PERFORMANCE OF SUGARCANE GENOTYPES UNDER WATER DEFICIT STRESS

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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MASTER OF SCIENCE
in
PLANT BREEDING AND GENETICS
(Minor Subject: Biotechnology)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **“Performance of sugarcane genotypes under water deficit stress”** submitted for the degree of **Master of Science,** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Rajwant Kaur (L-2018-A-112-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **“Performance of sugarcane genotypes under water deficit stress”** submitted by **Ms. Rajwant Kaur (L-2018-A-112-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science**, in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) has been approved by the Student’s Advisory Committee after an oral examination on the same.

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ABSTRACT

In this study, thirty-three diverse sugarcane (*Saccharum* spp. complex) genotypes were evaluated for drought tolerance. All 33 clones were planted in Randomized Block Design with three-replications under normal (E1), mild water stress (E2) and rainfed (E3) environments. Analysis of variance revealed significant differences among genotypes for all the traits under all environments. High magnitude of PCV, GCV, heritability and genetic advance were recorded for number of tillers (000/ha), number of shoots (000/ha), number of millable canes (000/ha), cane yield and CCS (t/ha) under both stress conditions. Correlation study showed that cane yield had significant positive association with number of tillers (000/ha), number of shoots (000/ha), NMC (000/ha) under all three environments while CMS%, proline and SOD showed significant positive association only under stressed conditions; whereas under normal environment, CCS (t/ha) exhibited significant positive association with brix%, pol%, purity%, CCS%. Regression study revealed that NMC (000/ha) is a main contributing trait for cane yield; whereas cane yield is major contributing trait for CCS (t/ha). Pol% and TSS% played major role for CCS%. Eberhart and Russel regression model analysis revealed that for cane yield, CCS% and CCS (t/ha), 10-genotypes, 12 genotypes and 12-genotypes are stable performing, respectively. GGE biplot analysis showed that genotypes with above average performances *i.e.* F 391/14 (26), CoPb 18181 (16), CoPb 94 (9) and F 301/11 (22) for cane yield; CoPb 18181 (16), CoPb 92 (7), CoPb 14185 (14) and CoPb 18181 (16) for CCSt/ha; F 391/14 (26), CoPb 16181 (15), SA 04-409 (31) and F 3/14 (23) for CCS% were more stable, comparatively. Expression of drought-responsive genes (P5CS, SOD, DEH, BADH, IGS, cAPX, LEA, TPS, PROT and DRP) were significantly higher in cultivar F 391/14 (resistant cultivar); whereas for cultivar CoJ 64 (susceptible cultivar) significant lower expression of genes (SOD, DRP, PROT and BADH) wereobserved.

Keywords: Cane yield, gene expression, physiological traits, sugarcane, water stress.

Signature of Major Advisor **Signature of the Student**

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ਮੁੱਖ ਸ਼ਬਦ: ਪਾਣੀ ਦੀ ਘਾਟ ਤੇ ਤਣਾਅ, ਗੰਨੇ, ਗੰਨੇ ਦੇ ਝਾੜ, ਜੀਨਾਂ, ਪ੍ਰਗਟਾਵਾ, ਫੀਜੀਓਲੋਜੀਕਲ ਗੁਣ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

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LIST OF ABBREVIATIONS

CCS	Commercial cane sugar
CG	Cane girth
CL	Cane length
Cm	Centimetre
CMS	Cell membrane stability
DAP	Days after planting
E1	Irrigated enviornment
E2	Mild water stress environment
E3	Rainfed environment
Env	Environment,
Ext%	Extraction %
GLM	Generalized linear model
Gm %	Germination
Kg	Kilogram
NMC	Number of millable canes
Rep	Replication
RWC	Relative water content
SOD	Superoxuide dismutase

CHAPTER-I

INTRODUCTION

Sugarcane (*Saccharum* spp. complex), an industrial crop for sugar and bio-energy (FAOSTAT 2014), is the world's major C4 crops with complex polyploidy (2n = 40 to 200) nature (Garsmeur *et al* 2018). It grows well in more than 120 countries of tropical and subtropical regions on both sides of the equator (up to 30 ^{O}N and 35 ^{O}S latitudes) (Ram 2017) with ~70 % world's sugar production (Singh *et al* 2017). It is native of tropical South Asia and Southeast Asia (Daniels *et al* 1975); which is world widely used for agro-industrial purposes. Sugarcane has the capacity to store sucrose levels in their stalks up to 50 per cent of dry weight (Botha and Black 2000).

In India, sugarcane is mostly used for the production of jaggery, and processed into sugar and alcoholic beverages. Brazil (9.9 million ha or 1 % of Brazil's cultivable land), followed by India (4.95mha), Thailand (1.52 mha), China (1.40 mha), Pakistan (1.23 mha) and Mexico (0.78 mha) are the major producer of sugarcane. Brazil and India account for 53% of global cane acreage (Ram 2017). Sugarcane was cultivated in an area of 2.61 mha with the production of 126.36 million tonnes at 48.3 t/ha productivity level during 1970-71 in India. However, till 2015-16 an improvement to the tune of 1.88, 2.7 and 1.43 times has been achieved in the area of 4.92 mha, production of 341.4 million tonnes and productivity of 69.4 t/ha, respectively. Besides during this period, sugar recovery at mill level has also increased from 9.78% to 10.62% in India (Annual Report 2017).

In Punjab, sugarcane is being cultivated on 92 thousand hectares with average cane yield 734 quintals per hectare and sugar recovery 9.78 per cent (Anonymous 2018). This improvement has been achieved with the adoption of improved varieties. However, still the sugarcane production is challenged by biotic stresses and abiotic stresses. Abiotic stresses including drought, heat, salinity and water logging are the key culprits of agricultural losses; which, depending on the crop, can reduce the average productivity by 65 % to 87 % (Battisti and Naylor 2009, Feng and Kobayashi 2009, Wassmann *et al* 2009).

Drought is one of the single most environmental stress factors restricting the production of sugarcane worldwide (Venkataramana *et al* 1986). Zingaretti *et al* (2012) described moisture stress in plants as the situation in which water reaches to plant in very tiny concentrations or when there is an extreme rate of transpiration in plants. Extreme drought combined with ensuing flooding and water-logging problems in Eastern U.P., Orissa, Bihar, coastal Andhra Pradesh (Nair 2011) and Maharashtra (Kolhapur and Marathwada) are around 2.13 lakh ha (Swami *et al* 2018). Seventy per cent of the yield as well as the productivity of sugarcane is reduced by the prolonged stress on the water during its lifespan (Gosal *et al* 2009, Morison *et al*, 2008, Swami *et al*, 2018). Stress is accentuated worldwide due to climate change leading to increased aridity in regions across the globe (Riaz *et al* 2010). This problem is accompanied by inadequate/ deficient rainfall in the sugarcane region, together with high temperatures limiting cane development and growth. Thus, the development of sugarcane varieties resistant to drought is important for maintaining production in areas where water supply is limited. The solution to these problems is the “region wise *in-vivo* evaluation and selection” of elite lines. This necessitates the need for specific objectives and methods, because each new objective naturally adds the complexity to the breeding programmes.

Weather and climatic factors i.e. temperature, rainfall, atmospheric humidity, photoperiod and other extreme weather play key role for vegetative growth and reproductive development of sugarcane. It requires high humidity (85% to 90%), high temperature (28 °C to 32°C) and bright sunshine day for best growth and development (Singh *et al* 2017). Water requirement of sugarcane crop varies from 1000 to 2900 mm (Robertson et al 1999) with an average of 2000 mm (Vasantha *et al* 2017). This requirement is enhanced with dry atmosphere and heavy water demand periods. Nearly 50% to 70% reduction in cane yield has been observed with moderate water stress by several researchers (Robertson *et al* 1999, Vinocur and Altman 2005, Arun *et al* 2017).

On an average 15% cane yield lossess per year has been reported in Australia despite the significant irrigation input (Inman-Bamber *et al* 2005). So, water deficit is one of the recurring productivity constraints. India has a wide range of agro-climatic regions; where varieties are required for multitude conditions (from sea level to about 2500 feet) with early maturity and tolerant to major stresses. The potential negative impact of stresses due to climate change, especially temperature and rainfall on sugarcane production, has been reviewed by several researchers (Gawander 2017, Chandiposha 2013, Zhao and Li 2015).

Sugarcane is long duration crop i.e. 10 to 12 months; hence crop suffers during any stage from germination to maturity. Cane yield at 360 days under normal and drought conditions have been utilized for construction of drought indices like Stress tolerance index (STI, Fernandez 1992), Tolerance Index (TOL, Rosielle and Hamblin 1981), Stress Susceptibility Index (SSI, Fischer and Maurer 1978), Yield Stability Index (YSI, Bouslama and Schapaugh 1984), Yield reduction Ratio (YRR, Golestani-Araghi and Assad 1998), Drought Resistance Index (DRI, Lan 1998) and Yield Index (YI, Gavuzzi *et al* 1997). However, selection of tolerant lines on the basis of these indices is generally is not conclusive as different indices identify different genotypes as tolerant. So, to determine the desirable clone according to all parameters of indices, ranking methods are generally followed by estimating the parameters like mean rank (Rm), standard deviation of rank (Rsd), and rank sum (RS). The clone with highest rank (least rank sum) with low Rsd is considered to be most drought tolerant. The cluster analysis based on Euclidian distance is also performed to classify the clones on the basis of drought tolerant indices, cane yield under stressed and non-stressed conditions.

Water stress is a multi-dimensional stress (Jamaux *et al* 1997) causing a variety of physiological and biochemical effects on plant (Singh and Reddy 1980). The morphological and physiological behaviours of sugarcane plants differ depending on the genotype, length (rapid or gradual) and severity of stress (severe or mild) as well as the type of tissue influenced. A variety of physiological studies have established certain characteristics associated with the adaptability of plants to drought-prone conditions. The juice quality deterioration i.e. decreases in sucrose content and increase in reducing sugars is attributed to more production of not fully formed internodes along with more juice weight throughout maturity phase following the release of moisture stress (Singh and Naidu 1985). Reactive oxygen species (ROS) are derivate of metabolic activities of the plant, like photosynthesis and respiration. Their biological activity depends on the number of molecules formed and their removal through cellular scavenging pathways (Mittler *et al 2011*). When plants experience long periods of stress from drought, variations in cellular homeostasis cause ROS outbursts. When ROS synthesis outweighs antioxidant mechanisms then cell membranes, DNA and proteins are damaged; which can lead to cell death (Gill and Tuteja 2010 and Miller *et al* 2010).

In plants, the antioxidant defense mechanism is classified into enzymatic and non-enzymatic scavenging of ROS. The operation of superoxide dismutase (SOD) plays very first step of oxidative ROS scavenging which catalyses the transformation of O−2 into H_2O_2 inside the cell. Activity of SOD in sugarcane is genotype-dependent and is also mediated by drought conditions (Hemaprabha *et al* 2004, Jain *et al* 2014, dos-Santos *et al* 2015). Non-enzymatic antioxidant compounds can function synergistically with enzymatic mechanisms for scavenging ROS to protect plant cells from oxidative harm. Proline is a highly effective OH scavenger. In addition, proline can act as a compatible reserve of osmolytes, molecular chaperones, carbon and nitrogen; and balances cytosolic pH (Verbruggen and Hermans 2008). The concentration of proline supports its role as an antioxidant while other amino acids behave as osmoprotectants during accumulation of sucrose in the stem. Free amino acid aggregation is commonly observed in stressed plants (Patade *et al* 2008 and Pagariya *et al* 2012), elevating osmotic pressure, and thus acting as an osmoregulator (Venekamp *et al* 1987, Molinari *et al* 2004 and Boaretto *et al* 2014). A link between water stress and the antioxidant system response in sugarcane is evident. ROS-scavenging enzyme activity can be used as an indicator of sugarcane water stress tolerance.

In order to manipulating the genetic variability in the production of more resistant cultivars, plant breeders have attempted to understand the tolerance mechanism. Use of such molecular methods has become a useful feature in this context in detecting genes involved in stress response in plants. These techniques can allow only the features of interest to be manipulated by breeders (Rodrigues *et al* 2009). Gene discoveries and genomic tools can help in speeding the genetic improvement of sugarcane. Variations in gene expression like activation and/or suppression of genes occur under abiotic and biotic stresses, and these modifications can be straightforwardly controlled by stress conditions or can result from secondary stresses and/or as a response to metabolic or cellular function injuries. Therefore, a particular biochemical reaction or physiological response to water stress arises from a mixture of previously molecular activities, triggered by the perception of the molecules that signal stress. With the incorporation of genes that are allowed to impart tolerance, crop plants may be genetically engineered, thus maintaining crop production under adverse conditions. This illustrates the relevance of researching a species multiple genotypes to help understand the gene profile involved in stress response.

At present, no fully resistant clone to water stress has been suggested or released. There is always the stress on growers to produce sugarcane with less amount of irrigation. With this kind of lacuna, researcher's main motive is to find out the morphological, physiological, biochemical as well as molecular characteristics; which can be exploited for developing tolerant cultivars (Domaingue 1996). By keeping all these points in consideration, the present study entitled "Performance of sugarcane genotypes under water deficit stress" was begun to screen diverse sugarcane (*Saccharm* spp. complex) genotypes for drought tolerant traits with the following objectives:

i) To assess the impact of water deficit stress on growth and physiological parameters of sugarcane,

ii) Identification of drought tolerant sugarcane genotypes through different morpho-biometrical analyses and

iii) Molecular characterization of sugarcane genotypes for drought tolerance.

CHAPTER-II

REVIEW OF LITERATURE

Sugarcane (*Saccharum* spp. complex) is a 10-14 months crop and having complex genome (Mukherjee 1957, Garsmeur *et al* 2018). Both biotic and abiotic stresses affect its productivity at different stage of the crop in one or more of the following ways: (i) by adversely affecting cane and sugar yield per unit area and time, (ii) by eliminating some of the outstanding varieties from commercial cultivation, and (iii) by discouraging multiple ratooning in areas where it can be practiced. Among abiotic stresses, water stress is the major constraint, which adversely affects the crop productivity. Drought stress is a foremost abiotic epidemic; it's widely spread over 1.2 billion ha worldwide in rainfed agricultural land (Chaves and Oliveira 2004, Kijne 2006, Passioura 2007) and not only causes disparities between the mean yield and the potential yield but also causes year-to-year fluctuations (yield instability). So, for this, drought tolerant varieties are the effective solution for this constraint.

Singh and Rao (1987) stated that 70%–80% of cane yield is manufactured during formative phase and water deficit during this phase affects cane production negatively (Venkataramana *et al* 1986). The "formative phase" of sugarcane, a combination of tillering and grand growth phase, has been recognized as critical crop stage which requires optimum soil water availability (Ramesh 2000). Sugarcane has been estimated to need around 20,000 kilo litres of water/ ha for cultivation in India (Bhattacharya 2010). Lysimeter studies have found that a sugarcane crop needs 88–118 kg of water/ kg of cane and 884–1157 kg of water/ kg of sugar produced, respectively (Shrivastava and Solomon 2011). A number of plant processes that occur at genetic, biochemical, cellular, and whole-plant level can be altered depending on the length and degree of drought stress. Drought tolerant genotypes can be identified by imposing drought condition during the growth stages, and by studying soil water relations to the photosynthetic responses (Silva *et al* 2007). Such studies have revealed that cultivars susceptible to drought are wilted early and showed less cane production, while tolerant cultivars are remained turgid for longer duration and possess near-optimal growth (Moore 1987); that entail detection of drought tolerant cultivars.

Effect of water deficit stress on field quantitative and quality traits

Crop plants are often exposed to various environmental factors, such as drought, high temperatures, and salinity, which limit sugarcane growth and affect productivity (Tas and Tas 2007). When exposed to water deficiency, plants possess physiological, biochemical, and molecular responses at both cellular and whole plant levels (Hasegawa *et al* 2000). According to Venkataramana (2003), more harmful is the tension of moisture associated with high temperatures. In many ways, drought shows its symptoms. Morphological changes are the first signs that are seen as having an effect on sugar when cane grown in drought and waterlogging. Drought has negative effects on the parameters of development. Reduction of tillering, discoloration of the leaves, rolling of the leaves, folding and shredding of the leaves are some of the morphological symptoms in sugarcane that are subject to drought (Gomathi *et al* 2018, Kariniki and Sahoo 2019). Some other characteristics of drought-affected canes are reduced leaf area and narrower leaves as well as decreased lipid peroxidation (Shrivastava and Srivastava 2006). Under water moisture stress, there is decline of assimilation of CO2 in sugarcane, that results into reduction in the photosynthetic activity which in turn reduces dry matter and yield (Alexander 1973).

Water stress affects germination, cane elongation, tillering and yield as according to Rao (2000). They also stated that the total output of dry matter, sucrose accumulation, photosynthate translocation, enzyme activation is also influenced. Ramesh and Mahadevaswamy (2000) found that water stress induces greater tillering reduction when it happens during the formative phase of the stool i.e. 60 to 150 days after sprouting.

According to Sugiharto (2004), the water deficit has a strong effect on tillering and height of the culm. The growth of tiller in sugarcane is the major sink for photosynthates while leaf canopy supported by the tiller population creates the potential photosynthate source. Factors influencing the tiller population also affect light interception and cane production. As a result, water (Inman-Bamber and Smith 2005) and genotype (SASEX 2001) are among these factors which play an important role. Inherent characteristics of that type are the number of tillers. The same numbers of tillers are not produced by the varieties even under the same environmental conditions and input (Hossain and Rahman 2009).

Kamat and Singh (2001) recorded high heritability and genetic advancement values as a percentage of the mean for tillers, leaf area, and cane height under moisture stress conditions, while moderate to lower values for cane girth. Kumar *et al* (2001) recorded high values for the cane girth and internodal length in a study with a clonal population under water stress.

Robertson *et al* (1999) recorded that the water deficits imposed during tillering had significant effects on the area of the leaf, tillering and development of biomass; but the final yield was impacted little. In many species, the amount of water consumed by a crop is closely related to photosynthetic behavior, dry matter development and yield (Qing *et al* 2001). Venkataramana (2003) and Vasantha *et al* (2005) documented decreases in root growth, tillering, leaf area, elongation of the leaf, cane, girth, and extension of the stalk due to moisture stress. Koonjah *et al* (2006) recorded a lower accumulation of biomass in the water-stressed cane due to reduced interception of light, plant extension rate, and photosynthesis rate.

Endres *et al* (2018) reported a decrease of 29.70 % and 27.00 %, respectively, in water stress during tillering and extreme growth process of SP 79-1011 and RB 855536 in cane and leaf length. Misra *et al* (2020) concluded the state of water deficit is prolonged, changes in leaf width have also been noted, suggesting that the plant is tolerant to such a state.

Domaingue (1996) also observed a marked reduction in the height of the cane and the mean length of internodes, indicating that the reduction in the elongation of the cells was greatly influenced by water stress. In the early growth phase, Silva *et al* (2008) did not observe the effect of the water stress regime on stalk diameter and brix, although the number of stalks and stalk heights during the initial growth phase was affected by the water stress. According to Raja *et al* (2006), millable cane length and amount of millable cane at harvest were found to be better indices for the recognition of clones with moisture stress tolerance.

Soares *et al* (2004) found that stalk height was the most affected biometric attribute by water stress in two genotypes; thus, irrigation had a beneficial impact on the height of stalks in plant cane as well as first ratoon. Manivannan *et al* (2007a) stated that the decrease in cell expansion and the increase in leaf senescence in the plant under water stress contributed to a decrease in plant height. Silva *et al* (2008) and Inman-Bamber and Smith (2005) showed that the height of the cane could be affected by drought. Due to drought treatment during the formative stage, Venkataramana *et al* (1986) reported a decrease in cane yield and in the number of millable canes.

Rajeshwari *et al* (2000) mentioned that the height and length of the millable cane were directly impacted by the stress of soil moisture relative to the number of internodes. Silva and Costa (2004) reported that the highest height values were always correlated with the most efficient genotypes under drought conditions, and the lowest efficient genotypes showed the lowest height values for stalks.

There are many reports showing physiological and morphological alterations such as the potential for leaf water, stomatal conductance, leaf area, and sugarcane productivity in response to drought stress, which are used as a possible and quick tool to test for drought tolerance (Silva and Costa 2004, Inman-Bamber and Smit 2005, Smit and Singels 2006, Silva *et al* 2007). Hemaprabha *et al* (2012) also found that the internode number decreased in water deficit conditions in most varieties such as Co 99008 (33.35%), Co 86032 (45.76%), while the internode number also decreased in only a few.

Silva *et al* (2008) had shown that under the stress of drought, the effect on the stalk diameter depends on the genotype. Meena *et al* (2013) observed that during the formative process, seven sugarcane cultivars were subjected to water deficiency, and genetic variability was observed for different growth, yield, and quality characteristics. Four cultivars possess better growth characters (Tillage and NMC/ ha), while three genotypes reported better yield for cane and sugar under induced water deficit. Genotype CoVC 99134 performed better at cane harvest (with 20.10 per cent juice brix, 18.83 per cent juice sucrose, and 12.99 per cent CCS. Genotypes CoVC 99134 and CoVC 99263 offer a potential for better results under minimal moisture conditions.

Hemaprabha *et al* (2013) assessed 28 elite sugarcane hybrids and 165 progenies, for sugar yield characteristics and other biochemical contributory attributes under normal and drought conditions. The genotypes displayed a higher decline in internode length (45.76%), single cane weight (25.5%), NMC (22.8%), cane diameter (20.10%), and sucrose content (18.73%), while the cane diameter (2.92%), internode number (3.58%) and Brix (-6.99%) displayed slight variability in normal and drought conditions.

Misra *et al* (2020) investigated a study of morphological losses under drought and waterlogging. Cane height and stalk diameter decrease by 18.28%, 7.52% , respectively, in drought-affected canes as opposed to normally grown canes. In drought-affected canes, the number of internodes was reduced more than in waterlogged canes. This study revealed that sugarcane has higher morphological losses in drought conditions compared to waterlogging conditions in comparison to normal grown canes.

Ramesh (2000) performed a field experiment at Coimbatore using four commercial sugarcane varieties examine the influence of three levels of water stress over the growth determinants. The decrease in dry matter content was respectively 60.8%, 52.4%, and 25.9% in extreme drought and 46.3%, 36.3%, and 15.1% in mild at the ends of the formative, grand growth, and maturity phases. Leaf area index and leaf area length under drought and crop growth rate, and stalk elongation rate under normal irrigated conditions, especially during the formative phase, may help to determine the total dry matter at harvest.

Silverio (2017) carried out experiments corresponding to seven varieties of sugarcane and five forms of soil water stress. The varieties RB073036 and RB073040 reveal longer stem lengths, linked with the greater diameter and higher water stress, and they show tolerance to the moderate water deficit. The variety RB92579 has a bigger diameter, with its longer length and higher water tension, as well as the variety RB855536. Both show no correlation with the other variables and indicate water deficit susceptibility.

Three varieties namely NC0376, Nl2, and N14 were assessed by Inman-Bamber *et al* (1988) under two water conditions. During the stress times, the dry matter and sucrose compositions of the stalk were reported to enhance significantly, and it seemed that moderate levels of stress could lead to a significant rise in the yield of sucrose. Significant decrease under moisture stress condition over the normal condition in cane yield, number of millable canes, CCS yield, and CCS% and mean cane weight was reported by Pawar *et al* (2006).

Ninety seven genotypes with high sugar content were assessed by Hemaprabha *et al* (2004) under the water deficit status and stated that cane yield was affected by the drop in single cane weight (64.16%) primarily by the reduction in cane height (48.79%); but on the other side juice quality traits was affected less in the condition of drought..Hemaprabha *et al* (2006) also evaluated the performance of sugarcane clones grown under a moisture-stress environment. Under moisture stress conditions for the improvement of quality, CoC 671, Co 740, Co 775, Co 6304, Co 6806, Co 7201, and Co 775 were recorded as valuable parents. They proposed that the use of these parents could contribute to the evolution of drought genotype varieties in the water shortage regions of India to boost sugarcane productivity.

Garcia *et al* (2020) performed an experiment under different water environments by using two cultivars, RB835486 (Tolerant) and RB855453 (Susceptible). The resistant genotype, however, displayed an increased reduction in leaf sugar content, while the susceptible genotype had increased non-photochemical quenching (qN). Multivariate analysis showed that reduction of leaf sugars was physiological parameters necessary for achieving homeostasis under conditions of water deficit

Effect of water deficit stress on physiological and biochemical parameters

Relative water content (RWC) is a typical measure of the condition of the plant water. RWC is the water content (percentage) relative to the water content of the same tissue, and the relationship depends on the species and growth stages, as well as the long-term environmental changes. RWC is a measure of the tissue water status and is in some cases explicitly related to the capacity of leaf water (Rahman *et al* 1999). Even Barrs (1968) mentioned that relative water content (RWC) under stress could be used as a measure of stress resistance.

Studies in other crops had indicated that leaf RWC and/or proline content are useful indicators for early detection of plant water-deficit stress (Bates *et al* 1973, Claussen 2005, Gonzalez *et al* 2008, Umebese *et al* 2009, Paknejad *et al* 2009). According to Bayoumi (2008), Nayyar and Gupta (2006) stated that leaves exhibit significant reductions in RWC and water potential when leaves are subjected to drought. Wahid and Close (2007) found that *Saccharum officinarum* exhibited a decline in RWC under higher temperature conditions.

Verma (2001) reported on the significance of RWC and leaf sheath moisture in 3rd to 6th leaf during the formative phase. Silva *et al* (2011) and Cia *et al* (2012) have also recorded a steady decline in RWC (ranging from 80 % to 60 %) in susceptible sugarcane cultivars subject to water deficit. These findings have already shown that higher RWC values are strongly indicative of tolerance in sugarcane cultivars during water shortages (Dossantos 2015).

The pressures of water have a detrimental effect on the cell membrane by disrupting its structural integrity in several respects. The damage to intracellular membranes can detrimentally affect the respiratory chain in the mitochondria, carbon fixation in the chloroplast, and cause chlorophyll degradation; while damage to the plasma membrane is related to the release of electrolytes and cell death. Cell membrane helps the plant adjust the stress environment, so the stability of the membrane implies a critical plant character under water stress pressure (Kumar 2019).

Blum *et al* (1981) documented the outflow of electrolytes through cell membrane, which regulates plant drought tolerance. Younger leaves exhibited lower injury values than older and younger leave tissue are more tolerant to drought than older (Blum 1981).

Venkataramana *et al* (1986) found that cellular membrane injury increased during stress, and increase of the membrane from 30.8% to 70.9% was associated with a decrease in leaf water potential from -0.97Mpa to -1.91Mpa. Tyagi *et al* (1999) reported that parameters relative water content, membrane stability, osmotic potential and ABA content could be used for identifying drought-tolerant genotypes.

Tripathy *et al* (2000) found that sugarcane varieties with lower membrane injury are relatively more tolerant to drought at the cellular level. Deshmukh and Kushwaha (2002) evaluated the possibility of using simple traits like relative water content (RWC) and membrane injury index for screening 20 genotypes for drought tolerance.

Quan *et al* (2004) found higher electrolyte leakage in drought-stressed maize plants than in plants grown under control conditions. The RWC and MII of a genotype measured during the early phase were found to provide an indication of its relative MII during reproductive stages. Osmolytes assist in protecting proteins and membranes from damage due to high concentrations of inorganic ions and oxidative damage under drought stress and multiple stresses, such as drought and salinity (khan *et al* 2015).

Augustine *et al* (2014) conducted a study to explore the *Erianthus* spp's physiological and molecular responses to the tension of drought. IK 76-81 has been found to be characterized by high thermostability of the cell membrane, stomatal conductance, transpiration rate, and relative water content under increased stress on soil moisture. Being a genus that is cross-compatible with cultivated sugarcane, the identification of abiotic stress-resistant accessions in *Erianthus* is beneficial for the sugarcane crop improvement project by introgression breeding.

Begum (2012) conducted a study examining the effects of drought stress at different levels with a view to study the physiological characteristics of sugarcane correlated with juice quality in drought. In drought treatments, the physiological parameters viz., total chlorophyll, chlorophyll stability index, leaf area index and relative water content, and biochemical parameters viz., brix, pol, purity, and sugar reduction were reduced. In terms of physiological adaptation linked with juice quality, genotypes I 95-01 and Isd 36 were determined to be better than other genotypes.

Silva *et al* (2011) observed that under water stress conditions, four physiological markers, PSII photochemical efficiency (Fv / Fm), SPAD unit, LT, and relative leaf water content (RWC) were identified to be correlated with drought in sugarcane. Under water deficiency, two drought-tolerant genotypes, HOCP01-523 and TCP89-3505, showed higher Fv / Fm, SPAD and RWC values and lower LT values, and could be categorized as tolerant.

Graca (2010) conducted an experiment to assess several physiological parameters in drought-tolerant and sensitive cultivars to evaluate the processes involved in the sensitivity of sugarcane plants to water deficit. In all cultivars submitted to water deficit, the photosynthetic rate and stomatal conductance dropped significantly. During the dry time, the Cultivar CTC 15 displayed the highest relative water content. In general, the tolerant cultivars exhibited better photosynthetic output under water-stressed conditions than the sensitive cultivar

Radha Jain (2014) investigated the impact of short-term water deficit on the physiological traits and expression of superoxide dismutase (SOD) and genes P5CS (pyrroline-5-carboxylate synthetase) using two varieties of sugarcane. CoLk 94184, exhibited higher aggregation of proline content with extreme wilting compared to variety BO 91. The quality of RWC, chlorophyll, carotenoids, and reading of SPAD decreased with water deficit levels. Higher expression of SOD and P5CS genes up to a certain water deficit level (48 h), proline content, and antioxidant enzyme activity (SOD and peroxidase) contribute to water deficit tolerance condition.

Kumar *et al* (2019) observed lowest percentage reduction of RWC in genotype Co 98014. Four other genotypes i.e. Co 0118, Co 0238, Co 05011 and CoPk 05191 also showed a lower reduction of RWC, comparatively, suggesting their resistance to water deficits under water stress of 30 days. The largest per cent reduction of RWC in genotypes CoJ 64, CoP 03220 and CoJ 85 indicates that these genotypes are more prone to water stress. The maximum percentage of MSI reduction was observed in the Co 89003, CoJ 64 and CoP 3220 genotypes. The highest accumulation of proline up to three-fold increament was found in the sugarcane genotypes Co 98014, and around two-fold increament in proline content were also reported in four other genotypes studied i.e. Co 0118 followed by CoPk 05191, Co 05011 and Co 0238 indicating their higher level of tolerance to water stress.

This research was carried out by Ribeiro *et al* (2013) to investigate the physiological reaction of sugarcane genotypes to drought and its effect on stalk yield. IACSP94-2094 and SP87-365 were considered to be drought-resistant genotypes since the production of stalk dry matter and the yield of soluble solids were not affected by the water deficit. In general, a decreased photosynthetic sensitivity to water deficit was an important physiological feature for the development of dry matter in sugarcane plants.

Zhao *et al* (2013) found that water stress decreased leaf relative chlorophyll level (SPAD), stomatal conductance (gs), leaf net photosynthetic rate (Pn) and transpiration use efficiency (TUE) of photosynthesis resulting in reduced shooting biomass. They concluded that physiological and growth characteristics such as SPAD, gs, Pn, Tr, TUE, GLA, tillering, and stalk length can be useful for early identification of water stress and for assessment of sugarcane genotypes in stress tolerance.

Proline is one of the essential organic osmolytes accumulating in different species of plants in response to environmental stresses such as drought, salinity, UV radiation, heavy metals and high temperatures (Verbruggen and Hermans 2008). Osmoprotectants are molecules that play a significant role in cell osmotic adjustment and are used in breeding programs to screen varieties that are tolerant to water deficiency (Chaum and Kirdmanee 2008 and Bartels and Nelson 1994). Nonetheless, biochemical and physiological studies of transgenic sugarcane improved with D1pyrroline-5-carboxylate synthetase indicate that the increased proline biosynthesis was more related to scavengers of reactive oxygen species than to osmoprotectors.

In sugarcane, proline aggregation and photosynthetic activity were used as efficient markers for selecting cultivar resistant to drought. The rise in proline under stress conditions may be caused by the stimulation or activation of enzymes engaged in its biosynthesis. This amino acid is the only molecule among many compatible solutes that have been proved to protect plants from damage caused by single oxygen and free radicals (Kishor *et al* 2005).

Proline plays an important role in stabilizing protein structures, DNA, membranes, and subcellular structures against denaturation (Kishor *et al* 2005). Additionally, this amino acid is related to a variety of other roles such as plasma membrane defense and macromolecule integrity (Silveira *et al* 2004) as well as a carbon and nitrogen source (Gupta and Huang 2014).)

It has been shown in several plants that proline increases proportionality faster than any other amino acid in water-stressed plants, and is an assessment parameter for selecting drought tolerance (Bates *et al* 1973). The increased concentration of proline in stressed plants may be an adaptation to overcome the stress condition, providing constant energy for survival and growth by acting on the osmotic change, helping to regulate the entry and exit of water in cells, cytoplasms, and vacuoles (Jaleel *et al* 2009).

In sugarcane under water stressed conditions the enhanced accumulation of proline in varieties indicates that such amino acid contributes to balanced ROS in leaves (Dossantos *et al* 2015). More proline accumulation in drought-resistant barley plants (Singh *et al* 1972), wheat, sorghum (Blum and Ebercron 1976) had been documented by many earlier investigators. Tuteja (2007) reported that higher proline accumulation in stressed environments might be an adjustment to overcome stress-related tension by providing constant energy for survival and development through osmotic adaptation.In sugarcane, Abbas *et al* (2014) observed a positive correlation between proline increase and drought stress tolerance when evaluating thirteen varieties under drought stress caused by greenhouse polyethylene glycol, distinguishing the most tolerant genotypes.

Cha-Um and Kirdmanee (2008) found similar findings in *S. officinarum* in the presence of mannitol and by Patade *et al* (2012) in the callus cultures of *S. Officeinarum* cultivar Co86032 with a 20 per cent polyethylene glycol content. Some authors, however, support the hypothesis that accumulation of proline is a symptom of stress rather than an adaptive property (Rampino *et al* 2006).

Yadav and Khare (1995) found that proline content of leaves and seeds in chickpea was higher for rainfed than for irrigated conditions. They also recorded higher content of leaf proline at the vegetative phase being correlated with high yield under rainfed conditions. The degree of resistance to the moisture stress has been positively associated with the proline level.

Zhang and Qiung (1998) found an increase in the proline content of sugarcane varieties leaves with an increase in the tension of the moisture. Once exposed to various abiotic pressures, many plant species naturally produce proline as the primary organic osmolyte. In pea cultivar, proline content increased under the stress of drought (Alexieva *et al* 2001). Raheleh *et al* (2012) reported that, in resistant genotypes of chickpea, drought stress significantly increased the content of proline.

Rizvi *et al* (2014) studied moisture stress tolerance in different genotypes of chickpea (Cicer arietinum L) and reported that RWC and chlorophyll, carotenoid and protein content decreased as a result of moisture stress, while proline content increased as moisture stress increased. Accumulation of amino acid proline was reported and was to be used as a physiological-biochemical indication of water stress (shao *et al* 2006) in order to associate an increase in concentrations with higher drought tolerance in sugar cane and wheat (Molinari *et al* 2007, vendruscolo *et al* 2007).

The word antioxidant can be regarded as representing any compound capable of extinguishing ROS without conversion to a harmful radical. ROS is extremely cytotoxic and can react with specific metabolites that cause oxidative damage to enzymes, proteins, lipids, and nucleic acids. Plants have the potential to counter oxidative stress by using ROS – removing mechanisms such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX). Various enzymes of antioxidant protection systems regulate the production of ROS (Karuppanapandian *et al* 2011).

SOD is the first enzyme in detoxification processes as a metalloenzyme that transforms O2- into H_2O_2. Plant's ability to overcome stress conditions depends on the efficiency and speed at which they perceive stress, produce signal molecules and trigger stress-protective mechanisms (Pasternak *et al* 2005).

Increased activity of antioxidant enzymes in sugarcane has been observed. The enzymatic pathways are assigned to reduce radical superoxide and hydrogen peroxide concentrations (Sairam and Tyagi 2004). In a broad range of plant species, the behavior of SOD, APX, and CAT under drought stress induced by tissue culture techniques has been documented.

Shehab *et al* (2010) studied rice plants under water stress and concluded that the double increase in enzyme activity is a clear indicator that oxidative stress was directly caused by the treatment of drought. Antioxidant enzyme activity is associated with the sugarcane variety tolerance system, and the tolerance behaviour has been established with higher antioxidant enzyme activity.

