

Unravelling the Genetic and Physiological Adaptations of Sugarcane to Water Deficit Conditions

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Abstract

Drought stress is a significant constraint affecting sugarcane (*Saccharum* spp.) growth, yield, and sucrose accumulation. This study investigates the physiological, biochemical, and molecular adaptations of sugarcane genotypes CoS 13235 and Co 15023 under water deficit conditions. Physiological assessments revealed that CoS 13235 maintained higher relative water content (RWC), membrane stability index (MSI), and chlorophyll content, indicating superior drought tolerance. Biochemical analysis showed significant accumulation of proline and increased antioxidant enzyme activities (*SOD*, *CAT*, and *POX*) under drought stress, with Co 15023 exhibiting higher proline accumulation and CoS 13235 showing stronger antioxidant responses. Gene expression analysis revealed differential responses in drought-related genes, with *DREB2A* being upregulated under stress, particularly in Co 15023, while *DREB1A* showed a more complex regulatory pattern. The *LEA* gene was downregulated under drought stress, whereas *SOD* gene expression increased significantly in CoS 13235, suggesting a stronger antioxidant defense mechanism. These findings highlight distinct drought adaptation strategies in sugarcane genotypes, emphasizing the need for targeted breeding approaches to improve drought resilience. Future studies should focus on the functional validation of key genes and their potential applications in sugarcane improvement programs.

1. Introduction

Sugarcane (*Saccharum* spp.) is a vital agro-industrial crop cultivated worldwide primarily for its high sucrose content, which serves as the principal raw material for the sugar and bioethanol industries (Goldemberg et al., 2008; Pillay, 2013). As a C_4 plant, sugarcane displays exceptional photosynthetic efficiency, high radiation-use efficiency, and remarkable biomass productivity, factors that collectively underpin its dominant role in global sugar production and in supplying renewable feedstocks for bioenergy applications (Waclawovsky et al., 2010). The crop's commercial value, however, depends heavily on adequate and timely water supply: water availability influences not only vegetative growth and cane tonnage but also sucrose partitioning, stalk quality, and final recoverable sugar. Consequently, prolonged or recurrent periods of water deficit have profound adverse effects on physiological processes, yield components, and the overall economic performance of sugarcane cultivation (Baksha et al., 2003; Rodrigues et al., 2009).

Water deficit stress disrupts an integrated network of plant processes — from stomatal control and photosynthetic carbon assimilation to nutrient uptake, phloem loading, and assimilate partitioning — that together determine cane growth and sucrose accumulation (Xiong and Zhu, 2002; Sengar et al., 2011). Under drought, sugarcane exhibits a spectrum of adaptive responses at morphological, physiological, and biochemical levels. Osmotic adjustment, achieved through accumulation of compatible solutes such as proline, soluble sugars, and certain sugar alcohols, helps maintain cell turgor and sustain metabolic activity during water shortage (Nezhadahmadi et al., 2013). Concomitantly, shifts in root architecture — including deeper rooting and increased root biomass or root-to-shoot ratios — enhance soil water exploration and uptake, while coordinated stomatal regulation reduces transpirational water loss at the expense of instantaneous photosynthetic rate (Kaur and Kapoor, 2016). Structural changes in leaves and canopy architecture, such as reduced leaf area and altered leaf angle, further contribute to minimizing evaporative demand and optimizing whole-plant water balance (ICAR-Sugarcane Report, 2022). At the cellular and molecular levels, drought perception and signalling rapidly activate complex regulatory networks. Absciscic acid (ABA) plays a central role as an upstream hormonal mediator, modulating stomatal behaviour, expression of stress-responsive genes, and cross-talk with other signaling

pathways (Rodrigues et al., 2011). Genes encoding late embryogenesis abundant (*LEA*) proteins, aquaporins that facilitate water transport across membranes, and a suite of transcription factors – notably *DREB*, *NAC*, and *bZIP* families – are frequently induced under water deficit and orchestrate downstream protective and adaptive responses (Andrad et al., 2015; Fang and Xiong, 2015). Activation of antioxidant defenses, including superoxide dismutase, catalase, and peroxidases, mitigates oxidative damage caused by drought-associated reactive oxygen species (ROS) bursts (Sandeep et al., 2009). Emerging evidence also implicates epigenetic modifications and small RNAs in modulating stress memory and phenotypic plasticity, offering potential routes for heritable adaptation to recurring drought episodes.

Genetic and genomic studies have accelerated the discovery of loci and molecular markers associated with drought tolerance. Quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), and transcriptome profiling have identified differentially expressed genes (DEGs) and candidate genomic regions correlated with traits such as root depth, water use efficiency (WUE), osmotic adjustment, and relative water content (RWC) (Jain et al., 2015; Wang et al., 2013). These insights are being translated into breeding pipelines by integrating molecular markers with conventional selection, high-throughput phenotyping, and physiological screening to develop cultivars that combine agronomic performance with stress resilience. In parallel, biotechnological interventions – including transgenic approaches, RNA interference (RNAi), and precise genome editing via CRISPR/Cas systems – hold substantial promise for the targeted modification of genes governing water relations, osmo-protection, and signal transduction to engineer drought-tolerant genotypes (Gentile et al., 2015; Hanlon et al., 2000). However, the complex polyploid genome of modern sugarcane, along with genotype × environment interactions, poses challenges that require integrative strategies.

Physiological comparisons of tolerant versus susceptible cultivars reveal consistent patterns: drought-tolerant genotypes tend to maintain higher RWC, sustain greater WUE, and exhibit more conservative water use through stomatal control, while allocating proportionally more biomass to roots (Melaku et al., 2016; Rashid and Lateef, 2016). These functional traits, together with metabolic adjustments and structural plasticity, underpin improved survival and yield stability under intermittent water shortages. Therefore, the interplay between molecular determinants and whole-plant physiological strategies is crucial to breeding and management programs aimed at mitigating the impacts of drought.

Given the mounting pressures of climate change, increased variability of rainfall, and growing demand for sugar and bioenergy, integrating genomic, transcriptomic, and physiological knowledge is essential to accelerate the development of high-yielding, drought-resilient sugarcane cultivars. Future research priorities include elucidating genotype-specific drought adaptation mechanisms across developmental stages, refining field-scale phenotyping methods, exploiting natural genetic variation from wild relatives, and deploying precision breeding and gene-editing tools in combination with improved crop management to sustain productivity in water-limited environments.

