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EVALUATION OF SUGARCANE GENOTYPES AND GENES EXPRESSION ASSOCIATED WITH DROUGHT TOLERANCE

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SUMMARY

Inadequate rainfall in the sugarcane-growing regions is a major constraint. This study evaluated 33 diverse sugarcane (*Saccharum* spp. complex) genotypes for drought tolerance to address this issue. Significant (< 0.05) variances across environments (normal, mild water stress, and rainfed) due to genotypes and environments along with higher heritability (> 60%) and genetic advance (> 20%) for CCS (t ha^{-1}) and related traits justified strong potential for genetic improvement. Novelty lies in the combined use of "Eberhart and Russell Regression" and "GGE biplot" analyses. The Regression's model better identified the adaptability of genotype(s), while the GGE biplot effectively characterized the environments for their discriminating power. Traits like number of millable canes (NMC), sucrose (Pol %), total soluble solids (TSS %), relative water content (RWC), membrane stability index (MSI), proline, and superoxide dismutase (SOD), emerged as the key yield contributor, highlighting their utility as selection indices. Significant higher expression of 10 drought-responsive genes (P5CS, SOD, DEH, BADH, IGS, cAPX, LEA, TPS, ProT, and DRP) in F 391/14 (CoPb 19182) and lower expression of four genes (SOD, DRP, ProT, and BADH) in CoJ 64 provided molecular insights into stress tolerance. These findings offer valuable strategies for breeding resilient cultivars.

Keywords: Sugarcane (*Saccharum* spp. complex), drought tolerance, genotype × environment interactions, stability and adaptability

Key findings: Sugarcane (*Saccharum* spp. complex) clone F 391/14 (CoPb 19182) exhibited tolerance under drought conditions.

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INTRODUCTION

Sugarcane (*Saccharum* spp. complex) is an industrial crop for sugar and bioenergy. It grows well in more than 120 countries of tropical and subtropical regions on both sides of the equator (up to 30° N and 35° S latitudes). About 75% of the world's sugar production comes from sugarcane (Singh *et al.*, 2022). However, sugarcane production still faces challenges from biotic and abiotic stresses. Among them, drought is the single most significant environmental stress that restricts the production of sugarcane worldwide. Seventy percent of the cane yield, as well as the productivity of sugarcane, gets reduced by the prolonged water deficit stress during its lifespan (Ferreira *et al*., 2017).

Various morphological, physiological, and biochemical studies have established particular characteristics associated with the adaptability of plants to drought-prone conditions (Tripathi *et al.*, 2019). Changes in relative water content, cell membrane permeability, osmotic regulators, and soluble protein content are some mechanisms connected with a plant's adaptations keeping plant cells in a state of homeostasis. The role of compatible osmolytes (Kumar *et al*., 2001), superoxide dismutase (SOD) (Santos and Silva, 2015), proline (Verbruggen and Hermans, 2008), and ROS-scavenging enzyme activities (Ferreira *et al.*, 2017) has reached well documentation in the context of drought tolerance in sugarcane.

Classically, a plant breeder performs multi-environment trials (METs) and analyzes the data with different statistical methods (Eberhart and Russell, 1966; Singh and Bhajan, 2016) pertaining to genotype-by-environment (G×E) interactions. This facilitates genotype recommendation in particular environments. Currently, the uses of molecular methods with understanding the plant tolerance mechanisms have been the effective tools in detecting the kind of gene or genes involved in response to stresses in plants. Gene discoveries and genomic tools help in speeding up the genetic improvement program of plants like sugarcane (Tripathi *et al*., 2019). Despite significant progress, a major gap exists in integrating multi-environment field performance with molecular insights for drought tolerance in sugarcane. Earlier studies largely focused on physiological responses or on G×E analyses in isolation, leaving a comprehensive study. This study evaluated diverse genotypes across environments to identify tolerant clones, responsive traits, and key molecular mechanisms, which could be beneficial for developing resilient cultivars.

MATERIALS AND METHODS

Experiment details

The experimental site (31.38° N, 75.38° E, 225 masl) comes under a semiarid (dry) climate zone with an average annual rainfall of 400 mm, where it receives up to 80% monsoon rain from July to September. The recorded weather data on different parameters (AICRPS-PI Report, 2019) came from the field observatory (located 200 meters from the experimental site) of the Punjab Agricultural University, Regional Research Station, Faridkot, for the years 2019–2020. Since conducting the experiments fell under different irrigation conditions, the differences in rainfall were the major concern.