According to Cia *et al* (2012) increased SOD activity in sugarcane under drought can be considered as proof of tolerance, while decreased activity can suggest vulnerability to drought. The relation between the rates of SOD activity and tolerance for sugar cane drought was previously observed.

Gomathi and Rakkiyapan (2011) found that SOD activity appeared to increase in the resistant cultivar when studying sugar cane under salt-induced stress. Plants with high levels of antioxidants were documented to have greater resistance to oxidative damage, either constitutive or induced (Moussa and Abdel-Aziz 2008). Tohidi-Moghadam *et al* (2009) documented a substantial increase in SOD, CAT, and GPX activities in canola leaves for plants under drought stress.

In Maize, Ali and Pessarakli (2010) noted that the stress from drought significantly increased the activity of superoxide dismutase (SOD). Dossantos (2015) revealed that oxidative stress in cultivars can mostly be prevented by maintaining equilibrium of antioxidant enzyme activity, photosynthetic effectiveness, stomatal balance, and water status. Stressed conditions have resulted in increased production of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes for most cultivars, confirming the significance of these enzymes interacting against oxidative stress damage.

Oliveira (2018) carried a study and observed that variation was present between species, and even within the same species of sugarcane when subjected to *in vitro* drought stress, and *S. officinarum* has proven to be the most tolerant. Proline can be used in sugarcane accessions as a biochemical predictor of the response to drought, and its accumulation in *S. robustum* and *S. spontaneum* accessions has been increased.

Hemaprabha *et al* (2013) observed under stress in the genotypes and progenies, proline aggregation, and superoxide dismutase and peroxidase activities become nearly doubled. Based on all of these observations, they grouped 165-progeny population into five groups with varying drought tolerance rates that followed a standard distribution curve.

Relative gene expression analyses of sugarcane under water deficit stress

In most breeding programs, genetic improvement for drought tolerance is achieved by yield selection, and traditional breeding methods are slow due to low yield heritability under stress and the spatial as well as temporal variability in the field climate. Molecular markers should promote the more successful production of drought-resistant genotypes, as their expressions are independent of environmental effects (Mitra 2001). Molecular markers have been considered tools for a variety of purposes ranging from the localization of a gene to the enhancement of plant varieties by marker assisted selection. The insufficient knowledge of the molecular and genetic processes involved in water stress tolerance and its quantitative background is a key challenge for the production of tolerant cultivars, despite significant advances in sugarcane breeding.

Identification and knowledge of genes and signaling networks is essential in sugarcane, for the production of new cultivars with increased tolerance in moisture-deprived conditions is important for overcoming drought conditions (De Andrade *et al* 2017). Quantitative PCR (qPCR), commonly referred to as real-time PCR, is an analytical approach that has revolutionized gene expression analysis exploration (Granados *et al* 2017). Higher sensitivity, real-time detection of transcripts, speed of analysis and reproducibility to acquire a gene expression profile are some of the benefits of using qPCR. In the literature, in different species such as rice, maize, wheat, sorghum, wheat and sugarcane, multiple normalization approaches under water stress have identified reference genes (Jain *et al* 2006, Lin *et al* 2010, Janska *et al* 2013, Aglawe *et al* 2012, Melloul *et al* 2014, Guo *et al* 2014, Ling *et al* 2014 and Silva *et al* 2014).

Recently, to find out genes and molecular markers associated with stress behaviors, several approaches and technologies, such as the serial study of gene expression is often used and the subsequent genetic modifications of these genes will be more useful for refinement of breeding. To describe these stress-related molecular pathways, the regulatory study of hundreds of genes related to stress is a key step. Because of the complexity of the sugarcane genome, studies of this crop are being improved by collections of ESTs, which promote the identification of genes and the study of their functions.

Molecular studies have identified a variety of genes that exhibit in sugarcane under conditions of water stress (Iskandar *et al* 2011; Rodrigues *et al* 2011; Li *et al* 2018). Rodrigues *et al* (2009) assessed 3,575 ESTs in a drought-tolerant cultivar of sugarcane and discovered 165 genes that differentially expressed, representing a significant number of genes linked with water stress tolerance. A specific physiological response to water stress results from a combination of previous molecular events, activated by the perception of the stress signalling molecules.

Isikander *et al* (2011) performed assays to comparing GAPDH, β-tubulin and β-actin as reference genes for RT-qPCR analysis of sugarcane, in addition to the widely used 25S rRNA gene. Expression levels of 25S RNA and GAPDH were higher and more consistent across tissues while GAPDH seems to be the strong protein-coding reference gene.

A study conducted by Hemaprabha *et al* (2012) stated that, during the formative phase of development, drought-resistant and susceptible progenies involving a resistant cultivar (Co 740) with a susceptible one (Co 775) were subjected to drought and by means of

RT-PCR analysis, differential expression of seventeen drought reactive candidate genes was examined. Specific activation in resistant clones of ten genes suggested their function in the tolerance of sugarcane to dehydration.

Tripathi *et al* (2018) showed that the variety CoPk 05191 exercised stronger drought safety over CoLk 94184 because certain physio-biochemical parameters were more desirable in order to escape the adverse effect of drought. This diversity has also been accentuated by increased expression of pyrroline5-carboxylase synthetase, betaine-aldehyde dehydrogenase, proline oxidase, and proline transporter genes under stress to perform better under stress conditions.

Augustine *et al* (2014) conducted a study to explore molecular response of *Erianthus* spp. to tension of drought. IK 76-81 also showed increases in DREB2 and expansin gene expression compared to the most common moderately drought-tolerant variety Co 86032. Being a genus that is cross-compatible with cultivated sugarcane, the identification in *Erianthus* of abiotic stress-resistant accessions is beneficial for the sugarcane crop improvement project by introgression breeding.

Water stress is a multi-dimensional stress (Jamaux *et al* 1997), causing a variety of physiological and biochemical effects on the plant (Singh and Reddy, 1980). The juice quality deterioration i.e., decreases in sucrose content and increase in reducing sugars is attributed to more production of not fully formed internodes along with more juice weight throughout the maturity phase following the release of moisture stress (Singh and Naidu, 1985). Gene discoveries and genomic tools can help in speeding the genetic improvement of sugarcane. DNA marker technologies have now reached at the point where major practical impacts are occurring in advanced breeding programs. With molecular markers, one can screen, select and make targeted recombination of desired genotypes. Plant breeders have aimed to understand the tolerance process in order to manipulate the genetic variability in the development of more tolerant cultivars. In this context, the use of molecular techniques has become a valuable tool in identifying genes involved in plant responses to stress. These techniques may allow breeders to manipulate only the characteristic of interest (Rodrigues *et al* 2009)

Genetic variability, Correlation, Eberhart & Russel regression stability, and GGE biplot analyses

Genetic variability and heritability are two valuable parameters that can assist the breeder at various stages of crop improvement. A population with a high degree of diversity offers opportunities for selection to generate a genotype of desirable traits (Arya *et al* 2013). To begin efficient breeding programs, it is important to estimate and research genetic variation and mode of inheritance in various physio-biochemical variables. In crop improvement breeding plans, heritability (in the broad sense) is critical. In a broad way, the coefficient of heritability is the proportion of total variance that is heritable (Lush 1940). If selection is practiced for the specific character, it indicates the repeatability of results. The most meaningful measure is genetic advance, which is the increase in genotypic value in the new population over the base population (Ebid *et al* 2015)

Koujalagi *et al* (2018) found that total carotenoids, Chl a, Chl b, Total chlorophyll, Proline content, RWC per cent, and Leaf firing all had a high degree of PCV with a mild GCV. High genetic advance and heritability was documented for parameters like proline content, total carotenoid, total chlorophyll and leaf firing.

Tadesse *et al* (2014) stated that existence of high PCV and GCV for various traits enables a breeder to practice successful selection based on these traits, as their phenotypic expression might provide a good indication of genotypic opportunity. Low GCV suggested that there would be less heterogeneity and manipulating these characteristics by plant breeding is more challenging.

Kashif and Khan (2007) found significant heterogeneity as well as high broad sense heritability in sugarcane for various quantitative traits such as cane height, internodal length, number of tillers per plant, number of leaves per plant, leaf area, cane girth, cane weight, sucrose content and brix value.

For effectual selection towards cane yield improvement in sugarcane, there is need of sound knowledge about the nature of relationship between cane yield and related traits. Natarajan *et al* (2020) conducted a study and found that among yield component characters studied, stalk number, displayed positive significant association with cane yield under rainfed conditions but not in other treatment.

Cane yield also exhibited positive correlation with stalk girth, and number of leaves across all irrigation conditions. Aitken *et al* (2008) also reported high genetic correlation between yield and girth, weight, and stalk number under normal water environment.

Silva *et al* (2008) found linear association between productivity and its yield components, but among cultivars instability for stalk girth was observed. Significant association was observed of cane height with number of canes, cane girth and cane weight. Cane girth was also positively correlated with cane weight.

Silva and Costa (2004) also observed significant positive association among productivity and cane height and cane weight, but a modest positive association was observed between productivity and number of canes, and no significant association to productivity against cane girth.

Bissessur *et al* (2001) did not discover any association between cane height and cane girth for both wet and dry conditions. Therefore, special concern should be given to number of canes and cane height for cane yield selection.

Kumar *et al* (2014) found cane yield was significantly positively associated with characters like germination (%), number of shoots, NMC, cane girth, cane weight and cane length while negative association of Cane yield was observed with brix in juice throughout all the stages of record.

According to Dias *et al* (2009) the method proposed by Eberhart and Russel (1966), classifies the genotypes for adaptability using the regression coefficient as a reference. It is a methodology based on simple linear regression and, therefore, genotypes with $\beta 1 = 1$ have general adaptability, $\beta 1 > 1$ specific adaptability to favorable environments and $\beta 1 < 1$ specific adaptability to unfavorable environments. The ideal genotype is one that has high productivity, $\beta 1 = 1$ e $\sigma^2_{di} = 0$.

The genotype main effect plus genotype-environment interaction (GGE) biplot model uses multi-region data to evaluate the environment and offers a useful graphical representation. GGE biplots can help researchers quickly identify complex GE interactions in breeding line trials. The GGE biplot is often used to determine crop cultivar performance in multiple stress environments, optimal cultivars, mega-environment and core testing sites. It has also been effectively utilized in crop trials including soybean, wheat, cotton, sunflower, and sugarcane (Zhou *et al* 2011, Chang *et al* 2010, Xu *et al* 2010, Sun *et al* 2010 and Ramburan *et al* 2012).

The consistency of a target environment when particularly compared to other environments or the mean of all test environments, is referred to as the representativeness of a test environment (Yan *et al* 2011a). The angle between the test environment vector and the average environment coordination in a GGE biplot indicates the representativeness of a target environment (AEC). The abscissa of the AEC is a single-arrowed line (Xu *et al* 2012). Cultivars chosen from optimal environments are more likely to have excellent mean performance in all or most of the test areas as well as greater adaptability.

Previous studies have found that comparing the discrimination power and representativeness of all the regions tested can help identify a desirable region for a cultivar. Yan *et al* (2011b) analysed the mega-environments and test-locations for oat in Quebec using GGE biplot. They discovered that the oat-growing areas of Quebec can be separated into two separate mega-environments.

Natarajan *et al* (2020) concluded that on the basis of GGE biplot, commercial genotypes Q208 and KQ228 had higher cane yield in both rainfed and irrigation conditions. While the commercial clones selected in normal conditions had the inherent ability to perform well under moisture-stress situations, this does not exclude the existence of chief water-stress adaption characters in another germplasm.

Gomathi and Kohila (2018) with water deficit experiments found that AN, CI, GS, TSS, POD, SS, NR and Chl A (assembled together with positive loading on the right upper side of the biplot) parameters had a high positive association among themselves whereas, total

Chl, CSI, SOD, RWC, NI, CHL B, SPAD, SPS and PRO (assembled on the right lower side of biplot) were detected, and indicating that these parameters had a positive association among themselves. While on the left upper portion of the biplot, LP and MII were detected indicating that these traits had a highly negative and significant association among themselves.

CHAPTER-III

MATERIALS AND METHODS

The present research entitled “Performance of sugarcane genotypes under water deficit stress” was carried out at experimental area of PAU, Regional Research Station (RRS) Faridkot. The Experimental site is located at of 31.38°N longitude and 75.38°E latitude at an elevation of 225 m above mean sea level (amsl) having loamy texture soils with pH of 8.3-8.7. Experimental materials and methodologies used in different experiments are presented in this chapter.

Experiment No. 1

Morpho-physio and biochemical evaluation of sugarcane (*Saccharum* spp. complex) genotypes under water deficit stress

Experimental plant material: The experimental material consisted of 33 diverse clones of sugarcane comprising nine commercial varieties released for Punjab state, eighteen elite selections and six ISH/ IGH clones collected from SBI, Coimbatore as listed popular cultivars, advanced breeding lines and interspecific/ intergeneric lines, respectively:

Sr. No	Genotypes and their parents
1.	Co 0238 = CoLk 8102 × Co 775
2.	Co 0118 = Co 8347 × Co 86011
3.	Co 64 = Co 976 × Co 617
4.	CoJ 85 = Q 63 × CoJ 70
5.	CoJ 88 =CoJ 82315 × Co1148
6.	CoPb 91 = CoH 110 × ISH 69
7.	CoPb 92 = Co 89003 PC
8.	CoPb 93= Co 1158 GC
9.	CoPb 94= Co 1148 GC
10.	CoPb 13181 = ISH 100 × Co 86011
11.	CoPb 13182 = BO 91 × Co 86002
12.	CoPb 14181 =Co 0238 GC
13.	CoPb 14184 =Co 86032 × CoSe 92423
14.	CoPb 14185 = CoS 8436 × CoPant 97222
15.	CoPb 16181 = CoSe 92423 PC
16.	CoPb 18181 = Co 0238 × CoS 92423
17.	CoPb 18182 =CoH 110 × Co 97015
18.	CoPb 15214 = Co 89003 GC
19.	CoPb 15213 = Co 89003 GC

20.	CoPb 15212 = Co 89003 GC
21.	F 3/14 = CoC 671 × CoSe 94423
22.	F 6/14 = CoC 671 × CoSe 92423
23.	F 362/14 = Co 98010 × Co 1148
24.	F 391/14 = Co 98010 × Co1148
25.	F 660/14 = Co 98010 × CoSe 92423?
26.	F 301/11= CoH 119 × CoC 8001
27.	F 404/13 = CoS 8436 PC
28.	BM 10-1068*
29.	SA 04-409*
30.	Co 98014 = Co 8316 × Co 8213
31.	MA 5/37*
32.	MA 5/51*
33.	AS 04-1687*

*Interspecific and intergeneric advance lines under testing in ICAR Trials

Field layout

During spring 2019-20, all 33 clones were planted at PAU, RRS, Faridkot in the first week of March in a randomized block design (*RBD*) with three replications under three different environments i.e. normal environment (E1), mild water stress environment (E2) and rainfed environment (E3). To get proper germination and establishment of all testing clones under all environments, all these three trials were kept under normal irrigation conditions up to germination phase i.e. up to 60 DAP (days after planting). After germination phase, E2 environment trial was completely avoided from irrigation and rain water during formative phase (i.e. 60 to 150 DAP); while E3 environment trial was completely avoided from irrigation water. Growing genotype was represented by a plot of two rows of 6 m length, each. The inter-row spacing was held 90 cm apart. In all the environments, the cane seed rate was 12 buds per meter row. To lift the ideal crop stand, standard agronomic practices as per PAU's package of practices for kharif field crops were followed.

Metrological data

Weather data on temperature (^{0}C, maximum and minimum), relative humidity (%), rainfall (mm), no. of rainy days, and evaporation were recorded for years 2019-20. Since, experiments were conducted under different water conditions; so only differences in rainfall were the major consideration. Highest mean Maximum temperature of 44.4ºC was recorded in the month of May-June and the lowest mean minimum temperature of 9.20ºC was recorded in the month of December 2019. The highest mean monthly relative humidity of 95.00 per cent was recorded in the month of December 2019. A total of 448.2 mm of rainfall was received during the crop duration in 33 rainy days. The highest mean weekly rainfall was in the month of July, 2019(102.4 mm)(Appendix table 1).

Observations recorded

Germination %

For each genotype, the germination (percentage) of three budded sets were reported on plot base at 60 DAP in each replication and expressed as per cent germination.

$$\text{Germination (\%)} = \frac{\text{Number of buds germinated per plot}}{\text{Total number of buds planted per plot}}$$

Nunber of Tillers (000/ha)

The number of tillers of each genotype in each replication was counted at 120 DAP. Mean values were taken from three replications and the number of tillers presented in thousand per hectare.

Number of Shoots (000/ha)

The number of shoots of each genotype in each replication was reported at 210 DAP and, also at 240 DAP. The average of three replications was determined and the number of shoots per hectare was expressed as thousand.

Number of millable canes (000/ha)

The number of millable canes (NMC) relates specifically to the completely formed canes that may be milled at the time of maturity. At maturity, the number of millable canes was counted by counting fully formed canes under each replication from each plot and expressed as thousand per hectare.

Single cane weight (kg)

The single cane weight was also recorded at the time of harvest by weighing five canes per plot selected randomly for each clone in each replication using digital weighing balance. The mean weight of single cane was taken and expressed in kilogram.

Cane length (cm)

The length of a cane was measured at harvest time. In each sample, five canes of each genotype were taken at random by removing the top and trash. Measuring tape was used to measure the length of each can. Mean of three replicates were taken and length of stalk was expressed in centimetres.

Cane diameter (cm)

With the aid of the Vernier calliper, the thickness of the canes was measured in centimetres using 5 canes from each genotype selected randomly from each sample. cane was measured in three positions, i.e. top, middle and bottom, and its mean stalk diameter was taken and expressed in centimetres.

Normal irrigated environment (E1)

Mild water stress environment (E2)

Rainfed environment (E3)

Cane yield (t/ha)

For each replication, total cane yield was recorded at the time of harvest by weighing all the canes harvested and expressed for tons per hectare for each genotype.

Quality Traits

Five healthy competitive canes of each genotype were taken randomly at harvest in each plot under each replication in all three conditions, and crushed for juice extraction with the aid of crusher. Lead acetate (1 mg) was added to the juice for its purification and shaking manually for thoroughly mixing. The juice was filtered after making the precipitation to collect distilled juice that was further used to estimate quality characters as follows:

Juice extraction (%)

Under each replication, the weight of five healthy competitive canes that was selected randomly from each genotype was registered. The weight of cane juice had also been documented after crushing these selected canes. The mean weight of five canes was measured for both the weight of the cane and the juice. At 11 months extraction was stated in per centage was determined as the ratio of mean juice weight determined in each replication and average cane weight calculated under each replication.

$$\text{Extraction (\%)} = (\text{Juice weight (kg)} / \text{Cane weight (kg)}) \times 100$$

Brix % at 11 months

The refractometer's prism face was cleaned and dried out. Distilled water‘s drop was mounted on the prism of the refractometer to standardize the reading by zero. A drop of juice, that was purified, was placed on the prism, and the reading was reported and the brix in cane juice was measured and expressed as percentage.

Pol % in juice at 11 months

One hundred ml of the juice that was filtered was shifted to 250 ml conical flask to which one gram of addition simple lead acetate was done, well stirred and permitted to stand for about an hour until pure supernatant was attained. This supernatant circulated into a filter paper called Whatman No. 1. The collection of filtrates was done, and digital automatic polarimeter registered its polarization (Chen and Chou 1993).

Purity % at 11 months

For the estimation of purity, the pol per cent and brix per cent measured above were taken and purity at and 11 months was measured using the following formula and expressed as per cent for juice of every genotype in each plot under each replication.

$$\text{Purity (\%)} = \frac{\text{Pol \%} \times 100}{\text{Brix \%}}$$

Commercial Cane Sugar % (CCS %) at 11 months

Commercial cane sugar (percentage) was measured at 11 months in juice from the pol (per cent) and purity (per cent) using the following formula:

$$\text{CCS (\%)} = \frac{0.292*\text{Pol \%}\ ((0.035*\text{Purity \%}) - 1))}{\text{Purity \%}} \times 100$$

Commercial Cane Sugar (CCS t/ha) at harvest

Using the following formula, commercial cane sugar (CCS) at harvest was determined using cane yield (t/ha) and commercial cane sugar per cent (CCS per cent), as described earlier:

$$\text{CCS (t/ha)} = [\text{Yield (t/ha)} \times \text{CCS\%}]$$

Physiological traits

Relative water content (%)

The relative water content (RWC) was computed in stressed moisture and unstressed samples of the leaf according to the Barrs and Weatherley method (1962). The fresh leaves were taken, cut into small disks, placed in petri plates for 24 hours on distilled water. Afterwards the discs were weighed and the oven dried. The dry weight was recorded, and the calculation for RWC was:

$$\text{RWC (percentage)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Dry weight})] \text{ x } 100$$

Relative water content (%) at 150 DAP and 240 DAP

Relative water content was recorded at 120 DAP, 150 DAP and 240 DAP and calculations was done by above formula.

Cell membrane stability (%)

Cell membrane stability/ membrane stability index (MSI) was calculated according to the method defined by Sairam and Srivastava (2002). For each sample, 100 mg of leaf tissue was weighed and excreted into small leaf discs put in 10 ml of double distilled water. These samples were first incubated at 40°C in the water bath for 30 min and the conductivity was monitored as C1. Again, these samples were incubated at 100°C in a water bath for 10 min and the conductivity was measured and reported as C2. Measurement of conductivity was conducted by the Wireless Conductivity Meter SE-238 (Scientech India).

The MSI was calculated using the following formula:

$$\text{MSI} = [(1 - C_1/C2) \times 100]$$

Biochemical traits

Proline (µ mol/ g)

The content of proline was calculated using the Bates *et al* (1973) test. Homogenization of one-gram leaf sample was done using mortar and pestle with sulphosalysilic acid 10 ml of 3 per cent. Filtration of homogenate was done through Whatman No.1. in a test tube 2 ml of aliquot, 2 ml of glacial acetate and 2 ml of ninhydrin acid reaction mixture was taken and incubated for one hour in a boiling bath of water. 6ml toluene was added after the reaction mixture had cooled, and the reaction mixture had been vortexed. The toluene-containing chromophore was isolated with Separate funnels and absorbance was read using UV at 520 nm on toluene spectrophotometer. Proline value was measured in the sample as:

$$\text{Proline } (\mu \text{ mol/ g}) = \frac{\mu\text{g proline x ml toluene x 5}}{\text{115.5 x fr.wt. of sample}}$$

Superoxide dismutase

The dismutation of superoxide radical has been catalysed by the superoxide dismutase (SOD) and production of hydrogen peroxide and oxygen takes place as a result. With some change, the Marklund and Marklund (1974) process, which is a test cantered on the ability of SOD to suppress autoxidation of pyrogallol is used for the determination of this enzyme 's operation.

The leaf sample was homogenized via pre chilled pestle mortar with 2ml 0f 100mM tris HCl buffer pH 8.2 containing 1 mM EDTA. At 10000 rpm at 4c, the mixture was centrifuged for 15 minutes and then supernatant had been used for enzyme assay. 100 mM Tris HCL (pH 8.2), 6mM EDTA, 0.6 mM pyrogallol solution and enzyme extract have been added to the reaction mixture of the cuvette. The absorbance was observed using a spectrophotometer at 420 nm for up to 2 mins at an interval of 30 seconds and pyrogallol was utilized as a blank. The enzyme activity unit was described as the quantity of enzyme causing a 50 per cent inhibition of the pyrogallol autoxidation observed in the blank.

Experiment No. 2

Molecular characterization of sugarcane (*Saccharum* spp. complex) genotypes for drought tolerance

Plant material

The one genotype performing better under stress conditions namely F 391/14 and one poor performing genotype CoJ 64 was selected based on per cent cane yield reduction and other morpho-physio-biochemical traits having similar trends of expression for gene expression study.

Rna extraction

Rna was extracted from the freshly opened leaves by BT-TRIZOL (Sigma Aldrich).

Reagents

1. Liquid nitrogen
2. BT-trizol
3. Chloroform
4. Isopropanol
5. Ethanol
6. Rnase free water

Procedure

1. Leaves of sugarcane that was freshly opened was collected from the field and were

crushed in liquid nitrogen for obtaining small particles.

2. 1 ml bt-zol was added to the finely ground tissue mass.
3. This mixture was allowed to stand at room temperature for 15 minutes.
4. for 5 min at 4^{o}C centrifuged the sample and supernatant was transferred to new 1.5 ml tube.
5. 0.2 ml chloroform was add to the freshly obtained supernatant and mixing was done gently and solution was kept at room temperature for 5 minutes.
6. This solution was at 4^{o}C centrifuged for 15 minutes at 12000 rpm.
7. Three layers were seen and uppermost layer was collected into a new tube.
8. 0.5ml Chilled isopropanol was added to this supernatant followed by inverting the tubes 5 - 6 times and kept this solution at -20^{o}C for one hour.
9. Centrifuged this solution at 4^{o}C for 10 minutes at 12000rpm precipitate the Rna.
10. Followed by immediate Washing of Rna pellet twice was done by 70% ethanol.
11. Drying of pellet was done by placing the tubes horizontally not upside down.
12. Then, the pellet was dissolved in 20 micro Rnase free water and Rna was stored at -40^{o}C.

cDNA synthesis

Prime Script 1st strand cDNA Synthesis Kit (Clonetech Takara) was used for preparing cDNA from 100 ng of total RNA. Until installation, the whole package portion was briefly centrifuged and put in ice. RNA was reverse transcribed into cDNA by using the following protocol. Before using kit, reaction component was briefly centrifuged and put in ice. The reaction mixture was prepared In the PCR strips (genetix).

Components

Oligo dT Primer (50 µM)	1 µl
dNTP Mixture (10 mM each)	1 µl
Template RNA (500ng/ reaction)	
Nuclease free water (up to 10 µl)	

The reaction mixture was heated at 65°C for 5 min, then immediately cooled on ice.

Following enlisted components were added to the above reaction mixture to make 20 µl final reaction volume.

5X Prime Script Buffer	4 µl
RNase Inhibitor	1 µl
Prime Script RT (200 Units)	0.5 µl
RNase-free dH2O	4.5 µl

The reaction components were gently mixed and incubated at 42 °C for one hour, followed by heat at 70 °C for 15 min and held at 4 °C at an infinite period.

Analysis of Real time data

Data normalization using the Ct values of the target gene and normalizer was done as per following

ΔCt target = Ct target- Ct normalizer

ΔCt calibrator= Ct calibrator – Ct normalizer

ΔΔCt = ΔCt target -ΔCt calibrator

Relative fold change in expression F= 2-ΔΔCt (Livak and Schmittgen 2001)

Student's t-test was used for performing statistical analysis. Dissociation curve or melt curve evaluation was carried to test for contamination or primer dimer in reaction.

Relative quantification of various genes of sugarcane (*Sachharum* spp. complex) under normal and drought conditions

Ten times dilution of cDNA samples was done before reaction setup. In Light cycler System (Roche life sciences, Mannheim, German) SYBR® Premix Ex TaqTMII, Takara, the qPCR reaction was performed as per manufacturer's instructions. In order to recognize any intrusion, a non-template control was added. The 25sRNA gene of *S. officinarum* was used as housekeeping gene for the normalization of data. Information of the primers used are mentioned in the list.

Reaction mixture (10µl) of each reaction consisted of the following components.

SYBR® Premix Ex TaqTMII	5µl
Forward primer	0.1µl
Reverse primer	0.1µl
cDNA template (diluted)	1µl
Nuclease free water	3.8µl

PCR thermal conditions

Stage 1: Initial denaturation at 95°C for 130 seconds

Stage2: Denaturation 95°C for 30 seconds

Annealing 60°C for 30 seconds

Final extension at 72°C for 20 seconds

Repeat stage 2 for 40 cycles

Stage3: Melt curve analysis 95°C for 5sec, 65°C for 60 sec, 95°C for 1sec

Stage4: 50°C for 30 sec (Cooling)

Statistical analysis

The experimental design was a randomized block (RBD) for the water (E1: normal irrigated; E2: withhold irrigation at formative stage and E3: rainfed) response studies. There were three replications for each environment. Analysis of variance was performed on genotypes using the GLM procedure in SAS for all traits. The probability threshold level was kept 0.05. Genotypes were treated as a fixed effect, and replication nested within year were treated as a random effect. Separation of means was done using the LSD test (P 0.05). The genotypic and phenotypic coefficients of variation, heritability in broad sense and expected

List of primers

Full name of gene	Name of gene used in text	Forward Primer (5’3’)	Reverse Primer (5’-3’)
25S RNA	25S rRNA	GCAGCCAAGCGTTCATAGC	CCTATTGGTGGGTGAACAATCC
Proline oxidase	Pox	CGAGCGTGTGCATCAAGATC	GTCTTCCATGGCAGGTTGAAC
Proline transporter	ProT	TCCCACTGACGTTTGTGCTC	AACCCAACAACATTCAGCCAG
Dehydrin	DEH	ACCAGTACGGCAATCCAGTTG	CGGAGCGATGCAGGATG
Betaine aldehyde dehydrogenase	BADH	GCTGCATGGGACATGGATG	CCATTGGAAGAGAAACTGGTGAG
Indole-3-glycerol-phosphate synthase	IGS	CAGCGTTTTGACAGACCAGA	CCAACAAGCTCGATTCCTTC
Superoxide dismutase	SOD	ACCACCTGTTCCACCACAAG	GCCTCCTTGTGGTCCTTCTT
Cytosolic ascorbate peroxidasec	APX	CCAACCGTGAGCGAAGATT	TAAGCATCAGCAAACCCAAG
Dehydration responsive element binding proteins	DREB	CCCGACGTACTCCTCAGTCC	CTTCTCGTCCTGGACTCCCAT
Late embryogenesis abundant	LEA	GCTTAGGATCAATGGCTTCCCACC	CCAAAGGGAAATCATTCACGGCGTC
Trehalose 6-phosphate synthase	TPS	GCACATGTCACAACTCACA	ACAGCTGCATTTGAGATCG
Drought-responsive protein 1	DRP	AGAAGAAATGTTGTGTCTGTGA	CGAGCTTGTACTCTGTCTTG
Pyrroline-5-carboxylase synthetase	P5CS	CCTGATGCCTTGGTCCAGA	TGCAATACTGTGTTTGATCTCATGG

genetic advance were calculated as suggested by Burton and Devane (1953) and Johnson *et al* (1955). Pearson's correlation coefficient and simple regression analyses of concerned traits as environment wise separately for E1, E2 and E3 were done to find out best selection index for cane yield and sucrose content. Pearson correlation procedure in "SPSS Statistics 22.0" (IBM Corp. 2013) was used to find out the correlation coefficients among all twenty-four traits. Simple regression procedure in MS Excel 2019 was used to regress Cane Yield (CY) on NMC and SCW; CCS% on TSS%, Pol% and Purity%; CCS t/ha on CY and CCS(t/ha). Eberhart & Russell's regression coefficient analysis using "SPAR 2.0" (IASRI 2012) and GGE Biplot analyses using "PB Tools 1.4" (http://bbi.irri.org/products) were carried out for the trait CY t/ha, CCS% and CCS t/ha.

CHAPTER-IV

RESULTS AND DISCUSSION

Experimental results of present study entitled “Performance of sugarcane genotypes under water deficit stress” are being presented in this chapter. The explanations of each experiment covered under different heads and sub heads are presented.

4.1 Analysis of variance

The analysis of variance under normal (E1), mild water stress (E2) and rainfed environment (E3) was carried out for eight field quantitative traits, six quality traits, two physiological and two biochemical traits. Mean sum of squares for all genotypes were found significant for all the traits under all three environments indicating high level of genetic diversity among them. Table 4.1 clearly revealing that there was significance difference among all three environments for all the qualitative, quantitative, physiological and biochemical traits except for germination %; because all three environments were similar duing germination period. Significance differences were also observed for Environment × Genotype interactions for all quality and quantitative traits except for germination %; while for physiological and biochemical traits, significance interaction for all traits were observed. RWC % and cell membrane stability % was reduced under mild water stressed and rainfed environment; while proline and SOD content was increased under water stressed conditions in comparison to normal environment (Table 4.2).

Significant differences for cane and component parameters have also been recognized by Nair *et al* (1999). Sanghera *et al* (2015) documented highly significant variability among thirteen sugarcane genotypes for the traits (germination % at 45 days, number of tillers at 120 days, stalk length, stalk girth, NMC and cane yield) under normal environment. The outcomes specified by Graca *et al* (2010) presented a significant difference in relative water content (RWC) in the stressed plants in contrast to irrigated plants. Similarly, of the six quality traits studied at harvest, genotypes performed significantly and differentially for all the traits in all the three environments (E1, E2 and E3) (table 4.1). Under normal water conditions, Tena *et al* (2016) described significant variability for all the quality parameters of sugarcane. The significant variability for various cane yield and component traits, physiological, biochemical and quality traits in all three environments discovered that there exists sufficient genetic variability in the experimental material used for the present study. It will help to isolate specific genotype fit for water stress and non-stress conditions.

4.1.2 Means, range and per cent increase/decrease of quantitative, qualitative, physiological and biochemical parameters under different water conditions

When exposed to water deficiency, plants possess physiological, biochemical,

Table 4.1 Analysis of variance for various quantitative and quality traits in sugarcane

Source of Variations	**DF**	**Gm %**	**Tillers (000/ha)**	**Shoots* (000/ha)**	**Shoots (000/ha)**	**NMC (000/ha)**	**CL (cm)**	**CG (cm)**	**SCW (kg)**	**Ext%**	**Brix%**	**Pol%**	**Purity%**	**CCS%**	**CY (t/ha)**	**CCS (t/ha)**
		Mean Square														
Environment	2	5.22	80992.08*	70279.67*	70310.97*	92135.55*	204590.86*	0.20*	8.05*	2524.12*	82.17*	174.36*	851.70*	117.67*	114803.61*	1706.95*
Rep(Env*Rep)	6	53.84*	202.50*	134.90	98.32	104.04	955.82*	0.11*	0.0142*	4.67	0.13	0.02	2.68	0.015*	260.42*	3.16*
Genotypes	32	248.01*	11815.21*	9988.42*	8521.98*	7262.23*	4548.80*	0.56*	0.095*	82.25*	13.55*	12.85*	18.22*	6.82*	1666.29*	15.60*
Env*Genotypes	64	33.01	647.68*	285.89*	350.15*	842.66*	476.46*	0.05*	0.02*	17.94*	1.46*	1.35*	6.63*	0.77*	302.02*	3.74*
Error		20.71	179.56	98.18	102.74	66.23	171.64	0.04	0.01	2.80	0.14	0.12	1.89	0.08	59.61	0.76
Summary of statistics																
R-Square		**0.72**	**0.94**	0.96	0.96	0.97	0.95	0.74	0.96	0.94	0.96	0.97	0.88	0.97	0.96	0.97
CV%		12.53	7.78	7.77	9.52	10.26	10.27	7.98	10.72	3.61	2.08	2.21	1.60	2.64	16.91	17.31
Root MSE		4.55	13.40	9.91	10.14	8.14	13.10	0.20	0.07	1.67	0.37	0.34	1.38	0.28	7.72	0.87
Mean		36.31	172.22	127.49	106.42	79.30	127.62	2.52	0.62	46.32	17.77	15.34	86.19	10.49	45.66	5.03
Range		21.3-68.61	66.67-286.67	22.22-253.34	17.78-233.89	11.67-236.11	54.77-234	1.86-3.16	0.21-1.27	30.72-55.49	12.13-21	10.06-19.12	80.72-93.22	6.74-13.41	3.33-145.46	0.31-12.78
% deviation under E2 over E1		0.38	23.26	20.04	20.01	24.76	37.34	2.72	41.94	11.00	7.13	11.64	4.76	13.74	53.88	60.31
%deviation under E3over E1		-0.85	24.93	34.08	40.05	56.08	48.55	3.11	59.14	19.53	9.31	15.08	6.28	17.75	80.73	83.32

Environment (Env), Replication (Rep), Germination % (Gm %), Number of Tillers (Tillers), Number of shoots at 210 DAP (Shoots*), Number of shoots at 240 DAP (Shoots), Number of millable canes (NMC), Cane length (CL), Cane girth (CG), Extraction (Ext%), CCS (commercial cane sugar).

