference indicates that these markers are significantly associated with respective candidate genes for drought tolerance. The significant association proves their worth for their use in marker assisted selection for drought tolerance. Similar approach was utilised to validate three marker for Pachymetra root rot and one for Brown rust in sugarcane (**McIntyre *et al.*, 2005**).



Summary and Conclusions



The present investigation entitled “Evaluation of drought tolerance potential in early generation progenies of sugarcane (*Saccharum* Sp. Complex)” was conducted with 65 Bi-parental progenies along with parents and six check varieties. The investigation comprised of two experiments viz., 1) evaluation of drought potential of sugarcane progenies based on physio-biochemical characters and studies of genetic parameters to aid breeding programme and 2) validation of candidate gene specific molecular markers for drought tolerance in sugarcane. The experimental material was evaluated in augmented block design- II in two seasons with two environments such as control at Norman-E-Borlaug Crop Research Center and stress at Pantnagar Centre for Plant Genetic Resources, G. B. Pant University of Agriculture & Technology, Pantnagar during Spring 2017 and Autumn 2017-18. Control environment referred to planting of sugarcane in field condition with normal course of irrigation and drought stress environment was provided in rainout shelter (Polyhouse) at PCPGR to evaluate the response of these genotypes 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. For evaluation of performances of clones, augmented design II with six check varieties viz., Co 0238, Co J 64 and Co Pant 03220 (early) and Co Pant 97222, Co Pant 05224, CoS 8436 (mid-late) was used. The data was recorded for 16 physiological and biochemical characters which were subjected for estimation of genetic parameters, correlation, path coefficients, genetic diversity analysis and selection indices. Further, Experiment 2 with the objective to validate 10 candidate gene specific molecular markers was carried out with 25 selected clones at Plant Biotechnology lab, Department of Genetics and Plant Breeding. The major findings of the investigation are given below.

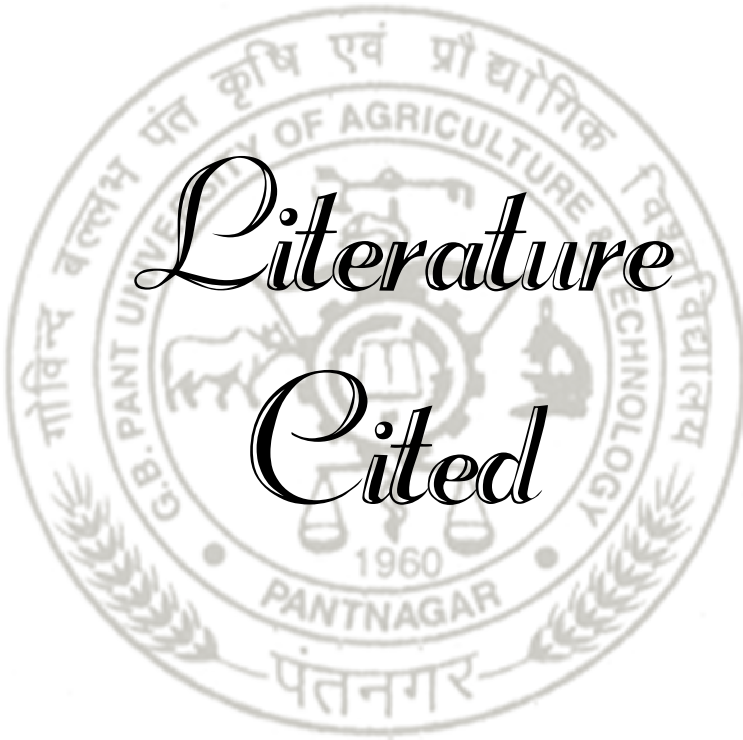
1. Analysis of Variance (ANOVA) for four environments revealed that traits like proline content and leaf firing exhibited significant differences in stress unlike control environment indicating that these traits are of very high utility in selection for drought tolerance.

2. F-test to test homogeneity of error variances between two seasons depicted that error variances for most of the traits were found to be homogeneous at 1 % level of significance except for CTD 180 under control environment. The homogeneity of almost all the error variances except CTD 180 satisfies the requirement for pooled analysis of variances.
3. Pooled analysis of variance for two different environments yielded similar results as of augmented ANOVA where, significant differences for proline content and leaf firing in stress environment unlike control environment were observed. This signifies that these two traits have significant role in water stress environment as they are exhibiting variable expression under stress.
4. Mean performance of all the 72 genotypes for 16 physiological and biochemical traits under both control and stress environment, exhibited an enormous effect of water stress. The effect of water stress was clearly reflected by the reduced values for almost all the traits except an increase in proline content under stress. A gradual reduction is observed in stress environment for SPAD readings and CTD over time interval. Similar observation were recorded for Chl a, Chl b, total chlorophyll and RWC percent. Genotypes with lower levels of reduction were considered to be tolerant to water stress and genotypes showing higher levels of reductions indicate their sensitivity towards water stress.
5. 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.
6. Significant associations between all the traits except for days to germination were observed under stress environment. Proline content, total carotenoids and Leaf firing recorded significant associations only under stress environment indicating that these traits might get triggered due to water stress and expressed at higher levels which make their presence counted only in stress environment.