Two experiments, i.e., field evaluation of sugarcane genotypes during 2019–2020 and expression analyses of gene(s) linked with drought tolerance during 2020–2021, succeeded completion in two years. The field evaluation had three different field trials conducted in the first week of March in the spring of 2019–2020 in a randomized complete block design with three replications. One was under a normal irrigated environment (ENV1), the second under a mild water stress environment (ENV2), and the third under a rainfed environment (ENV3). With this, 33 diverse sugarcane clones (Table 1) underwent evaluation by repetition of providing two different levels of drought environments. In ensuring proper germination and establishment of all testing clones under all environments, all three of these trials received normal irrigation

conditions up to the germination phase—up to 60 DAP (days after planting). After the germination phase, the ENV2 environment trial was completely without irrigation and rainwater during the formative phase, i.e., 60 to 150 DAP, with rainout shelter on all over three replications. Meanwhile, the ENV3 environment trial completely had no irrigation water, remaining rainfed. The plot size of each genotype was two rows of 6 m length with 90-cm apart row-to-row spacing.

Data recorded

Data on germination % (Gm %) at 45 DAP, the number of tillers (000/ha) at 120 DAP, the number of shoots at 210 and 240 DAP (000/ha), and the number of millable canes (NMC, 000/ha) at 300 DAP came from field conditions. Data recording on cane yield (CY, t ha^{-1}), cane length (CL, cm), cane girth (CG, cm), single cane weight (SCW, kg), juice extraction % (Extn %), Brix/total soluble solids (TSS %), sucrose (Pol %), purity %, commercial cane sugar % (CCS %), and commercial cane sugar tons per hectare (CCS t ha^{-1}) occurred at crop harvest (300 DAP). Agro-morphological traits data recording was on a plot basis before converting into hectares. The cane juice quality trait's data comprised TSS %, Pol %, purity %, and CCS %, including cane yield attributes—cane length, cane girth, and single cane weight. These data came from 10 randomly selected competitive millable canes from each plot of each replication. Using the "Biquartz Sodium Lamp Polarimeter" for cane juice analyses followed standard protocols (Meade and Chen, 1977).

Relative water content (RWC) (Barrs and Weatherley, 1962), membrane stability index (MSI) (Sairam and Srivastava, 2002), proline content (Bates *et al.*, 1973), and SOD activities (Marklund and Marklund, 1974) entailed estimation from fresh and clean first fully opened (+1) leaves collected from each replication of each genotype. The measurement of conductivity employed the Wireless Conductivity Meter SE-238, Scientech-India.

Gene expression analyses through RT-PCR

The observation of F 391/14 (CoPb 19182) as a drought-tolerant clone and CoJ 64 as a susceptible cultivar (Table 1, Figure 1) succeeded in 2019–2020. For validating this, both genotypes underwent testing again in drought as well as well-irrigated conditions in 2020–2021, with the genotypes from well-irrigated conditions taken as the control. RNA extraction proceeded from the fully opened fresh leaves (+1) by the BT-TRIZOL method. In the Light Cycler System (Roche Life Sciences, Mannheim, Germany), SYBR® Premix Ex TaqTMII, Takara, performing the qPCR reaction continued as per the manufacturer's instructions. The 25-sRNA gene of *S. officinarum* served as a housekeeping gene for the normalization of data. Relative expression calculation of the target gene used the formula by Livak and Schmittgen (2001). Details of the sequence of gene-specific primers appear in Table 2.

Statistical analyses

Following the PROC MIXED procedure for statistical analyses had a 0.05 probability threshold level. The research treated genotypes as a fixed effect, while the replication nested within the year served as a random effect. Separation of means ensued using the LSD test (*P* 0.05). The calculation of coefficients of variation, heritability, and genetic advance followed the suggestion by Burton and DeVane (1953) and Johnson *et al.* (1955). Pearson's correlation coefficient concerning traits, considering the environment, entailed separate calculation. The application of Eberhart and Russell's regression coefficient and GGE biplot analyses continued for cane yield (t ha^{-1}), CCS %, and CCS (t ha^{-1}). All statistical analyses performed employed the R statistical software (R Core Team, 2021).