*Significant at 5% level

Table 4.2 Analysis of variance for various physiological and biochemical traits in sugarcane

		CMS% (150 DAP)	**CMS% (240 DAP)**	**RWC% (120 DAP)**	**RWC% (150 DAP)**	**RWC% (240 DAP)**	**SOD(eu/100ml/ gfw) (150 DAP)**	**SOD(eu/100ml/ gfw)(240 DAP)**	**Proline(µg/ gfw) (150 DAP)**	**Proline (µg/gfw) (240 DAP)**
Source of variations	DF	**Mean squares**								
Env	2	6733.20*	3795.03*	1248.63*	4335.28*	2942.05*	43917.01*	19550.56*	35673.30*	30983.77*
Rep(Env*Rep)	6	1.71	4.64	9.25	7.82	3.92	1.87*	0.03	0.90	1.48
Genotypes	32	100.79*	46.89*	143.51*	147.91*	85.69*	22.68*	9.738*	44.83*	54.88*
Env*Genotypes	64	18.73*	8.63*	8.92*	21.43*	9.32*	22.52*	9.68*	25.52*	28.27*
Error		3.38	2.94	5.75	7.01	6.79	0.55	0.29	0.77	0.87
Summary of statistics										
R-Square		0.96	0.94	0.87	0.92	0.88	1.00	1.00	1.00	1.00
CV%		2.82	2.48	3.67	3.93	3.42	6.02	6.49	4.38	4.94
Root MSE		1.84	1.71	2.40	2.65	2.61	0.74	0.54	0.88	0.93
Mean		65.17	69.20	65.25	67.32	76.14	12.31	8.24	19.99	18.86
Range		52.81-80	58.27-80	53.14-76.76	49.69-88.21	62.80-90.51	0.103-0.203	0.101-0.166	15.28-51.97	14.74-47.65
% deviation under E2 over E1		17.96	6.38	6.57	8.68	6.21	41.67	27.27	68.18	15.81
% deviation under E3 over E1		20.13	16.40	10.13	17.92	13.38	50.00	36.36	74.52	67.06

Env; Environment, Rep; Replication, CMS; Cell membrane stability, RWC; Relative water content, SOD; Superoxide dismutase

*Significant at 5% level

and molecular responses at both cellular and whole plant levels (Hasegawa *et al* 2000). In plants, water limitedcondition have been shown to reduce both growth and yield (Flagella *et al* 2000). It is now well demonstrated that choice of suitable genotype from germplasm using specific morpho-physiological traits is a viable way forward for crop improvement for water stress tolerance (Reynolds *et al* 2005 and Kiani *et al* 2007). Therefore, in the present study, the emphasis was laid down to evaluate the lines from different sources for various morpho-physiological, yield and quality parameters as discussed below:

4.1.2.1 Quantitative parameters

4.1.2.1a Germination %

Under normal environment (E1), range of germination % among the different genotypes was varied from 25.00 to 44.91% with mean value 36.25%. Under mild stress environment, it was ranged from 21.30-47.22% with mean value 36.11%; and from 24.07-48.61% with mean value 36.50% under rainfed environmentSugarcane variety Co 0118 (44.91) exhibited maximum germination % closely followed by CoPb 91 (43.52%), Co 0238 (42.59%), CoPb 15212 (42.13%), respectively. Under mild stress environment, maximum germination % was observed for cultivar CoPb 18181 (47.22%) followed by F 660/14 (46.3%); and under rainfed environment, maximum germination % was recorded for clone F 660/14 (48.61%) while minimum for Co 98014 (24.07%) (Appendix table 2). Variations in the germination % among clones may be due to variation in genetic make-up of each clone (Hossain *et al* 2010).

4.1.2.1b Number of tillers (000/ha)

The number of tillers was ranged from 113.89-286.67 (000/ha) with mean value of 205.18 (000/ha). Maximum number of tillers under normal environment was recorded for variety AS 04-1687 (286.67) followed by CoPb 15214, CoPb 18181, and minimum number of tiller count was found for genotype MA 5/37, followed by MA 5/51. Similarly, under mild stress and rainfed environment, minimum number of tillers was recorded for MA 5/51 followed by MA 5/37. Under mild stress and rainfed environment maximum number of tillers was found for AS 04-1687(241.11 and 212.78, respectively) followed by CoPb 18181(Appendix table 2). Tiller count (0000/ha) decreased by 23.26% and 24.93% under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1). Ramesh and Mahadevaswamy (2000) detected that water deficit causes greater tiller decrease. Vasantha *et al* (2005) and Rajeshwari *et al* (2000) also described significant decrease in tiller population due to water deficit.

4.1.2.1c Number of shoots (000/ha)

In this study, number of shoots at 210 DAP under normal, mild stress and rainfed environment was ranged from 71.67 to 253.33(000/ha), 52.22 to 208.33(000/ha) and 22.22 to 170(000/ha), respectively. Among genotypes, maximum no. of shoots was recorded for genotype AS 04-1687 under all environments. Minimum number of shoots was recorded for genotype MA 5/37 followed by MA 5/51 under normal environment. Under mild stress and rainfed environment, minimum number of shoots was noted for MA 5/51 followed by MA 5/37.

Number of shoots at 240 DAPS in present study was ranged from 51.67-233.89 (000/ha) with mean value 133.06 (000/ha) under normal environment. Maximum number of shoots in E1 was noted for cultivar AS 04-1687 followed by CoPb 18181 while minimum number of shoots was observed for genotype MA 5/51 followed by MA 5/37(Appendix 2). It was ranged from 37.78 to 179.44 (000/ha) and 17.78 to 146.11 (000/ha) with mean value 106.43 (000/ha) and 79.76 (000/ha), respectively, under mild water stress and rainfed environment. Maximum and minimum number of shoots under both mild water stress and rainfed environment was observed for cultivars AS 04-1687 and MA5/31, respectively. Shoot count (000/ha) was decreased by 20.04% and 34.08% and 20.01 & 40.05% (Table 4.1) under mild water stress and rainfed environment in comparison to normal environment, respectively.

4.1.2.1c Number of millable canes (000/ha)

Number of millable canes **(**NMC) is most important yield contributing parameter for cane yield and it depends on number of tillers converted into economic shoots. At maturity, NMC were ranged from 45 to 236.11 (000/ha), with mean value 108.5 (000/ha) under normal conditions. Under mild stress and rainfed conditions, range of NMC were from 24.44 to 210 (000/ha), 11.67 to 102.78(000/ha) with mean value of 81.67(000/ha) and 47.68(000/ha), respectively (Appendix table 2). Under normal conditions, genotype AS 04-1687 showed maximum NMC closely followed by CoPb15214 CoPb15214, CoPb18181. Similarly, under mild stress condition, AS 04-1687 exhibited maximum number of millable canes closely followed by CoPb15214, CoPb15214, CoPb18181. Under rainfed conditions, maximum NMC were reported for clone AS 04-1687 closely followed by CoPb18181. Under all three environments, minimum NMC were reported for cultivar MA 5/51 and MA 5/37. 24.76 % and 56.08 % NMC reduction was reported for mild water stress and rainfed environment, respectively (Table 4.1). Number of millable cane is decreased under water deficit conditions (Silva and Costa 2004). Silva (2008) also reported reduced number of millable canes under waters stress.

4.1.2.1d Cane length (cm)

Maximum height was recorded for genotype BM101068. Clones SA 04-409, AS 04-1687, CoPb 15212, CoPb 92, CoPb 18182 and Co 98014 were recorded > 200cm height under normal condition. Under mild stress conditions, cane length was ranged from 75.11 to 165.11 cm with mean value 112.05 cm. Maximum height under this environment was recorded for AS 04-1687 closely followed by SA 04-409, and minimum height was noted for

CoJ 85 followed by F 6/14. Under rainfed environment, range of height was from 54.77 to132.11 cm with mean value 92 cm. Maximum and minimum height were recorded for SA 04-409 and Co 0238, respectively (Appendix table 3). CL (cm) was decreased by 37.34% and 48.55%, under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1).

Misra *et al* (2020) observed decreased cane length under water deficit condition. Furthermore, Silva *et al* (2008) and Inman-Bamber and Smith (2005) had demonstrated that cane length might be more affected under drought.

4.1.2.1e Cane girth (cm):

Cane girth under normal environment was ranged from 1.86 to 3.09 cm with mean value 2.57 cm; and under this environment, maximum girth was recorded for clone F 391/14 closely followed by F 3/14. Minimum girth was registered for genotype AS 04-1687 followed by BM 101068 under both normal and mild stress environment. Genotype CoPb 91 closely followed by CoPb 94 exhibited maximum cane girth under mild stress and rainfed environment, respectively. Clones BM 101068 followed by AS 04-1687 exhibited minimum cane girth under rainfed environment (Appendix table 2). CG (cm) was decreased by 2.72 and 3.11 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1).

Misra *et al* (2020) observed decreased pattern of cane diameter under drought against normal conditions; and Silva *et al* (2008) also observed drought stress effect on stalk diameter is dependent on genotype.

4.1.2.1f Single cane weight (kg)

In the present study, SCW was ranged from 0.63 to 1.27 kg with a mean value of 0.93 kg under normal (E1) conditions. In this environment, genotype CoPb 91 Closely followed by F 391/14 possessed maximum SCW; and minimum SCW was registered for AS 04-1687 closely Followed by CoPb 15214. Under mild stress environment, SCW was ranged from 0.37 to 0.76 kg with a mean value of 0.54 kg. Maximum SCW for this environment was reported for F 391/14 followed by F 3/14, while minimum SCW for clones CoJ 64, CoJ 85 was estimated. Under rainfed environment, maximum SCW was reported for clone F 391/14 followed by SA 04-409, CoPb 94, while minimum SCW was noted for genotype CoJ 64 followed by Co 0238 and CoPb 14181(Appendix table 3). Cane weight was reported to be affected by the irrigation amount. Silva & Costa (2004) and Silva *et al* (2008) observed reduced cane weight under water limited environment. SCW (kg) was decreased by 41.94 and 59.14 % under mild water stress and rainfed environment in comparison to normal environment, respectively (table 4.1).

4.1.2.1g Cane yield (t/ha)

In present study, cane yield was ranged from 36.67 to 145.56 (t/ha), 7.78 to 87.78

(t/ha) and 3.33 to 38.33 (t/ha) with mean value of 82.8, 38.2 and 15.96 (t/ha) under normal environment, mild water stress and rainfed environment, respectively. Maximum cane yield under normal condition was recorded for cultivar AS 04-1687 followed by CoPb 91, F 660/14 and F 301/11, however, minimum yield was recorded for clone MA 5/51 followed by MA 5/37. Under mild water stress environment, highest yield was recorded for clone AS 04-1687 followed by CoPb 18182 and F 391/14, while lowest cane yield was reported for cultivar MA 5/51 followed by CoJ 85 and MA 5/37. Genotype F 391/14 was recorded highest yield under rainfed environment closely followed by CoPb 94 and AS 04-1687, while lowest yield for CoPb 14181 followed by CoJ 64(Appendix table 3). CY (t/ha) was decreased by 53.88 % and 80.73 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1).

It is expected that all the above characteristics are affected by water deficit conditions; and consequently, the cane yield was affected. Silva *et al* (2008) reported the decline productivity under water deficit condition in comparison to normal conditions in sugarcane.

4.1.2.2 Quality parameters

4.1.2.2a Brix % at 11 months

Brix % was ranged from 12.7 % to 21.0 % with mean value of 18.78 % under normal environment. Under E1, highest and lowest values for brix % was observed for clone Co 0118 and AS 04-1687, respectively. Under mild stress environment, range of brix % was varied from 12.30 % to 18.73 % with mean value of 17.45 %. Maximum brix % under E2 was recorded for clone CoJ 88 followed by F 3/14, however cultivar AS 04-1687 followed by BM 101068 exhibited minimum percentage of brix value. Brix % was ranged from 18.03 % to 14.7 % under rainfed environment, and maximum brix % was noted for clone F 6/14 closely followed by F 6/14; while minimum percentage of brix was recorded for clone AS 04-1687 closely followed by MA 5/51(Appendix table 4). Brix % was decreased by 7.13 % and 9.31 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1). Patil (2008) also reported high brix% under normal conditions in comparison to water stressed conditions.

4.1.2.2b Pol % at 11 months

In the present study, range of pol % under E1, E2 and E3 environments were varied from 10.25 % to 17.74 %, 10.06 % to 16.33 % and 12.19 % to 15.48 % with mean value of 16.80 %, 14.88 % and 14.30 %, respectively. Under E1, highest percentage of Pol was recorded for clone SA 04-409 followed by MA 5/51, while cultivar AS 04-1687 followed by BM 101068 showed minimum pol%. Under E2, maximum Pol % was reported for clone CoJ 88 closely followed by F 404/13, however minimum pol% under this environment was recorded for clone AS 04-1687 closely followed by BM 101068. Under E3, maximum pol%

was noted for clone CoJ 88 followed by CoJ 85, while cultivar MA 5/51 followed by AS 04-1687 exhibited minimum value of Pol % (Appendix table 4). Pol % was decreased by 11.64 % and 15.08 % (Table 4.1) under mild water stress and rainfed environment in comparison to normal environment, respectively; but Hossain and Rahman (2009) observed that sucrose % was not significantly affected by irrigation.

4.1.2.2c Purity % at 11 months

The purity % among clones at 11 months (%) was varied from 83.14 % to 92.23 % with a mean value of 89.48 % under normal (E1) conditions with maximum Purity (%) for the clone F 404/13 closely followed by clones CoPb 94. However, minimum purity (%) was recorded for clone AS 04-1687. It was ranged from 80.72 % to 88.89 % under mild water stress environment with mean value of 85.21 %. Maximum purity % was observed for clone Co 0238 closely followed by Co 98014, while genotype MA 5/51 followed by CoPb 13182 exhibited minimum purity percentage. Under E3, it was ranged from 80.84 % to 86.56 % with mean value of 83.85 %. Clone CoJ 88 closely followed by CoJ 85 and Co 0238 exhibited highest purity % under this environment; however, clone F 6/14 followed by MA 5/51 possessed minimum value of purity % (Appendix table 4). Purity was decreased by 4.76 % and 6.28 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1). Drought has deleterious effects on the sugarcane quality particularly brix, sucrose and purity of juice (Mukunda *et al* 2000).

4.1.2.2d CCS %

CCS % was ranged from 6.95 to 13.14 (%) with average value of 11.71 (%) under normal (E1) conditions whereas under mild water stress (E2) condition and rainfed conditions, it was varied from 6.74 % to 11.22 % and 8.08 % to 10.67 % with mean value of 10.11 % and 9.63 %, respectively. The highest and lowest CCS (%) at 11 months (%) were recorded for clones Co 0118 and AS 04-1687, respectively, under normal (E1) conditions; while under mild water stress (E2), maximum CCS% was recorded for clone CoJ 88 and minimum value for clone AS 04-1687. Under rainfed environment, maximum value for CCS% was observed for cultivar CoJ 88; while clone MA5/51 exhibited minimum value (Appendix table 4). CCS% was decreased by 13.74 % and 17.75 % under E2 and E3 environment in comparison to E1 environment, respectively (Table 4.1). Pawar *et al* (2006) also observed variation in CCS% among the clones with the similar trends as observed in present study.

4.1.2.2e Extraction % at 11 months

Under normal environment, extraction % was ranged from 39.62 % to 55.49 % with mean value of 51.56 %. Maximum extraction % was observed for cultivar CoPb 14185 closely followed by Co 98014 while minimum extraction % for cultivar AS 04-1687. Under mild stress environment range of extraction % was varied from 36.01 to 51.26 with mean value 45.88 %. Maximum extraction % under this environment was reported for the genotype F 404/13 followed by CoPb16181, while cultivar SA 04-409 followed by MA 5/51 exhibited minimum extraction %. Under rainfed environment, range of extraction % was varied from 30.72 % to 49.33% with mean value 41.49 %. Highest extraction % was reported for clone F 3/14 followed by CoPb 15214 while lowest extraction % was recorded for genotype MA 5/51 followed by Co 0238 (Appendix table 4). Extn % was decreased by 11.00 % and 19.53 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1). Differences in extraction % among the genotypes were also observed by Pawar et al (2006).

4.1.2.2f CCS (t/ha)

CCS (t/ha) under normal (E1) conditions was ranged from 3.84 to 12.78 with overall mean value of 9.64 t/ha, while, under mild water stress (E2) conditions and rainfed (E3) conditions; it was varied from 0.67 t/ha to 6.51 t/ha and 0.31 t/ha to 4.16 t/ha with population mean value of 3.83 t/ha and 1.6 t/ha, respectively. Under E1, maximum CCS (t/ha) was recorded for cultivar CoPb 91 followed by CoPb 18182 while minimum CCS (t/ha) was recorded for the cultivar MA 5/51. Under E2 and E3 environment, maximum and minimum CCS (t/ha) was registered for cultivar F 391/14 and MA 5/51, respectively. CCS (t/ha) was decreased by 60.31 % and 83.32 % under mild water stress and rainfed environment in comparison to normal environment, respectively (Table 4.1). Significant reduction in CCS (mt/ha) due to moisture stress was also reported by Pawar *et al* (2006), they further stated that the reduction in CCS (mt/ha) might be due to adverse effect of moisture stress on sucrose synthatase activity.

4.1.2.3 Physiological parameters

4.1.2.3a Relative water content (%) at 120 DAP, 150 DAP and 240 DAP

The relative water content (RWC) in leaf at 120 DAP was ranged from 60.8 to 76.76 (%), 54.7 to 70.72 (%) and 53.13 to 67.96 (%) with mean value of 69.09 %, 65.55 % and 62.09 % under normal (E1), mild water stress (E2) and rainfed (E3) environments, respectively (Appendix Table 6). Clones Co 98014 followed by F 660/14 and CoJ 85 exhibited higher value of RWC under E1; while, clones F 6/14 followed by F 3/14 and CoPb 18181 showed lowest values for RWC. Under E2 and E3 environments, maximum RWC (%) was reported for clone CoJ 88 (70.72 % and 67.96 %, respectively) followed by CoPb 92 (70.35 % and 67.86 %, respectively); while, lowest values under both environments were recorded for cultivar F 3/14 (54.7 % and 53.13 %, respectively) followed by CoPb 18181 and CoPb 18182, respectively (Fig 4.1).

At 150 DAP; RWC % was ranged from 66.40 to 88.21, 57.32 to 73.75 and 49.69 to 69.53 with mean value of 73.86 %, 67.45 % and 60.63 % under E1, E2 and E3 environments, respectively (Appendix Table 6). Clone CoJ 64 followed by F 362/14 exhibited highest RWC values while clone CoPb 13182 followed by CoPb 93 exhibited lowest values for RWC under normal environment. Under E2 and E3 environment, cultivar MA 5/51 (57.32 % and 49.69 %, respectively) followed by Co 98014 (57.6 % and 50.8 %, respectively) showed minimum values while for cultivar F 391/14 (73.75 % and 69.53%, respectively) highest values of RWC was recorded (Fig 4.2).

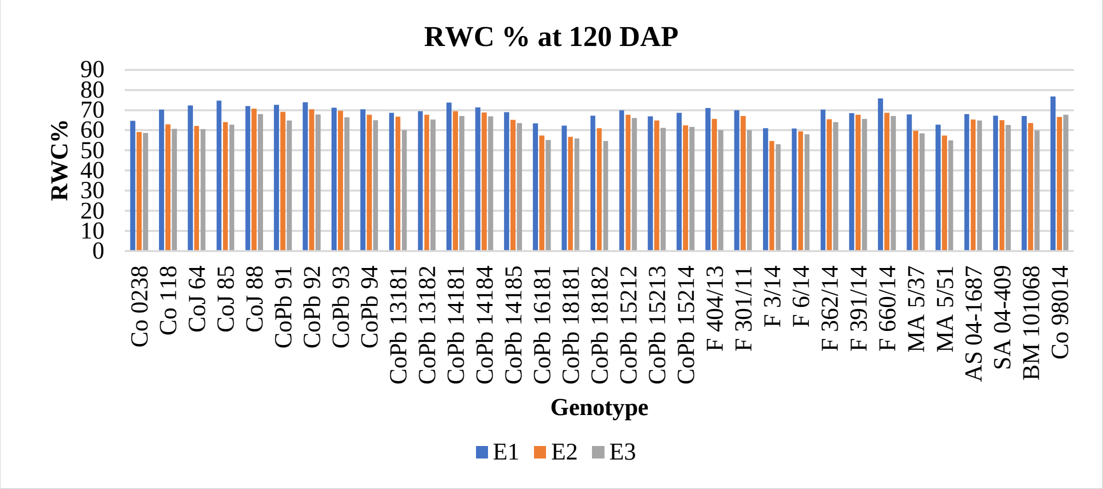

Fig 4.1: Average value of relative water content at 120 days after planting

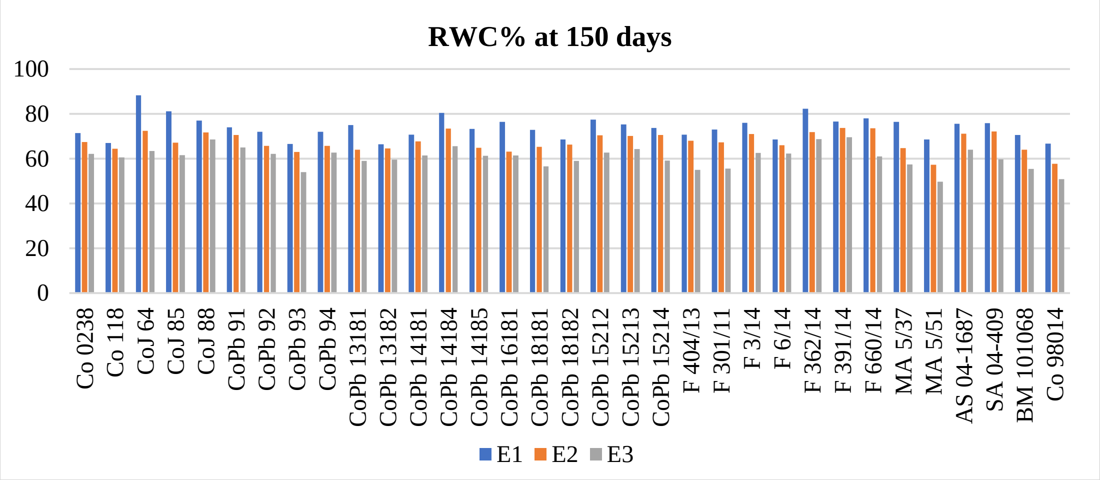

Fig 4.2: Average value of relative water content at 120 days after planting

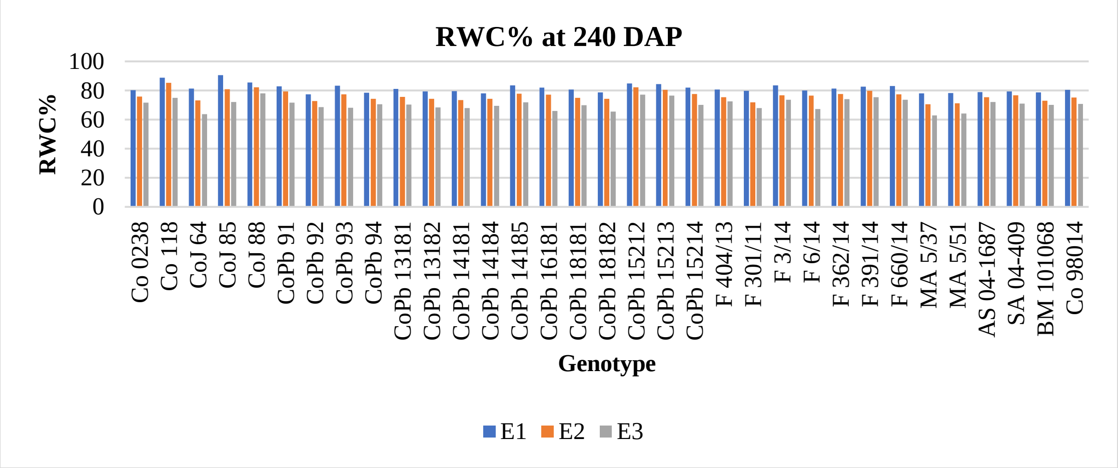

Fig 4.3: Average value of relative water content at 240 days after planting

At 240 DAP, RWC (%) was ranged from 77.37 % to 90.91 % to, 70.62 % to 85.24 % and 62.80 % to 78.07 % with mean values of 81.45 %, 76.40 % and 70.56 % under normal, mild water stress and rainfed environment, respectively (Appendix Table 6). Clone CoJ 85 followed by Co 0118 exhibited highest RWC values; while, clone CoPb 92 followed by MA 5/37 exhibited lowest values of RWC under normal environment. Under mild water stress and rainfed environment, cultivar MA 5/37 (70.62 % and 62.80 % respectively) showed minimum values; while, Co 0118 and CoJ 88, respectively, was recorded highest values for RWC (%) (Fig 4.3). Reddy *et al* (2003) reported that stressed plants have lower RWC than non-stressed plants. A decrease in the RWC in response to water stress has been observed in variety of plants as stated by Nayyar and Gupta (2006) that when leaves are exposed to water stress, leaves display large decrease in RWC and water potential. Silva *et al* (2007) stated that water stress-tolerant sugarcane cultivars displayed higher RWC as compared to the drought sensitive cultivars.

4.1.2.3b Cell membrane stability (%) at 150 DAP and 240 DAP

CMS % was ranged from 68.72 % to 80.77 %, 52.81 % to 71.00 % and 52.99 % to 67.45 % with mean value of 76.64 %, 61.23 % and 59.62 % under E1, E2 and E3 environments, respectively (Appendix Table 6). Highest value was reported for genotype CoPb 14185 followed by CoPb 93 under normal environment; whereas, lowest value was recorded for CoPb 94 followed by CoPb 91. Under E2 and E3 environments, cultivar CoPb 14181 (71.00 % and 67.43 %, respectively) showed highest CMS (%) value while clone F 404/13 (52.81 % and 52.9 %, respectively) exhibited lowest CMS (%) values (Fig 4.4).

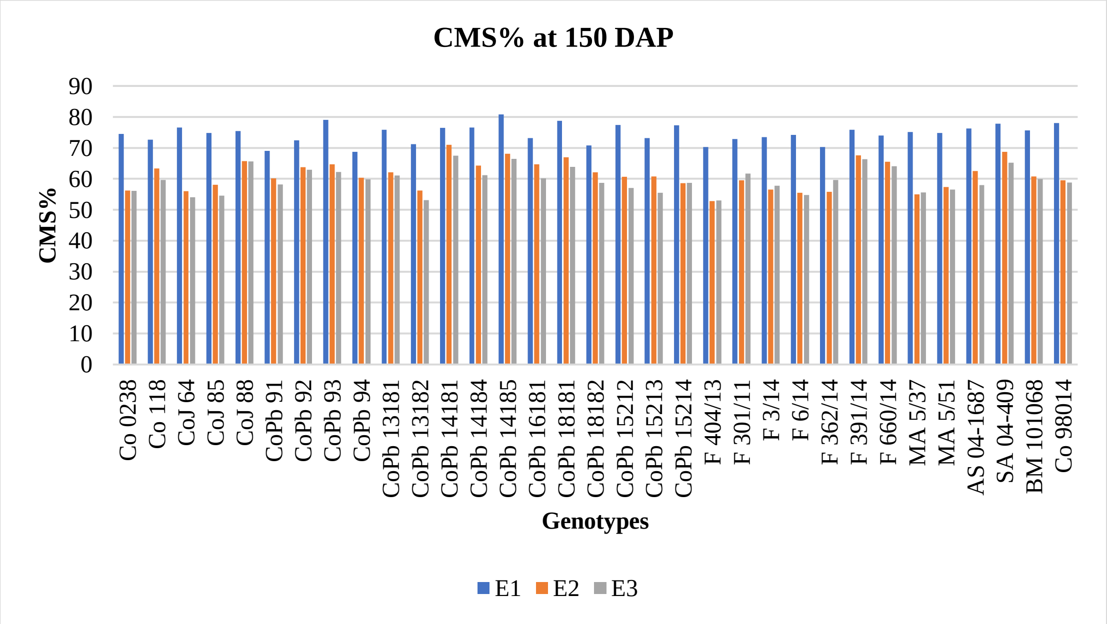

Fig 4.4: Average value of cell membrane stability (%) at 150 days after planting

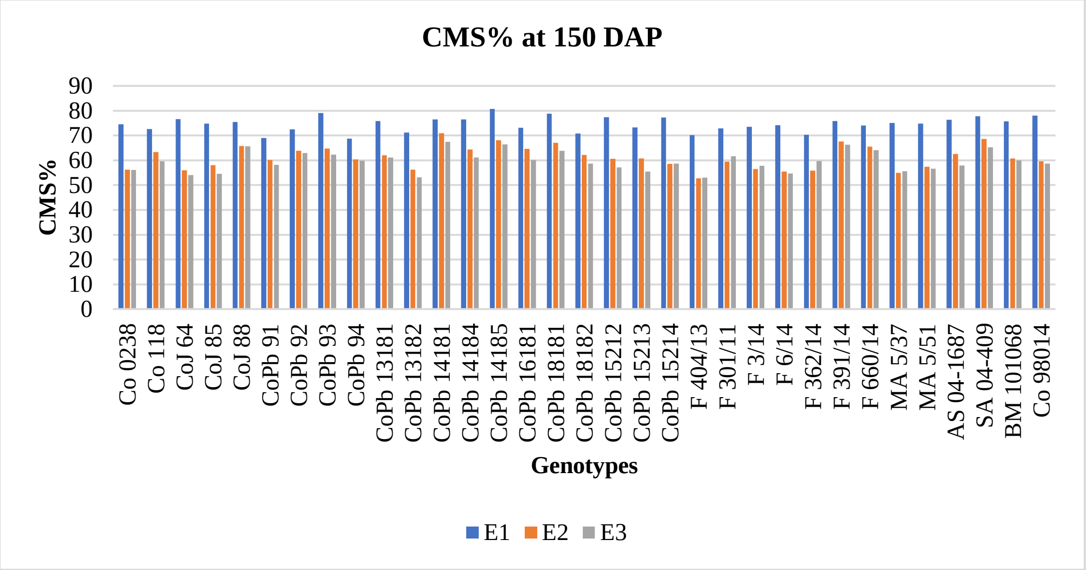

Fig 4.5: Average value of cell membrane stability (%) at 240 days after planting

At 240 DAP, it was ranged from 69.83 % to 80.00 %, 65.03 % to 75.00 % and 58.27 % to 69.73 % with average value of 74.89 %, 70.00 % and 62.60 % under E1, E2 and E3 environments, respectively (Appendix Table 6). Under E1 environment, highest CMS (%) values were reported for clone CoPb 93 followed by CoPb 18181 and CoPb 14185 however clone CoPb 94 followed by CoPb 91 exhibited lowest CMS (%) values. Under E2 environment, varieties CoPb 93 followed by CoJ 88 and CoPb 18181 were recorded highest values of CMS (%) whereas CoPb 91 followed by CoPb 94 exhibited lowest CMS (%) values. Under E3 environment, genotype F 391/14 followed by CoJ 88 exhibited maximum CMS (%) value while CoPb15213 followed by CoJ 64 reported lowest values (Fig 4.5). A relatively sharp reduction in membrane stability index under stress conditions recommends sensitivity of these genotypes to water deficit. Our results under present study are in conformity with Augustine *et al* (2014).

4.1.2.4 Biochemical parameters

4.1.2.4a Proline (µg/gfw) at 150 days after planting (DAP) and 240 DAP

Proline contents in leaves were recorded at 120 DAP, and it was ranged from 15.28 to 25.15(µg/gfw), 23.33 to 51.28 (µg/gfw) and 24.49 to 51.97(µg/gfw) with mean value of 21.7, 36.64 and 38.01 (µg/gfw) under E1, E2 and E3 environments, respectively (Appendix Table). Under normal environment, clone BM 101068 had the highest proline content (25.15 µg/gfw), followed by clones F 404/13(24.82), CoPb15214(24.7) and CoPb15212 (24.39) while cultivar CoPb13181 (15.28) followed by Co 0118 (16.58) exhibited lowest value of proline. Under mild water stress environment, variety F 391/14 followed by Co 0238 showed maximum proline content whereas clone CoJ 64 followed by MA 5/51 exhibited lowest proline value. Similarly, under rainfed environment cultivar CoJ 64 followed by MA 5/51exhibited lowest value of proline while cultivar F 391/14 exhibited highest proline value

(Fig 4.6).

Proline was also recorded at 240 DAP to assess the water stress tolerance level of the clones under study. The mean values for proline at 240 DAP among clones was ranged from 14.74 to 24.94 (µg/gfw) with an average of 21.13 (µg/gfw) under normal conditions (E1), 17.56 to 31.35 (µg/gfw) with an average of 24.47 (µg/gfw) under mild water stress (E2) environment, 20.58 to 47.65 (µg/gfw) with an average value of 35.3 (µg/gfw) under rainfed environment (Appendix Table 5).

Highest proline content was observed for clone BM 101068 followed by CoPb 14184 and CoPb 15214 under E1 environment whereas CoPb 13181 followed by Co 0118 and CoJ 64 possessed minimum value of proline. Under E2 environment, clone F 391/14 followed by F 404/13 and BM 10-1068 showed high proline value while clone CoPb 13181 followed by CoJ 64 and Co 0118 had lowest proline value. Under E3 environment, clone F 391/14 followed by F 362/14 and CoPb 15212 exhibited highest proline value whereas CoJ 64 followed CoPb 13181 exhibited lowest value of proline (Fig 4.7).

Maximum increase in proline was observed in cultivar F 391/14 i.e. 116.65 % followed CoPb 13181 (113.22 %) and Co 0118(109.59 %) and minimum content was observed for cultivar BM 101068 (37.10 %) followed by CoJ 64 (39.78 %) under mild water stress. Under rainfed environment, maximum increase in proline contents were estimated for cultivar CoPb 13181 (126.64 %) followed by F 391/14 (119.56%) while lowest proline contents were observed for clone BM 10-1068 (39.24 %) followed by CoJ 64(46.73 %) (Appendix Table 8).

Our findings under study have been supported by the study done by Kumar *et al* (2019) and Hemaprabha *et al* (2013). Silva (2015) also described higher rise proline accumulation in water deficit tolerant genotypes.