7. Estimates of path coefficients revealed that Traits like Chl a, Chl b, total chlorophyll, SPAD 120, SPAD 180 and proline content were found to confer positive direct as well as indirect effects on DTC which supports their utilization as a reliable tool in selection for drought tolerance in sugarcane. Path coefficients under both the environments and at both phenotypic and genotypic levels explained 100 percent of variations in DTC as revealed by the higher coefficient of determinants. However, the residual effects under control environment was recorded to be > 0.5 which, indicates some more factors need to be included in the analysis to account fully for the variation in DTC.
8. Estimates of genetic diversity using Mahalanobis D^2 analysis revealed that in both the environments there were two or three major clusters consisting of approximately 40 and 30 percent clones exhibiting a normal distribution of clones. The presence of tolerant and sensitive parents in different clusters along with other clones indicates corresponding response of those clones to water stress. Under both the environmental conditions highest inter-cluster distances were found to be between a drought tolerant cluster and a drought sensitive cluster. Thus, hybridization of these two groups could result in transgressive recombinants for drought tolerance as well as for agronomically important traits.
9. Cluster I, III and IV were found to comprise desirable clones for most of the traits. Therefore, it is suggested to use clones from these clusters for inter-crossing to generate good number of sugarcane progenies having greater potentiality for breeding purpose by virtue of their desirable characters. Percent contribution of characters indicated that the maximum contribution towards divergence was conferred by germination percent. Along with germination percent, Chl a, RWC percent, leaf firing, total carotenoids, SPAD 60, Chl b, DTC and proline content contributed approximately 70 percent of the total divergence.
10. Relative efficiency of the selection index for most of the traits were found to be >40 percent. Based on the estimates of selection indices and selection scores the genotypes namely PC (2015-16)-55, PC (2015-16)-26, PC (2015-16)-40,

PC (2015-16)-54, and PC (2015-16)-1 were found best ones among drought tolerant genotypes.

11. Estimates of percent change revealed that genotypes like PC (2015-16)-9, PC (2015-16)-18, PC (2015-16)-20, PC (2015-16)-28, PC (2015-16)-30, PC (2015-16)-37, PC (2015-16)-38, PC (2015-16)-50, PC (2015-16)-51, PC (2015-16)-54 and PC (2015-16)-57 were found to record lower reductions for SPAD readings, CTD, Chl a, Chl b, total chlorophyll and RWC percent and recorded increased values for proline content under stress indicating their tolerant response towards water stress.
12. The genotyping of 25 genotypes revealed that 11 genotypes showed varied marker presence. The genotypes PC (2015-16)-28 and PC (2015-16)-34 showed presence of all five markers. PC (2015-16)-9, PC (2015-16)-31, PC (2015-16)-64, PC (2015-16)-65 recorded presence of four marker such as DREB 1A, NAC 2, Snac 1 and LEA 3. PC (2015-16)-29 recorded presence of three markers NAC 1, Snac 1 and ABF 2, ISH 100 recorded DREB 1A, NAC 2 and LEA 3 and PC (2015-16)-54 recorded DREB 1A, NAC 2 and Snac 1. PC (2015-16)-33 and Co 1148 recorded presence of single marker i.e. DREB 1A.
13. Validation analysis of the marker trait association with respect to candidate gene specific markers and drought tolerance through t-test revealed that four markers DREB 1A, NAC 2, Snac 1 and LEA 3 recorded significant difference between the means of the two groups (marker presence and absence). The significant association proves their worth for future use of these markers in marker assisted selection for drought tolerance in sugarcane.