Table 1. List of primers used for gene expression analyses by RT-PCR in sugarcane.

No.	Name of gene	Gene's details	Forward Primer (5'-3')	Reverse Primer (5'-3')
1	25S rRNA	25S rRNA	GCAGCCAAGCGTTCATAGC	CCTATTGGTGGGTGAACAATCC
2	Pox	Proline oxidase	CGAGCGTGTGCATCAAGATC	GTCTTCCATGGCAGGTTGAAC
3	ProT	Proline transporter	TCCCACTGACGTTTGTGCTC	AACCCAACAACATTCAGCCAG
4	DEH	Dehydrin	ACCAGTACGGCAATCCAGTTG	CGGAGCGATGCAGGATG
5	BADH	Betaine aldehyde dehydrogenase	GCTGCATGGGACATGGATG	CCATTGGAAGAGAAACTGGTGAG
6	IGS	Indole-3-glycerol-phosphate synthase	CAGCGTTTTGACAGACCAGA	CCAACAAGCTCGATTCCTTC
7	SOD	Superoxide dismutase	ACCACCTGTTCCACCACAAG	GCCTCCTTGTGGTCCTTCTT
8	cAPX	Cytosolic ascorbate peroxidase	CCAACCGTGAGCGAAGATT	TAAGCATCAGCAAACCCAAG
9	DREB	Dehydration responsive element binding proteins	CCCGACGTACTCCTCAGTCC	CTTCTCGTCCTGGACTCCCAT
10	LEA	Late embryogenesis abundant	GCTTAGGATCAATGGCTTCCCACC	CCAAAGGGAAATCATTCACGGCGTC
11	TPS	Trehalose 6-phosphate synthase	GCACATGTCACAACTCACA	ACAGCTGCATTTGAGATCG
12	DRP	Drought-responsive protein 1	AGAAGAAATGTTGTGTCTGTGA	CGAGCTTGTACTCTGTCTTG
13	P5CS	Pyrroline-5-carboxylase synthetase	CCTGATGCCTTGGTCCAGA	TGCAATACTGTGTTTGATCTCATGG

Table 2. Selection parameters for agro-morphological and cane juice quality traits in sugarcane under normal (ENV1), mild water stress (ENV2), and rainfed (ENV3) environments.

Selection parameters		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^{-1})	CCS (t ha^{-1})
GCV%	ENV1	13.63	17.43	22.81	26.27	32.12	15.52	9.86	16.74	5.34	9.02	10.07	2.31	10.66	20.44	18.49
	ENV2	15.77	25.2	26.3	27.68	42.46	20.51	11.02	18.67	8.56	7.75	8.52	2.02	9.05	46.33	43.44
	ENV3	14.24	23.74	32.24	37.23	46.83	20.75	8.41	23.22	8.88	4.75	5.37	1.4	5.81	61.77	61.76
PCV%	ENV1	18.66	18.79	23.69	27.79	33.28	17.75	11.98	18.71	6.07	9.3	10.32	2.5	10.92	24.64	23.14
	ENV2	19.79	26.56	27.39	29.22	43.55	23.01	12.85	22.74	9.32	7.98	8.81	2.82	9.52	48.9	45.99
	ENV3	19.20	25.10	33.75	38.53	48.95	24.42	13.17	26.19	9.91	5.14	5.77	2.24	6.38	65.89	65.87
h^2	ENV1	53.00	86.00	93.00	89.00	93.00	76.00	68.00	80.00	77.00	94.00	95.00	85.00	95.00	69.00	64.00
	ENV2	64.00	90.00	92.00	90.00	95.00	80.00	74.00	67.00	84.00	94.00	94.00	51.00	90.00	90.00	89.00
	ENV3	55.00	90.00	91.00	93.00	92.00	72.00	41.00	79.00	80.00	85.00	87.00	39.00	83.00	88.00	88.00
GA (%)	ENV1	20.52	33.32	45.22	51.15	63.84	27.93	16.73	30.84	9.67	18.00	20.25	4.39	21.45	34.94	30.42
	ENV2	25.90	49.25	52.03	54.00	85.27	37.68	19.47	31.57	16.20	15.49	16.97	2.98	17.73	90.44	84.50
	ENV3	21.76	46.26	63.46	74.09	92.28	36.31	11.07	42.40	16.39	9.05	10.30	1.81	10.88	119.29	119.31
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*#

Env: Environment, Gm %: Germination %, Tillers: Number of tillers, Shoots@: Number of shoots at 210 days after planting (DAP), Shoots: Number of shoots at 240 DAP, NMC: Number of millable canes, CL: Cane length, CG: Cane girth, Ext %: Cane juice extraction %, CCS %: Commercial cane sugar, GCV %: Genetic coefficient of variations, PCV %: Phenotypic coefficient of variations, h^2: Broad sense heritability, GA %: Genetic advance, and CCS: Commercial cane sugar.