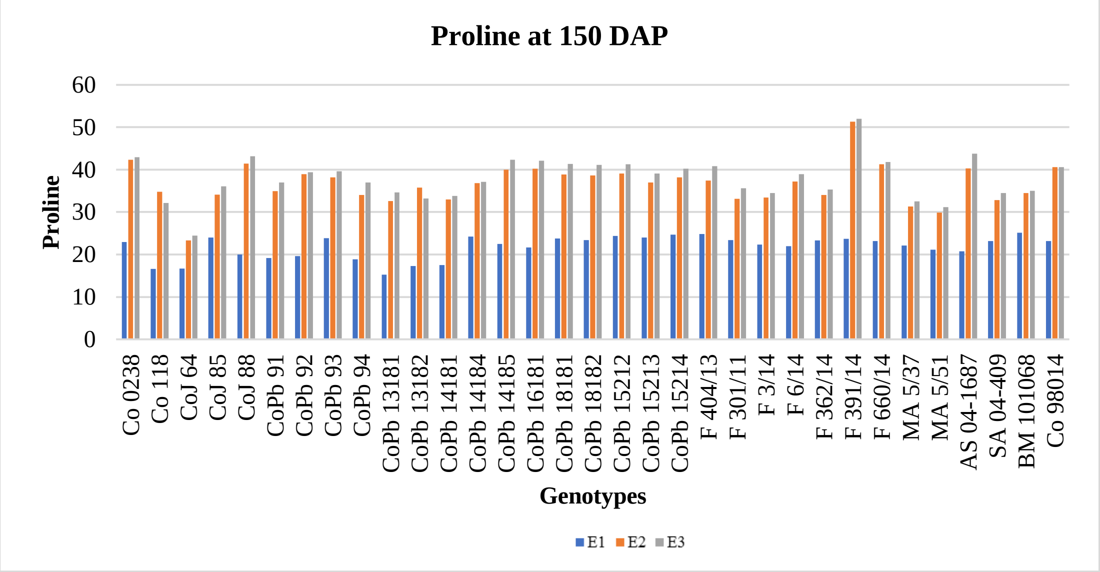

Fig 4.6: Average value of proline at 150 days after planting

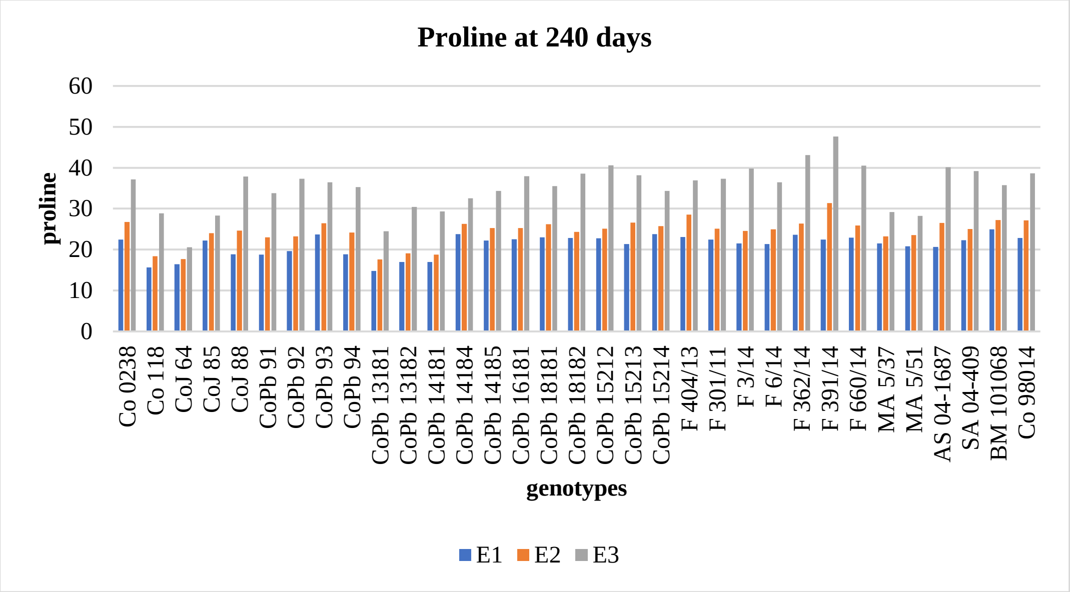

Fig 4.7: Average value of proline at 240 days after planting

4.1.2.4b SOD (eu/100ml/gfw) at 150 DAP and 240 DAP

SOD **(eu/100ml/gfw)** content was ranged from 0.103 to 0.122, 0.135 to 0.196 and 0.137 to 0.203 (eu/100ml/gFW) with mean value of 0.146, 0.172 and 0.177 (eu/100ml/gFW) under normal, mild water stress and rainfed environments, respectively (Appendix Table 5). Highest SOD value under normal environment was recorded for clone Co 0238 followed by F 3/14 and F 301/11 whereas clones SA 04-409 followed by CoJ 64 exhibited minimum value (Fig 4.8). Clone F 391/14 followed by F 3/14 and Co 0118 were reported highest SOD value while minimum value was recorded for clone CoJ 64 followed by MA 5/51 under E2 environment. Under E3 environment, maximum proline content was observed for clone

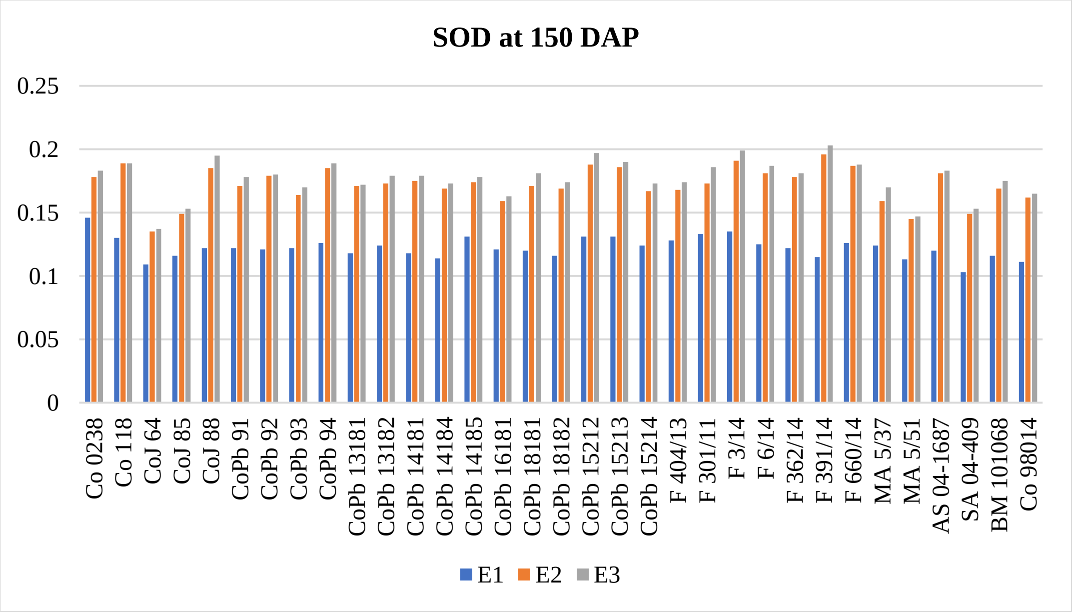

Fig 4.8: Average value of superoxide dismutase at 150 days after planting

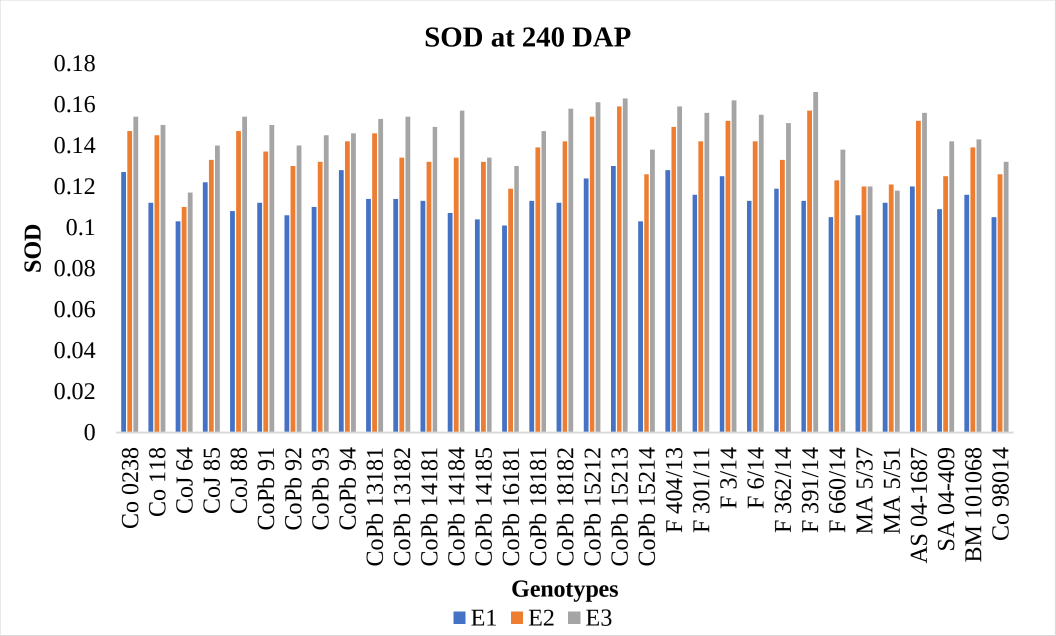

Fig 4.9: Average value of at superoxide dismutase at 240 days after planting

F 391/14 followed by F 391/14 while minimum values were observed for clone CoJ 64 followed by MA 5/51 and CoJ 85.

At 240 DAP, SOD contents were ranged from 0.101 to 0.130, 0.110 to 0.159 and 0.117 to 0.166 (eu/100ml/gFW) with mean value of 0.113, 0.137 and 0.146 (eu/100ml/gFW) under normal, mild water stress and rainfed environments, respectively (Appendix Table 5). Highest SOD value under normal environment was recorded for clone CoPb15213 followed by F 404/13 and CoPb 94 whereas clone CoPb 16181 followed by CoJ 64 exhibited minimum value of proline. Clone CoPb 15213 followed by F 391/14 and CoPb 15212 were reported of having highest SOD value while minimum values were recorded for clones CoJ 64 followed by CoPb 16181 under E2 environment. Under E3 environment, maximum proline content was observed for clone F 391/14 followed by CoPb 15213 and minimum values were observed for clones CoJ 64 followed by MA 5/51 and MA 5/37 (Fig 4.9).

Tripathi *et al* (2018) observed the increased activity of SOD in CoPk 05191 over CoLk 94184 as indicating its better performance.

Per cent increase over normal environment was estimated for SOD. Under E2 environment, maximum increase of SOD was observed for cultivar F 391/14 (70.43 %) while cultivar Co 0238 followed by CoJ 64 accumulated minimum amount of SOD (Appendix Table 8). Similarly, under E3 environment, maximum SOD accumulation was observed for cultivar F 391/14 followed by CoJ 88 while cultivar Co 0238 followed by CoJ 64 accumulated minimum amount of SOD. At 240 days under E2 and E3 environments, maximum accumulation of SOD was observed for cultivar F 391/14 (38.93 % and 46.90 %, respectively). Under E2, minimum value of SOD was observed for genotype CoJ 64; whereas under E3, minimum SOD value was recorded for MA 5/51 followed by MA 5/37.

In sugarcane, SOD activity is genotype-dependent and is also modulated by water stress (Hemaprabha et al 2004). Moreover, (Jangpromma et al 2012) showed that sugarcane cultivars classified as drought-tolerant exhibit higher levels of SOD activity under water deficit conditions. Non-enzymatic antioxidant molecules can work synergistically with enzymatic ROS scavenging mechanisms to protect plant cells against oxidative damage. According to Guimaraes et al (2008) free-proline content is correlated to water stress tolerance in different sugarcane cultivars. Taken all together, a correlation between water stress and the response of the antioxidant system in sugarcane is evident. The activity of ROS-scavenging enzymes may be used as a marker of water stress tolerance in sugarcane.

So, we may conclude that cultivar F 391/14, CoJ 88, CoPb 13182 performed better, and are of tolerant type; while clones CoJ 64 and CoJ 85 performed poor, and behaved as susceptible type under moisture deficit stress.

4.2 Selection parameters for quality, quantitative, physiological and biochemical traits

Any experiment can only be improved if there is genetic variability. The range of average scores based on phenotypic expression can only be a rough approximation of the variance or magnitude of divergence between genotypes. Estimates of phenotypic and genotypic coefficients of variability are more accurate indicators of the level of variability in the experimental materials. Similarly, breeders need to know about character heritability because it shows the scope and likelihood of change that can be accomplished through selection. So, for cane yield and it’s component characters, physiological, biochemical and quality traits studied in the current study under all three normal (E1), mild water stress(E2) and rainfed (E3) environments (Table 4.21 and 4.22), the genetic variability parameters like phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance are being discussed below:

4.2.1 Quantitative parameters

4.2.1a Germination %

Genetic variability studies revealed that under normal (E1) environment, genotypic & phenotypic coefficients of variation for germination percentage were reported to be 13.63 % & 18.66 %, respectively, whereas under mild water stressed (E2) and rainfed (E3) environments, the corresponding values were 15.77 % & 19.79 % and 14.24 % & 19.20 %, respectively (Table 4.3). Moderate value of heritability (broad sense) for germination percentage under E1 and E3 environment to the tune of 53.00 %, and 55.00 %, respectively; whereas under E2, higher values (64.00 %) were observed. Genetic advance (per cent of mean) was found to be moderate under all three environments i.e. E1, E2 and E3 with 20.52 %, 20.90 % and 21.76 %, respectively. Singh *et al* (2002) documented coefficient of variation was moderate for percentage of germination. Moderate to high heritability and genetic advance for germination (%) has been reported in earlier studies (Singh *et al* 2010). So, this characteristic should be considered while making selection for higher cane yield; whether it is irrigated or drought environments.

4.2.1b Number of tillers (000/ha)

The number of tillers exhibited moderate magnitude of PCV (18.79 %) and GCV (17.43 %) under E1; while under E2 and E3 high magnitude of GCV (25.20 % and 23.75 %, respectively) and PCV (26.56 % and 25.10 %, respectively) was reported. The heritability and genetic advance calculated for this trait were higher under all three environments i.e. 86.00 % and 33.32 % (E1), 90.00 % and 49.25 % (E2) and 90.00 % and 46.26 %(E3), respectively (Table 4.3).

Gowda *et al* (2016) had described high magnitude of phenotypic and genotypic coefficient of variation for tiller count, and values of heritability and genetic advance were also high.

4.2.1c Number of shoots at 210 DAP and 240 DAP

At 210 DAP, number of shoots exhibited high magnitude for all the variability parameters like PCV (23.69 %), GCV (22.81 %), heritability (93.00 %) and genetic advance (per cent of mean) (45.22 %) under normal (E1) environment and the corresponding figures in water stress (E2) and rainfed (E3) environment were PCV (27.39 % and 33.75 %, respectively) and GCV (26.30 % and 32.24 %, respectively), heritability (92.00 % and 91.00 %) and genetic advance (per cent of mean) (45.22 % and 52.03 %), respectively (Table 4.3). At 240 DAPfor number of shoots, moderate GCV, PCV, heritability and GA % in sugarcane was verified by Sanghera *et al* (2018) under irrigated conditions.

Similarly at 240 DAP, number of shoots exhibited high magnitude of all the variability parameters like PCV (27.79 %), GCV (26.27 %), heritability (89.00 %) and genetic advance (per cent of mean) (451.15 %) under E1 and the corresponding figures in E2 and E3 environments were PCV (29.22 % and 38.53 %, respectively) and GCV (27.68 % and 37.23 %, respectively), heritability (90.00 % and 93.00 %) and genetic advance (per cent of mean) (54.00 % and 74.09 %), respectively (Table 4.21).

4.2.1d Number of millable canes (000/ha)

High magnitude of GCV, PCV, heritability and GA was observed under normal, mild water stress and rainfed environments i.e. GCV (32.12 %, 42.46 % and 46.83 %, respectively), PCV (33.28 %, 43.55% and 48.95 %, respectively), heritability (93.00 %, 95.00 % and 92.00 %, respectively) and GA (63.84 %, 85.27 % and 92.28 %, respectively). Chaudhary *et al* (1970) reported high PCV %, GCV %, GA and high heritability value for NMC in sugarcane (Table 4.3). Gowda *et al* (2016) and Tena *et al* (2016) also reported high heritability values for this trait. This recommends that simple selection for this trait might be effective for potential cane yielding genotypes.

Table 4.3: Selection parameters for quality and quantitative traits in sugarcane under normal (E1), mild water stress (E2) and rainfed (E3) environments

Traits	GCV%			PCV%			h² (Broad Sense)			GA (%)		
	E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
Cane yield and Component traits												
Germination %	13.63	15.77	14.24	18.66	19.79	19.2	53.00	64.00	55.00	20.52	25.9	21.76
No. of tillers (000/ha)	17.43	25.2	23.74	18.79	26.56	25.1	86.00	90.00	90.00	33.32	49.25	46.26
No. of shoots at 210 DAP (000/ha)	22.81	26.3	32.24	23.69	27.39	33.75	93.00	92.00	91.00	45.22	52.03	63.46
No. of shoots at 240 DAP (000/ha)	26.27	27.68	37.23	27.79	29.22	38.53	89.00	90.00	93.00	51.15	54	74.09
No. of millable canes (000/ha)	32.12	42.46	46.83	33.28	43.55	48.95	93.00	95.00	92.00	63.84	85.27	92.28
Cane length (cm)	15.52	20.51	20.75	17.75	23.01	24.42	76.00	80.00	72.00	27.93	37.68	36.31
Cane girth (cm)	9.86	11.02	8.41	11.98	12.85	13.17	68.00	74.00	41.00	16.73	19.47	11.07
Single cane weight (kg)	16.74	18.67	23.22	18.71	22.74	26.19	80.00	67.00	79.00	30.84	31.57	42.4
Cane yield (t/ha)	20.44	46.33	61.77	24.64	48.9	65.89	69.00	90.00	88.00	34.94	90.44	119.29
Quality traits												
Extraction (%) at 11 months	5.34	8.56	8.88	6.07	9.32	9.91	77.00	84.00	80.00	9.67	16.2	16.39
Brix (%) at 11 months	9.02	7.75	4.75	9.3	7.98	5.14	94.00	94.00	85.00	18	15.49	9.05
Pol (%) at 11 months	10.07	8.52	5.37	10.32	8.81	5.77	95.00	94.00	87.00	20.25	16.97	10.3
Purity (%) at 11 months	2.31	2.02	1.4	2.5	2.82	2.24	85.00	51.00	39.00	4.39	2.98	1.81
CCS (%) at 11 months	10.66	9.05	5.81	10.92	9.52	6.38	95.00	90.00	83.00	21.45	17.73	10.88
CCS (t/ha)	18.49	43.44	61.76	23.14	45.99	65.87	64.00	89.00	88.00	30.42	84.5	119.31

Genetic coefficient of variations (GCV %), Phenotypic coefficient of variations (PCV %), Broad sense heritability (h², Broad Sense), Genetic advance (GA %), Commercial cane sugar (CCS)

Table 4.4: Selection parameters for physiological and biochemical traits in sugarcane under normal (E1), mild water stress (E2) and rainfed (E3) environments

Traits	GCV%			PCV%			h^2 (Broad Sense)			GA (%)		
	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3
Physiological traits												
Relative water content (%) at 120	5.67	6.24	6.54	6.2	7.57	7.73	83.00	68.00.	72.00	10.66	10.6	11.4
Relative water content (%) at 150	6.57	5.79	6.96	6.99	7.02	8.81	88.00	68.00	62.00	12.71	9.84	11.31
Relative water content (%) at 240	3.41	4.01	4.68	4.25	5.08	6.55	64.00	62.00	51.00	5.64	6.51	6.89
Cell membrane stability (%) at 150 DAP	3.93	7.17	6.46	4.02	8.07	7.4	96.00	79.00	76.00	7.92	13.11	11.63
Cell membrane stability (%) at 240 DAP	3.43	2.92	4.42	3.55	3.68	5.88	94.00	63.00	57.00	6.85	4.76	6.85
Biochemical traits												
Superoxide dismutase at 150 DAP	6.61	12.81	8.15	7.31	13.29	9.23	82.00	93.00	78.00	12.32	25.46	14.83
Superoxide dismutase at 240 DAP	7.29	12.54	8.28	8.32	13.1	10.49	77.00	92.00	62.00	13.16	24.73	13.48
Proline at 150 days DAP	12.39	7.93	12.87	12.73	8.81	13.37	95.00	81.00	93.00	24.82	14.71	25.52
Proline at 150 days DAP	12.21	7.83	15.42	12.67	9.48	15.95	93.00	68.00	93.00	24.23	13.3	30.7

Genetic coefficient of variations (GCV %), Phenotypic coefficient of variations (PCV %), Broad sense heritability (h^2, Broad Sense), Genetic advance (GA %)

4.2.1e Cane length (cm)

The PCV, GCV, heritability and GA values for cane length were moderate (17.75 %, 15.52%, 76 % and 27.93 %, respectively) under E1 while high values of GCV, PCV, heritability and GA under E2 and E3 environments were found i.e. GCV (20.51 % and 20.75 %), PCV (23.01 % and 24.42 %), heritability (80.00 % and 72.00 %) and GA (37.68 % and 36.31 %), respectively (Table 4.3). Chaudhary *et al* (2001) reported moderate magnitude for heritability for this trait. Similarly, Moderate values of PCV %, GCV % and GA were recorded by Sanghera *et al* (2015).

4.2.1f Cane girth (cm)

Variability studies discovered that, under normal (E1) environment, cane girth was having moderate PCV (11.98 %), low GCV (9.86 %), high heritability (68.00 %) and low GA (11.98 %). Similarly, under E2, this trait showed moderate magnitude of PCV (12.85 %), GCV (11.02 %), high heritability (74.00 %) and low GA (19.47 %). Under E3, low GCV (8.41 %), moderate PCV (13.17 %), low heritability (41.00 %) and low GA (11.07 %) was reported (Table 4.3). Since, stalk girth is a characteristic which directly affect the cane yield, so moderate heritability and low genetic advance for this character would not give noteworthy breeding gain for cane yield as well as sucrose % selection cycles in different generations. The results under E1 conditions are in corroboration with former study of Singh *et al* (2010).

4.2.1g Single cane weight (kg)

For this trait, moderate magnitude was reported of GCV under E1 and E2 i.e. 16.74 % and 18.67 %, respectively, whereas higher value was noted under E3 i.e. 23.22 %. PCV magnitude under E1 was moderate (18.71 %) while high PCV values under both E2 and E3 environment was recorded i.e. 22.74 % and 26.19 %, respectively. High values of heritability i.e. 80.00 %, 67.00 % and 79.00 % and high value of GA i.e. 30.84 %, 31.57 % and 42.40 % was observed under E1, E2 and E3, respectively (Table 4.3). For SCW under water stress conditions, moderate heritability value was described by Kumar *et al* (2001). Sanghera *et al* (2018) reported high magnitude of PCV, GCV and GA whereas heritability was moderate under both normal and stress environments. Tena *et al* (2016) and Arora *et al* (2014) had documented high magnitude of PCV, GCV and GA for SCW.

4.2.1h Cane yield (t/ha)

In context of variability studies, high PCV (24.64 %, 48.90 % and 65.89 %, respectively), GCV (20.44 %, 46.33 % and 61.77 %, respectively) and GA (34.94 %, 90.44 % and 119.29 %) were recorded under E1, E2 and E3 environments, respectively. High heritability values were observed under E1, E2 and E3 i.e. 69.00 %, 90.00 % and 88.00 %, respectively (Table 4.3). Kumar *et al* (2001) also stated high heritability and GA as per cent of mean for cane yield under water stress environment. Under irrigated conditions, Arora *et al* (2014) documented high genetic advance and high to moderate variability coefficients for cane yield. Sanghera *et al* (2018) reported high magnitude of PCV and GA under both normal and stressed environment; whereas moderate value of heritability was recorded under normal condition and high heritability was noted under stressed environment. This recommends that a large proportion of the total variance is heritable and selection of this trait would be effective under all three enivronments (E1, E2 and E3).

4.2.2 Quality traits

4.2.2a Brix % at 11 months

Studies on variability parameters for this trait revealed low PCV, GCV, GA and high heritability values under all three environments (Table 4.3). In sugarcane, Tena *et al* (2016) observed low magnitude of GCV, PCV and GA, and Sanghera *et al* (2018) reported high heritability values for brix%.

4.2.2b Pol % at 11 months

Pol (%) was recorded with moderate GCV, PCV, GA and high heritability under E1 environment, whereas low GCV, PCV, moderate GA and high heritability was observed under both E2 and E3 environments (Table 4.23. The results on variability parameters obtained in this study under mild water stress (E2) and rainfed (E3) environment were in agreement with the results described by Tena *et al* (2016) and Mehareb *et al* (2017).

4.2.2c Purity% at 11 months

This trait exhibited low GCV, PCV, GA under all three environments. High, moderate and low value of heritability was recorded under E1, E2 and E3 environments, respectively (Table 4.3) Tena *et al* (2016) reported low values of GCV, PCV, GA and heritability. Sanghera *et al* (2018) found low magnitude of GCV, PCV and GA under both normal and stress environment; whereas low heritability was observed under normal condition and moderate value was observed under stress condition. Gowda *et al* (2016) also registered low heritability coupled with low GCV, PCV and GA for purity %.

4.2.2d CCS %

This trait exhibited moderate PCV, GCV and GA value under normal condition while low value of GCV, PCV and GA were observed for both mild water stress and rainfed environment. High heritability was observed under all environments (Table 4.3). Gowda *et al* (2016) reported moderate magnitude for all genetic variability traits. Moderate estimations of GCV, PCV joined with high heritability have been reported by Sanghera *et al* (2014) for CCS%. Therefore, this character should be taken into consideration while making selection for higher sugar yield.

4.2.2e Extraction % at 11 months

Under normal, mid water stress and rainfed environment for extraction% low magnitude of PCV i.e. 5.34 %, 8.56 % and 8.88 %, respectively, and GCV i.e. 6.07 %, 9.32 % and 9.91 %, respectively; whereas moderate value of GA i.e. 9.69 %, 16.20 % and 16.39 %, respectively, was recorded in present study. Heritability was moderate under E1 and E3 i.e. 77.00 % and 80.00 %, respectively; whereas under E2 environment, high value of heritability was observed i.e. 84.00 % (Table 4.3). Sanghera *et al* (2018) also reported low magnitude of PCV, GCV and GA coupled with low to moderate heritability for juice extraction %.

4.2.2f CCS (t/ha)

This trait exhibited moderate GCV value (18.49 %), high PCV value (23.14 %) , high GA (30.42 %) and moderate heritability value (64.00 %) under E1, while under E2 and E3 environment, high GCV(43.44 % and 61.76 %, respectively) , PCV (45.99 % and 65.87 %, respectively), GA(84.5 % and 119.31 %, respectively) and high heritability value (89.00 % and 88.00 %, respectively) was observed (Table 4.21). Krishna *et al* (2017) reported moderate magnitude of GCV, PCV and high GA coupled with high heritability for this trait. Gowda *et al* (2016) also observed high magnitude of heritability. Anbanandan and Saravanan (2010) and Arora *et al* (2014) also reported high PCV, GCV and GA for CCS (t/ha).

4.2.3 Biochemical parameters

4.2.3a Proline (µg/gfw) at 150 DAP and 240 DAP

Variability parameters for proline content at 150 DAP and 240 DAP were observed of having moderate value of GCV and PCV, moderate GA and high heritability under E1 environment. Under E2 environment, low GCV, low PCV, low GA and moderate to high heritability were estimated; whereas under E3 environment, moderate value of GCV, PCV, GA and high heritability (Table 4.4) were observed. Under salt stress, Hien *et al* 2003 also reported many folds amplified proline content.

4.2.3b SOD at 150 DAP and 240 DAP

Variability study for this trait showed low PCV, low to moderate GCV, low genetic advance and moderate to high heritability values under normal (E1) conditions whereas under mild water stress (E2) conditions its exhibited moderate PCV, GCV and high heritability coupled with moderate GA values. Under rainfed (E3) condition, low GCV, low to moderate PCV, low genetic advance coupled with moderate heritability value were reported (Table 4.4). Saed *et al* (2021) found SOD activity had the highest heritability and GCV under drought stress condition, while it had almost low values of these parameters under normal condition. These results indicate the importance of this enzyme in triticale genotypes under water shortage condition,

4.2.4 Physiological parameters

4.2.4a Relative water content (RWC, %) at 120 DAP, 150 DAP and 240 DAP

The RWC exhibited low magnitude of PCV, GCV and GA coupled with moderate to high heritability under all three environments (Table 4.4).

Sanghera *et al* (2018) reported, under normal conditions, low magnitude of GCV and PCV; while GA was low under both normal and stress environment.

4.2.4b Cell membrane stability (%) at 150 DAP and 240 DAP

Studies on variability parameters for this trait revealed low PCV, GCV and GA with high heritability values under normal (E1) environment whereas under mild water stress (E2) and rainfed conditions (E3) low PCV, GCV, GA and moderate heritability were recorded (Table 4.4).

The conclusive discoveries of the study relating to genetic variability parameters for cane yield & its components traits, physiological, biochemical and quality characters are additionally concised below with appropriate supportive literature. High heritability values for major economic traits like cane yield, CCS t/ha, number of tillers (000/ha), number of shoots (000/ha), brix %, purity % and Pol % and CCS % at 11 months under mild water stress (E2) and rainfed (E3) conditions specifies that significant improvement can be expected by giving emphasis on the selection of these characters. The relative water content (%) at 120 DAP (32.27) had the lowest h^2bs values followed by relative water content (%) at 60 DAP (32.48) under water stressed (E2) environment and under normal (E1) environment. The lowest h^2bs value was recorded for purity % at 11 months (24.14) followed by specific leaf weight in grams (30.87). This could be due to the environmental influence on the expression of these traits as it is also indicated by the differences in PCV and GCV values.

The results of genetic advance (GA) showed that the characters namely NMC, cane yield (t/ha), CCS (t/ha) and SCW were having high GA values under all the environments (E1, E2 and E3), and considerable improvement can be predictable by practicing selection for these characters. Low to moderate values of GCV, PCV and GA for characters like RWC, CMS, proline and SOD along with some quality traits show that direct selection could not be effective for these characters. The low levels of GA attained for some agronomic and quality traits could be attributed due to low levels of GCV. Balasundaram and Bhagyalakshmi (1978) also found low level of GA for cane girth and juice quality traits; which is attributed with low level of variability. Jain *et al* (2001) and Chaudhary (2001) had also accentuated on the significance of single cane weight, number of millable canes and cane yield at the time of selection because these were found of having high heritability and GA.

Based on the results of genetic variability, heritability and GA, it can be concluded that cane yield, number of tillers, NMC, SCW and CCS are the characters which should be given due weightage at the time of selection under water stress conditions for achieving the improvements in a crop breeding.

For cane juice purity %, lowest value of GCV and PCV and lowest heritability (39.00 %) was observed. Arora *et al* (2014) assessed the coefficient of variations of various characters like number of tillers, NMC, cane yield, SCW, CCS % and CCS (t/ha); which explain that these characters had high GCV joined with high heritability and high GA. High levels of genotypic variations for cane yield and it’s contributing traits like NMC have been described in numerous previous reports (Bhide 1971, Khairwal and Babu 1976). Ram (1994) had also described high coefficient of variation for SCW, cane yield and sugar yield. High amount of variability in cane weight, cane yield and sugar yield, and low variability in brix % and Pol (%) rose per cent has also been stated by Hapase and Repale (1999).

4.3. Correlation and regression coefficient among the traits

Under all three environments i.e. normal (E1), mild water stress (E2) and rainfed (E3), cane yield regarding it's contributing traits was observed to be significantly positively ($p < 0.01$, $r > 0.48$) correlated with tillers, shoot and NMC (Table 4.5); while it was negatively ($p < 0.01$, $r > 0.04$) correlated with quality traits i.e. brix %. Pol % and CCS % (Table 4.5). However, under E2 and E3 environment, juice extraction % and purity % was non-significantly positively correlated with cane yield.

Single cane weight was significantly positively ($p > 0.01$, $r > 0.43$) correlated with cane yield under both stressed conditions (E2 & E3); while under E1, there was non-significant negative association among these two traits. Under all three environments (E1, E2 & E3) single cane weight was significantly positively ($p < 0.01$, $r > 0.36$) correlated with cane girth. Silva *et al* (2008) observed, under normal as well as drought), a positive association of cane yield with single cane weight, number of stalks, cane length. Sanghera *el al* (2017) found significant positive association of cane yield with SCW. Kumar *et al* (2001) observed positive association of cane yield with tillers, shoots, NMC and SCW. Tena el al (2014) observed significant positive correlation of cane yield with NMC and SCW. Gomathi *et al* (2020) observed significant positive association of yield with shoots, NMC and SCW.

Under both stressed environments (E2 & E3), cane yield was significantly ($p < 0.01$) positively associated with CMS % at 150 DAP ($r > 0.37$), SOD at 150 DAP ($r > 0.47$), SOD at 240 DAP ($r > 0.37$), proline at 150 DAP ($r > 0.50$) and proline at 240 DAP ($r > 0.43$) (Table 4.6).

In sugarcane, cane yield (t/ha) is decided by the NMC and SCW; while these two traits are directly linked a number of other traits i.e. Gm %, tiller counts, shoot count, cane length and cane girth. In our present study, NMC was observed to be main contributing trait with $R^2 > 0.63$ while role of SCW was not so pronounced specially under normal environment (Fig 4.10 & 4.11). Begum *et al* (2012) found linear relationship among cane yield and millable canes. Silva *et al* (2008) also observed linear relation of cane yield with number of millable stalk and SCW.