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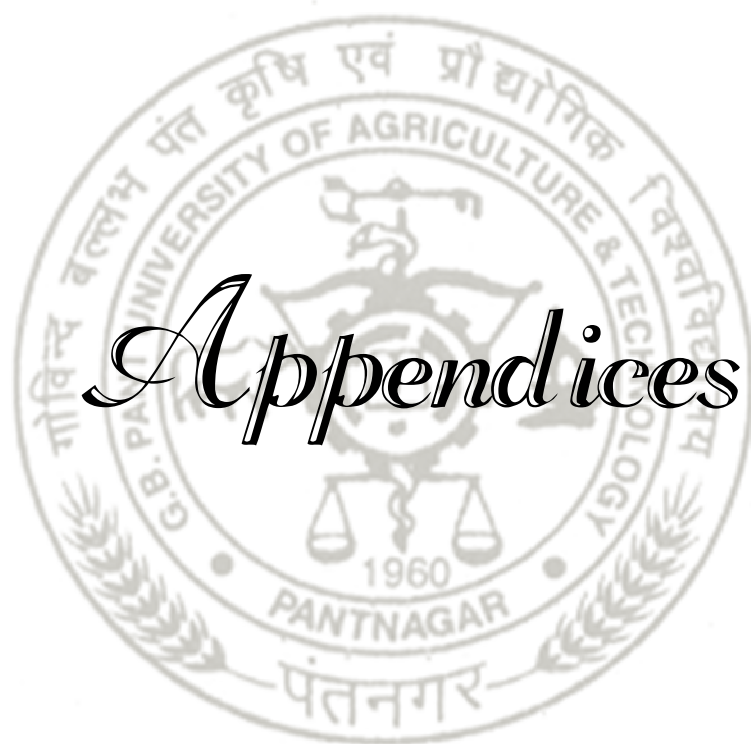
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Appendices

APPENDIX

Appendix I: Meteorological data for the year 2016-17 and 2017-18

Month	Temperature °C		Rel. Humidity		Rainfall (mm)	No. of R/days
	Max.	Min.	712	1412		
Jan. 2016	20.56	6.48	93.52	52.03	0.00	0.00
Feb. 2016	9.45	88.97	40.10	0.09	2.50	1.00
March 2016	30.93	14.05	81.39	33.52	0.90	1.00
April 2016	37.62	18.86	67.50	29.73	0.00	0.00
May 2016	35.33	22.66	68.84	41.23	135.40	8.00
June 2016	34.42	26.21	78.10	58.40	171.20	13.00
July 2016	31.50	25.58	90.32	74.68	499.10	23.00
Aug. 2016	33.15	26.12	88.42	67.71	201.60	15.00
Sept. 2016	32.57	24.34	89.23	67.33	166.20	5.00
Oct. 2016	31.70	18.53	86.03	52.42	0.00	0.00
Nov. 2016	27.97	11.02	91.30	38.87	0.00	0.00
Dec. 2016	22.7	9.3	94	55	0	0
Jan. 2017	20.50	7.60	93.00	57.10	60.40	3.00
Feb. 2017	24.80	9.10	91.80	47.50	0.00	0.00
Mar. 2017	28.90	11.80	84.50	39.30	3.60	1.00
April 2017	35.9	19.4	65	28	4.0	2
May 2017	37.4	22.8	64	36	39.0	1
June 2017	36.9	25.2	71	50	73.0	5
July 2017	32.3	25.8	90	78	505.0	21
Aug. 2017	32.0	25.7	90	75	440.6	19
Sept. 2017	32.5	24.3	89	69	336.8	7
Oct. 2017	32.5	18.7	85	51	0.0	0
Nov. 2017	27.1	10.9	92	46	0.0	0
Dec. 2017	22.7	9.3	94	63	2.8	1
Jan. 2018	16.7	5.6	97	74	6.8	1
Feb. 2018	24.6	8.9	93	54	4.0	1
Mar. 2018	30.9	11.8	85	41	0.0	0
April 2018	34.5	17.7	74	39	42.2	2

The author of this manuscript was born on Jan. 05, 1991 in Belagavi district (Karnataka). He passed secondary and higher secondary examination (1st divisions) from C.B.S.E in 2007 and 2009, respectively. He completed his B.Sc. in Agriculture (1st division) in the year 2013 from University of Agricultural Sciences, Dharwad. Thereafter, he pursued his M.Sc. (Ag.) (distinction) with major in Genetics and Plant Breeding and minor in Plant pathology year in the 2015 from University of Agricultural Sciences, Dharwad. Then he joined PhD programme with major in Genetics and Plant Breeding and minor in Molecular Biology and Biotechnology at G.B. Pant University of Agriculture & Technology, Pantnagar in year 2015. He qualified ICAR SRF in 2015 and ARS NET in 2016 and was Project Coordinator, in Department of Genetics and Plant Breeding in 2017-18.