*Significant mean square value due to environments, genotypes and environments × genotypes at 5% level; #Significant mean square value due to Replication (environment × Replication) at 5% level; and $Significant mean square value due to genotypes at 5 % level.

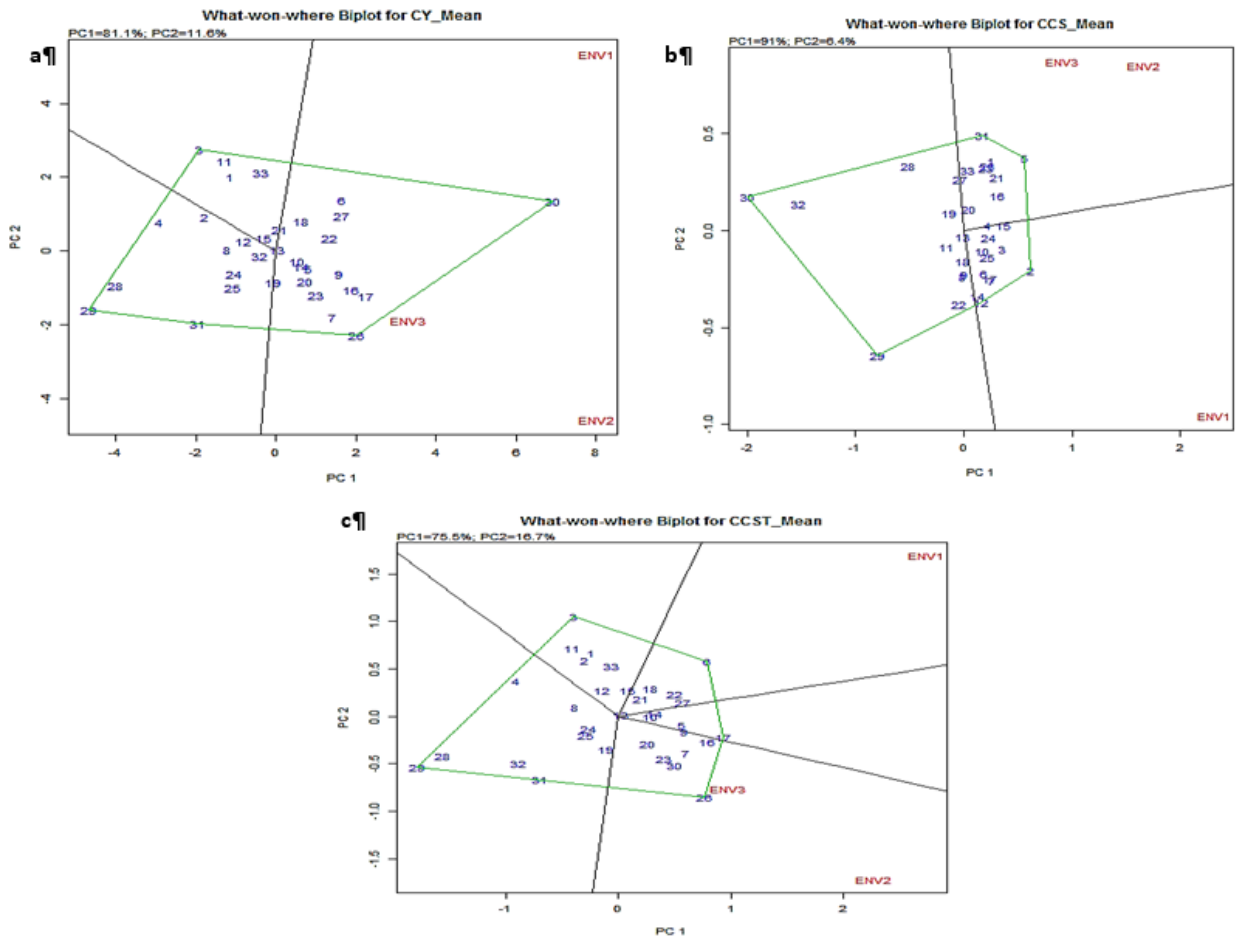

Figure 1. The "What-won-where" view of the GGE biplot for (a) cane yield (t/ha), (b) CCS %, and (c) CCS (t/ha) in sugarcane.