2. Materials and Methods

The present study involved the evaluation of sugarcane genotypes for drought tolerance and the validation of the functional expression of drought-responsive genes.

2.1. Plant Material and Growth Conditions

Commercially cultivated sugarcane (*Saccharum officinarum* L.) genotypes exhibiting contrasting responses to drought stress – one known for higher tolerance and another for relative susceptibility – were selected for the present study to enable comparative physiological and biochemical analyses. Uniform, disease-free, and healthy single-budded setts were prepared from the middle portion of mature stalks, ensuring uniformity in size and bud viability. These setts were planted in cylindrical plastic pots measuring 30 cm in diameter and 40 cm in height, each filled with a thoroughly homogenized and well-aerated growth medium composed of topsoil, river sand, and decomposed farmyard compost in a ratio of 2:1:1 (v/v/v). This mixture was chosen to provide adequate drainage, aeration, and nutrient availability while simulating field-like growth conditions in a controlled environment. The experiment was carried out under regulated greenhouse conditions at Sardar Vallabhbhai Patel University of Agriculture and Technology (SVPUA&T), Modipuram, Meerut, Uttar Pradesh, India, with environmental parameters continuously monitored and maintained at an average temperature of $28 \pm 2^{\circ}\text{C}$, relative humidity of 60–70%, and a photoperiod of 14 h light and 10 h dark to mimic optimal tropical growth conditions. The photosynthetically active radiation (PAR) at canopy height was maintained, and ventilation was provided to prevent heat buildup and ensure consistent air circulation. During the initial establishment phase (first 45 days after planting), all plants were irrigated uniformly to field capacity using tap water to eliminate variability in soil moisture and to promote uniform seedling emergence and early growth. The pots were arranged in a completely randomized design (CRD) with 3 replicates per treatment to minimize positional effects within the greenhouse. Throughout the establishment period, emerging weeds were manually removed, and no nutrient deficiencies were allowed to develop, ensuring that subsequent physiological responses could be attributed solely to imposed drought stress treatments rather than to confounding environmental or nutritional factors. The list of genotypes used in the present study, along with their source of procurement, is given in Table 1.

Table 1
List of all fifteen genotypes used in the present study and their procurement source.

S. No.	Genotypes	Collection site
1	Co98014	Sri Ram Sugarcane Farm, Lakhimpur Kheri, Uttar Pradesh
2	CoS13231	
3	Co15023	
4	Co0118	
5	Co0238	SVPUA&T, Meerut, Uttar Pradesh, India
6	CoLK14201	ICAR-Indian Sugarcane Research Institute, Lucknow, Uttar Pradesh, India
7	CoP9301	
8	CoS9232	
9	CoPB092	
10	CoSE11453	
11	CoS13236	
12	CoJ64	
13	CoJ85	
14	CoS13235	
15	Co8272	

2.2. Physiological and Biochemical Assessment of Drought

To evaluate genotype-specific responses to water deficit, drought stress treatments were initiated at the 6-leaf growth stage, a critical phase for sugarcane vegetative development and biomass accumulation. Stress was imposed by withholding irrigation until the soil moisture content in the pots declined to predetermined fractions of the soil's field capacity (FC), corresponding to different drought intensities. Specifically, moderate drought stress was defined as 40% of FC moisture, whereas severe drought stress corresponded to 20% of FC moisture. Control plants were maintained under well-watered conditions at approximately 80% of FC throughout the experimental period to serve as the reference baseline for all measurements. Soil moisture content was monitored daily using a calibrated soil moisture sensor inserted to a uniform depth near the plant base in each pot to ensure accurate and consistent readings. Irrigation was adjusted promptly to maintain the target FC levels throughout the stress period. This gradual, precisely regulated depletion of soil moisture enabled the simulation of realistic drought scenarios without causing abrupt plant mortality. Following stress imposition, an array of physiological and biochemical parameters was assessed to capture both immediate and cumulative effects of water deficit on plant function. Leaf Water Potential (Ψ_{leaf}) was measured at both pre-dawn (representing baseline hydration status) and midday (representing peak evaporative demand) using a Scholander-type pressure chamber. These measurements provided quantitative insight into the degree of water tension in the leaf xylem and the plant's overall water status. Relative Water Content (RWC) was determined as an indicator of tissue hydration and tolerance to drought-induced dehydration. Leaf discs were weighed for fresh weight (FW), fully rehydrated in distilled water for 4–6 hours to obtain turgid weight (TW), and subsequently oven-dried at 70°C for 48 hours to

obtain dry weight (DW). RWC (%) was calculated using the formula, where a higher RWC under stress conditions indicates better water retention capacity and osmotic adjustment.

$$RWC (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Non-destructive chlorophyll estimation was carried out using a SPAD-502 chlorophyll meter (Konica Minolta, Japan), with readings taken from the mid-section of the third fully expanded leaf from the top. SPAD values serve as a rapid proxy for chlorophyll concentration and photosynthetic potential under drought conditions. Electrolyte leakage was used as an indicator of plasma membrane stability and integrity. Leaf discs (1 cm diameter) were incubated in deionized water, and initial electrical conductivity was measured using a digital conductivity meter. Samples were then autoclaved to release all electrolytes, and the final conductivity was recorded. The percentage leakage was calculated relative to total conductivity, with higher values reflecting greater membrane injury due to drought-induced oxidative stress. Proline accumulation was quantified following the acid-ninhydrin assay (Bates et al., 1973), with absorbance measured at 520 nm using a spectrophotometer. This integrated physiological–biochemical framework ensured a comprehensive assessment of plant performance under water deficit, enabling the identification of tolerance-associated traits and their correlation with drought intensity. Such multi-dimensional evaluation provides valuable insight into the mechanisms underlying genotype-specific drought adaptation in sugarcane.