CCS % was significantly positively ($p < 0.01$, $r > 0.41$) correlated with it's other quality contributing traits (extraction %, brix %, Pol % and purity %); while it's negative association was observed with cane yield and it's contributing traits except Gm%, cane girth and SCW.(Table 4.5) Cane girth was significantly correlated with CCS %; while for Gm% and SCW, this association was non-significant (Table 4.5). The main quality traits

Table 4.5 Correlation coefficients among different quantitative and qualitative traits of sugarcane under normal (E1), mild water stressed (E2) and rainfed (E3) environment

		Gm %	Tillers (000/ha)	Shoots* (000/ha)	Shoots (000/ha)	NMC (000/ha)	CY (t/ha)	CL (cm)	CG (cm)	SCW (kg)	Ext%	Brix%	Pol%	Purity%	CCS%	CCS (t/ha)
Gm %	E1	1.00	0.29	0.35*	0.34	0.31	0.41*	-0.05	0.11	0.12	-0.09	0.09	0.14	0.21	0.15	0.45*
	E2	1.00	0.57*	0.47*	0.44*	0.34	0.29	-0.15	-0.16	-0.20	0.26	-0.09	-0.02	0.26	0.02	0.25
	E3	1.00	0.26	0.13	0.05	-0.07	-0.02	-0.29	0.05	-0.13	0.06	0.03	0.11	0.28	0.11	0.00
Tillers (000/ha)	E1		1.00	0.90*	0.92*	0.88*	0.75*	-0.16	-0.34	-0.49*	-0.05	-0.11	-0.10	-0.05	-0.09	0.63*
	E2		1.00	0.94*	0.94*	0.86*	0.84*	0.07	-0.17	0.17	0.26	-0.24	-0.16	0.21	-0.12	0.80*
	E3		1.00	0.92*	0.82*	0.63*	0.50*	-0.05	0.05	0.02	0.53*	-0.02	-0.01	0.04	-0.16	0.45*
Shoots* (000/ha)	E1			1.00	0.92*	0.92*	0.81*	-0.09	-0.38*	-0.47*	-0.14	-0.20	-0.17	-0.07	-0.16	0.63*
	E2			1.00	0.97*	0.92*	0.87*	0.09	-0.29	0.09	0.19	-0.32	-0.23	0.21	-0.19	0.80*
	E3			1.00	0.96*	0.66*	0.48*	-0.15	0.08	-0.06	0.40*	-0.02	0.00	0.07	-0.19	0.42*
Shoots (000/ha)	E1				1.00	0.94*	0.81*	-0.04	-0.45*	-0.51*	-0.17	-0.23	-0.22	-0.16	-0.21	0.60*
	E2				1.00	0.89*	0.84*	0.06	-0.23	0.13	0.22	-0.31	-0.22	0.22	-0.17	0.79*
	E3				1.00	0.71*	0.51*	-0.07	-0.02	-0.08	0.33	-0.07	-0.04	0.08	-0.24	0.44*
NMC (000/ha)	E1					1.00	0.79*	0.08	-0.55*	-0.60*	-0.34*	-0.36*	-0.36*	-0.29	-0.35*	0.47*
	E2					1.00	0.91*	0.33	-0.41*	0.14	-0.04	-0.49*	-0.40*	0.13	-0.35*	0.78*
	E3					1.00	0.85*	0.22	-0.02	0.19	0.15	-0.08	-0.02	0.14	-0.29	0.79*
CY (t/ha)	E1						1.00	0.15	-0.30	-0.16	-0.29	-0.28	-0.26	-0.14	-0.25	0.76*
	E2						1.00	0.31	-0.20	0.43*	0.04	-0.28	-0.21	0.15	-0.18	0.95*
	E3					.	1.00	0.33	0.17	0.59*	0.16	-0.04	-0.01	0.06	-0.18	0.96*
CL (cm)	E1							1.00	-0.56*	0.06	-0.40*	-0.38*	-0.40*	-0.35*	-0.41*	-0.10
	E2							1.00	-0.59*	0.22	-0.61*	-0.56*	-0.53*	-0.14	-0.51*	0.18
	E3							1.00	-0.47*	0.41*	-0.28	-0.41*	-0.46*	-0.32	-0.53*	0.23
CG (cm)	E1								1.00	0.68*	0.41*	0.47*	0.50*	0.47*	0.51*	0.06
	E2								1.00	0.47*	0.46*	0.63*	0.57*	0.06	0.53*	-0.03
	E3								1.00	0.36*	0.31	0.39*	0.39*	0.17	0.54*	0.30

SCW (kg)	E1									1.00	0.18	0.36*	0.40*	0.44*	0.42*	0.14
	E2									1.00	0.04	0.27	0.25	0.05	0.24	0.53*
	E3									1.00	0.08	0.10	0.06	-0.07	0.09	0.63*
Ext%	E1										1.00	0.53*	0.55*	0.50*	0.55*	0.18
	E2										1.00	0.56*	0.59*	0.33	0.58*	0.21
	E3									.	1.00	0.50*	0.42*	-0.04	0.41*	0.24
Brix%	E1											1.00	0.98*	0.54*	0.96*	0.36*
	E2											1.00	0.96*	0.29	0.93*	-0.01
	E3											1.00	0.95*	0.23	0.86*	0.15
Pol%	E1												1.00	0.68*	0.99*	0.40*
	E2												1.00	0.52*	0.99*	0.07
	E3												1.00	0.52*	0.84*	0.17
Purity%	E1													1.00	0.74*	0.36*
	E2													1.00	0.61*	0.29
	E3													1.00	0.25	0.13
CCS%	E1														1.00	0.41*
	E2														1.00	0.10
	E3														1.00	0.05
CCS (t/ha)	E1															1.00
	E2															1.00
	E3															1.00

***. Correlation is significant at the 0.05 level (2-tailed).**

Table 4.6 Correlation coefficients among different physiological traits with cane yield (t/ha) of sugarcane under normal (E1), mild water stressed (E2) and rainfed (E3) environments

Traits	Env	CMS at 150 DAP	CMS at 240 DAP	SOD(at 150DAP	SOD at 240 DAP	Proline at 150 DAP	Proline at 240 DAP	RWC at 120DAP	RWC at 150 DAP	RWC at 240 DAP	Cane yield
CMS at 150DAP	E1	1.00	0.96*	-0.26	-0.376*	0.22	0.21	-0.03	0.08	0.10	-0.07
	E2	1.00	0.64*	0.38*	0.04	0.20	0.02	0.357*	0.14	0.22	0.37*
	E3	1.00	0.73*	0.22	0.15	0.35*	0.30	0.365*	0.19	0.21	0.40*
CMS at 240 DAP	E1		1.00	-0.26	-0.345*	0.25	0.23	-0.02	0.05	0.14	-0.01
	E2		1.00	0.519*	0.31	0.13	0.11	0.13	0.06	0.24	0.22
	E3		1.00	0.426*	0.30	0.58*	0.44*	0.456*	0.34	0.47*	0.59*
SOD(at 150DAP	E1			1.00	0.59*	0.09	0.04	-0.13	-0.14	0.22	0.20
	E2			1.00	0.66*	0.58*	0.44*	0.21	0.14	0.38*	0.47*
	E3			1.00	0.73*	0.51*	0.58*	0.14	0.40*	0.58*	0.52*
SOD at 240 DAP	E1				1.00	0.16	0.07	-0.20	0.00	0.06	0.12
	E2				1.00	0.25	0.29	-0.03	0.18	0.09	0.37*
	E3				1.00	0.45*	0.50*	0.15	0.43*	0.59*	0.40*
Proline at 150 DAP	E1					1.00	0.97*	-0.16	0.04	0.01	-0.06
	E2					1.00	0.66*	0.24	0.39*	0.46*	0.50*
	E3					1.00	0.75*	0.23	0.26	0.47*	0.57*
Proline at 240 DAP	E1						1.00	-0.18	0.03	-0.10	-0.03
	E2						1.00	0.09	0.22	0.51*	0.43*
	E3						1.00	0.16	0.28	0.51*	0.55*
RWC at 120DAP	E1							1.00	0.17	0.20	0.20
	E2							1.00	0.27	0.19	0.14
	E3							1.00	0.32	0.39*	0.37*
RWC at 150 DAP	E1								1.00	0.21	0.04
	E2								1.00	0.32	0.37*
	E3								1.00	0.49*	0.41*
RWC at 240 DAP	E1									1.00	-0.04
	E2									1.00	0.03
	E3									1.00	0.47*

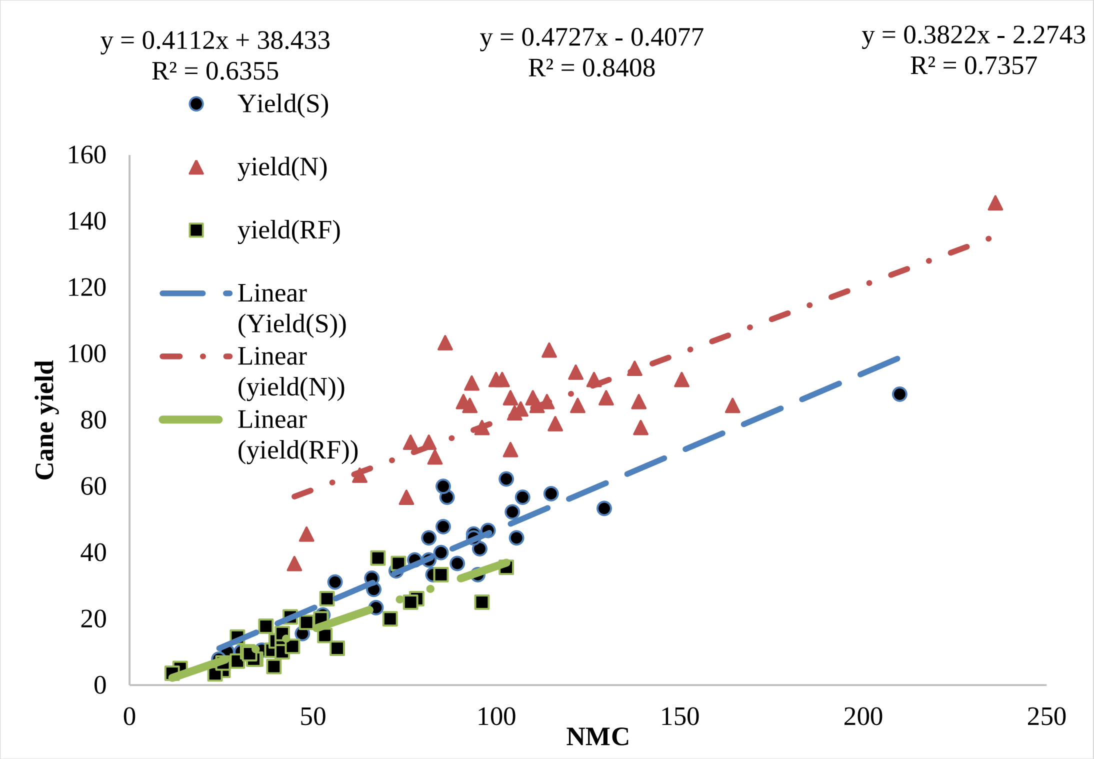

Fig 4.10: Relationship of Cane yield with NMC under Normal (N), Mild stress (S) and Rainfed (RF) environments within the set of 33 sugarcane genotypes

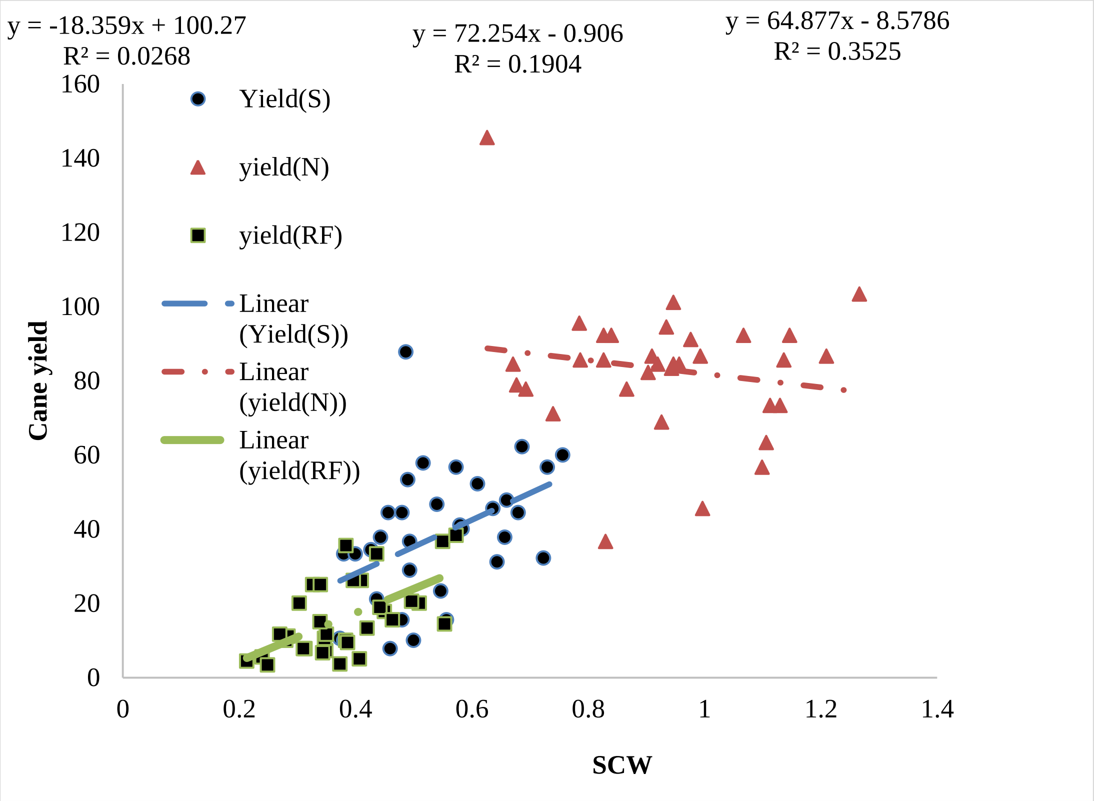

Fig 4.11: Relationship of Cane yield with SCW under Normal (N), Mild stress (S) and Rainfed (RF) environments within the set of 33 sugarcane genotypes

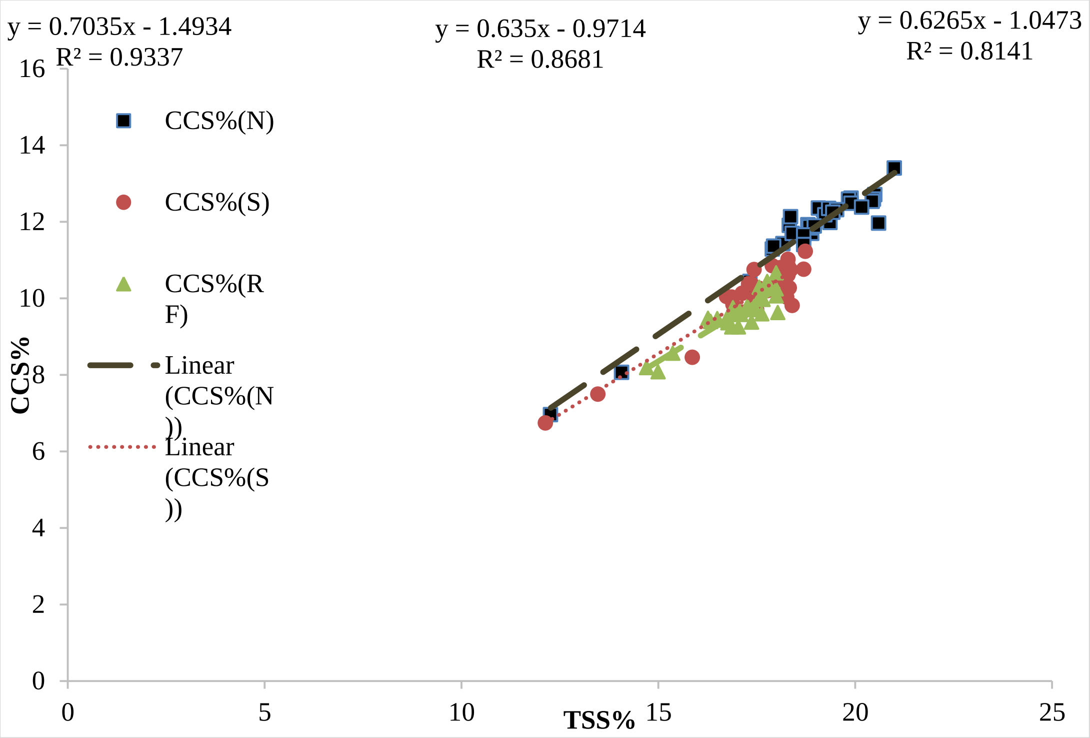

Fig 4.12: Relationship of CCS % with TSS % under Normal (N), Mild stress (S) and Rainfed (RF) environments within the set of 33 sugarcane genotypes

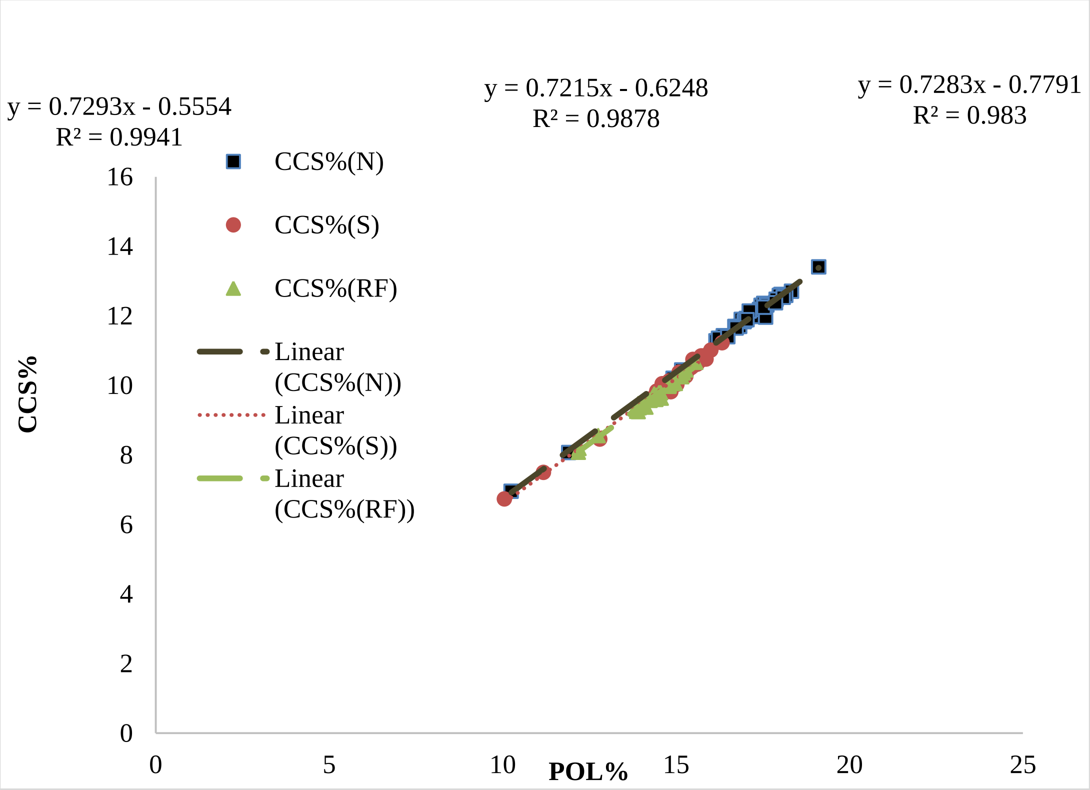

Fig 4.13: Relationship of CCS % with Pol % under Normal (N), Mild stress (S) and Rainfed (RF) conditions within the set of 33 sugarcane genotypes

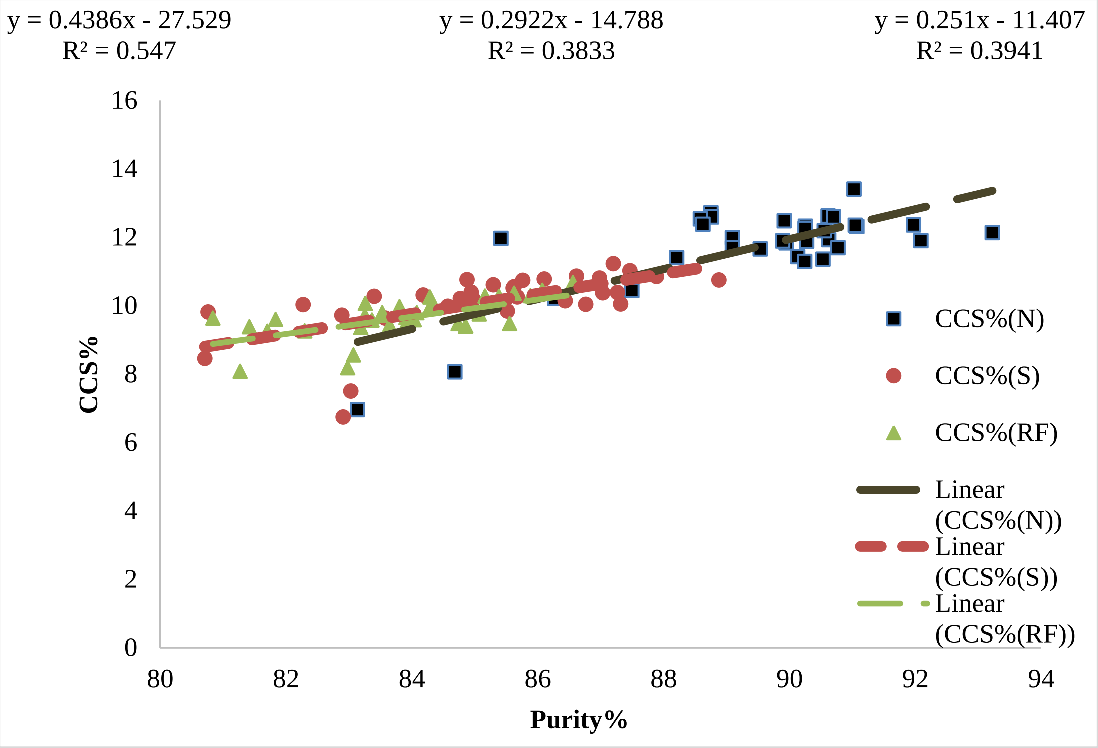

Fig 4.14: Relationship of CCS % with Purity % under Normal (N), Mild stress (S) and Rainfed (RF) environments within the set of 33 sugarcane genotypes

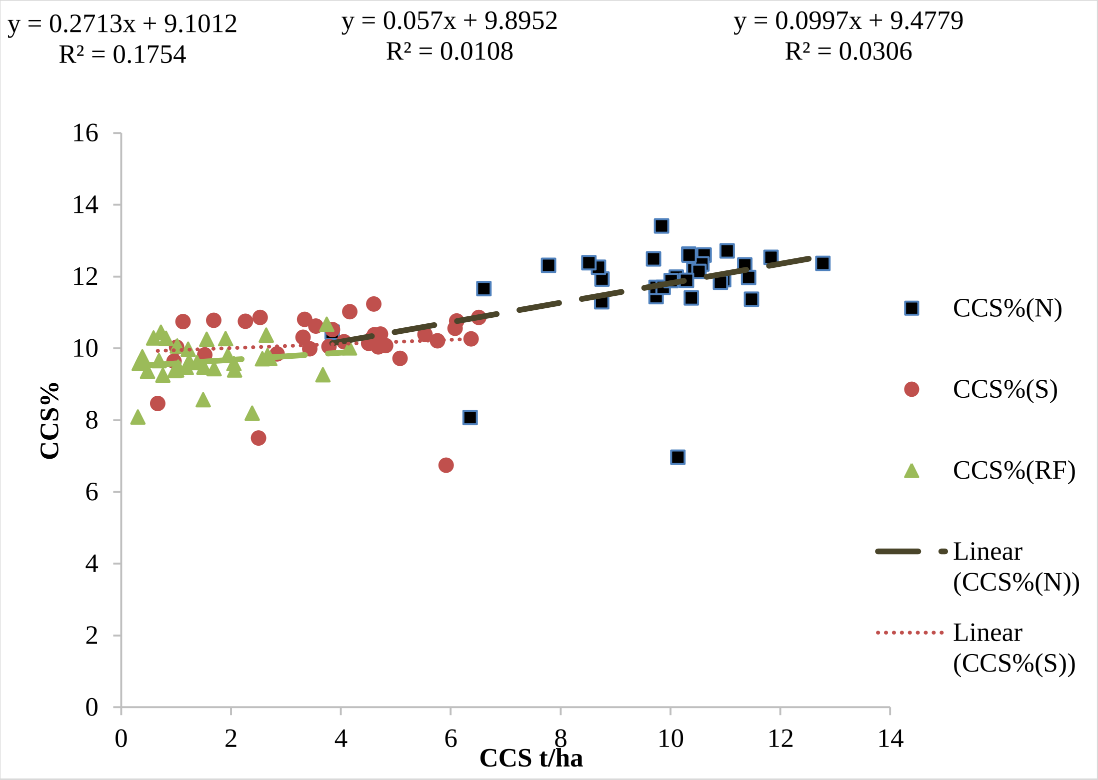

Fig 4.15: Relationship of CCS t/ha with CCS % under Normal (N), Mild stress (S) and Rainfed (RF) conditions within the set of 33 sugarcane genotypes

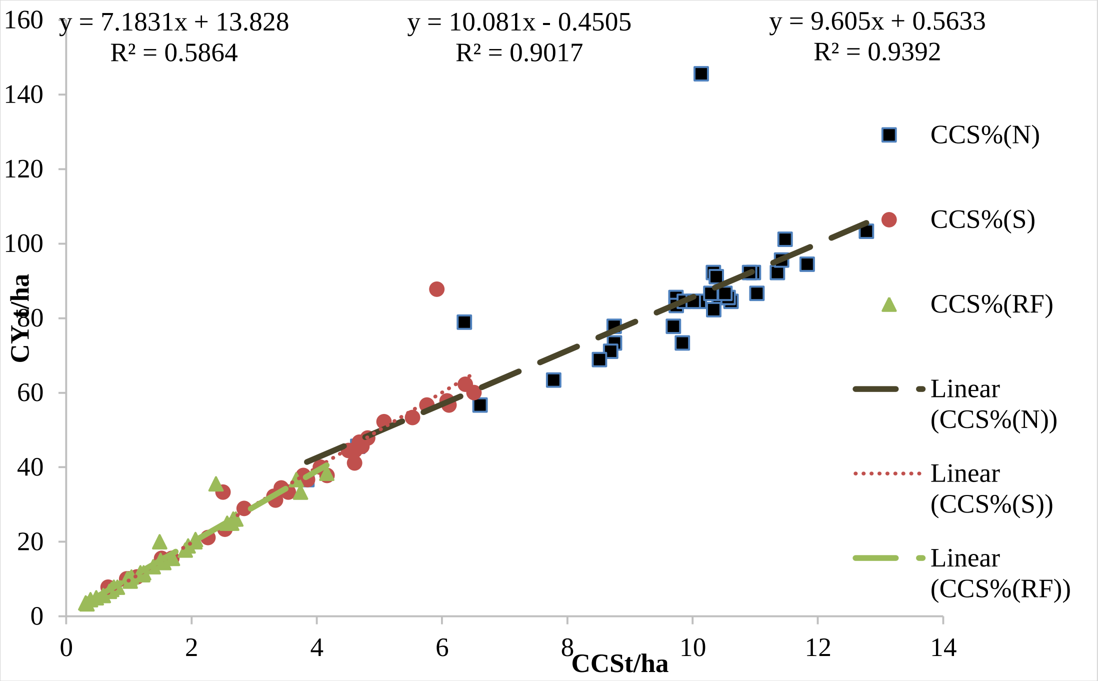

Fig 4.16: Relationship of CCS t/ha with Cane yield under Normal (N), Mild stress (S) and Rainfed (RF) environments within the set of 33 sugarcane genotypes

deciding CCS % are TSS % (brix %), Pol % and purity % (all having major role in CCS % with R^2 value > 0.81, > 0.98% and > 0.39, respectively) (Fig 4.12, 4.13 & 4.14). However, Pol% was observed to be main traits determining CCS % under all environments.

CCS (t/ha) was also observed having positive association with its contributing traits i.e. cane yield ($p < 0.01$, $r > 0.76$) and CCS% ($r > 0.05$) under all environments. CCS (t/ha) was significantly positively correlated with CCS % only under E1 environment.

Under all three environments, CCS (t/ha) was significantly positively ($p < 0.01$, $r > 0.46$) correlated with other yield contributing traits i.e. no. of tillers, no. of shoots and NMC. CCS (t/ha) was positively correlated with quality traits i.e. brix %, Pol %, purity %, however this association was non-significant. Under both stressed (E2 & E3), SCW was significant positively associated with CCS (t/ha), whereas under normal conditions this association was non-significant. Here in this study, cane yield (t/ha) was observed to be more pronounced traits with comparatively more role ($R^2 > 0.58$) in determining the CCS (t/ha) as in compare to CCS % ($R^2 > 0.01$) under all three environments (Fig 4.15 & 4.16).

Sanghera *et al* (2017) found negative association of cane yield with quality traits i.e. brix %, Pol % and purity %. Pol % had also been observed of high direct effect on CCS (t/ha) and CCS % by Sanghera *et al* (2017). Gomathi *et al* (2020) observed significant positive correlation of cane yield with sugar yield. Tena *et al* (2014) found of negative association of Pol % with cane yield; while it's positive association with sugar yield is a key problem in the improvement of sugarcane.

4.4 Eberhart and Russell regression coefficient analyses for cane yield, CCS % and CCS (t/ha)

Based on Eberhart and Russell's regression coefficient parameters (1966), all the 33 sugarcane genotypes were evaluated for cane yield (t/ha), CCS % and CCS t/ha (Table 4.4). The significant ($p \leq 0.01$) value of Environment (linear) components and G × E linear component were observed. So, the observed high magnitude of environmental (linear) effect in comparison to G × E (linear) suggested that high magnitude of environmental (linear) effect might be responsible for high adaptation of these genotypes in relation to quality and cane yield. Pooled deviations differed significantly. Eleven sugarcane genotypes out of 33 were observed stable as deviated non-significantly from zero ($S_d^2{\sim}0$) for cane yield along with high per se mean performance (> 45.66 t/ha). Among these genotypes, four average responsive ($\beta_{ii}{\sim}1$) genotypes i.e. CoPb18181, CoPb13181, CoPb1418 and CoPb15212 and four low responsive ($\beta_{ii}<1$) genotypes i.e. CoJ 88, CoPb 92, CoPb 94 and F 391/14 to water stress were identified (Table 4.7). Two genotypes namely F 301/11 and F 660/14 were highly responsive ($\beta_i> 1$) to water conditions.

For CCS %, three average responsive ($\beta_i{\sim}1$) genotypes i.e. CoJ 88, CoPb 18181 & CoPb 15214, while eleven high responsive ($\beta_i > 1$) genotypes i.e. Co 0118, CoJ 64, CoPb 91, CoPb 92, CoPb 13181, CoPb 14181, CoPb 14185, CoPb 16181, CoPb18182, F 6/14 & F 362/14, and four genotypes namely F 391/14, F3/14, Co 98014 & Co 0238 were reported for water deficite stress responsive as having high *per se* stable performance (>10.49 CCS%, $S_d^2{\sim}0$).

For the trait CCS t/ha, five average responsive ($\beta_i{\sim}1$) genotypes i.e. CoJ 88, CoPb 92, CoPb 94, CoPb 13181 & CoPb 18181, while five high responsive ($\beta_i > 1$) genotypes i.e. CoPb 91, CoPb 14185, CoPb18182, CoPb 15212, F 404/13 & F 660/14, and one low responsive genotype F391/14 was identified to water deficite stress (Table 4.7).

Khan *et al* (2004) observed that based on the estimation of stability parameters, it is possible to infer that the clone AEC 86-347 has good adaptation ability under both favourable and unfavourable environmental growing conditions in Sindh. Tiawari *et al* (2011) reported that UP 05233 and CoS 05263 performed better than the majority of the elite genotypes studied for cane yield and sucrose per cent in juice due to having higher average values of genotype across all three environments. As a result, these genotypes can be commercially grown in a wide range of environments. Koli (2016) concluded that genotypes CoH-7261 and Co-6032 were found stable for cane yield (q/ha) and CCS q/ha yield based on the linear component (bi), non-linear response (s2di) and high mean performance, while genotype Co 7025 was considered stable for CCS %.

Table 4.7: Regression coefficient, deviation from regression and mean value of cane yield (t/ha), CCS % and CCS t/ha over the environments

S. no.	Genotype	Cane yield (t/ha)			CCS % (Commercial Cane Sugar %)			CCS t/ha (Commercial Cane Sugar t/ha)		
		Mean	βi	s^2di	Mean	βi	s^2di	Mean	βi	s^2di
1	Co 0238	37.04	1.21	51.42	11.00	0.80	-0.02	4.32	1.22	0.30
2	Co 118	35.56	0.90	253.43*	11.48	1.54	0.01	4.48	1.07	3.37*
3	CoJ 64	32.13	1.24	215.01*	11.04	1.32	0.04	3.95	1.31	2.26*
4	CoJ 85	26.85	0.89	140.23*	10.93	1.03	0.40*	3.17	0.94	1.47*
5	CoJ 88	53.70	0.83	41.85	11.53	0.97	-0.02	6.46	0.95	0.56
6	CoPb 91	55.93	1.26	-15.03	10.73	1.33	-0.02	6.47	1.35	-0.15
7	CoPb 92	55.74	0.84	58.07	10.86	1.38	0.01	6.35	0.96	0.22
8	CoPb 93	37.59	0.95	-17.74	10.41	1.21	-0.01	4.21	0.97	-0.21
9	CoPb 94	58.89	0.85	12.96	10.41	1.24	0.01	6.49	0.94	0.21
10	CoPb 13181	48.15	1.05	21.34	10.72	1.21	0.01	5.51	1.10	0.15
11	CoPb 13182	37.04	1.19	224.28*	10.21	0.99	-0.02	4.08	1.15	1.93*
12	CoPb 14181	38.52	1.09	4.03	10.68	1.44	0.02	4.49	1.15	-0.12
13	CoPb 14184	45.00	1.06	-8.89	10.44	1.04	0.01	4.99	1.05	-0.12
14	CoPb 14185	49.07	1.03	21.33	10.62	1.40	-0.02	5.59	1.10	0.01
15	CoPb 16181	42.22	1.10	-10.94	11.13	1.16	0.03	5.00	1.17	-0.18
16	CoPb 18181	58.33	0.97	47.89	11.08	0.98	0.01	6.69	1.05	0.38
17	CoPb 18182	60.56	0.99	106.34*	10.83	1.37	-0.02	6.92	1.11	0.68
18	CoPb 15212	50.19	1.09	6.20	10.43	1.16	-0.02	5.59	1.13	0.06
19	CoPb 15213	44.07	0.98	69.15*	10.27	0.86	0.01	4.76	0.92	0.86*
20	CoPb 15214	48.15	1.10	256.12*	10.57	0.94	0.02	5.36	1.06	3.01*
21	F 404/13	45.19	1.13	-20.38	11.04	0.95	0.16*	5.30	1.14	-0.19
22	F 301/11	51.85	1.26	127.58	10.31	1.33	-0.01	5.75	1.28	0.8824*
23	F 3/14	50.19	1.05	297.01*	10.90	0.83	0.02	5.72	1.03	3.94*
24	F 6/14	38.70	0.88	-19.59	10.83	1.20	0.06	4.49	0.92	-0.24
25	F 362/14	40.00	0.76	-14.92	10.82	1.26	-0.02	4.59	0.83	-0.15
26	F 391/14	61.67	0.70	-1.80	10.92	0.84	0.08	6.99	0.74	-0.01
27	F 660/14	57.22	1.14	6.18	10.48	0.75	0.02	6.27	1.11	-0.10
28	MA 5/37	20.19	0.63	24.40	9.73	0.39	-0.02	2.03	0.54	0.01
29	MA 5/51	16.02	0.52	8.05	8.99	1.16	-0.01	1.61	0.46	-0.04
30	AS 04-1687	89.63	1.59	135.27*	7.29	-0.41	0.80*	6.15	0.92	0.91*
31	SA 04-409	34.07	0.62	-17.47	10.90	0.64	0.01	3.84	0.61	-0.18
32	BM 101068	44.07	0.90	3.22	8.04	-0.09	0.52*	3.45	0.62	-0.21
33	Co 98014	43.33	1.19	171.56*	10.56	0.79	0.55	4.87	1.13	1.26

*Significant at 0.05 probability level; $^{+}$Significantly deviate from unity; $^{@}$, $^{\#}$ and $^{\$}$ Average, High and Low responsive genotypes to water availability, respectively, with high mean value for CY, CCS% and CCS t/ha; βi Regression Coefficient, mGeneral Mean for concerned traits, S_d^2Mean Square Deviation from Linear Regression.

4.5 GGE Biplot analyses for cane yield, CCS % and CCS (t/ha)

Under this section of genotype by environment data (GED) analyses for three major traits (cane yield t/ha, CCS % and CCS t/ha) of sugarcane, three major aspects were studied i.e. mega environment analyses, test environment evaluation and genotype evaluation (Yan and Kang 2003). For this, the genotype main effect (G) + genotype bye environment (GE) interaction (GGE) biplots were constructed by plotting the first principle component (PC1) scores of genotypes and environments against their respective scores for the second principal component (PC2) that result from the singular value decomposition (SVD) of environment centered GED (Yan *et al* 2007).

Based on mean value of each genotypes in each environment over replications; three environments fell into one sector (one mega environment) ENV1, ENV2 and ENV3 however these are located far apart from each other for cane yield traits as ENV1 was on the right upper side of the biplot while ENV2 & ENV3 were on right lower side of the biplot. For other traits i.e. CCS%, CCS (t/ha) three environments fell into two sectors (two mega environments) i.e. one with ENV1 (irrigated, E1) and another with ENV2(Mild water stress, E2) & ENV3 (Rainfed, E3).

For cane yield (t/ha), CCS %, CCS (t/ha), it was reported that five and nine genotypes, six and eight genotypes, nine and ten genotypes, respectively, as winning genotypes in the 'what won where' view of mega environments analysis (Yan *et al* 2000). This is indicating that these genotype(s) had the highest cane yield or sugar yield potential in that concerned environments. Specifically, AS 04-1687 (30) & F 391/14 (26), Co 118(2), CoJ 88 (5) & SA 04-409 (31), CoPb 91 (6), CoPb 18182(17) & F 391/14 (26) were the higher yielding genotypes than others for the traits cane yield (t/ha), CCS % and CCS (t/ha), respectively. Here crossover GE suggested that target environments may be grouped into two mega environments (Yan *et al* 2007). The corner genotypes (most responsive to water availability) can be visually determined i.e. AS 04-1687 (30), F 391/14 & MA 5/51, CoJ 64 (30, 26 & 29, 3), Co 0118, CoPb 14181, CoJ 88, SA 04-409 & AS 04-1687, MA 5/51 (2, 12, 5, 31 & 30, 29), CoJ 88 , F 391/14 & CoJ 64, MA 5/51 (5, 26 & 3, 29), as most favourable and lowest yielding for the traits cane yield (t/ha), CCS% and CCS (t/ha), respectively (Fig 8A, 8B & 8C).

Fig 4.20, 4.21, 4.22 is the average environment coordination (AEC) view of the GGE biplots with three environments in the niche of clones namely AS 04-1687 (30), F391/14 (26) and Co 0118 (2) as identified in Fig 4.17, 4.18, 4.19. This AEC view facilitated the genotype comparisons based on the mean performance and stability across environments within the mega-environment (Yan 2002). The genotypes were ranked according to genotype main effect (G, i.e. proportional to the rank two approximations of the genotype means; Yan 2002) as follows:

For trait cane yield (t/ha): AS - 041687 (30) > CoPb 18182 (17) > F 391/14 (26) > CoPb 18181 (16) > CoPb 94 (9) = F 660/14 (27) > CoPb 91 (6) > CoPb 92 (7) > F 301/14 (22) > F 3/14 (23) > CoPb 14185 (14) > CoPb 13181 (10) > CoPb 15212 (18) > Mean = F 404/13 (21) = CoPb 15213 (19) > CoPb 1818 (16) > BM 101068 (32) > Co 98014 (33) > CoPb 93 (8) > F 6/14 (24) > F 362/14 (25) > CoJ64 (4) > MA 5/37 (28) > MA 5/51 (29).

For trait CCS t/ha: F 391/14 (26) > CoPb 18182 (7) > CoPb 94 (9) > CoPb 91 (6) > F 660/14 (27) > AS 04-1687 (30) > CoPb 181811 (16) > CoPb 92 (7) > CoPb 14185 (14) > CoPb 15214 (20) > CoPb 15212 (18) > F 404/13 (21) > Mean > CoPb 15213 (19) > Co 98014 (33) > CoPb 13181 (10) > F 6/14 (24) > F 404/13 (21) > Co 0238 (1) > Co 0118 (2) > CoPb 93 (8) > CoJ 64 (3) > SA 04-409 (31) > BM 101068 (32) > CoJ 85 (4) > M 5/37 (28) > MA 5/51 (29).