RESULTS AND DISCUSSION

Variances and selection parameters

Genetic variability, heritability, and genetic advance are the valuable parameters for crop improvement. A population with a higher degree of variance offers opportunities for selection to generate the desirable genotype(s) (Singh *et al.*, 2022). With pooled variance analyses (Tables 3 and 4), significant differences were notable due to Gen and ENV for all traits. This explained the existence of a high level of diversity. Remarkable differences were also evident due to the ENV×Gen interactions for all traits, except germination (%). Higher ranges of trait-specific value in context to their mean value appeared for all traits, except germination (%) along with higher percent deviations under ENV2 and ENV3 over ENV1 environment (Tables 3 and 4). The percent reduction in the formation of NMC from shoots at 240 DAP was higher in ENV3 (67.30%) than in ENV2 (30.32%) and ENV1 (22.58%). This represented the significant impact of drought on the growth, development, and sugar accumulation of cane, as also observed by Ferreira *et al*. (2017).

Medium to higher magnitudes of coefficient of variations (PCV and GCV, Burton and DeVane, 1953) for agro-morphological traits including CCS (t ha^{-1}) were noteworthy under all three environments, while the number of tillers, cane length, and cane yield showed higher values under stressed environments, comparatively (Table 3). This

Table 3. Selection parameters for physiological and biochemical traits in sugarcane under normal (ENV1), mild water stress (ENV2), and rainfed (ENV3) environments.

Selection parameters		MSI (120 DAP)	MSI (1500 DAP)	RWC % (2400 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)
GCV%	ENV1	3.93	3.43	5.67	6.57	3.41	6.61	7.29	12.39	12.21
	ENV2	7.17	2.92	6.24	5.79	4.01	12.81	12.54	7.93	7.83
	ENV3	6.46	4.42	6.54	6.96	4.68	8.15	8.28	12.87	15.42
PCV%	ENV1	4.02	3.55	6.2	6.99	4.25	7.31	8.32	12.73	12.67
	ENV2	8.07	3.68	7.57	7.02	5.08	13.29	13.10	8.81	9.48
	ENV3	7.40	5.88	7.73	8.81	6.55	9.23	10.49	13.37	15.95
h^2	ENV1	96.00	94.00	83.00	88.00	64.00	82.00	77.00	95.00	93.00
	ENV2	79.00	63.00	68.00	68.00	62.00	93.00	92.00	81.00	68.00
	ENV3	76.00	57.00	72.00	62.00	51.00	78.00	62.00	93.00	93.00
GA (%)	ENV1	7.92	6.85	10.66	12.71	5.64	12.32	13.16	24.82	24.23
	ENV2	13.11	4.76	10.60	9.84	6.51	25.46	24.73	14.71	13.30
	ENV3	11.63	6.85	11.40	11.31	6.89	14.83	13.48	25.52	30.70
Mean		65.17*	69.20*	65.25*	67.32*	76.14*	12.31*#	8.24*	19.99*	18.86*

Env: Environment, MSI: Membrane stability index, RWC: Relative water content, SOD: Superoxide dismutase, GCV %: Genetic coefficient of variations, PCV %: Phenotypic coefficient of variations, h^2: Broad sense heritability, GA %: Genetic advance, and DAP: Days after planting.

*Significant mean square value due to environments, genotypes, and environments × genotypes at 5% level; #Significant mean square value due to Replication (environment × Replication) at 5% level.

represented the sensitivity of traits to the stress. For SCW (kg), a higher magnitude of PCV appeared in both stress environments, while GCV values were higher under ENV3 only. For cane juice quality traits, physiological traits, and biochemical traits, low to medium values of GCV and PCV emerged (Tables 3 and 4).