2.3. RNA isolation for gene expression analysis

To elucidate the molecular responses of sugarcane genotypes to drought stress, total RNA was extracted from fully expanded leaf tissue collected from plants subjected to various water-deficit treatments and from well-watered controls. Leaf samples were harvested at the formative stage at the same time of day to minimize diurnal variation in gene expression, immediately frozen in liquid nitrogen, and stored at – 80°C until analysis. RNA isolation was performed using TRIzol reagent (Invitrogen, USA) following the manufacturer’s recommended protocol. Approximately 100 mg of fresh or snap-frozen leaf tissue was finely ground in liquid nitrogen using a pre-chilled mortar and pestle, and homogenized thoroughly in TRIzol to ensure complete cell lysis and solubilization of nucleic acids. Chloroform was added to the homogenate, followed by vigorous mixing and centrifugation at 12,000 RPM for 15 min at 4°C to achieve phase separation. The aqueous phase containing RNA was carefully transferred to a fresh tube, precipitated with an equal volume of isopropanol, and centrifuged to pellet the RNA. The pellet was washed twice with 75% ethanol, air-dried briefly, and resuspended in RNase-free water. The quality of RNA was verified through both purity and integrity assessments. Purity was determined spectrophotometrically using a NanoDrop™ (Thermo Fisher Scientific, USA), with the A260/A280 ratio maintained between 1.9–2.1 and A260/A230 above 2.0 to confirm minimal protein and polysaccharide contamination. Integrity was confirmed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide, where distinct 28S and 18S rRNA bands without smearing indicated intact RNA. First-strand complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), employing oligo(dT) primers to selectively target mRNA poly-A tails. Reverse transcription was carried out according to the manufacturer’s instructions, and the resulting cDNA was diluted and stored at – 20°C until further use in gene expression assays.

2.4. Quantitative Real-Time PCR (qRT-PCR)

Expression profiling of selected drought-responsive genes — including *DREB2A*, *DREB1A*, *MnSOD*, and *LEA* — was conducted using SYBR Green chemistry on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA). Gene-specific primers were designed to span exon–exon junctions to avoid amplification of genomic DNA. Each 20 µL reaction mixture contained 10 µL SYBR Green Master Mix (Applied Biosystems), 0.4 µL each of forward and reverse primers (10 µM), 2 µL of cDNA template, and nuclease-free water to volume. The amplification protocol included an initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. Actin and GAPDH genes served as internal reference controls for normalization. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, and melting curve analysis was performed to confirm the specificity of amplicons.

2.5. Statistical Analysis

All physiological and morphological data were subjected to analysis of variance (ANOVA) using SPSS v25.0 (IBM, USA), and means were compared using Tukey's HSD test at a significance threshold of $p < 0.05$.

3. Results

The results obtained from the present investigation provide comprehensive insights into the morphological, physiological, biochemical, and molecular responses of selected sugarcane genotypes under drought stress. The observations were recorded under controlled, moderate (T1), and severe (T2) drought conditions to assess the extent of variation among genotypes in their adaptive mechanisms. The findings highlight distinct genotypic differences in growth performance, physiological regulation, biochemical response, and gene expression patterns, which collectively contribute to their drought tolerance or susceptibility. The results are presented and discussed under the following subheadings

3.1. Morphological Responses of Sugarcane Genotypes under Drought Stress

Fifteen sugarcane genotypes were collected from various locations as mentioned in Table 1. Morphological screening revealed substantial variation in responses to water deficit, indicating inherent differences in drought adaptation strategies under experimental conditions. Under control conditions, plant height ranged from 116.00 cm in CoS13231 to 138.33 cm in CoP9301, whereas leaf area varied between 182.40 cm² in Co0238 and 246.24 cm² in CoS13231. The number of tillers ranged from 4.33 in CoLK14201 to 6.67 in Co98014. Drought stress significantly affected these traits in all genotypes. Under moderate drought (T1), plant heights declined by 10–15%, and under severe drought (T2), reductions were more pronounced, with plant heights ranging from 94.78 cm to 115 cm. Leaf area decreased by approximately 20–45% under T1 and T2, while tiller number showed the most drastic reduction, particularly in susceptible genotypes. Among all genotypes, CoS13235, collected from IISR, Lucknow, demonstrated relatively better performance under drought stress. Its plant height declined from 118.67 cm under the control to 107.33 cm under T1 and 99.11 cm under T2, indicating a moderate reduction compared with other genotypes. Leaf area in CoS13235 decreased from 200.64 cm² under control to 186.22 cm² under T1 and 174.34 cm² under T2. The number of tillers declined from 5.33 to 4.67 under T1 and 3.65 under T2. These observations indicate that CoS13235 maintains a considerable proportion of vegetative growth under stress. In contrast, Co15023, a genotype from Sri Ram Sugarcane Farm, exhibited sharper reductions in growth. Plant height decreased from 124.00 cm under control to 107.00 cm under T1 and 97.00 cm under T2. Leaf area declined from 228.23 cm² to 166.44 cm² and further to 154.33 cm², while the number of tillers dropped from 6.33 under control

to 4.33 under T1 and 2.32 under T2. These data highlight Co15023's greater susceptibility to drought and its limited capacity to maintain vegetative growth. Figure 1 (A-C) explains the mean performances of morphological traits for all the 15 genotypes under Control (Irrigated on 110th day), T1 (15 days' water stress with irrigation on 125th day), and T2 (30 days' water stress with irrigation on 140th day).

3.2. Physiological Responses

Physiological evaluation focused on relative water content (RWC), membrane stability index (MSI), and chlorophyll content (SPAD values), which are critical indicators of drought tolerance (Table 2). Drought stress significantly affected all three parameters in both genotypes, revealing clear contrasts in their adaptive responses. CoS13235 maintained relatively high RWC under stress, with values of 73.26% under T1 and 65.22% under T2, compared to 84.05% under control. This indicates efficient water retention mechanisms, possibly facilitated by osmotic adjustment and deeper root water extraction. Co15023 exhibited higher absolute RWC under stress (88.99% in T1 and 78.92% in T2) compared to its control value of 95.49%, but the proportional decline indicates a weaker capacity for osmotic regulation under severe stress. Membrane stability also differed markedly between genotypes. CoS13235 showed a decline from 54.55% under control to 51.38% under T1 and 45.23% under T2, suggesting moderate membrane damage and a relatively stable cellular environment. In contrast, Co15023 experienced a sharp decrease from 41.86% under control to 30.77% under T1 and 25.77% under T2, indicating higher susceptibility to drought-induced cellular injury. Chlorophyll content, measured as SPAD values, was maintained better in CoS13235, with readings of 37.88 under control, 33.32 under T1, and 25.23 under T2. In Co15023, chlorophyll content decreased from 45.45 under control to 39.62 under T1 and 25.44 under T2. These findings suggest that CoS13235 preserves photosynthetic capacity under stress, whereas Co15023 is more prone to pigment degradation and impaired photosynthesis under drought conditions. Figure 1 (D-F) explains the mean performances of physiological traits for all the 15 genotypes under Control (Irrigated on 110th day), T1 (15 days' water stress with irrigation on 125th day), and T2 (30 days' water stress with irrigation on 140th day).