For trait CCS%: Co 0118 (2) > CoJ 88 (5) > F 391/14 (26) > F 362/14 (25) > Co 13181 (10) > CoPb 16181 (15) > CoPb 18181 (16) > F 404/13 (21) > Co 0238 (1) > CoJ 64 (3) > Co 0118 (2) > SA 04-1687 (31) > F 3/14 (23) > F 6/14 (24) > CoPb 18182 (17) > F 362/14 (25) > CoPb 91 (6) > CoPb 13181 (10) > CoPb 14181 (12) > CoPb 14185 (14) > CoPb 15214 (20) > Co 98014 (33) > F 660/14 (27) > CoPb 14185 (13) > CoPb 15212 (18) = Mean > CoPb 93 (8) > F 6/14 (22) > CoPb 15213 (19) > CoPb 13182 (11) > MA 5/37 (28) > MA 5/51 (29) > BM 101068 (32) > AS 04-1687 (30)**.**

Here since GGE represents G+GE while AEC abscissa approximates the genotype’s contributions to GE, which is a measure of their stability/instability. Thus AS 04-1687 (30) for cane yield, F 391/14 (26) for CCS t/ha and Co 0118 (2) for CCS % were identified ideal cultivars (Yan 2001). While other genotypes with above average performances i.e. F 391/14 (26), CoPb 18181 (16), CoPb 94 (9) and F 301/11 (22) for cane yield, CoPb 18181 (16), CoPb 92 (7), CoPb 14185 (14) and CoPb 18181 (16) for CCS t/ha, F 391/14 (26), CoPb 16181 (15), SA 04-409 (31) and F 3/14 (23) for CCS % were exhibited more stable because located almost on the AEC abscissa. In contrast, genotypes CoPb 91 (6), CoPb 92 (7) genotypes for cane yield CoPb 91 (6), AS 04-1687 (30), MA 5/37 (28) for CCS t/ha, Co 0238 (1), Co 0118 (2), SA 04-409 (31), CoPb 13182 (11) for CCS % were the least stable genotypes with above average performance.

Fig 4.23, Fig 4.24 & Fig 4.25 are the same as Fig 4.20, Fig 4.21& Fig 4.22, respectively, except that Fig 10 are based on environment focused scaling for studying the relationships among test environments. So, stress environment i.e. ENV2 & ENV3 (mild water stress & rainfed) for cane yield, CCS % and CCS t/ha was observed to be more representative of mega environments with high discriminating power of genotypes. Because of these environment(s) are having long vectors (i.e. vector length is proportional to standard deviation of genotypes mean in the environment, which is the measure of discriminating power of environment experiment errors) and small angles (i.e. more representative of mega environment because angle cosine between any environment vector and average environment axis approximates the correlation coefficient between the genotypes values in that environment and the genotype means across the environment) with the AEC abscissa. This is useful for selecting superior genotypes with stable performance. Blanche and Myers (2006) had used GGE biplot approach to classify research locations with the highest discriminating ability and representativeness for genotype selection. Glaz and Kang (2008) had used this approach to classify an organic-soil site in Florida that would be replaced with a sand site for studying final-stage sugarcane clones. Sandhu *et al* (2014) used GGE biplot analysis and two advanced sugarcane genotypes, Co 0238 and CoPb 08214, have been determined to be stable and could be commercially grown in all major sugarcane-growing areas of Punjab.

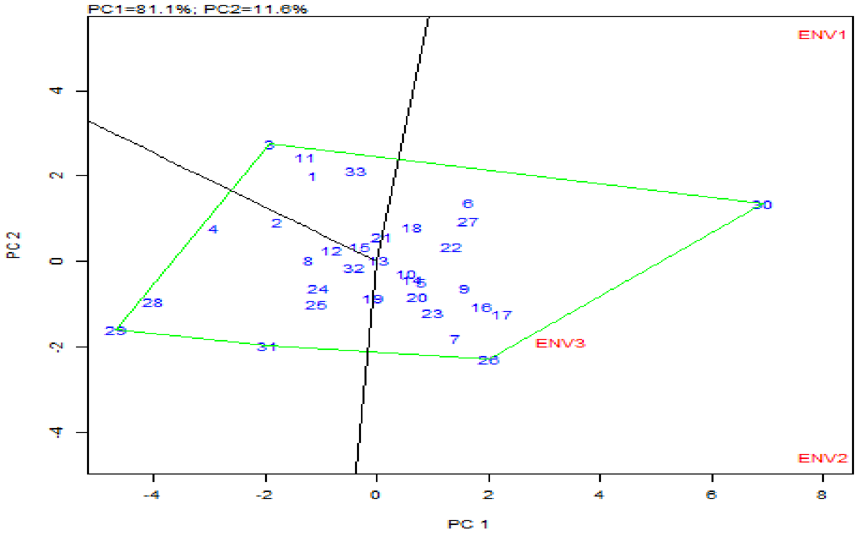

Fig 4.17: “Which-won-where” view of GGE biplot for cane yield (t/ha) in sugarcane

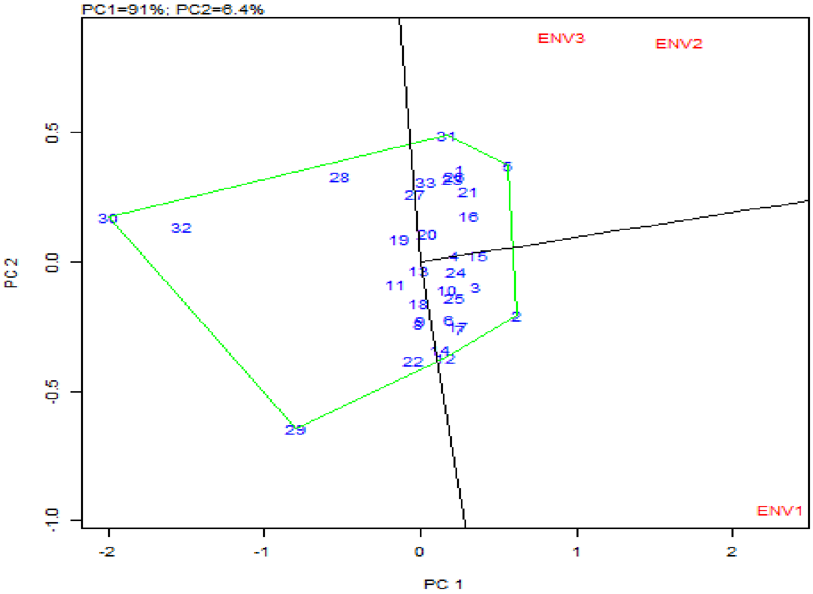

Fig 4.18: “Which-won-where” view of GGE biplot for CCS % in sugarcane

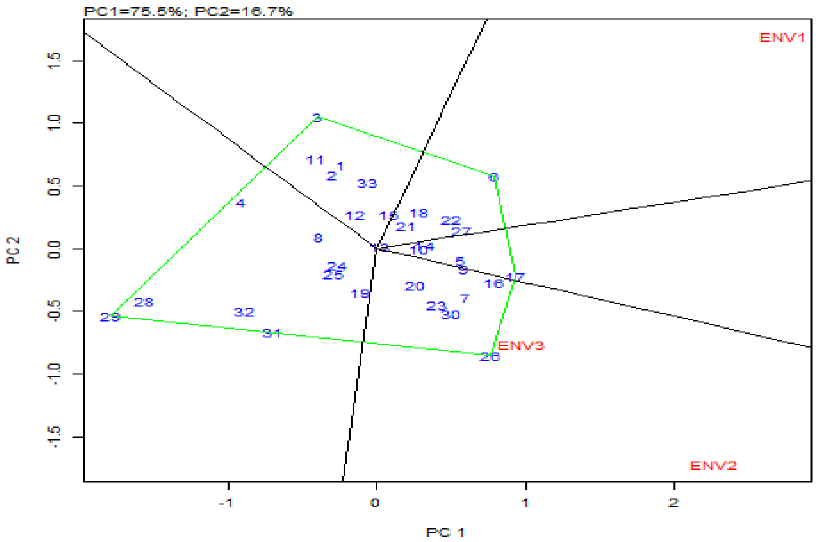

Fig 4.19:"Which-won-where" view of GGE biplot for CCS (t/ha) in sugarcane

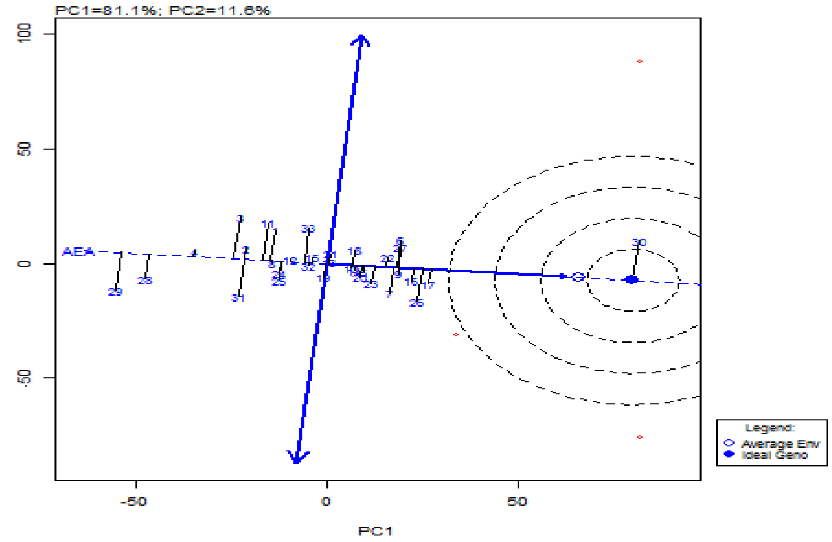

Fig 4.20: "Mean vs. stability" view of GGE biplot for cane yield (t/ha) in sugarcane

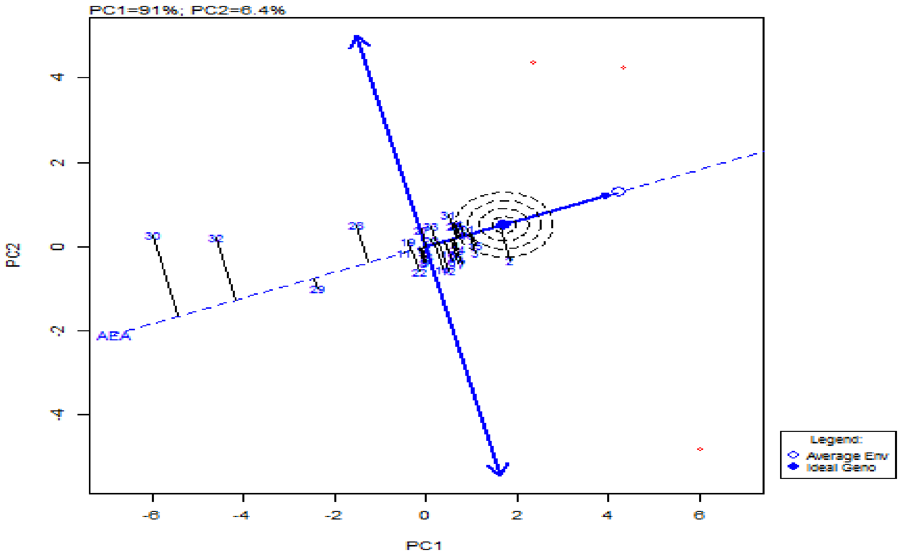

Fig 4.21: "Mean vs. stability" view of GGE biplot for CCS % in sugarcane

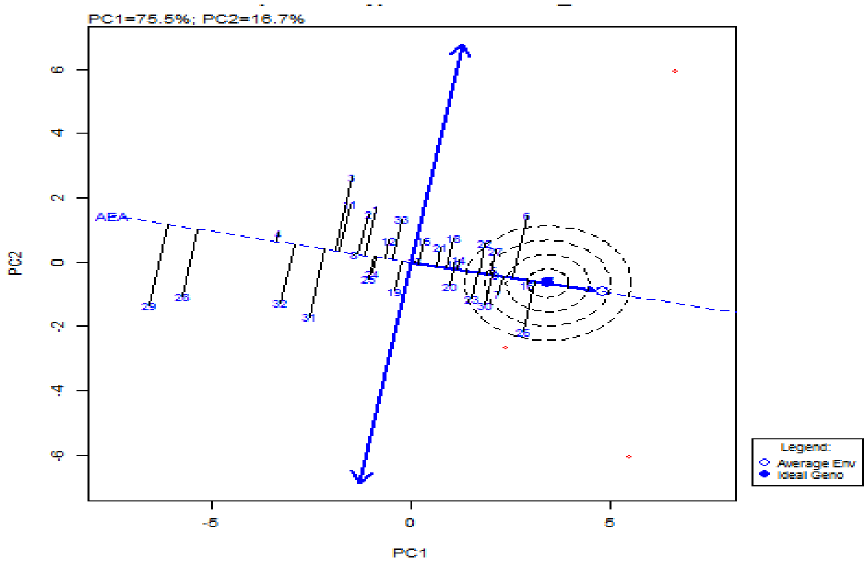

Fig 4.22: “Mean Fvs. stability” view of GGE biplot for CCS % in sugarcane

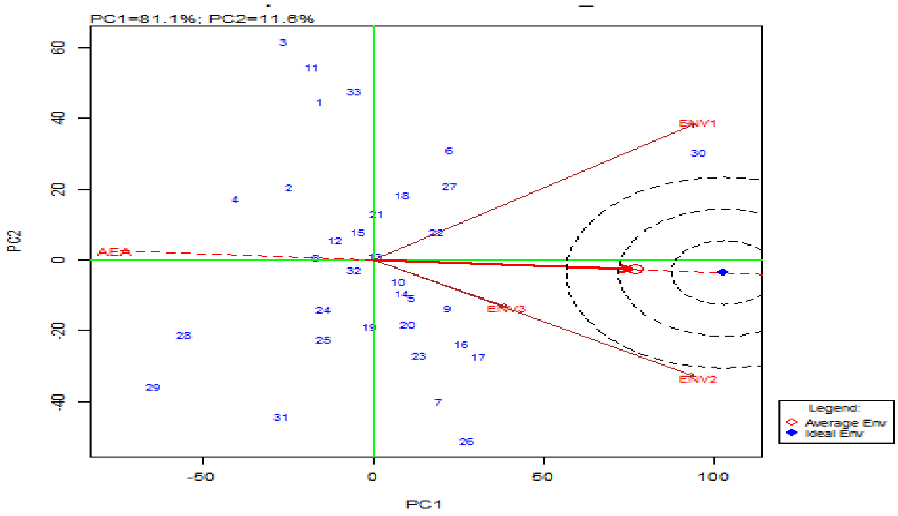

Fig 4.23: “Discriminating power vs. represenativeness” view of GGE biplot for cane yield (t/ha) in sugarcane

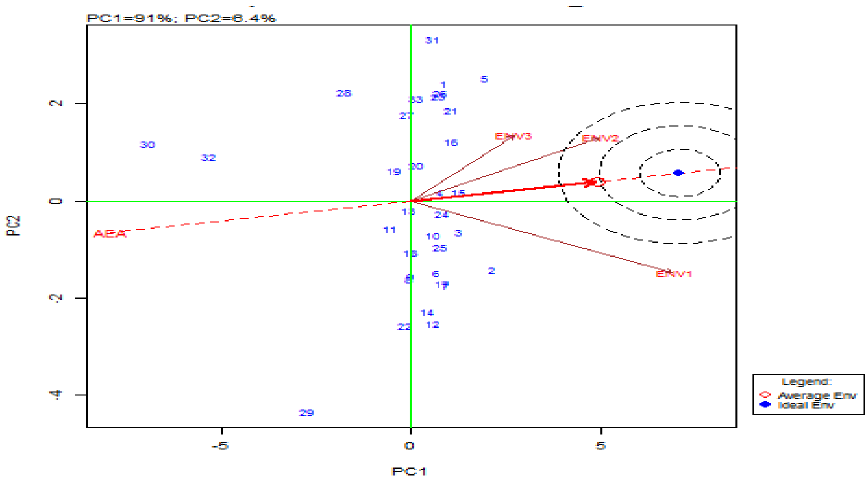

Fig 4.24: “Discriminating power vs. represenativeness” view of GGE biplot for CCS % in sugarcane

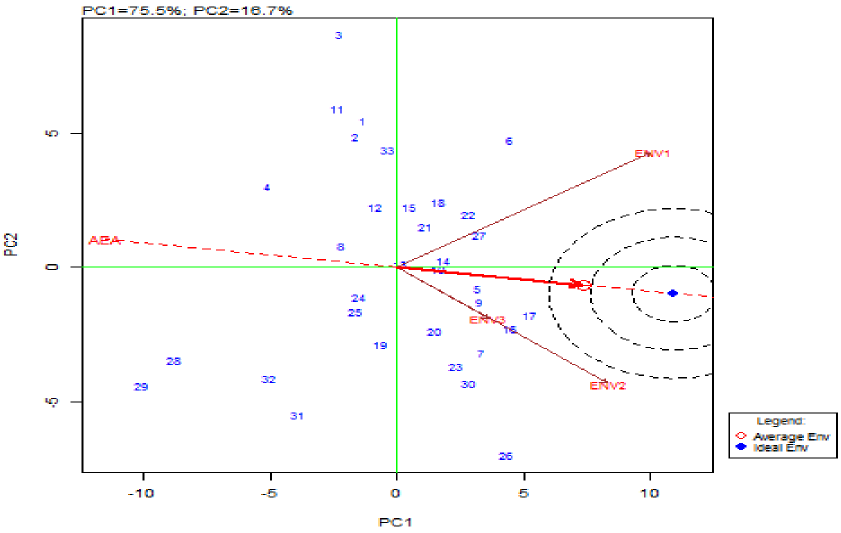

Fig 4.25: "Discriminating power vs. represenativeness" view of GGE biplot for CCS (t/ha) in sugarcane

4.5 Expression of target genes in response to water deficit stress

In the present study, 33 genotypes were subjected to water limited conditions and screening was done by analysing quantitative (germination %, no. of tillers, no. of shoots at 210 DAP, No. of shoots at 240 DAP, NMC, cane yield, cane length, cane diameter, SCW, extraction %), qualitative (brix %, Pol %, purity %, CCS % and CCS t/ha), physiological (leaf rolling, RWC % and CMS %) and biochemical (proline and SOD) traits with primarily based on per cent yield reduction of cane and sugar under stress conditions. F 391/14 was reported as tolerant type to water deficit stress and CoJ64 as susceptible type. As from Table it can be clearly observed that maximum percent reduction in yield while minimum percent reduction was observed for F391/14. Furthermore, maximum proline accumulation and higher SOD activity has been observed for F391/14, whereas in CoJ64 minimum proline accumulation and lowest SOD activiy has been reported in present study. The selected genotypes were subjected to drought as well as raised in well irrigated conditions. The genotypes from well irrigated conditions were taken as control. Fifteen different stress responsive genes (P5CS, PROT, POX, LEA, DEH1, DRP, SOD, IGS, TPS, DEH2, DREB1, DREB2, cAPX, SPS, BADH) were used along with 25sRNA as internal control to illustrate the expression pattern of genes associated with water deficit stress tolerance. Differential expression patterns revealed a significant and high expression of proline transporter (PROT), dehydrin (DEH), late embryogenic abundant (LEA), drought responsive protein (DRP), betaine aldehyde dehydrogenase (BADH), Cytosolic ascorbate peroxidase (cAPX), pyrroline-5-carboxylasesynthetase(P5CS), Trehalose 6-phosphate synthase (TPS) and Dehydration responsive element binding proteins (DREB1) genes in variety F 391/14. Non-significant differences in gene expression of superoxide dismutase (SOD) and Indole-3-glycerol-phosphate synthase (IGS) were observed in F 391/14 under water deficit stress conditions.

Upregulation of these genes under water limited environments showed their part in defensive or adapting the genotype to the water stress condition. Expression of Proline oxidase (POX), Dehydrin (DEH2), Dehydration responsive element binding proteins (DREB2), Sucrose phosphate synthase B (SPS) genes were not observed in any of the cultivar.

Expression of indol-3-glycerol phosphate synthase (IGS) gene

The IGS gene plays a key role in active biosynthesis of auxin via the independent pathway of tryptophan. The expression of indole-3-glycerol phosphate synthase (IGS) through RT-PCR analysis has been overserved in both cultivars; and the tolerant genotype showed more expression of this gene as compared to susceptible cultivar. F 391/14 showed 62 % higher expression over control while CoJ64 exhibited 41.5 % higher expression over control (Fig 4.26).

Earlier studies have suggested that IGS gene could be served as a potential and emerging candidate gene for an intermediate role in stressed environments such as phytohormone auxin (Pasternak *et al* 2005). Simon and Hemprabha (2012) observed the expression of the indol-3-glycerol phosphate synthase (IGS) in drought tolerant parent (Co 740) and progeny of sugarcane. Srivastava and Kumar (2020) also observed the high expression of IGS gene in tolerant type (Co 740).

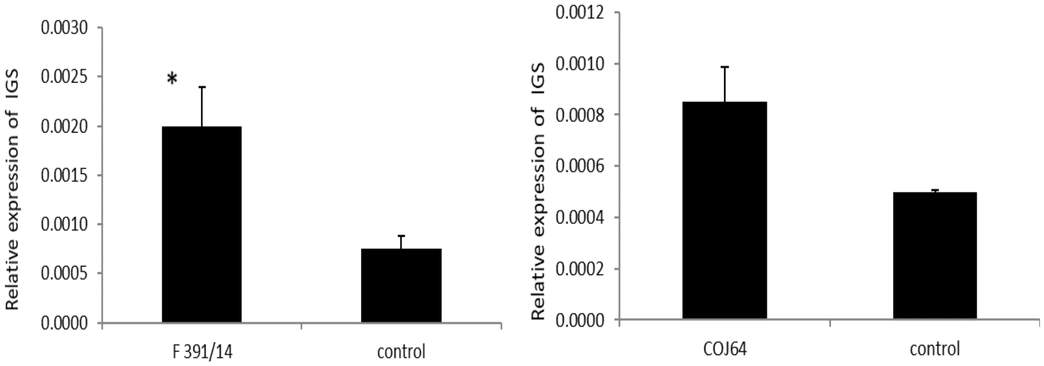

Fig 4.26: Relative expression of IGS gene in cultivar F 391/14 and CoJ 64 subjected to water deficit stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F 391/14 compared to the control ($P \leq 0.05$, Student's t-test)

Expression profile of P5CS gene

The synthesis of a primary precursor for proline biosynthesis in plants is catalyzed by P5CS (Isikander 2011). The expression of P5CS was much significant high in case of F 391/14 i.e. 93.39 % over the control; whereas non significantly high expression i.e. 24.1 % was recorded in CoJ 64 (Fig 4.27). By sustaining cell turgidity, the higher magnitude of proline can enable plants to tolerate dehydration. In sugarcane under water deficit stress, improved proline content has previously been observed in rice (Chaum and Kirdmanee 2008).

In rice, increased content of proline and P5CS activities in both tolerant and susceptible cultivar was reported by Choudhary *et al* (2005), and tolerant variety showed more activity of P5CS. Increased proline content in tolerant genotype was because of raised P5CS activities. In another study by Bagdi and shawv (2013), it was deciphered that enhanced proline content in plants exposed to 425 mM Nacl solution resulted in proline accumulation. Srivastava and Kumar (2020) checked the expression of P5CS in drought tolerant (Co 740) and susceptible cultivars (Co 7219), and gene activities in both was detected. However highest expression was noticed at 48 hours after stress treatment in Co 740 i.e. 2.5-fold expression over control. Numerous studies have confirmed that over-expression of Pyrroline-5-carboxylase synthetase (P5CS) gene rises proline production and confer abiotic stress tolerance in transgenic plants (Kishor & Sreenivasulu 2013).

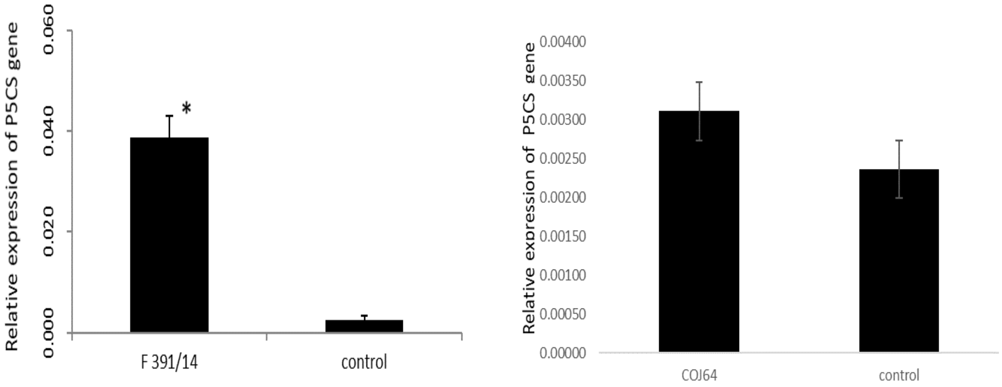

Fig 4.27: Relative expression of P5CS gene in cultivar F 391/14 and CoJ 64 subjected to water deficit stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F 391/14 cultivar compared to the control ($P \leq 0.05$, Student's t-test).

Expression profile of cAPX (ascorbate peroxidase)

Ascorbate peroxidase (cAPX) is a non-glycosylated heme-containing enzyme, that kills injurious hydrogen peroxide in plants through the ascorbate glutathione route (Breusegem *et al* 1995). Our results indicated that F 391/14 cultivar showed significant high expression i.e. 61.68 % over control however CoJ 64 revealed non-significantly high expression as compared to control i.e. 16.67 % (Fig 4.28). Srivastava and Kumar (2020) also reported the expression of cAPX gene in both sensitive and tolerant cultivars and showed 3.17 fold increased expression in cultivar Co 740. It was manifested that expression increases as the duration of stress increases.

Lin and Pu (2010) revealed that the change in enzyme activity under salt stress in sweet potato plants during ROS scavenging; and concluded that the activities of APX was increased in salt stress tolerant genotypes. The ascorbate-glutathione cycle occurs in plant cells under abiotic stresses, in which APX isoenzyme contributes in ROS scavenging by transforming H_2O_2 into H2O using a particular donor.

Hemaprabha *et al* (2012) discovered the utility of this gene for screening drought tolerance by its up-regulation in parents and progeny resistant to drought, while it was down-regulated in parents and progeny susceptible to drought, indicating its role in the mechanism of drought tolerance in sugarcane. Hemaprabha *et al* (2013) detected 92.98 % enhancement in peroxidase activity in tolerant sugarcane genotype under drought. The significant role of APX during response of plant to drought has also been depicted by Singh *et al* (2014).

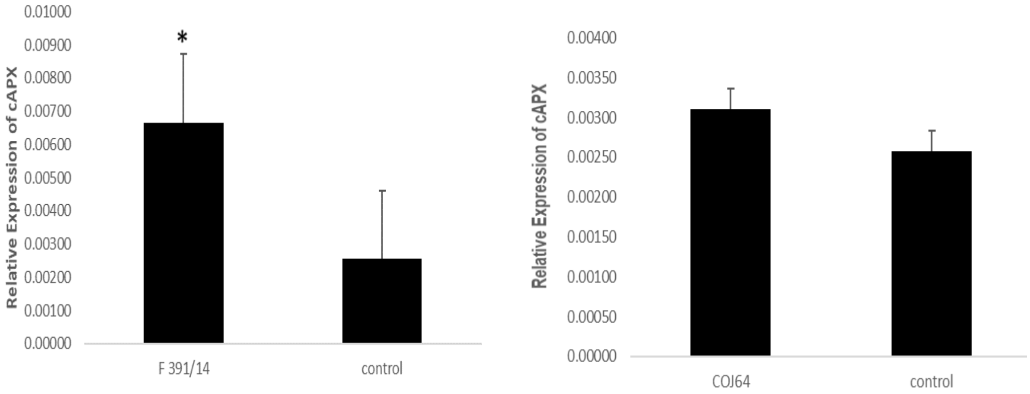

Fig. 4.28: Relative expression of (cAPX) gene in cultivar F391/14 and CoJ 64 subjected to water deficit stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F 391/14 cultivar compared to the control ($P \leq 0.05$, Student's t-test).

Expression profile of superoxide dismutase gene

The dismutation of superoxide to hydrogen peroxide (H_2O_2) and O2 is believed to be catalyzed by the SOD. SOD is found in most aerobic species and is thought to play a major role in the defense against oxidative stress (Roychowdhury *et al* 2019). In the present study, we found that expression of Mn-SOD was significantly increased in variety F 391/14 in comparison to its control i.e. 72.33 % while CoJ 64 cultivar exhibited significant lower expression i.e. 43.58 % than its internal control (Fig 4.29).

Swapna and Hemaprabha (2010) ascertained that cultivar Co 740 showed raised expression of SOD and no expression was recorded in susceptible cultivar Co775. Enhanced activities of superoxide dismutase i.e. almost doubled (79.72 %) has been recorded in sugarcane progenies grown under water stress (Hemprabha *et al* 2013). Another study by Srivastava and Kumar (2020) recorded upregulation of SOD gene in both cultivars but expression was high in tolerant i.e. 1.6 folds in contrast to susceptible cultivar i.e. 1.2 folds. Ahmed *et al* (2013) observed the activities of SOD in wild *Hordeum vulgare* cultivars. They deciphered that the SOD expression was significantly amplified in all through the anthesis period during salinity and water stress alone and also in associated treatments.

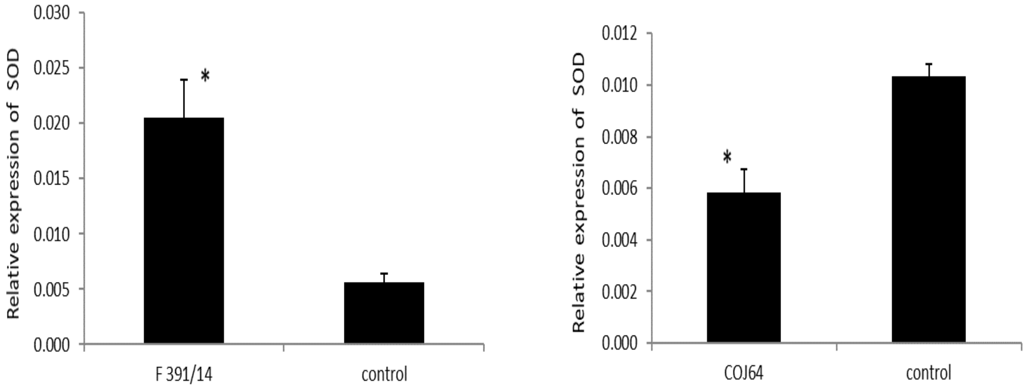

Fig 4.29: Relative expression of SOD gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in both ciltivars compared to the control (P ≤ 0.05, Student's t-test).

Expression profiling of LEA gene

LEA proteins have a protective function in avoiding desiccation and aggregation of protein by functioning as molecular chaperones and also display a part in maintaining structure of membrane during extreme stress. In our study, it is determined that F 391/14 exhibited significantly high expression of LEA gene i.e. 9.51 % over its internal control, while it was non significantly high to its control in CoJ 64 i.e.12.60 % (Fig 4.30).

Kumar (2020) also observed the expression of LEA and found it was higher in Co 740 than Co 7279. Co 740 showed 2 folds expression while Co 7279 showed 1.5 folds expression under severe stress conditions. Tripathi *et al* (2018) also reported that during drought conditions expression of LEA gene was significantly induced in cultivars CoPk 0519 and CoLk 94184. In *Arabidopsis thaliana*, overexpression of transgenic LEA proteins exhibited improved salt and drought tolerance (Brini *et al*. 2007; Garcia *et al*. 2015). Liang *et al* (2013) reported that the expression of target genes during salinity and water stress increases in transgenic *Arabidopsis* plants. They examined physiological traits and hypothesized that the LEA gene expression significantly improves the resistance of transgenic *Arabidopsis* plants to dehydration and salt stress. Devi *et al* (2019) documented upregulation of LEA in sugarcane cultivars at different days of stress interval, and relative quantification showed LEA 1.5 folds higher in Co 06022 leaf in contrast to Co 8021 leaf. Iskandar *et al* (2011) stated LEA were intensely increased in moisture stress by more than a100-folds in immature sugarcane culms.

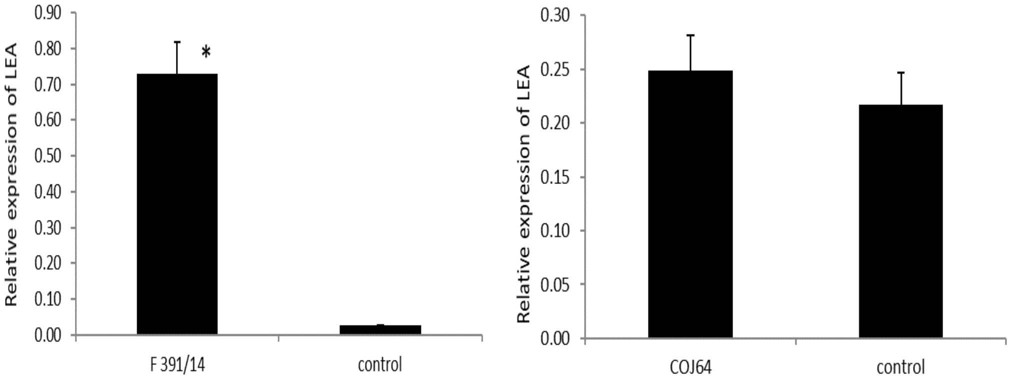

Fig 4.30: Relative expression of LEA gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F 391/14 compared to the control ($P \leq 0.05$, Student's t-test).

Expression profile of Dehydrin gene

Owing to their unfolded state, dehydrins belonged to group 2 LEA proteins serve as filler particles, leading to high aggregation and improved binding ability to water;thus, avoiding cellular collapse because of dehydration. Our results showed that significantly higher expression in F 391/14 i.e. 75.79 % in contrast to it's internal control; whereas in CoJ 64 non-significant higher i.e. 34.95 % expression was observed in comparison to its control (Fig 4.31). Previous studies suggested that dehydrin genes are significantly induced in sugarcane under drought stress (Iskandar *et al* 2011).

Devi *et al* (2019) reported at ten days of water deficit stress, expression of DEH gene was high in Co 06022 in comparison to Co 8021; and analysis presented that expression levels were 3.7 folds higher in Co 06022 shoot compared to water stress sensitive genotype Co 8021. Srivastava and Kumar (2020) also presented upregulation of dehydrin gene in both tolerant and sensitive cultivar i.e. 3.5 folds and 3.46 folds, respectively. Tripathi *et al* (2018) also mentioned the upregulation of dehydrin gene under drought. Kumar *et al* (2014) stated that overexpression of OsDHN1 gene in rice conferred tolerance to salt stress and drought. Isikander *et al* (2011) reported that dehydrin gene was up regulated in older culm internodes in response to water stress. Dehydrin is well known for being expressed under the pressures of dehydration. Hassan *et al* (2015) reported that at ten days of stress 4 folds upregulation of dehydrin gene in stress-tolerant wheat plants. Dehydrins are well known to be expressed under dehydration stresses. Dehydrins were described to be upregulated in barley as reported by Wehner *et al* (2016). Dehydrins are thought to be associated with crucial protective functions (Hassan *et al* 2015).

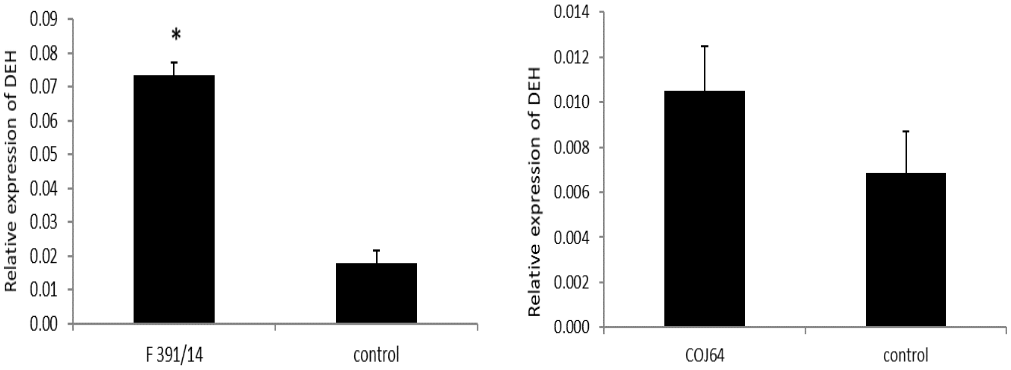

Fig 4.31: **Relative expression of DEH gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F 391/14 compared to the control (P ≤ 0.05, Student's t-test).**

Expression profile of Trehalose 6-phosphate synthase gene

Trehalose is a non-reducing sugar that functions in most desiccation resistance plants as an osmolyte and is produced by action of TPS and TPP (trehalose phosphate phosphatase) from UDP glucose 6-phosphate. Expression of TPS was significantly high in tolerant genotype F 391/14 i.e. 96.60 % but sensitive cultivar CoJ 64 possessed 26.78 % reduced expression in comparison to its control (Fig 4.32) .