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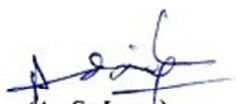
ABSTRACT

The present investigation entitled “Evaluation of drought tolerance potential in early generation progenies of sugarcane (*Saccharum* Sp. Complex)” was conducted with 65 Bi-parental progenies along with parents and six check varieties viz., Co 0238, Co J 64 and Co Pant 03220 (early) and Co Pant 97222, Co Pant 05224, CoS 8436 (mid-late) to investigate drought tolerance potential based on physio-biochemical characters, studies on genetic parameters and revalidation of candidate gene specific molecular markers to aid breeding programme. The experiment was conducted in augmented block design- II in two seasons with two environments such as control at N.E.B.C.R.C. and stress at P. C.P.G.R. G.B.P.U.A&T, Pantnagar during spring 2017 and autumn 2017-18. The drought stress was created by withdrawing the irrigations during the 50 DAS to 200 DAS. The data was recorded on 16 physiological and biochemical characters for estimation of genetic parameters, genetic diversity analysis and selection indices. Further, Twenty five selected clones were utilized for candidate gene specific molecular markers validation study at Plant Biotechnology lab, Department of Genetics and Plant Breeding.

Analysis of Variance (ANOVA) for four environments revealed that traits like proline content and leaf firing exhibited significant differences in stress unlike control environment indicating that these traits are of very high utility in selection for drought tolerance. The effect of water stress was clearly reflected by the reduced mean values for almost all the traits except an increase in proline content under stress. High PCV and GCV along with high heritability and high genetic advance as percent of mean was observed for CTD 60, CTD 120, CTD 180, Chl a, Chl b, Total chlorophyll, Total carotenoids Proline content, RWC % and Leaf firing indicating the prevalence of additive gene action in expression of these characters.

Estimates of path coefficients revealed that Traits like Chl a, Chl b, total chlorophyll, SPAD 120, SPAD 180 and proline content were found to confer positive direct as well as indirect effects on DTC which supports their utilization as a reliable tool in selection for drought tolerance in sugarcane. Under both the environmental conditions highest inter-cluster distances were found to be between a drought tolerant cluster and a drought sensitive cluster. Cluster I, III and IV were found to comprise desirable clones for most of the traits. Germination percent, Chl a, RWC percent, leaf firing, total carotenoids, SPAD 60, Chl b, DTC and proline content contributed approximately 70 percent of the total divergence. Based on the selection scores the genotypes namely PC (2015-16)-55, PC (2015-16)-26, PC (2015-16)-40, PC (2015-16)-54, and PC (2015-16)-1 were found best ones among drought tolerant genotypes.

Validation analysis of the marker trait association with respect to candidate gene specific markers and drought tolerance through t-test revealed that four markers DREB 1A, NAC 2, Snac 1 and LEA 3 recorded significant difference between the means of the two groups. The significant association proves their worth for future use of these markers in marker-assisted selection for drought tolerance in sugarcane.


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प्रमुख विषय	: आनुवांशिकी एवं पादप प्रजनन	विभाग	: आनुवांशिकी एवं पादप प्रजनन
गौण विषय	: आणविक जीव विज्ञान एवं जैव प्रौद्योगिकी		
शोध का विषय	: "गन्ने (सैक्रेम स्पसीज काम्लैक्स) की प्रारम्भिक वंशीय संततियों में सूखे हेतु सहिष्णुता के सामर्थ्य का मूल्यांकन"		
सलाहकार	: डा० आनन्द सिंह जीना		

सारांश

वर्तमान अध्ययन गन्ने (सैक्रेम स्पसीज काम्लैक्स) की प्रारम्भिक वंशीय 65 द्वि-पैतृक संततियों में सूखे की स्थिति हेतु सहिष्णुता के सामर्थ्य के मूल्यांकन के लिए पैतृक किस्मों व मानक किस्मों को. 0238, को. जे. 64 और को. पंत 03220 (अगेती) और को. पंत 97222, को. पंत 05224, को. 8436 (सामान्य) के साथ 65 के साथ आयोजित की गयी थी। प्रायोगिक सामग्री का मूल्यांकन संवर्धित ब्लॉक डिजाइन-2 में दो सत्रों क्रमशः वसंत 2017 और शरद 2017-18 की ऋतु के दौरान फसल अनुसंधान केन्द्र (सामान्य वातावरण) तथा पंतनगर पादप जैव संपदा केन्द्र (सूखे का स्थिति), गो. ब. पंत कृषि एवं प्रौ. विश्वविद्यालय, पंतनगर में किया गया। पंतनगर पादप जैव संपदा केन्द्र के संरक्षित गृह में बुवाई के बाद 50 दिन से लेकर 200 दिनों के दौरान सिंचाई बंद कर सूखे की स्थिति बनायी गयी। सोलह कार्यांक एवं जैव रासायनिक लक्षणों के लिए आंकड़े दर्ज किये गये और आनुवांशिक मानकों, सहसम्बन्ध, पथ गुणांक, आनुवांशिक विभिन्नता विश्लेषण और चयन सूचकांक का आंकलन किया गया। इसके अलावा, पादप जैवप्रौद्योगिकी प्रयोगशाला, आनुवांशिकी एवं पादप प्रजनन विभाग में गन्ने में सूखे के प्रति सहिष्णुता के लिए उम्मीदवार जीन विशिष्ट आणविक चिन्हकों (मार्कर) को सत्यापित करने के लिए 25 चयनित प्रतिरूपकों (क्लोन) के साथ प्रयोग किया गया।