Table 2
Mean performance of 15 sugarcane genotypes for morpho-physiological characters under control and drought stress conditions.

Genotype name	Plant height (cm)			Leaf area (cm ²)			Number of Tillers		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
CoS9232	126.67	112.00	108	186.20	143.26	132.22	4.67	2.33	1.51
CoP9301	138.33	126.00	116	222.30	175.56	165.24	5.00	3.67	2.55
CoSE11453	129.67	114.67	104.67	206.72	158.69	148.66	6.33	3.33	2.22
CoS13231	116.00	100.67	94.78	246.24	193.19	184.11	5.67	3.67	2.36
CoPB092	122.15	109.33	103.24	221.07	179.46	166.36	5.33	3.33	3.33
CoJ64	128.45	113.33	104.11	209.93	171.54	162.43	5.67	3.67	1.67
CoJ85	116.33	100.33	95.12	218.20	170.24	162.12	5.00	2.67	1.66
Co8272	124.67	111.00	101	223.44	180.12	171.21	6.33	3.33	2.32
Co0238	131.67	122.33	109.22	182.40	166.48	155.32	5.67	5.33	4.23
Co98014	123.33	117.67	109.11	239.40	222.52	204.33	6.67	6.00	5.1
CoLK 14201	127.67	110.00	98	204.29	160.06	149.02	4.33	3.33	2.33
Co13236	129.67	107.33	95.11	207.94	153.22	145.11	5.67	4.33	3.5
Co0118	118.00	111.00	101	229.98	205.01	198.03	5.67	5.00	4.8
Co15023	124.00	107.00	97	228.23	166.44	154.33	6.33	4.33	2.32
CoS 13235	118.67	107.33	99.11	200.64	186.22	174.34	5.33	4.67	3.65
RANGE HIGHEST	138.33	126.00	115	246.24	222.52	204.33	6.67	6.00	5.1
RANGE LOWEST	116.00	100.33	94.78	182.40	143.26	132.22	4.33	2.33	1.51
GRAND MEAN	125.01	111.33	102.66	215.13	175.46	165.25	5.57	3.93	2.95
CD 1%	7.74	7.69	7.62	14.69	12.56	11.24	1.62	1.35	1.22
CV	0.05	0.06	0.07	0.09	0.12	0.13	0.12	0.25	0.35
SE(m)	1.97	1.96	1.94	3.74	3.18	2.99	0.41	0.34	0.26
SE(d)	2.79	2.77	2.75	5.29	4.49	3.88	0.58	0.48	0.36
SD	6.35	7.21	8.11	18.47	21.08	23.55	0.68	1.02	1.36

Control: Irrigated (110th day), T1: Treatment-I of 15 days water stress (125th day), T2: treatment-II of 30 days water stress (140th day). The values in columns with different superscripts differ significantly ($p < 0.05$)

3.3. Biochemical Responses

Drought stress induced substantial changes in biochemical markers, including *catalase (CAT)*, *peroxidase (POX)*, and proline accumulation (Table 3). CoS13235 exhibited higher *CAT* activity compared to Co15023, with values of 773.99 units/g FW/min under T1 and 788.85 units/g FW/min under T2, reflecting enhanced detoxification of reactive oxygen species. Co15023 showed lower activity, with 670.11 units/g FW/min under T1 and 684.11 under T2. Peroxidase activity in CoS13235 increased from 834.57 units/g FW/min under control to 895.43 under T1 and 910.55 under T2, while Co15023 recorded 554.57 units/g FW/min under control, rising to 698.29 under T1 and 710.35 under T2. The higher *POX* activity in CoS13235 likely contributes to more efficient ROS scavenging under drought. Proline accumulation, a key osmoprotective mechanism, was also higher in CoS13235, with 17.96 $\mu\text{mol/g FW}$ under T1 and 24.88 $\mu\text{mol/g FW}$ under T2, compared to Co15023 with 14.84 and 21.78 $\mu\text{mol/g FW}$ under the respective stress conditions. These biochemical responses indicate that CoS13235 effectively combines osmotic adjustment with oxidative stress mitigation, while Co15023, although accumulating proline, shows comparatively lower antioxidant enzyme activity, leaving it more vulnerable to oxidative damage. Figure 2 (A-C) explains the mean performances of biochemical traits for all the 15 genotypes under Control (Irrigated on 110th day), T1 (15 days' water stress with irrigation on 125th day), and T2 (30 days' water stress with irrigation on 140th day).

Table 3
Mean performance of 15 sugarcane genotypes for physio-biochemical characters under control and drought stress conditions

Genotype	Catalase Activity (unit/g FW/min)			Proline content (μmoles/g FW)			Peroxidase enzyme activity (unit/g FW/min)		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
CoS9232	486.24	525.7	570.5	9.35	15.64	18.68	741.86	974.55	985.44
CoP9301	571.26	643.75	654.75	10.57	17.68	19.65	524.86	668.29	675.23
CoSE11453	606.24	721.5	750.3	7.55	15.94	22.99	688.29	855.65	865.34
CoS13231	551.78	632.21	677.44	7.09	15.78	21.75	751.29	921.3	938.22
CoPB092	532.29	623.01	655.22	8.9	16.28	20.33	711.33	826.36	834.46
CoJ64	508.83	670.99	698.33	9.9	15.54	23.54	721.57	845.66	854.33
CoJ85	497.84	538.95	544.24	8.28	16.65	18.64	844.29	955.71	965.74
Co8272	447.86	574.32	588.23	7.41	17.25	24.33	648.86	738	738
Co0238	527.24	635.46	650.44	9.26	25.12	29.11	803.29	856.29	856.29
Co98014	477.27	585.66	600.56	9.41	24.83	30.73	725.57	785	793.33
CoLK 14201	487.22	596.8	608.9	7.81	14.2	19.3	525	678.57	687.45
Co13236	520.22	650.11	675.11	9.87	15.8	20.8	788.43	922.14	933.15
Co0118	470.11	550.3	565.4	7.23	19.96	22.88	876.57	913.46	922.34
Co15023	567.88	670.11	684.11	9.28	14.84	21.78	554.57	698.29	710.35
CoS 13235	623.02	773.99	788.85	10.13	17.96	24.88	834.57	895.43	910.55
Grand Mean	7875.3	9392.86	647.492	8.80	17.56	22.62	716.02	835.64	844.68
Range Highest	623.02	773.99	788.85	10.57	25.12	30.73	876.57	974.55	985.44
Range Lowest	447.86	525.7	544.24	7.09	14.2	18.64	524.86	668.29	675.23
SD	110.92	140.96	152.033	1.03	4.36	4.97	107.51	102.32	102.25
CV	10.262	13.099	13.146	11.77%	25.00%	22.26%	15.13%	12.36%	12.16%
SE	28.618	36.465	39.329	0.27	1.12	1.28	27.75	26.4	26.37
CD	101.05	136.01	135.487	0.75	3.1	3.55	77.07	73.21	73.09
Control: Irrigated (110th day), T-I: treatment-I of 15 days water stress (125th day), T-II: treatment-II of 30 days water stress (140th day). The values in columns with different superscripts differ significantly ($p < 0.05$)									