Devi *et al* (2019) verified the expression levels of Trehalose 6-phosphate synthase (TPS) gene and detected 17.4 folds higher expression in leaves of drought stressed Co 06022 compared with its control indicating its tolerance capacity to moisture stress. Almeida *et al* (2007) established that expression of trehalose in sugarcane was two times more in treated leaves as compared with control leaves during water stress. Under drought stress, increasing amounts of accumulation of trehalose was found that indicates the influence of osmotic adjustment of species, genotypes or cultivars in grasses (Hongbo *et al*. 2006).

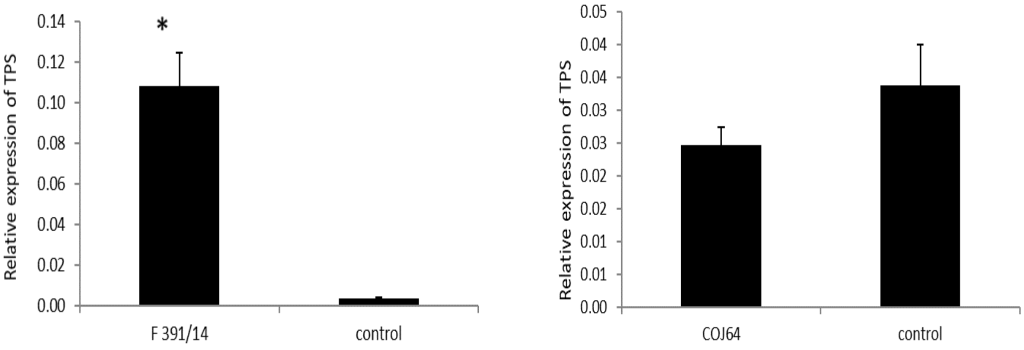

Fig 4.32: **Relative expression of TPS gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in F391/14 compared to the control (P ≤ 0.05, Student's t-test).**

Expression profile of DREB gene

DREB is known to play a vital regulatory role in abiotic stress tolerance in plants. As a transcription factor in the ABA-independent pathway of signal transduction (Shinozaki and Yamaguchi-Shinozaki, 2000), the expression of stress-responsive genes is regulated by this gene. In present investigation, the DREB gene expressed in both F 391/14 and CoJ 64 genotypes. The expression of DREB was significantly higher in F 391/14 and CoJ 64 i.e. 88.18 % and 88.20 % than their respective controls (Fig 4.33). Devi *et al* (2018) found that the upregulation of DREB2 in stressed genotypes compared to respective controls. Higher expression levels were noticed in genotypes Co 06022, Co 99004, Co 7336 and Co 8021, while expression levels were comparatively lower in genotypes of Co 0315 and Co 06015. Expression of DREB2 was 25.7 folds higher in Co 06022 compared with control.

Hemprabha *et al* (2013) concluded that expression of DREB was seen in drought resistant progeny but null expression was seen in drought susceptible genotypes. Augustine *et al* (2014) reported up regulation of DREB2 gene 2300 folds in contrast to controls in transgenic sugarcane cultivar Co 86032. Under drought ABA independent gene expression is regulated by the involvement of DREB motifs and protein attaches to DRE/LTRE/CRT components in the promoter region and controls the expression of stress-responsive gene. DREB has shown promise as a gene that can enhance drought tolerance and as an indicator for sugarcane drought screening. Reis *et al* (2014) also observed that induced expression of AtDREB2A CA in sugarcane improved its moisture stress tolerance without biomass penalty. The over-expression of DREB2A gene in *Arabidopsis* and several other transgenic plants such as maize, rice, tobacco resulted in improved stress tolerance (Qin *et al* 2007, Bihani *et al* 2011)

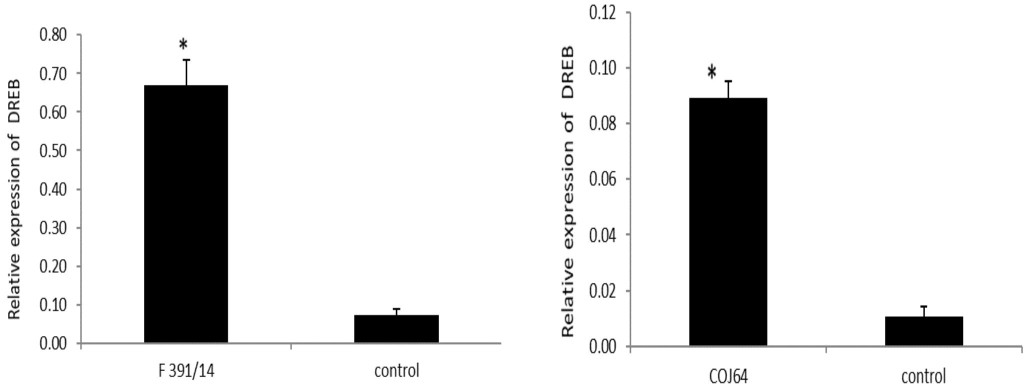

Fig 4.33: Relative expression of DREB gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in both cultivars compared to the control (P ≤ 0.05, Student's t-test).

Expression profile of drought responsive protein

Gosal *et al*. (2009) illustrated that the main regulator of gene expression in transgenic plants that are better to cope up with moisture deficit stress are dehydration-responsive transcription factors. 69.25 % increased expression of DRP was recorded in F 391/14 but 86.00 % less expression to its control was found in CoJ 64 cultivar (Fig 4.34). Devi *et al* (2018) stated that qRT analysis revealed that at severe stress, expression levels of drought responsive protein were 15.5 folds higher in Co 06022 leaf as compared to control.

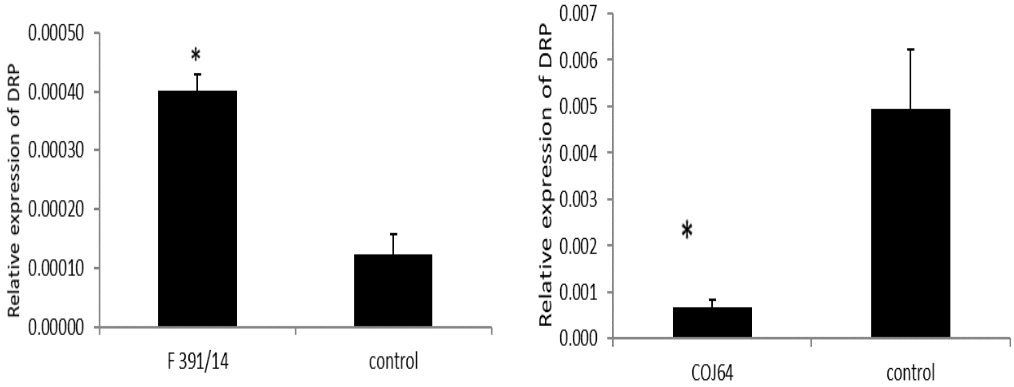

Fig 4.34: Relative expression of DRP gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in both cultivars compared to the control (P ≤ 0.05, Student's t-test).

Expression profile of PROT gene

Proline transporter that mediates proline and glycine betaine transport supposed to be involved in long-distance transport of proline and required for phloem loading, retrieval of proline leaking from the phloem, or in xylem-to-phloem transfer. Significant high expression of PROT was reported in F 391/14 genotype i.e. 69.36 % in contrast to its control but significantly reduced expression i.e. 59.00 % less than its control was reported in CoJ 64 (Fig 4.35).

Devi *et al* (2019) documented the expression of proline transporter (PROT) which was higher in CoPk 05191 exposed to stress. High expression of PROT gene in drought tolerant cultivars was also recorded by Tripathi *et al* (2018). Firstly, PROT was thought to transference only proline (Rentsch *et al* 1996) but, further studies discovered that the ProT2 of *Arabidopsis* and barley also transported glycine betaine (Rentsch *et al* 1996). Chen *et al* (2016) observed the highest level of proline content and PvProT mRNA in the leaves of common beans. Further evaluation of PvProT gene expression profiles specified that water stress tolerant cultivars showed higher expression levels of PvProT than did drought sensitive landraces. These results proposed that following the expression rise of PvProT, proline conveyance may increase quickly.

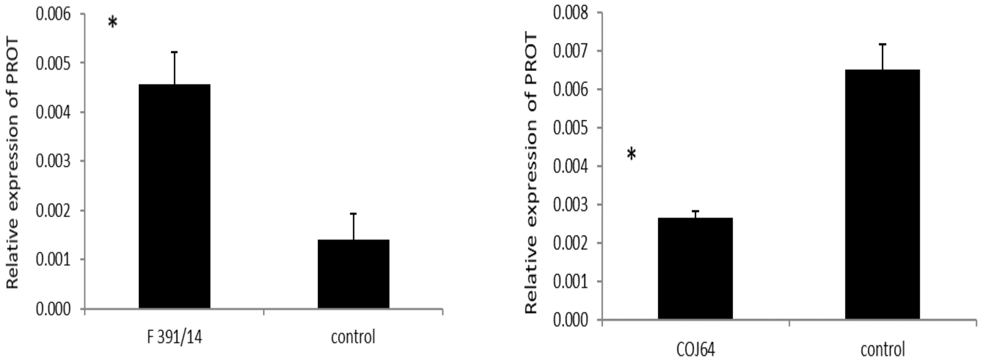

Fig 4.35: **Relative expression of PROT gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in both cultivars compared to the control ($P \leq 0.05$, Student's t-test).**

Expression profile of BADH gene

Glycine betaine (GB) is a compatible substance that is considered to function in many plants, including sugar cane, as an osmoprotective and transforming plant to adapt to the condition of water deficit. F 391/14 showed significant high expression of betaine aldehyde dehydrogenase (BADH) i.e. 67.64 % high compared to its internal control, however significant reduced expression was depicted in CoJ 64 i.e. 82.00 % less expression than its control (Fig 4.36). Devi *et al* (2019) found that with increased stress in sugarcane cultivar CoPk 0519 BADH genes were selectively up -regulated over control. The expression of BADH was also found higher in stressed plants at 6th week of stress. Glycine betaine protects the enzyme function and protein structure and preserves membrane integrity against disruption induced by climatic stresses (Sugiharto 2018). Betaine is synthetized by two enzymes: choline monooxygenase (CMO), and betaine aldehyde dehydrogenase (BADH) (Sonia Zingaretti 2014).

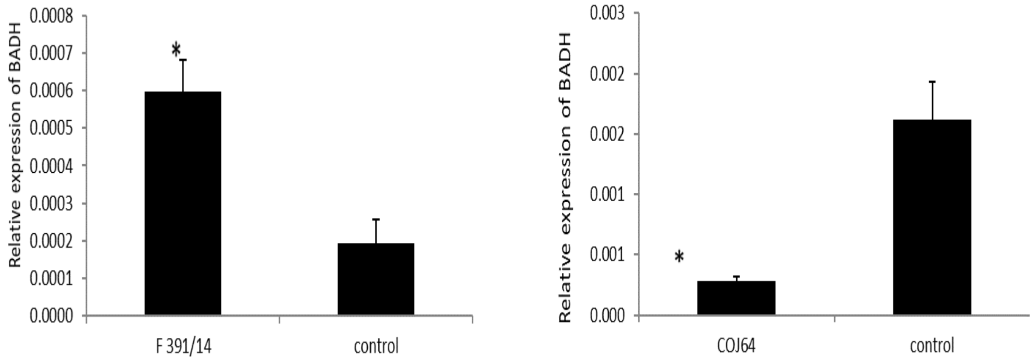

Fig 4.36: **Relative expression of BADH gene in cultivar F 391/14 and CoJ 64 subjected to water stress in comparison to control. Here control is well irrigated plant. The error bars represent the standard deviation (n=3) and * represents significant differences the expression of gene in both cultivars compared to the control ($P \leq 0.05$, Student's t-test).**

CHAPTER V

SUMMARY

Sugarcane (*Saccharum spp.* complex) is an important industrial crop of the country mainly because of it's ability to store high concentrations of sugar in the stem; and more recently for the production of ethanol, which is an important renewable biofuel source. Abiotic and biotic stresses are the main causes of low productivity that cause negative impacts on crop adaptation and production. Among abiotic stresses, drought is major stress, and may reduces sugarcane yield up to 50.00 % or even more. It is being a multi-dimensional stress, causes various physiological and biochemical effects on plant growth and development. So, there is the need to identify sugarcane clones adapted to moisture stress in order to sustain sugarcane production and sugar recovery.

At present, no completely drought tolerant clone has been recomFmended or released. Therefore, the present study was designed to evaluate different elite sugarcane varieties, intergenic and interspecific clones against water deficit stress. This investigation was carried out during spring season 2019-20 using 33 diverse clones of sugarcane. All the 33 clones were planted in a *RBD* design with three replications under normal irrigated (E1), mild water stress (E2) and rainfed (E3) environments. Under mild water stress (E2) environment, irrigation was suspended at critical growth stage of sugarcane viz. formative stage. Standard agronomic were followed to raise the ideal crop stand. The observations on eight cane yield and component traits, three physiological, two biochemical and six quality traits were recorded at appropriate stages for each clone under all three environments. Gene expression study was also carried out. The mean values of all the traits were subjected to statistical analysis for genetic variability, correlation and regression coefficient, Eberhart and Russel regression stability and GGE biplot. The results on various aspects of study are summarized below:

Analysis of variance unveiled significant differences among clones for all the quantitative and qualitative traits recorded in this study under all three environments except for the germination (%); because of normal irrigated environments were maintained in all trials for uniform better crop stands. For physiological traits, RWC (at 120, 150 and 240 DAP), CMS (at 150 & 240 DAP) and for biochemical traits, proline (150 & 240 DAP) and SOD (150 & 240 DAP) were found to be significantly influuenced under all three environments. Similarly, six qualitative traits studied at 11 months genotypes behaved significantly different under all three environments i.e. E1, E2 & E3.

Per cent decrease was observed for all qualitative and quantitative traits. Under E2 and E3 environments, maximum per cent reduction for cane yield was observed over E1 environment. For physiological traits, there was observed per cent reduction for traits RWC %

and CMS % under E2 and E3 environments over E1. For biochemical traits, there was increase of proline content and SOD values under E2 and E3 environment over E1.

Higher magnitudes of coefficient of variation i.e. PCV and GCV for quantitative traits were recorded for shoot count and number of millable canes under all three environments; while tiller count, stalk length and cane yield showed high magnitude of differences under E2 and E3 environments. For SCW, high magnitude of PCV was observed under E2 and E3 environments while GCV values was higher under E3, only. For quality traits, CCS (t/ha) showed higher magnitude of differences under all three environments. For physiological and biochemical traits, low to medium values of differences for GCV and PCV were recorded.

High heritability values for major economic traits like NMC, shoot counts, cane yield, CCS (%), CCS (t/ha), brix % and Pol% under stressed environments indicated that substantial improvement can be expected by giving emphasis on the selection of these traits under water deficit stress. In this study, environmental effect on the expression of various characters was observed as specified by the differences in parameters of variability. Medium to higher magnitude of GA observed for traits cane yield, CCS (t/ha), NMC and SCW under all three environments (E1, E2 and E3). So consequently, substantial improvement can be expected by practicing selection for these characters. Higher heritability coupled with higher magnitude of GA recorded for number of tillers at 120 days (000/ha), number of shoots, number of millable canes, cane yield (t/ha) and CCS (t/ha) under water stress (E2) and rainfed(E3) environment indicated that direct selection of these traits under stress could be effective. Low to moderate values of h^2bs and GA for traits like relative water content (%), CMS (%), proline and SOD at 150 and 240 days, and some quality traits indicated that direct selection could not be much effective for these traits.

Correlation of cane yield was significant positively correlated with tiller count, shoot count, Number of millable canes; while cane length and cane girth did not show a valid association. Cane yield and CCS (t/ha) exhibited significant positive association. Physiological traits like RWC and CMS showed significant positive association with yield under E2 and E3 environments.

From regression study, it is cleared that number of millable canes was observed to be main contributing trait with $R^2 > 0.63$ while role of SCW was not so pronounced specially under irrigated environment. The main quality traits deciding the CCS % are TSS %, Pol % and purity% i.e. all having major role in on CCS %; however, Pol % was observed to be main traits determining the CCS % under all three environments. Here in this study, cane yield (t/ha) was observed to be more pronounced traits as comparatively more role in determining the CCS (t/ha) as in compare to CCS % under all three environments were observed.

Based on Eberhart and Russell regression coefficient analysis for cane yield, four average responsive (β_{ii}~1) genotypes i.e. CoPb 18181, CoPb 13181, CoPb 1418 and CoPb 15212 and four low responsive ($\beta_{ii} < 1$) genotypes i.e. CoJ 88, CoPb 92, CoPb 94 and F 391/14 to water stress were identified. Two genotypes namely F 301/11 and F 660/14 were highly responsive ($\beta_i > 1$) to water conditions. For CCS %, three average responsive ($\beta_i \sim 1$) genotypes i.e.CoJ 88, CoPb 18181, CoPb 15214 and eleven high responsive ($\beta_i > 1$) genotypes i.e. Co 0118, CoJ 64, CoPb 91, CoPb 92, CoPb 13181, CoPb 14181, CoPb 14185, CoPb 16181, CoPb 18182, F 6/14, F 362/14 and four genotypes namely F 391/14, F 3/14 , Co 98014 and Co 0238 were reported for stress responsive as having high *per se* stable performance (> 10.49 CCS %, S_d^2~0). For the trait CCS t/ha, five average responsive ($\beta_i \sim 1$) genotypes i.e. CoJ 88, CoPb 92, CoPb 94, CoPb 13181, CoPb 18181 and five high responsive ($\beta_i > 1$) genotypes i.e. CoPb 91, CoPb 14185, CoPb 18182, CoPb 15212, F 404/13 and F 660/14 one low responsive genotype F 391/14 was identified.

AS 04-1687 (30) & F 391/14 (26), Co 0118 (2), CoJ 88 (5) & SA 04-409 (31), CoPb 91 (6), CoPb 18182 (17) & F 391/14 (26) were the higher yielding genotypes than others for the traits cane yield (t/ha), CCS % and CCS (t/ha), respectively. AS 04-1687(30) for cane yield, F 391/14 (26) for CCS t/ha and Co 0118 (2) for CCS % were identified ideal cultivars (Yan 2001). While other genotypes with above average performances i.e. F 391/14 (26), CoPb 18181 (16), CoPb 94 (9) and F 301/11 (22) for cane yield, CoPb 18181 (16), CoPb 92 (7), CoPb 14185 (14) and CoPb 18181 (16) for CCS t/ha, F 391/14 (26), CoPb 16181 (15), SA 04-409 (31) and F 3/14 (23) for CCS % were exhibited more stable because located almost on the AEC abscissa. In contrast, genotypes CoPb 91 (6), CoPb 92 (7) genotypes for cane yield; CoPb 91 (6), AS 04-1687 (30), MA 5/37 (28) for CCS t/ha, Co 0238 (1), Co 0118 (2), SA 04-409 (31), CoPb 13182 (11) for CCS % were the least stable genotypes with above average performance.

For study the expression of twelve drought responsive genes, two cultivars namely CoJ 64 (susceptible) and F 391/14 (resistant) were selected. Expression of drought responsive genes i.e. P5cs, SOD, DEH, BADH, IGS, cAPX, LEA, TPS, PROT, DRP was significantly high in comparison to its control in cultivar F 391/14; whereas for cultivar CoJ 64, significant low expression of genes i.e. SOD, DRP, PROT and BADH was observed.

- Analyses of variances revealed significance differences among clones for all qualitative, quantitative, physiological and biochemical traits under all three environments except for germination (%).
- Under all three environments, higher magnitude of coefficient of variations were calculated for shoot count (000/ha), number of millable canes (000/ha), cane yield (t/ha). Under mild water stress and rainfed environment, high PCV and GCV was recorded for cane length and tiller count (000/ha).

- High genetic advance coupled with high heritability for the traits like tillers (000/ha), shoots (000/ha), SCW (kg), NMC (000/ha) and CCS (t/ha) indicated direct selection of these traits under water deficit stress could be effective.
- Correlation study revealed that cane yield was positively correlated with tiller (000/ha), shoots (000/ha), NMC (000/ha). However, CCS (t/ha) was also positively correlated with these traits. Yield and CCS (t/ha) were positively associated with SOD and proline under mild water stress and rainfed conditions.
- Regression study revealed that NMC (000/ha) is a main contributing trait for cane yield; where as cane yield is major contributing trait for CCS (t/ha). Quality trait i.e. Pol % and TSS % has major role for CCS %.
- From Eberhart and Russel regression stability analysis, ten genotypes were reported stable performing for cane yield as deviated non significantly from zero, while twelve genotypes for CCS % and twelve genotypes for CCS (t/ha).
- It can be clearly observed from GGE biplots that there were two mega environments, as normal irrigated environment (E1) was in one cluster whereas mild water stress (E2) and rainfed (E3) environment were come into one cluster for traits CCS % and CCS (t/ha).
- Further for gene expression study two genotypes i.e. F 391/14 (tolerant type) and CoJ 64 (susceptible type) were selected.
- Expression of drought responsive genes i.e. P5cs, SOD, DEH, BADH, IGS, cAPX, LEA, TPS, PROT, DRP was significantly high in comparison to its control for cultivar F 391/14.

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APPENDIX-1

Mean Weekly meteorological data of 2019-20 at PAU, RRS, Faridkot

Date	Temperature (°C)		Rainfall		Relative Humidity (%)		Evaporation (mm)
	Max	Min	Rain	Rainy-day	RH1	RH2	Evap.
(12-Mar to 18-Mar)	24.20	10.00	3.60	1.00	89.00	44.00	2.60
(19-Mar to 25-Mar)	26.70	13.20	0.00	0.00	83.00	41.00	3.90
(26-Mar to 01-Apr)	31.70	15.00	0.00	0.00	79.00	34.00	5.30
(02-Apr to 08-Apr)	34.80	17.70	0.00	0.00	71.00	30.00	6.00
(09-Apr to 15-Apr)	35.60	20.40	0.00	0.00	69.00	31.00	6.10
(16-Apr to 22-Apr)	32.70	17.60	24.60	2.00	82.00	34.00	5.60
(23-Apr to 29-Apr)	40.00	21.70	0.00	0.00	55.00	18.00	9.50
(30-Apr to 06-May)	39.40	22.20	0.00	0.00	39.00	17.00	10.40
(07-May to 13-May)	39.90	23.10	0.00	0.00	52.00	30.00	8.50
(14-May to 20-May)	33.60	20.90	51.40	2.00	74.00	41.00	6.30
(21-May to 27-May)	37.20	22.10	3.80	1.00	60.00	28.00	7.50
(28-May to 03-Jun)	44.40	25.60	0.00	0.00	49.00	20.00	10.40
(04-Jun to 10-Jun)	43.70	26.20	0.00	0.00	48.00	24.00	11.30
(11-Jun to 17-Jun)	40.80	26.50	12.80	2.00	55.00	26.00	10.50
(18-Jun to 24-Jun)	35.90	25.70	18.80	1.00	68.00	44.00	7.40
(25-Jun to 01-Jul)	39.40	28.30	0.00	0.00	62.00	37.00	7.80
(02-Jul to 08-Jul)	37.10	28.00	26.70	2.00	72.00	54.00	7.70
(09-Jul to 15-Jul)	33.60	28.00	3.70	0.00	78.00	64.00	5.90
(16-Jul to 22-Jul)	32.40	26.30	102.40	3.00	87.00	69.00	5.60
(23-Jul to 29-Jul)	33.90	27.20	0.90	0.00	81.00	64.00	4.40
(30-Jul to 05-Aug)	34.00	27.00	25.10	3.00	84.00	68.00	4.60
(06-Aug to 12-Aug)	34.80	27.80	12.40	2.00	83.00	64.00	4.80
(13-Aug to 19-Aug)	33.30	26.10	20.20	2.00	87.00	66.00	4.50
(20-Aug to 26-Aug)	35.20	26.60	0.00	0.00	83.00	58.00	5.10
(27-Aug to 02-Sep)	35.90	27.00	6.60	1.00	82.00	63.00	4.80
(03-Sep to 09-Sep)	35.20	27.20	45.80	1.00	86.00	61.00	4.20
(10-Sep to 16-Sep)	35.60	27.20	0.00	0.00	86.00	24.00	4.60
(17-Sep to 23-Sep)	33.50	25.00	0.00	0.00	84.00	61.00	4.60

Date	Temperature (°C)		Rainfall		Relative Humidity (%)		Evaporation (mm)
	Max	Min	Rain	Rainy-day	RH1	RH2	Evap.
(24-Sep to 30-Sep)	31.90	23.70	1.00	0.00	84.00	60.00	4.60
(01-Oct to 07-Oct)	30.00	20.40	3.70	1.00	90.00	59.00	2.90
(08-Oct to 14-Oct)	32.70	18.30	0.00	0.00	86.00	40.00	3.60
(15-Oct to 21-Oct)	31.00	18.10	4.00	1.00	82.00	43.00	3.20
(22-Oct to 28-Oct)	31.50	15.80	0.00	0.00	86.00	37.00	2.70
(29-Oct to 04-Nov)	30.10	15.40	0.00	0.00	89.00	40.00	2.30
(05-Nov to 11-Nov)	27.00	12.30	8.40	1.00	85.00	39.00	2.70
(12-Nov to 18-Nov)	23.70	12.50	10.00	2.00	91.00	57.00	0.80
(19-Nov to 25-Nov)	25.00	10.20	0.00	0.00	93.00	40.00	1.80
(26-Nov to 02-Dec)	22.90	9.80	8.40	1.00	93.00	47.00	1.20
(03-Dec to 09-Dec)	23.10	5.10	0.00	0.00	93.00	33.00	1.50
(10-Dec to 16-Dec)	16.00	8.30	15.30	2.00	95.00	70.00	0.80
(17-Dec to 23-Dec)	12.60	6.10	0.00	0.00	95.00	82.00	0.50
(24-Dec to 31-Dec)	9.20	4.50	0.00	0.00	91.00	81.00	0.40

APPENDIX-2

Mean performance of 33 sugarcane genotypes for different yield and component traits under normal (E1) and mild water stress (E2) and rainfed (E3) environments

S. No.	Genotype	Gm%			Tiller (000/ha)			Shoot at 210 DAP (000/ha)			Shoot at 240 DAP (000/ha)			NMC (000/ha)		
		E1	E2	E3	E1	E1	E2	E3	E2	E3	E1	E2	E3	E1	E2	E3
1	Co 0238	42.59	42.59	43.52	193.33	138.89	138.89	163.89	119.44	101.67	130.56	103.33	74.44	92.78	52.78	39.44
2	Co 118	44.91	41.20	42.59	161.11	131.11	125.00	132.78	97.22	90.00	102.78	80.00	66.11	76.67	47.22	37.22
3	CoJ 64	42.13	38.43	43.52	217.78	99.44	100.00	172.78	81.11	50.00	147.78	55.00	36.11	121.67	36.11	22.22
4	CoJ 85	25.93	31.48	28.24	167.78	97.78	95.56	127.78	87.78	63.33	100.00	70.00	46.67	62.78	30.56	29.44
5	CoJ 88	41.67	38.89	38.43	228.89	184.44	186.67	165.00	128.89	115.00	147.22	120.56	95.00	130.00	95.56	85.00
6	CoPb 91	43.52	33.80	41.20	203.89	171.67	168.33	161.11	129.44	112.78	116.67	111.11	79.44	86.11	81.67	52.22
7	CoPb 92	30.09	31.94	25.93	196.67	164.44	161.11	167.22	139.44	126.67	135.00	119.44	102.22	111.11	107.22	78.33
8	CoPb 93	41.67	42.13	38.43	164.44	158.33	144.44	132.78	113.89	96.11	119.44	103.89	75.56	81.67	66.67	38.89
9	CoPb 94	31.94	31.48	34.72	230.00	193.89	185.56	163.89	132.22	108.89	141.11	118.33	88.33	100.00	85.56	73.33
10	CoPb 13181	40.28	40.74	37.04	243.33	188.33	186.11	186.11	140.00	120.00	166.11	113.89	92.22	138.89	93.89	40.00
11	CoPb 13182	40.28	39.35	30.09	175.00	151.11	147.78	122.78	102.22	87.78	106.11	92.22	41.67	91.11	41.67	32.22
12	CoPb 14181	39.35	40.74	47.22	182.78	150.00	138.89	126.11	110.00	72.22	121.67	101.11	52.78	96.11	72.78	23.33
13	CoPb 14184	36.57	33.80	33.33	194.44	168.33	162.22	152.78	128.89	106.11	130.00	115.56	95.00	106.67	85.00	41.67
14	CoPb 14185	42.13	40.74	39.81	229.44	191.67	186.67	178.33	141.67	134.44	143.33	130.00	99.44	113.89	97.78	53.33
15	CoPb 16181	28.24	29.63	31.02	213.33	147.78	147.22	150.56	122.78	100.00	125.56	106.67	80.56	105.00	89.44	34.44
16	CoPb 18181	38.43	47.22	35.19	250.56	223.89	223.33	208.33	166.67	150.56	190.00	136.67	131.11	150.56	115.00	96.11
17	CoPb 18182	36.11	35.19	34.26	206.67	171.11	162.22	160.00	147.78	126.11	148.33	130.56	107.78	121.67	102.78	76.67
18	CoPb 15212	42.13	42.13	43.98	207.22	152.22	166.11	135.00	121.11	100.00	125.00	104.44	63.33	101.67	77.78	43.89
19	CoPb 15213	36.11	41.67	39.81	241.67	194.44	182.78	189.44	170.00	150.00	167.78	134.44	111.11	139.44	105.56	41.67

S. No.	Genotype	Gm%			Tiller (000/ha)			Shoot at 210 DAP (000/ha)			Shoot at 240 DAP (000/ha)			NMC (000/ha)		
		E1	**E2**	**E3**	**E1**	**E1**	**E2**	**E3**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
20	CoPb 15214	37.96	39.35	38.43	250.56	215.56	208.33	206.67	175.00	151.67	176.11	158.33	130.00	164.44	129.44	25.56
21	F 404/13	30.09	31.94	39.35	205.56	148.89	146.67	147.22	130.00	92.78	117.78	98.33	67.78	103.89	81.67	56.67
22	F 301/11	31.02	34.26	31.02	246.11	174.44	172.78	185.00	150.56	123.89	166.67	128.33	100.56	137.78	104.44	33.89
23	F 3/14	32.87	29.63	42.13	221.67	170.56	173.33	171.11	135.56	105.56	136.11	112.78	70.00	122.22	86.67	32.78
24	F 6/14	35.19	38.43	33.80	218.33	161.11	148.33	155	135.56	119.44	142.22	123.33	91.11	103.89	82.78	44.44
25	F 362/14	34.26	26.85	31.02	226.67	98.89	90	134.44	91.11	77.22	102.22	78.89	68.33	83.33	66.11	48.33
26	F 391/14	37.04	36.57	37.04	207.78	172.22	162.22	170.56	129.44	112.78	153.33	116.67	85.56	110.00	85.56	67.78
27	F 660/14	39.81	46.30	48.61	217.22	189.44	190.56	169.44	147.78	119.44	147.22	120	95.56	114.44	93.89	53.89
28	MA 5/37	28.70	27. 78	29.63	113.89	78.89	90.56	71.67	53.89	28.89	55.56	37.78	19.44	48.33	26.67	13.89
29	MA 5/51	25.00	29.17	33.33	121.11	66.67	70.56	73.89	52.22	22.22	51.67	37.78	17.78	45.00	24.44	11.67
30	AS 04-1687	41.67	44.44	35.65	286.67	241.11	212.78	253.33	208.33	170.00	233.89	169.44	146.11	236.11	210	102.78
31	SA 04-409	38.89	34.26	38.43	157.22	125	119.44	102.78	82.22	65.00	77.78	66.67	46.67	75.56	56.11	29.44
32	BM 101068	31.48	28.24	35.65	205.56	165.56	168.89	157.78	135.56	111.11	140.56	128.33	92.78	116.11	95	71.11
33	Co 98014	28.24	21.3	24.07	184.44	108.33	119.44	137.78	96.67	72.22	125.56	88.33	61.67	93.33	67.22	41.67
	Mean	36.25	36.11	36.56	205.18	157.44	154.02	155.56	124.38	102.54	133.06	106.43	79.76	108.55	81.67	47.68
	SEm	2.67	2.50	2.72	8.30	7.63	7.24	5.76	5.4	5.91	6.97	5.7	4.58	5.47	4.56	3.93
	Range Lowest	25.00	21.30	24.07	113.89	66.67	70.56	71.67	52.22	22.22	51.67	37.78	17.78	45.00	24.44	11.67
	Range Highest	44.91	47.22	48.61	286.67	241.11	223.33	253.34	208.33	170.00	233.89	169.44	146.11	236.11	210	102.78
	C.V. %	12.74	11.95	12.88	7.01	8.4	8.14	6.42	7.64	9.97	9.07	9.37	9.94	8.73	9.68	14.27
	C.D. at 5%	7.53	7.04	7.68	23.45	21.57	20.45	16.28	15.5	16.68	19.7	16.27	12.93	15.47	12.9	11.09

APPENDIX- 3

Mean performance of 33 sugarcane genotypes for different yield and component traits under normal (E1) and mild water stress (E2) and rainfed (E3) environment

Sr No	Genotype	Cane length(cm)			Cane girth(cm)			SCW(kg)			Yield(t/ha)		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
1	Co 0238	149.00	100.33	54.77	2.66	2.65	2.56	0.95	0.44	0.24	84.44	21.11	5.56
2	Co 118	173.22	82.22	79.33	2.90	2.61	2.42	1.11	0.48	0.45	73.33	15.56	17.78
3	CoJ 64	166.00	81.88	76.89	2.65	2.38	2.37	0.84	0.37	0.21	82.22	10.56	3.61
4	CoJ 85	159.00	75.11	70.77	2.65	2.49	2.83	1.11	0.38	0.35	63.33	10.00	7.22
5	CoJ 88	180.45	103.44	98.11	2.62	2.64	2.61	0.99	0.58	0.44	86.67	41.11	33.33
6	CoPb 91	192.67	94.33	82.88	2.87	3.16	3.01	1.27	0.68	0.51	103.33	44.44	20.00
7	CoPb 92	213.33	131.00	106.55	2.29	2.35	2.39	0.96	0.57	0.41	84.44	56.67	26.11
8	CoPb 93	175.22	89.67	71.22	2.90	2.75	2.57	1.13	0.49	0.35	73.33	28.89	10.56
9	CoPb 94	175.55	101.55	97.11	2.81	3.03	2.99	1.15	0.66	0.55	92.22	47.78	36.67
10	CoPb 13181	183.89	123.00	94.66	2.34	2.44	2.33	0.79	0.64	0.42	85.56	45.56	13.33
11	CoPb 13182	192.44	98.43	80.22	2.84	2.69	2.43	1.14	0.56	0.38	85.56	15.56	10.00
12	CoPb 14181	170.44	100.98	79.66	2.57	2.50	2.07	0.87	0.43	0.25	77.78	34.44	3.33
13	CoPb 14184	170.78	98.33	79.11	2.70	2.81	2.48	0.94	0.58	0.35	83.33	40.00	11.67
14	CoPb 14185	148.22	86.78	66.89	2.54	2.50	2.89	0.83	0.54	0.34	85.56	46.67	15.00
15	CoPb 16181	195.67	120.33	78.11	2.43	2.33	2.43	0.90	0.49	0.31	82.22	36.67	7.78
16	CoPb 18181	198.22	132.11	91.44	2.23	2.10	2.31	0.83	0.52	0.33	92.22	57.78	25.00
17	CoPb 18182	213.11	140.77	106.44	2.34	2.30	2.43	0.94	0.69	0.34	94.44	62.22	25.00
18	CoPb 15212	216.11	138.78	106.53	2.44	2.42	2.63	1.07	0.66	0.50	92.22	37.78	20.56
19	CoPb 15213	173.78	100.67	85.33	2.32	2.31	2.56	0.69	0.46	0.28	77.78	44.44	10.00
20	CoPb 15214	163.89	105.11	69.11	2.39	2.30	2.40	0.67	0.49	0.34	84.44	53.33	6.67
21	F 404/13	173.22	88.77	82.55	2.60	2.46	2.42	0.91	0.44	0.28	86.67	37.78	11.11
22	F 301/11	155.11	109.44	96.44	2.52	2.44	2.64	0.79	0.61	0.31	95.56	52.22	7.78
23	F 3/14	133.22	111.00	88.22	3.04	2.88	2.63	0.92	0.73	0.39	84.44	56.67	9.44