भिन्नता (एनोवा) के विश्लेषण से पता चला कि प्रोलीन की मात्रा और पत्ती का जलना जैसे लक्षणों के लिए सामान्य की अपेक्षा सूखे की स्थिति में सार्थक अंतर पाया गया जो यह दर्शाता है कि सूखे हेतु सहिष्णुता के चयन में ये लक्षण बहुत अधिक उपयोगी हैं। सामान्य और सूखे की स्थिति के तहत कार्यांक एवं जैव रासायनिक लक्षणों के लिए सभी जीन प्रारूपों के औसत प्रदर्शन पर पानी की कमी का नकारात्मक प्रभाव दिखा। सीटीडी 60, सीटीडी 120, सीटीडी 180, पर्णहरित ए, पर्णहरित बी, कुल पर्णहरित, कुल कैरोटीनोइड, प्रोलीन, आरडब्ल्यूसी और पत्तियों का जलना के लिए उच्च कार्यांक एवं जीनिक विभिन्नता के साथ उच्च वंशागतत्व एवं उच्च आनुवांशिक प्रगति यह दर्शाती है कि, इन लक्षणों की अभिव्यक्ति में योजक जीन क्रिया अधिक प्रभावी है इसलिए चयन द्वारा इनका उन्नयन सफलतापूर्वक किया जा सकता है। पथ गुणांक के अनुमानों से पता चला कि पर्णहरित ए, पर्णहरित बी, कुल पर्णहरित, एसपीएडी 120, एसपीएडी 180 और प्रोलीन जैसे लक्षण डीटीसी पर सकारात्मक प्रत्यक्ष और अप्रत्यक्ष प्रभाव प्रदर्शित करते हैं जो कि गन्ने में सूखे हेतु सहिष्णुता के चयन में एक विश्वसनीय कारक के रूप में उनके उपयोग का समर्थन करते हैं। दोनों पर्यावरणीय परिस्थितियों में सूखा-सहिष्णु समूह और सूखा-संवेदनशील समूह के बीच उच्च अंतर-सामूहिक दूरी पाई गई। समूह 1, 3 और 4 में अधिकांश लक्षणों के लिए वांछनीय प्रतिरूप शामिल थे। अंकुरण प्रतिशत, पर्णहरित ए, आरडब्ल्यूसी प्रतिशत, पत्तियों का जलना, कुल कैरोटीनोइड, एसपीएडी 60, सीएचएल बी, डीटीसी और प्रोलीन ने कुल विविधता में लगभग 70 प्रतिशत योगदान दिया। चयन गुणांक के आधार पर जीन प्रारूप अर्थात् पीसी (2015-16)-55, पीसी (2015-16)-26, पीसी (2015-16)-40, पीसी (2015-16)-54, और पीसी (2015-16) -1 सूखे हेतु सहिष्णु जीन प्रारूपों के बीच सबसे अच्छे पाए गए।

टी-टेस्ट द्वारा उम्मीदवार जीन विशिष्ट चिन्हकों एवं सूखे हेतु सहिष्णुता क्षमता के संबंध में चिन्हक लक्षण सम्बन्धों के सत्यापन विश्लेषण से पता चला कि डीआरईबी 1 ए, एनएसी 2, एसएनएसी 1 और एलइए 3 नामक चार चिन्हकों ने सूखा-सहिष्णु समूह और सूखा-संवेदनशील समूह के औसतों के बीच सार्थक अंतर दर्ज किया है। भविष्य में गन्ने में सूखा-सहिष्णुता के लिए चिन्हक सहायित चयन हेतु इन चिन्हकों का उपयोग सार्थक सिद्ध होगा।

(आनन्द सिंह जीना)
सलाहकार

(दीपक कौजलगी)
लेखक