3.4. Gene Expression Responses

Transcriptional analysis of stress-responsive genes—*LEA*, *DREB2A*, *DREB1A*, and *SOD*—revealed distinct drought-adaptive strategies in the two genotypes (Table 4). *LEA* expression decreased under drought in both genotypes. In CoS13235, fold expression reduced from control to 0.453 under T1 and 0.19 under T2, whereas in Co15023, it decreased to 0.366 under T1 and 0.189 under T2, indicating that *LEA*-mediated protection is not a decisive factor in differentiating drought tolerance in these genotypes. *DREB2A*, a key transcription factor regulating drought-responsive genes, was strongly upregulated under stress. CoS13235 exhibited fold changes of 1.972 under T1 and 4.626 under T2, whereas Co15023 showed higher induction, reaching 3.052 under T1 and 7.21 under T2. This pronounced transcriptional activation indicates engagement of stress-inducible pathways, with Co15023 showing stronger *DREB2A* activation despite being physiologically more susceptible. *DREB1A* expression displayed contrasting dynamics. In CoS13235, fold expression increased to 3.052 under T1 and slightly decreased to 1.972 under T2, reflecting a sustained adaptive response. In Co15023, *DREB1A* increased to 1.753 under T1 but drastically dropped to 0.167 under T2, suggesting an inability to maintain long-term transcriptional responses under severe drought. *SOD*, a key antioxidant enzyme, exhibited a dual-phase response. CoS13235 showed slight downregulation under T1 (0.453) followed by strong upregulation under T2 (2.948), indicating robust activation of antioxidant defense under severe stress. Co15023 displayed moderate increases from 1.753 under T1 to 1.972 under T2, suggesting less effective oxidative stress management. A heat map showing differential expression in leaf of *LEA*, *DREB2A*, *DREB1A*, and *SOD* genes in contrasting sugarcane cultivar CoS13235 (Tolerant) and Co15023 (Susceptible) is given in Fig. 3.

Table 4

Quantitative-Real Time PCR (qRT-PCR) expression analysis showing Cycle-Threshold (Ct) value and fold expression of LEA, DREB2A, DREB1A, and SOD genes in Sugarcane Cultivar Cos 13235 and Co 15023.

Gene	Genotype	Cos 13235			Co 15023		
	Treatment	Control	T1	T2	Control	T1	T2
LEA	Δ ct actin	12.54	12.62	11.65	14.46	13.62	14.35
	Δ ct sample	19.3	20.52	20.8	21.31	21.92	23.6
	$\Delta\Delta$ ct	0.00923	0.00419	0.00176	0.00867	0.00317	0.00164
	Fold Expression	1	0.453	0.19	1	0.366	0.189
DREB 2A	Δ ct actin	5.93	6.33	7.64	6.13	6.76	7.04
	Δ ct sample	18.21	17.63	17.71	17.01	16.03	15.07
	$\Delta\Delta$ ct	0.0002	0.0004	0.00093	0.00053	0.00162	0.00383
	Fold Expression	1	1.972	4.626	1	3.052	7.21
DREB 1A	Δ ct actin	6.61	6.76	6.33	6.13	7.02	10.95
	Δ ct sample	19.67	16.03	17.63	17.01	19.27	21.59
	$\Delta\Delta$ ct	0.00012	0.00162	0.0004	0.00053	0.00021	0.00054
	Fold Expression	1	3.052	1.972	1	1.753	0.167
SOD	Δ ct actin	8.76	12.62	15.34	6.13	1.753	6.33
	Δ ct sample	21.62	20.52	22.86	17.01	1.753	17.63
	$\Delta\Delta$ ct	0.00014	0.00419	0.00545	0.00053	1.753	0.0004
	Fold Expression	1	0.453	2.948	1	1.753	1.972

4. Discussion

Morphological evaluation of fifteen sugarcane genotypes under control and drought stress conditions revealed substantial variation in growth performance, highlighting inherent differences in drought adaptation strategies. Drought stress significantly reduced plant height, leaf area, and tiller number in all genotypes, with reductions being more pronounced under severe drought (T2). Leanasawat et al., (2021) had also studied the importance of several physiological and biochemical traits in response to early drought stress in sugarcane. CoS13235 maintained relatively better vegetative growth, with moderate reductions in plant height (118.67 cm to 99.11 cm), leaf area (200.64 cm² to 174.34 cm²), and tiller number (5.33 to 3.65) under severe drought (T2), indicating its capacity to preserve growth under water deficit. Ribeiro et al., (2013) had identified IACSP94-2094 and SP87-365 as drought-resistant genotypes, based on stalk dry matter production and yield of soluble solids under water deficit conditions. In contrast, Co15023 showed sharp reductions in all morphological traits, reflecting higher drought susceptibility (plant height: 124.00 cm to 97.00 cm; leaf area: 228.23 cm² to 154.33 cm²; tiller number: 6.33 to 2.32). These results are consistent with previous reports indicating genotypic variation in sugarcane under

drought stress (Kumar et al., 2023), emphasizing the importance of morphological traits as indicators of drought tolerance.