Sr No	Genotype	Cane length(cm)			Cane girth(cm)			SCW(kg)			Yield(t/ha)		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
24	F 6/14	98.89	76.33	74.22	2.53	2.51	2.59	0.74	0.38	0.27	71.11	33.33	11.67
25	F 362/14	144.56	102.99	77.66	2.99	2.91	2.64	0.93	0.72	0.44	68.89	32.22	18.89
26	F 391/14	151.67	106.11	88.66	3.09	2.75	2.83	1.21	0.76	0.57	86.67	60.00	38.33
27	F 660/14	161.11	113.88	95.66	2.67	2.58	2.52	0.95	0.48	0.40	101.11	44.44	26.11
28	MA 5/37	198.89	145.33	131.00	2.68	2.46	2.30	1.00	0.50	0.41	45.56	10.00	5.00
29	MA 5/51	185.22	141.33	119.33	2.50	2.30	2.24	0.83	0.46	0.37	36.67	7.78	3.61
30	AS 04-1687	218.11	165.11	132.11	1.86	1.73	1.94	0.63	0.49	0.38	145.56	87.78	35.56
31	SA 04-409	226.44	156.22	132.11	2.42	2.33	2.36	1.10	0.64	0.55	56.67	31.11	14.44
32	BM 101068	234.00	148.55	121.89	2.11	1.92	1.93	0.68	0.40	0.30	78.89	33.33	20.00
33	Co 98014	209.45	127.74	120.55	2.39	2.41	2.34	0.98	0.55	0.46	91.11	23.33	15.56
	Mean	178.81	112.05	91.99	2.57	2.50	2.49	0.93	0.54	0.38	82.83	38.20	15.96
	SEm	8.91	6.70	6.84	0.10	0.09	0.15	0.05	0.41	0.03	6.58	3.45	2.11
	Range Lowest	98.89	75.11	54.77	1.86	1.73	1.93	0.63	0.37	0.21	36.67	7.78	3.33
	Range Highest	234.00	165.11	132.11	3.09	3.16	3.01	1.27	0.76	0.57	145.46	87.78	38.33
	C.V. %	8.63	10.42	12.88	6.80	6.61	10.13	8.37	12.99	12.12	13.76	15.62	22.94
	C.D. at 5%	25.17	19.04	19.33	0.29	0.27	0.41	0.13	0.11	0.07	18.59	9.73	5.97

APPENDIX-4

Mean performance of 33 sugarcane genotypes for different quality traits under normal (E1) and water stress (E2) environment

Sr. no	Genotype	Extraction at 11 months (%)			Brix at 11 months (%)			TSS in juice at 11 months (%)			Purity at 11 months (%)			CCS%			CCS(t/ha)		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
1	Co 0238	53.05	49.80	36.26	19.37	17.43	17.57	17.25	15.50	15.08	89.10	88.89	85.88	11.98	10.75	10.29	10.11	2.26	0.59
2	Co 118	51.10	48.55	39.78	21.00	18.33	17.70	19.12	15.78	15.10	91.03	86.10	85.39	13.41	10.77	10.27	9.84	1.69	1.90
3	CoJ 64	50.27	47.44	39.37	19.90	18.37	16.90	18.03	15.75	14.37	90.62	85.76	85.07	12.62	10.74	9.75	10.34	1.13	0.39
4	CoJ 85	51.24	45.09	39.30	19.20	18.27	17.77	17.46	15.03	15.29	91.07	82.27	86.07	12.31	10.03	10.44	7.78	1.01	0.73
5	CoJ 88	50.27	48.49	42.76	20.50	18.73	18.00	18.34	16.33	15.58	88.76	87.20	86.56	12.71	11.22	10.67	11.03	4.60	3.74
6	CoPb 91	51.96	49.02	41.65	19.07	18.33	17.10	17.54	15.29	14.25	91.98	83.41	83.37	12.36	10.27	9.57	12.78	4.57	2.06
7	CoPb 92	48.20	44.88	41.66	19.83	17.77	17.27	17.99	15.06	14.52	90.71	84.77	84.08	12.59	10.20	9.79	10.62	5.76	2.67
8	CoPb 93	52.81	47.37	40.43	18.80	16.90	16.50	17.04	14.45	13.98	90.63	85.52	84.74	11.92	9.84	9.47	8.75	2.84	1.04
9	CoPb 94	52.98	48.05	45.84	18.33	17.50	16.87	16.88	14.85	13.87	92.09	84.87	82.30	11.90	10.07	9.25	10.97	4.82	3.67
10	CoPb 13181	54.81	47.76	43.20	19.23	18.03	17.63	17.42	15.32	14.43	90.56	84.94	81.84	12.18	10.39	9.60	10.43	4.72	1.39
11	CoPb 13182	52.18	49.18	45.34	18.17	18.40	17.37	16.37	14.86	14.14	90.13	80.76	81.42	11.43	9.81	9.38	9.74	1.52	0.98
12	CoPb 14181	50.00	45.03	40.84	19.90	17.43	16.90	17.90	14.74	14.20	89.92	84.58	84.04	12.48	9.98	9.58	9.70	3.43	0.33
13	CoPb 14184	48.88	45.01	41.35	18.90	17.67	16.73	16.84	15.01	14.04	89.10	84.98	83.91	11.69	10.18	9.46	9.74	4.06	1.19
14	CoPb 14185	55.49	47.74	42.72	19.33	16.87	16.27	17.60	14.63	13.92	91.05	86.76	85.56	12.35	10.03	9.47	10.57	4.68	1.51
15	CoPb 16181	53.32	50.91	44.57	20.47	18.03	17.77	18.17	15.44	15.13	88.76	85.61	85.17	12.59	10.51	10.27	10.34	3.85	0.82
16	CoPb 18181	51.75	47.95	43.66	19.53	18.10	17.77	17.63	15.50	15.21	90.26	85.63	85.63	12.31	10.55	10.36	11.36	6.08	2.64
17	CoPb 18182	51.78	45.51	41.12	20.43	17.57	17.37	18.10	15.05	14.46	88.59	85.68	83.26	12.53	10.25	9.70	11.83	6.37	2.57
18	CoPb 15212	50.92	45.11	37.03	18.87	16.73	16.33	16.97	14.61	13.86	89.95	87.32	84.86	11.83	10.05	9.40	10.91	3.79	2.07
19	CoPb 15213	52.31	40.75	39.47	17.90	17.13	16.33	16.16	14.81	13.86	90.24	86.44	84.86	11.29	10.13	9.40	8.75	4.51	1.01
20	CoPb 15214	55.17	49.62	47.24	18.40	17.30	17.07	16.70	15.10	14.32	90.77	87.27	83.91	11.69	10.38	9.65	9.87	5.53	0.69
21	F 404/13	53.02	51.26	46.13	18.37	18.30	17.67	17.12	16.01	14.80	93.23	87.47	83.81	12.13	11.02	9.97	10.52	4.17	1.22

Sr. no	Genotype	Extraction at 11 months (%)			Brix at 11 months (%)			TSS in juice at 11 months (%)			Purity at 11 months (%)			CCS%			CCS(t/ha)		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
22	F 301/11	52.42	48.16	45.96	20.60	17.50	17.03	17.59	14.50	13.92	85.42	82.89	81.71	11.97	9.71	9.25	11.42	5.08	0.76
23	F 3/14	50.56	47.42	49.33	18.83	18.70	18.00	17.00	15.87	14.99	90.28	84.88	83.26	11.87	10.76	10.06	10.01	6.11	1.02
24	F 6/14	52.09	47.32	39.79	19.43	18.30	18.03	17.54	15.61	14.58	90.25	85.30	80.84	12.25	10.61	9.63	8.69	3.55	1.24
25	F 362/14	53.39	42.33	37.49	20.17	18.13	17.43	17.87	15.26	14.56	88.63	84.18	83.53	12.38	10.30	9.79	8.52	3.32	1.95
26	F 391/14	54.55	48.49	45.53	18.97	18.30	17.57	17.05	15.85	14.81	89.90	86.62	84.31	11.89	10.86	10.01	10.30	6.51	4.16
27	F 660/14	52.99	49.76	46.05	17.93	17.37	17.30	16.24	15.11	14.45	90.54	87.04	83.53	11.36	10.37	9.71	11.48	4.61	2.71
28	MA 5/37	50.56	43.89	39.55	17.30	17.13	16.77	14.92	14.32	13.95	86.27	83.58	83.19	10.20	9.63	9.36	4.66	0.97	0.48
29	MA 5/51	50.75	36.17	30.72	17.33	15.87	15.00	15.17	12.81	12.19	87.50	80.72	81.27	10.44	8.46	8.08	3.84	0.67	0.31
30	AS 04-1687	39.62	37.37	36.62	12.27	12.13	14.70	10.25	10.06	12.21	83.14	82.91	82.98	6.95	6.74	8.19	10.14	5.92	2.39
31	SA 04-409	47.90	36.01	38.39	18.70	18.10	18.00	16.74	15.74	15.17	89.54	86.98	84.29	11.65	10.80	10.25	6.61	3.35	1.56
32	BM 101068	50.07	40.11	39.18	14.07	13.47	15.37	11.91	11.18	12.77	84.69	83.03	83.07	8.06	7.50	8.56	6.36	2.50	1.50
33	Co 98014	55.23	42.79	40.94	18.70	17.90	16.73	16.50	15.73	14.00	88.21	87.89	83.65	11.40	10.85	10.29	10.38	2.53	1.70
	Mean	51.56	45.89	41.49	18.80	17.46	17.05	16.84	14.88	14.30	89.48	85.22	83.86	11.72	10.11	9.63	9.65	3.83	1.61
	SEm	0.86	0.97	1.05	0.25	0.19	0.19	0.22	0.19	0.17	0.50	0.96	0.84	0.16	0.17	0.15	0.78	0.33	0.21
	Range Lowest	39.61	36.01	30.72	12.47	12.13	14.70	10.25	10.06	12.19	83.14	80.72	80.84	6.95	6.74	8.08	3.84	0.67	0.31
	Range Highest	55.49	51.26	49.33	21.00	19.12	18.03	19.12	16.33	15.58	93.22	88.89	86.56	13.41	11.22	10.67	12.78	6.51	4.16
	C.V. %	2.89	3.67	4.40	2.29	1.92	1.97	2.24	2.25	2.11	0.96	1.97	1.74	2.35	2.95	2.65	13.92	15.13	22.88
	C.D. at 5%	2.43	2.75	2.98	0.70	0.55	0.55	0.62	0.55	0.49	1.40	2.73	2.39	0.45	0.49	0.42	2.19	0.95	0.60

APPENDIX-5

Mean performance of 33 sugarcane genotypes for different biochemical traits under normal (E1), mild water stress (E2) and rainfed (E3) environments

Sr.no	Genotype	Proline(µg/gfw) (at120 days)			Proline(µg/gfw) (at 240 days)			SOD (eu/100ml/gFW) (at120 days)			SOD (eu/100ml/gFW) (at 240 days)		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
1	Co 0238	22.94	42.33	42.91	22.47	26.73	37.14	0.146	0.178	0.183	0.127	0.147	0.154
2	Co 118	16.58	34.75	32.13	15.59	18.36	28.86	0.13	0.189	0.189	0.112	0.145	0.15
3	CoJ 64	16.69	23.33	24.49	16.43	17.68	20.58	0.109	0.135	0.137	0.103	0.11	0.117
4	CoJ 85	23.97	34.09	36.06	22.18	23.99	28.34	0.116	0.149	0.153	0.122	0.133	0.14
5	CoJ 88	19.97	41.4	43.13	18.86	24.61	37.85	0.122	0.185	0.195	0.108	0.147	0.154
6	CoPb 91	19.2	34.94	36.97	18.74	23.01	33.79	0.122	0.171	0.178	0.112	0.137	0.15
7	CoPb 92	19.66	38.94	39.39	19.63	23.25	37.33	0.121	0.179	0.18	0.106	0.13	0.14
8	CoPb 93	23.86	38.21	39.6	23.7	26.4	36.48	0.122	0.164	0.17	0.11	0.132	0.145
9	CoPb 94	18.86	34.07	36.94	18.86	24.13	35.29	0.126	0.185	0.189	0.128	0.142	0.146
10	CoPb 13181	15.28	32.58	34.63	14.74	17.56	24.48	0.118	0.171	0.172	0.114	0.146	0.153
11	CoPb 13182	17.28	35.74	33.17	16.93	19.08	30.43	0.124	0.173	0.179	0.114	0.134	0.154
12	CoPb 14181	17.55	32.94	33.79	16.93	18.79	29.36	0.118	0.175	0.179	0.113	0.132	0.149
13	CoPb 14184	24.24	36.79	37.15	23.78	26.24	32.56	0.114	0.169	0.173	0.107	0.134	0.157
14	CoPb 14185	22.51	39.97	42.3	22.17	25.26	34.36	0.131	0.174	0.178	0.104	0.132	0.134
15	CoPb 16181	21.64	40.25	42.1	22.55	25.23	37.9	0.121	0.159	0.163	0.101	0.119	0.13
16	CoPb 18181	23.78	38.84	41.36	22.97	26.18	35.52	0.12	0.171	0.181	0.113	0.139	0.147
17	CoPb 18182	23.43	38.67	41.14	22.82	24.28	38.56	0.116	0.169	0.174	0.112	0.142	0.158
18	CoPb 15212	24.39	39.09	41.28	22.78	25.11	40.56	0.131	0.188	0.197	0.124	0.154	0.161
19	CoPb 15213	23.97	36.98	39.07	21.32	26.6	38.14	0.131	0.186	0.19	0.13	0.159	0.163
20	CoPb 15214	24.7	38.21	40.23	23.74	25.74	34.36	0.124	0.167	0.173	0.103	0.126	0.138
21	F 404/13	24.82	37.44	40.8	23.03	28.55	36.9	0.128	0.168	0.174	0.128	0.149	0.159

Sr.no	**Genotype**	**Proline(µg/gfw) (at120 days)**			**Proline(µg/gfw) (at 240 days)**			**SOD (eu/100ml/gFW) (at120 days)**			**SOD (eu/100ml/gFW) (at 240 days)**		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
22	F 301/11	23.42	33.09	35.58	22.46	25.13	37.33	0.133	0.173	0.186	0.116	0.142	0.156
23	F 3/14	22.32	33.4	34.48	21.5	24.55	39.83	0.135	0.191	0.199	0.125	0.152	0.162
24	F 6/14	21.97	37.17	38.92	21.36	24.94	36.48	0.125	0.181	0.187	0.113	0.142	0.155
25	F 362/14	23.32	34.02	35.33	23.63	26.31	43.14	0.122	0.178	0.181	0.119	0.133	0.151
26	F 391/14	23.67	51.28	51.97	22.43	31.35	47.65	0.115	0.196	0.203	0.113	0.157	0.166
27	F 660/14	23.2	41.24	41.79	22.9	25.86	40.52	0.126	0.187	0.188	0.105	0.123	0.138
28	MA 5/37	22.09	31.31	32.54	21.47	23.23	29.18	0.124	0.159	0.17	0.106	0.12	0.12
29	MA 5/51	21.15	29.89	31.17	20.78	23.51	28.23	0.113	0.145	0.147	0.112	0.121	0.118
30	AS 04-1687	20.78	40.27	43.76	20.66	26.47	40.16	0.12	0.181	0.183	0.12	0.152	0.156
31	SA 04-409	23.2	32.81	34.52	22.24	25.05	39.17	0.103	0.149	0.153	0.109	0.125	0.142
32	BM 101068	25.15	34.48	35.02	24.94	27.19	35.75	0.116	0.169	0.175	0.116	0.139	0.143
33	Co 98014	23.17	40.6	40.63	22.82	27.15	38.64	0.111	0.162	0.165	0.105	0.126	0.132
	Mean	21.78	36.63	38.01	21.13	24.47	35.3	0.12	0.17	0.18	0.11	0.14	0.15
	SEm	0.36	0.74	0.79	0.42	0.53	.0.83	0.01	.013	.17	0.11	0.14	0.18
	Range Lowest	15.28	23.33	24.49	14.74	17.56	20.58	0.103	0.135	.137	0.101	0.110	.117
	Range Highest	25.15	51.28	51.97	24.94	31.35	47.65	0.146	0.196	0.203	0.130	0.16	0.166
	C.V. %	2.94	3.51	3.62	3.38	3.79	4.1	3.12	3.83	4.34	4.02	5.36	6.43
	C.D. at 5%	1.05	2.09	2.24	1.17	1.51	2.36	0.01	0.01	0.01	0.01	0.01	0.02

APPENDIX-6

Mean performance of 33 sugarcane genotypes for different physiological traits under normal (E1), mild water stress (E2) and rainfed (E3) environments

Sr No	Genotype	RWC % at 120 DAP			RWC % at 150DAP			RWC % at 240 DAP			CMS % at 150 DAP			CMS % at 240 DAP		
		E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
1	Co 0238	64.71	59.09	58.58	71.38	67.36	62.13	80.2	75.78	71.68	74.55	56.23	56.12	74.37	70.2	62.13
2	Co 118	70.31	62.99	60.64	66.93	64.36	60.61	88.82	85.24	75	72.67	63.35	59.65	72.97	69.4	65.13
3	CoJ 64	72.38	62.09	60.63	88.21	72.34	63.34	81.43	73.12	63.72	76.61	55.98	54.08	75.33	68.63	58.59
4	CoJ 85	74.66	64.09	62.72	81.17	67.13	61.6	90.51	81.01	72.14	74.81	58.05	54.54	75.33	69	59.6
5	CoJ 88	72.07	70.72	67.96	77	71.72	68.57	85.53	82.18	78.07	75.41	65.73	65.63	77.07	73.83	68.97
6	CoPb 91	72.69	69.17	64.89	74.01	70.54	64.92	82.87	79.43	71.74	69.06	60.09	58.2	70.37	65.03	59.47
7	CoPb 92	73.89	70.35	67.86	72	65.66	62.05	77.37	72.79	68.66	72.43	63.79	62.98	73.31	69.67	64.93
8	CoPb 93	71.23	69.54	66.43	66.57	63	54.01	83.42	77.34	68.08	79.09	64.69	62.24	80	75	64.27
9	CoPb 94	70.41	67.72	64.93	72.01	65.69	62.68	78.5	74.21	70.62	68.72	60.36	59.8	69.83	66.48	62.2
10	CoPb 13181	68.66	66.81	60.05	74.97	63.98	58.9	81.02	75.74	70.29	75.89	62.06	61.11	76.1	70.23	60.63
11	CoPb 13182	69.41	67.63	65.38	66.44	64.47	59.52	79.46	74.21	68.39	71.22	56.23	53.15	72.27	68.57	59.73
12	CoPb 14181	73.74	69.52	67.08	70.63	67.65	61.37	79.66	73.53	67.93	76.45	71	67.43	76.23	70.4	65.77
13	CoPb 14184	71.43	68.86	66.93	80.44	73.43	65.61	78.07	74.4	69.55	76.54	64.3	61.17	76.73	70.3	62
14	CoPb 14185	68.97	65.2	63.56	73.22	64.83	61.21	83.62	77.85	71.84	80.77	68.12	66.46	78.3	72.56	67.5
15	CoPb 16181	63.34	57.44	55.18	76.42	63.13	61.38	82.1	77.09	66.06	73.17	64.65	60.12	73.73	70.47	60.93
16	CoPb 18181	62.28	56.66	55.91	72.78	65.26	56.61	80.65	74.92	69.88	78.77	67.01	63.84	79.6	73.8	65.47
17	CoPb 18182	67.24	61.1	54.63	68.57	66.3	59	78.66	74.4	65.42	70.77	62.12	58.64	71.47	69.03	59.53
18	CoPb 15212	69.88	67.63	66.17	77.47	70.43	62.62	84.88	82.25	77.13	77.44	60.62	57.08	78.07	72.29	63.5
19	CoPb 15213	66.89	64.86	61.25	75.27	70.09	64.25	84.34	80.48	76.54	73.2	60.78	55.52	74.37	69.45	58.27
20	CoPb 15214	68.67	62.48	61.72	73.71	70.58	59.05	82.04	77.63	70.2	77.28	58.54	58.67	77.6	71.2	61.4

Sr No	Genotype	RWC % at 120 DAP			RWC % at 150DAP			RWC % at 240 DAP			CMS % at 150 DAP			CMS % at 240 DAP		
		E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3
21	F 404/13	71.03	65.59	60.07	70.67	67.99	54.94	80.63	75.33	72.65	70.22	52.81	52.99	71.07	67	63.43
22	F 301/11	69.98	67.05	60	73.02	67.26	55.54	79.82	71.94	68	72.83	59.5	61.66	73.63	69.07	60.87
23	F 3/14	61.04	54.7	53.13	75.93	71	62.58	83.58	76.75	73.76	73.47	56.53	57.76	72.8	68.4	61.23
24	F 6/14	60.8	59.5	58	68.55	65.93	62.23	80.04	76.59	67.35	74.2	55.46	54.73	74.5	69.23	60.67
25	F 362/14	70.24	65.52	64	82.22	71.81	68.62	81.25	77.66	74.04	70.23	55.79	59.66	70.8	67.43	62.1
26	F 391/14	68.57	67.72	65.68	76.48	73.75	69.53	82.6	79.81	75.48	75.84	67.57	66.32	76.53	73.63	69.73
27	F 660/14	75.79	68.71	67.03	78.01	73.52	60.94	83.18	77.49	73.59	74	65.52	64.05	73.9	70.56	66.13
28	MA 5/37	67.82	59.79	58.49	76.37	64.75	57.42	78.03	70.62	62.8	75.09	54.96	55.54	74.47	69.07	60.23
29	MA 5/51	62.83	57.41	55.02	68.57	57.32	49.69	78.33	71.19	64.09	74.78	57.36	56.57	74.23	67.77	58.73
30	AS 04-1687	68.09	65.29	64.85	75.58	71.09	63.97	79	75.33	72.05	76.29	62.56	57.92	76.5	71.6	67.13
31	SA 04-409	67.3	64.95	62.61	75.85	72.09	59.74	79.4	76.64	70.98	77.83	68.68	65.25	77.13	72.37	65.03
32	BM 101068	67	63.52	59.96	70.48	64.03	55.46	78.78	73.08	70.14	75.69	60.81	59.92	75.97	70.8	60.33
33	Co 98014	76.76	66.59	67.7	66.66	57.65	50.8	80.37	75.2	70.78	78.01	59.56	58.75	76.87	71.03	60.43
	Mean	69.09	64.55	62.09	73.87	67.46	60.63	81.46	76.40	70.56	74.65	61.24	59.62	74.89	70.11	62.61
	SEm	1.04	2.25	3.11	1.06	2.50	2.78	1.17	2.41	2.12	0.34	0.53	0.69	1.06	1.77	1.71
	Range Lowest	60.80	59.09	53.14	66.44	57.32	49.69	77.37	70.61	62.80	68.72	52.81	52.99	69.83	65.03	58.27
	Range Highest	76.76	66.59	67.96	88.21	73.75	69.53	90.51	85.24	78.08	80.00	71.00	67.43	80	75.00	69.73
	C.V.	2.53	4.29	4.12	2.40	3.97	5.41	2.54	3.12	4.58	0.83	3.71	3.60	0.89	2.24	3.88
	C.D. 5%	2.85	4.52	4.17	2.89	4.36	5.35	3.37	3.89	5.27	1.01	3.71	3.50	1.09	2.57	3.96

APPENDIX-7

Per cent decrease in mean under E2 and E3 environments of sugarcane genotypes for cane yield, CCS % and CCS (t/ha) over E1 environments

S. No.	Genotype	% deviation Under E2 over E1	% deviation Under E3 over E1	% deviation Under E2 over E1	% deviation Under E3 over E1	% deviation Under E2 over E2	% deviation Under E3 over E2
		Cane yield(t/ha)		CCS%		CCS(t/ha)	
1	Co 0238	75	93.42	10.27	14.14	77.65	94.16
2	Co 118	78.78	75.75	19.69	23.44	82.83	80.69
3	CoJ 64	87.16	95.61	14.90	22.70	89.07	96.23
4	CoJ 85	84.21	88.6	18.52	15.18	87.02	90.62
5	CoJ 88	52.57	61.54	11.72	16.08	58.30	66.09
6	CoPb 91	56.99	80.64	16.91	22.54	64.24	83.88
7	CoPb 92	32.89	69.08	18.98	22.21	45.76	74.86
8	CoPb 93	60.6	85.6	17.45	20.56	67.54	88.11
9	CoPb 94	48.19	60.24	15.38	22.24	56.06	66.55
10	CoPb 13181	46.75	84.42	14.70	21.22	54.75	86.67
11	CoPb 13182	81.81	88.31	14.17	17.96	84.39	89.94
12	CoPb 14181	55.72	95.72	20.03	23.23	64.64	96.60
13	CoPb 14184	52	86	12.92	19.05	58.32	87.78
14	CoPb 14185	45.45	82.47	18.79	23.30	55.72	85.71
15	CoPb 16181	55.4	90.54	16.52	18.39	62.77	92.07
16	CoPb 18181	37.35	72.89	14.30	15.84	46.48	76.76
17	CoPb 18182	34.12	73.53	18.20	22.56	46.15	78.28
18	CoPb 15212	59.03	77.71	15.05	20.58	65.26	81.03
19	CoPb 15213	42.86	87.14	10.27	16.78	48.46	88.46
18	CoPb 15212	59.03	77.71	15.05	20.58	65.26	81.03

19	CoPb 15213	42.86	87.14	10.27	16.78	48.46	88.46
20	CoPb 15214	36.84	92.1	11.21	17.44	43.97	93.01
21	F 404/13	56.41	87.18	9.15	17.8	60.36	88.4
22	F 301/11	45.35	91.86	18.88	22.73	55.52	93.35
23	F 3/14	32.89	88.82	9.35	15.25	38.96	89.81
24	F 6/14	53.13	83.59	13.39	21.37	59.15	85.73
25	F 362/14	53.23	72.58	16.8	20.92	61.03	77.11
26	F 391/14	30.77	55.77	8.66	15.84	36.8	59.61
27	F 660/14	56.05	74.18	8.71	14.5	59.84	76.39
28	MA 5/37	78.05	89.03	5.59	8.26	79.18	89.7
29	MA 5/51	78.78	90.16	18.97	22.59	82.55	91.93
30	AS 04-1687	39.69	75.57	3.02	-17.79	41.62	76.43
31	SA 04-409	45.1	74.52	7.3	12.04	49.32	76.4
32	BM 101068	57.75	74.65	6.95	-6.21	60.69	76.42
33	Co 98014	74.39	82.92	4.82	17.32	75.63	83.62
	Mean	55.31	81.28	13.38	16.97	61.21	83.71

APPENDIX-8

Per cent decrease in mean under E2 and E3 environments of sugarcane genotypes for biochemical traits over E1 environment

S. No.	**Genotypes**	**Proline (µg/gfw) at 150DAP**		**Proline (µg/gfw) at 240 DAP**		**SOD (eu/100ml/gFW) at 150DAP**		**SOD (eu/100ml/gFW) at 240DAP**	
		% increase in mean under E2% over E1	**% % increase in mean under E3 over E1**	**% increase in mean under E2**	**% increase in mean under E3**	**% increase in mean under E2 over E1**	**% increase in mean under E3 over E1**	**% increase in mean under E2 over E1**	**increase in mean under E3 over E1**
1	Co 0238	84.52	87.05	18.96	65.29	21.92	25.34	15.75	21.26
2	Co 118	109.59	93.79	17.77	85.12	45.38	45.38	29.46	33.93
3	CoJ 64	39.78	46.73	7.61	25.26	23.85	25.69	6.80	13.59
4	CoJ 85	42.22	50.44	8.16	27.77	28.45	31.90	9.02	14.75
5	CoJ 88	107.31	115.97	30.49	100.69	51.64	59.84	36.11	42.59
6	CoPb 91	81.98	92.55	22.79	80.31	40.16	45.90	22.32	33.93
7	CoPb 92	98.07	100.36	18.44	90.17	47.93	48.76	22.64	32.08
8	CoPb 93	60.14	65.97	11.39	53.92	34.43	39.34	20.00	31.82
9	CoPb 94	80.65	95.86	27.94	87.12	46.83	50.00	10.94	14.06
10	CoPb 13181	113.22	126.64	19.13	66.08	44.92	45.76	28.07	34.21
11	CoPb 13182	106.83	91.96	12.70	79.74	39.52	44.35	17.54	35.09
12	CoPb 14181	87.69	92.54	10.99	73.42	48.31	51.69	16.81	31.86
13	CoPb 14184	51.77	53.26	10.34	36.92	48.25	51.75	25.23	46.73
14	CoPb 14185	77.57	87.92	13.94	54.98	32.82	35.88	26.92	28.85
15	CoPb 16181	86.00	94.55	11.88	68.07	31.40	34.71	17.82	28.71
16	CoPb 18181	63.33	73.93	13.97	54.64	42.50	50.83	23.01	30.09
17	CoPb 18182	65.04	75.59	6.40	68.97	45.69	50.00	26.79	41.07
18	CoPb 15212	60.27	69.25	10.23	78.05	43.51	50.38	24.19	29.84

S. No.	Genotypes	Proline (µg/gfw) at 150DAP		Proline (µg/gfw) at 240 DAP		SOD (eu/100ml/gFW) at 150DAP		SOD (eu/100ml/gFW) at 240DAP	
		% increase in mean under E2% over E1	**% % increase in mean under E3 over E1**	**% increase in mean under E2**	**% increase in mean under E3**	**% increase in mean under E2 over E1**	**% increase in mean under E3 over E1**	**% increase in mean under E2 over E1**	**increase in mean under E3 over E1**
19	CoPb 15213	54.28	63.0	24.77	78.89	41.98	45.04	22.31	25.38
20	CoPb 15214	54.70	62.87	8.42	44.73	34.68	39.52	22.33	33.98
21	F 404/13	50.85	64.38	23.97	60.23	31.25	35.94	16.41	24.22
22	F 301/11	41.29	51.92	11.89	66.21	30.08	39.85	22.41	34.48
23	F 3/14	49.64	54.48	14.19	85.26	41.48	47.41	21.60	29.6
24	F 6/14	69.19	77.15	16.76	70.79	44.80	49.60	25.66	37.17
25	F 362/14	45.88	51.50	11.34	82.56	45.90	48.36	11.76	26.89
26	F 391/14	116.65	119.56	39.77	112.44	70.43	76.52	38.94	46.9
27	F 660/14	77.76	80.13	12.93	76.94	48.41	49.21	17.14	31.43
28	MA 5/37	41.74	47.31	8.20	35.91	28.23	37.1	13.21	13.21
29	MA 5/51	41.32	47.38	13.14	35.85	28.32	30.09	8.04	5.36
30	AS 04-1687	93.79	110.59	28.12	94.39	50.83	52.50	26.67	30.00
31	SA 04-409	41.42	48.79	12.63	76.12	44.66	48.54	14.68	30.28
32	BM 101068	37.10	39.24	9.02	43.34	45.69	50.86	19.83	23.28
33	Co 98014	75.23	75.36	18.97	69.33	45.95	48.65	20.00	25.71
	Mean	69.9	76	15.98	67.56	40.92	45.05	20.62	29.16

APPENDIX-9

Per cent decrease in mean under E2 and E3 environments of sugarcane genotypes for physiological traits over E1 environment

S. N0.	Genotype	RWC (%) at 120DAP		RWC (%) at 150DAP		RWC (%) at 240DAP		CMS (%) at 150DAP		CMS (%) at 240DAP	
		%↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3
1	Co 0238	8.68	9.47	5.63	12.96	5.51	10.62	24.57	24.72	5.61	16.46
2	Co 118	10.41	13.75	3.84	9.44	4.03	15.56	12.83	17.92	4.89	10.74
3	CoJ 64	14.22	16.23	17.99	28.19	10.21	21.75	26.93	29.41	8.89	22.22
4	CoJ 85	14.16	15.99	17.3	24.11	10.5	20.3	22.4	27.1	8.4	20.88
5	CoJ 88	1.87	5.7	6.86	10.95	3.92	8.72	12.84	12.97	4.2	10.51
6	CoPb 91	4.84	10.73	4.69	12.28	4.15	13.43	12.99	15.73	7.59	15.49
7	CoPb 92	4.79	8.16	8.81	13.82	5.92	11.26	11.93	13.05	4.97	11.43
8	CoPb 93	2.37	6.74	5.36	18.87	7.29	18.39	18.21	21.3	6.25	19.66
9	CoPb 94	3.82	7.78	8.78	12.96	5.46	10.04	12.17	12.98	4.8	10.93
10	CoPb 13181	2.69	12.54	14.66	21.44	6.52	13.24	18.22	19.48	7.71	20.33
11	CoPb 13182	2.56	5.81	2.97	10.42	6.61	13.93	21.05	25.37	5.12	17.35
12	CoPb 14181	5.72	9.03	4.22	13.11	7.7	14.73	7.13	11.8	7.65	13.72
13	CoPb 14184	3.6	6.3	8.71	18.44	4.7	10.91	15.99	20.08	8.38	19.2
14	CoPb 14185	5.47	7.84	11.46	16.4	6.9	14.09	15.66	17.72	7.33	13.79
15	CoPb 16181	9.31	12.88	17.39	19.68	6.1	19.54	11.64	17.84	4.42	17.36
16	CoPb 18181	9.02	10.23	10.33	22.22	7.1	13.35	14.93	18.95	7.29	17.75
17	CoPb 18182	9.13	18.75	3.31	13.96	5.42	16.83	12.22	17.14	3.41	16.71

S. N0.	Genotype	RWC (%) at 120DAP		RWC (%) at 150DAP		RWC (%) at 240DAP		CMS (%) at 150DAP		CMS (%) at 240DAP	
		%↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3	% ↓ in mean under E2	% ↓ in mean under E3
18	CoPb 15212	3.22	5.31	9.09	19.17	3.1	9.13	21.72	26.29	7.4	18.66
19	CoPb 15213	3.03	8.43	6.88	14.64	4.58	9.25	16.97	24.15	6.62	21.65
20	CoPb 15214	9.01	10.12	4.25	19.89	5.38	14.43	24.25	24.08	8.25	20.88
21	F 404/13	7.66	15.43	3.79	22.26	6.57	9.90	24.79	24.54	5.73	10.75
22	F 301/11	4.19	14.26	7.89	23.94	9.87	14.81	18.30	15.34	6.19	17.33
23	F 3/14	10.39	12.96	6.49	17.58	8.17	11.75	23.06	21.38	6.04	15.89
24	F 6/14	2.14	4.61	3.82	9.22	4.31	15.85	25.26	26.24	7.07	18.56
25	F 362/14	6.72	8.88	12.66	16.54	4.42	8.87	20.56	15.05	4.76	12.29
26	F 391/14	1.24	4.21	3.57	9.09	3.38	8.62	10.9	12.55	3.79	8.89
27	F 660/14	9.34	11.56	5.76	21.88	6.84	11.53	11.46	13.45	4.52	10.51
28	MA 5/37	11.84	13.76	15.22	24.81	9.5	19.52	26.81	26.04	7.25	19.12
29	MA 5/51	8.63	12.43	16.41	27.53	9.12	18.18	23.29	24.35	8.7	20.88
30	AS 04-1687	4.11	4.76	5.94	15.36	4.65	8.8	18	24.08	6.41	12.25
31	SA 04-409	3.49	6.97	4.96	21.24	3.48	10.6	11.76	16.16	6.17	15.69
32	BM 101068	5.19	10.51	9.15	21.31	7.24	10.97	19.66	20.83	6.81	20.59
33	Co 98014	13.25	11.8	13.52	23.79	6.43	11.93	23.65	24.69	7.6	21.39
	Mean	6.55	10.12	8.54	17.8	6.21	13.36	17.94	20.08	6.37	16.36

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