Physiological parameters, including relative water content (RWC), membrane stability index (MSI), and chlorophyll content (SPAD values), revealed contrasting adaptive responses between the two genotypes. CoS13235 maintained higher RWC (73.26% under T1; 65.22% under T2) and moderate membrane stability (MSI: 51.38% under T1; 45.23% under T2), reflecting efficient water retention and cellular homeostasis. Masoabi et al., (2023) had also reported that enhanced expression of cytokinin oxidase/dehydrogenase (CKX) gene in transgenic plants has delayed leaf senescence and maintained high leaf water content under water deficit stress. In our study, Co15023 exhibited higher absolute RWC under stress but experienced a sharper proportional decline (95.49% to 78.92%), accompanied by significant membrane damage (MSI: 41.86% to 25.77%), indicating weaker osmotic adjustment and greater cellular susceptibility. Chlorophyll retention was also better in CoS13235 (SPAD: 37.88 to 25.23) than in Co15023 (45.45 to 25.44), suggesting preservation of photosynthetic capacity under drought. Zhang et al., (2020) has reported that drought tolerance in Yunzhe 05–51 was because of enhanced antioxidant activities, increased osmolyte accumulation and increased rate of photosynthesis. These findings align with previous studies highlighting RWC, MSI, and chlorophyll content as critical indicators of drought tolerance in sugarcane and other crops (Varone et al., 2012; Yordanov et al., 2003; Kumar et al., 2023).

Biochemical assays demonstrated enhanced antioxidant defense and osmotic adjustment in CoS13235. *Catalase* (CAT) activity increased under drought stress (773.99 units/g FW/min under T1; 788.85 under T2), supporting effective H₂O₂ detoxification (Mittler, 2002; You et al., 2015). Cia et al. (2012) also reported that the drought-sensitive varieties have the highest lipid peroxidation and lowest leaf relative water content, increased hydrogen peroxide (H₂O₂), and proline contents during the progression of the drought-stress condition. Peroxidase (POX) activity (834.57 to 910.55 units/g FW/min), facilitating ROS scavenging under stress (Cia et al., 2012). Proline accumulation, a key osmoprotectant, was higher in CoS13235 (17.96 µmol/g FW under T1; 24.88 under T2) compared to Co15023 (14.84 and 21.78 µmol/g FW), indicating superior osmotic adjustment (Bates et al., 1973; Hare et al., 2003; Roychoudhury & Tripathi, 2020). Oliveira et al., (2018) had also reported that proline can be used as biochemical indicator of response to drought in sugarcane accessions. In Co15023, although proline levels increased, lower antioxidant enzyme activities suggest a limited capacity to mitigate oxidative stress, corroborating its higher drought susceptibility (Cia et al., 2012; Kumar et al., 2023).

Analysis of drought-responsive genes revealed distinct transcriptional adaptations. LEA expression decreased under stress in both genotypes, suggesting limited involvement in drought tolerance differentiation. Devi et al., (2019) had also performed a gene expression profiling of key candidate genes affecting drought stress and rehydration under drought stress in sugarcane. Kaura et al., (2023) had characterised the expression response of *LEA* proteins under several abiotic stresses and identified its differential expression in response of drought stress. *DREB2A*, a key regulator of stress-responsive pathways, was strongly upregulated in both genotypes, with Co15023 showing higher induction (7.21-fold under T2) despite physiological sensitivity. *DREB1A* expression in CoS13235 remained relatively stable (3.052 under T1; 1.972 under T2), indicating sustained transcriptional adaptation, whereas in Co15023, it sharply declined under severe stress (0.167), reflecting impaired long-term gene regulation. Chen et al., (2022) indicated that *ScDREB2B-1* confers drought tolerance in sugarcane by modulating the ABA Signal, ROS Level and Stress-Related Gene Expression in sugarcane. *SOD* expression, crucial for oxidative stress mitigation, was strongly upregulated under drought stress in CoS13235 under T2 (2.948), while Co15023 exhibited moderate increases (1.972), consistent with biochemical observations of antioxidant activity (Rodriguez et al., 2006; Rocha et al., 2007). These findings suggest that drought tolerance in sugarcane

involves a coordinated physiological, biochemical, and transcriptional response, with CoS13235 exhibiting a more balanced and effective adaptive strategy compared to Co15023.

The present study highlights the significant genotypic variation in sugarcane (*Saccharum* spp.) in response to water deficit, emphasizing the interplay between morphological, physiological, biochemical, and molecular mechanisms in conferring drought tolerance. Among the evaluated genotypes, CoS13235 demonstrated superior adaptive capacity under both moderate and severe drought conditions, maintaining relatively higher plant height, leaf area, and tiller number. Physiologically, it exhibited better water retention, membrane stability, and chlorophyll content, indicating efficient osmotic adjustment and sustained photosynthetic potential. Biochemically, CoS13235 showed enhanced antioxidant enzyme activities (*CAT* and *POX*) and greater proline accumulation, suggesting effective mitigation of oxidative stress and osmotic regulation. At the molecular level, coordinated expression of drought-responsive genes, particularly *DREB1A*, *DREB2A*, and *SOD*, further supported its stress resilience. In contrast, Co15023, while showing some induction of stress-responsive genes, experienced sharper declines in morphological and physiological traits, lower antioxidant defense, and impaired long-term transcriptional adaptation, reflecting higher drought susceptibility.

5. Conclusion

Collectively, these findings indicate that drought tolerance in sugarcane is a complex, multi-layered trait requiring integrated physiological, biochemical, and molecular responses. CoS13235 represents a promising candidate for breeding programs aimed at enhancing drought resilience, and its traits could serve as valuable markers for selection. This study reinforces the importance of combining morpho-physiological screening with molecular analyses to accurately identify and develop sugarcane genotypes capable of sustaining productivity under water-limited environments, a critical objective in the context of climate change and increasing demand for sugar and bioenergy crops.

Declarations

6. Funding

No funding was obtained for this research.

Author Contribution

Author Contributions Statement Krishanu: Conceptualized and performed the experiments, collected the data, and wrote the initial draft of the manuscript. Shailendra Pratap Singh: Assisted with data analysis and visualization. Nitish Ranjan Prakash: Performed data analysis, critical editing, and proofreading of the manuscript. Rakesh Singh Sengar: Conceptualized, designed, and supervised the research project, and critically revised and proofread the manuscript. All authors have read and approved the final version of the manuscript.

Data Availability

Included in Tables and Figures All data supporting the findings of this study are available within the article and its tables and figures files.

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Figures

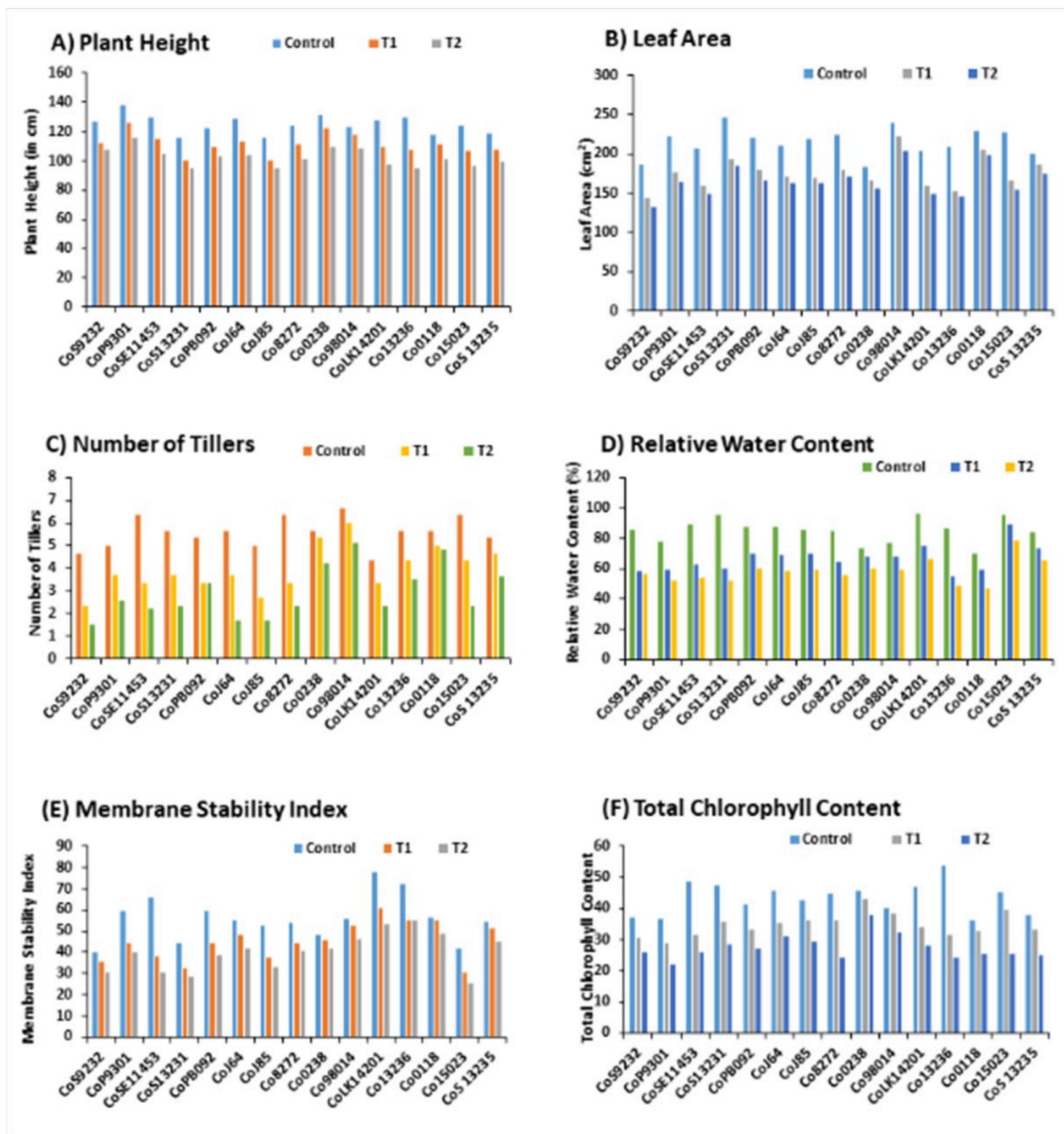


Figure 1

Change in morphological as well as physiological traits of all 15 genotypes evaluated under three different conditions (Control - Irrigated on 110th day; T1 - 15 days' water stress with irrigation on 125th day; T2 - 30 days' water stress irrigation on 140th day).

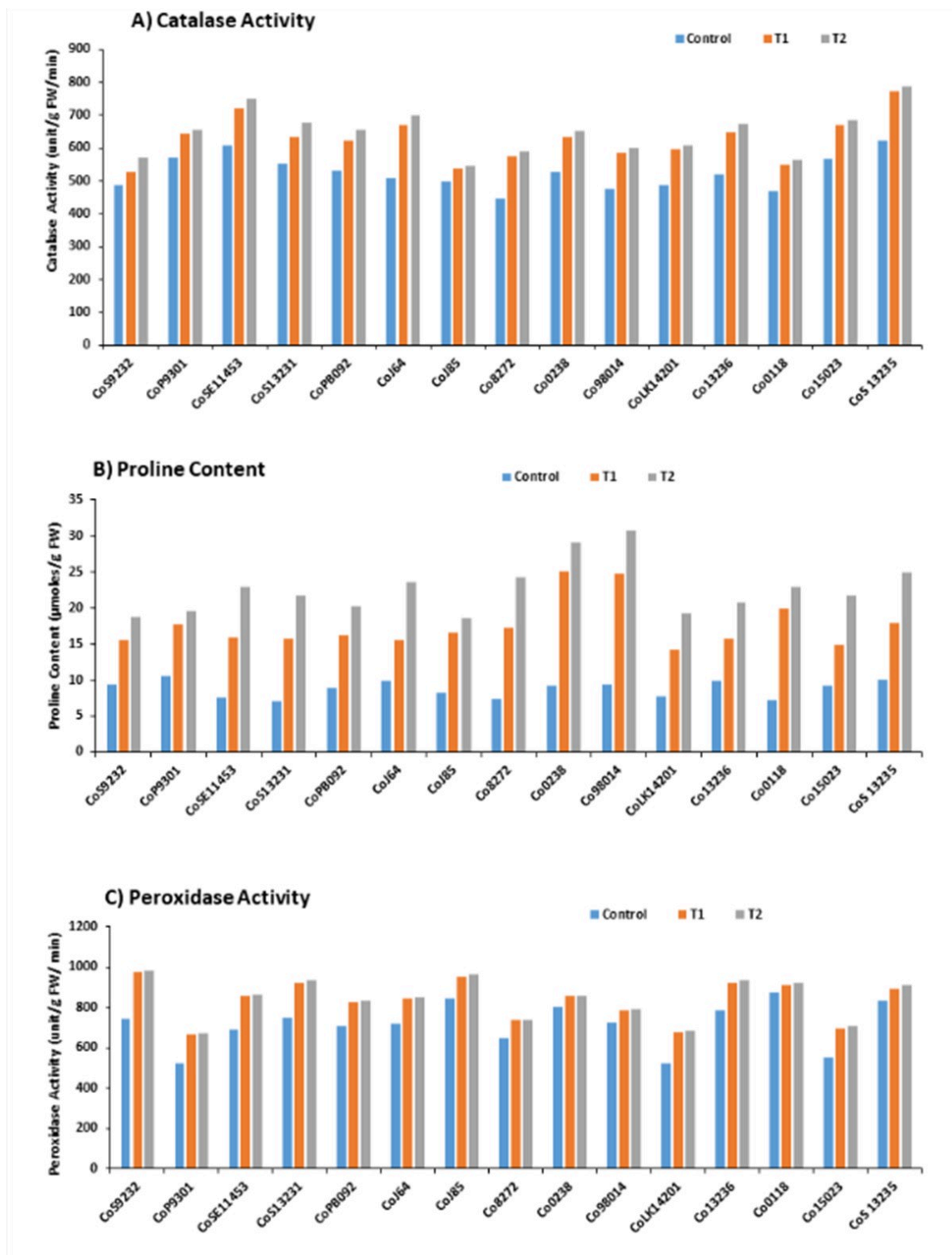


Figure 2

Change in biochemical traits of all 15 genotypes evaluated under three different conditions (Control - Irrigated on 110th day; T1 - 15 days' water stress with irrigation on 125th day; T2 - 30 days' water stress irrigation on 140th day).

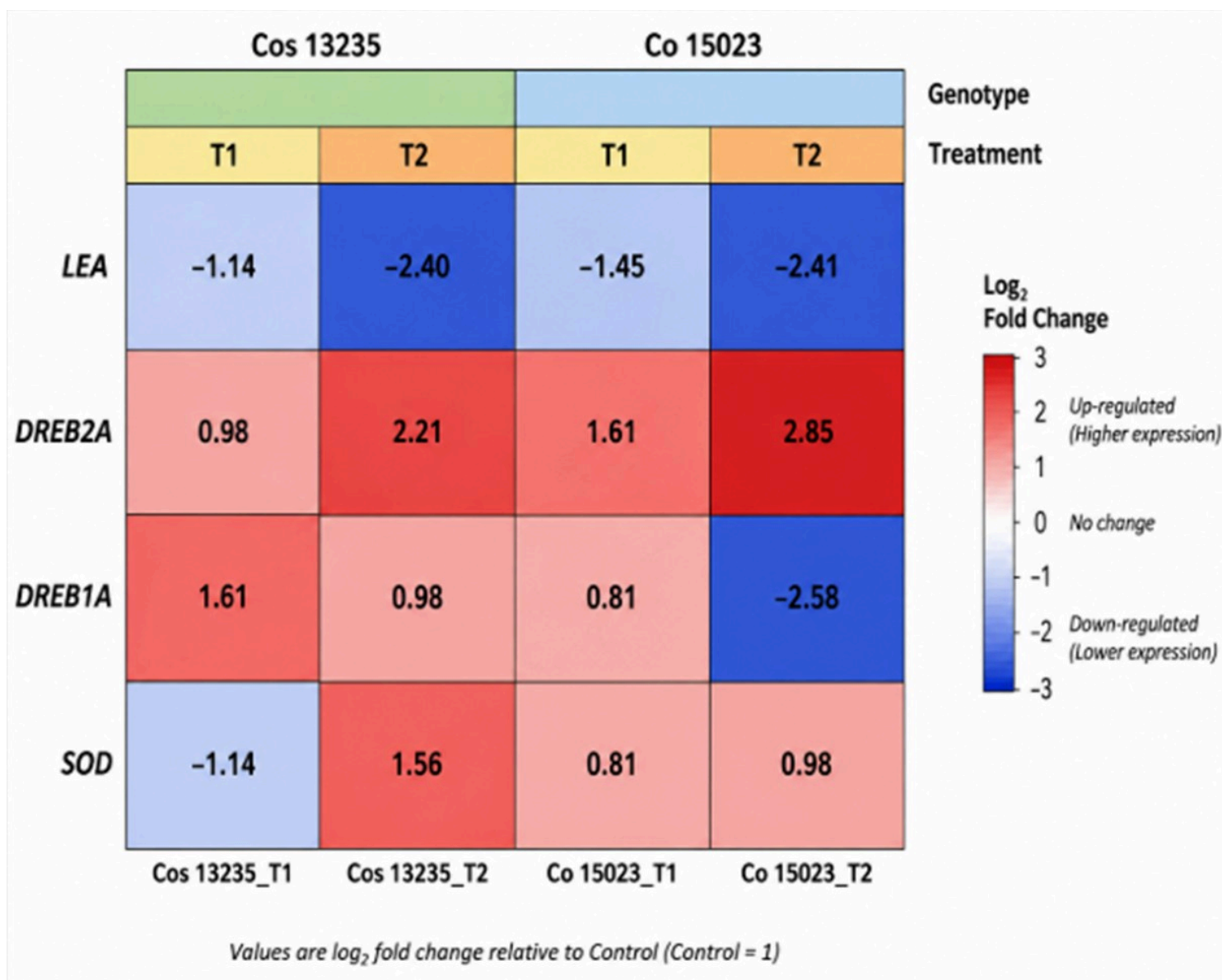


Figure 3

Change in expression of *LEA*, *DREB2A*, *DREB1A*, and *SOD* genes in leaves after drought stress in contrasting drought tolerant cultivars Cos13235 AND Co15023 expressed as log2 Fold change under three different conditions (Control - Irrigated on 110th day; T1 - 15 days' water stress with irrigation on 125th day; T2 - 30 days' water stress irrigation on 140th day).