Recording high heritability and a higher range of GA (Johnson *et al.*, 1955) for traits like NMC, the number of shoots, cane yield, CCS (%), CCS (t ha^{-1}), Brix (%), and Pol (%) occurred (Tables 3 and 4). Singh *et al.* (2022) also mentioned that substantial improvement could happen by giving emphasis to the selection of these traits. High heritability coupled with high genetic advance for the number of tillers (000/ha), number of shoots, NMC, cane yield (tha^{-1}), and CCS (tha^{-1}) under ENV2 and ENV3 revealed the positive impact of direct selection if based on these traits under drought. Direct selection could not be an effective option for the traits like RWC (%), MSI, proline, and SOD at 150 and 240 days, and for some quality traits because of having low to moderate values of h^2 and GA (Tables 2 and 3).

Changes in physiological and biochemical parameters

Noting alterations in physiological and biochemical parameters continued to authenticate the impact of drought stress on sugarcane genotypes (Table 4). Plants use the physio-biochemical mechanisms as a defense system to protect themselves against water stress (Cha-Um and Kirdmanee, 2009). Under the ENV2 environment, the leaf RWC sustained a decrease of 8.68% and 6.21% at 150 and 240 DAP, respectively, as compared with the control. However, under the ENV3 environment, RWC declined by 17.92% and 13.38% at 150 and 240 DAP, respectively, in comparison with the control. The MSI always tends to be an important measurement of cell membrane injuries (Cha-Um and Kirdmanee, 2009). Under the ENV2 environment, the decrease in MSI was 17.96% and 6.380% at 150 and 240 DAP, respectively. Meanwhile, under the ENV3 environment, MSI reduction reached 20.13% and 16.40% at 150 and 240 DAP, respectively.

Table 4. Regression coefficient, deviation from regression, and mean value of cane yield (t ha^{-1}), CCS % and CCS t ha^{-1} over the environments.

S#	Genotype	Cane yield (t ha^{-1})			CCS % (Commercial Cane Sugar %)			CCS t ha^{-1} (Commercial Cane Sugar t ha^{-1})		
		Mean	βi	s²di	Mean	βi	s²di	Mean	βi	s²di
1	Co 0238	37.04	1.21	51.42	11.00	0.80	-0.02	4.32	1.22	0.30
2	Co 0118	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	0.88*
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 deviating from unity; $^{@}$, $^{\#}$ and $^{\$}$ Average, High, and Low responsive genotypes to water availability, respectively, with high mean value for Cane Yield, CCS %, and CCS t ha^{-1}; βi Regression Coefficient, m General Mean for concerned traits, Mean square Deviation from Linear Regression, ☺Named as CoPb 19182.

Proline contents of the genotypes under drought were remarkably higher than from the control (Munawarti *et al.*, 2014). Notably, under the ENV2 environment, proline content was 68.18% and 15.81% higher than in the control (ENV1) at 150 and 240 DAP. As for the rainfed (ENV3), proline content was 74.52% and 67.06% higher than in the control (ENV1) at 150 and 240 DAP, respectively. Osmotic substances inside the cells gain accumulation due to drought stress, and these accumulated osmotic substances play a key role in the plant's tolerance mechanism (Munawarti *et al.*, 2014). Under the mild water stress (ENV2), increases of the SOD were 41.67% and 27.27% at 150 and 240 DAP, respectively, compared with the control (ENV1). Meanwhile, under the rainfed (ENV3), SOD enhancement reached 50.00% and 36.36% at 150 and 240 DAP, respectively, versus the control environment (ENV1).

Correlation and regression coefficients among the traits

Cane yield expressed a positive and significant correlation ($p < 0.05$, $r > 0.48$) with the number of tillers and shoots and NMC; however, it was a negative correlation ($p < 0.05$, $r > 0.04$) with juice quality traits across the environments (Figure 2). Kumar *et al.* (2001) and Begum *et al.* (2012) also observed linear associations of cane yield with the number of tillers and shoots, NMC, and SCW. CCS% gave a negative correlation with all major agro-morphological traits, except cane girth, SCW, and all cane juice quality traits. Meanwhile, the CCS (t ha^{-1}) expressed a positive correlation with all the traits under all environments (Figure 2). In both drought environments (ENV2 and ENV3), cane yield had a significant positive association with MSI (at 150 DAP, $p < 0.05$, $r > 0.37$), SOD (at 150 DAP, $p < 0.05$, $r > 0.47$ and at 240 DAP, $p < 0.05$, $r > 0.37$), and proline (at 150 DAP, $p < 0.05$, $r > 0.50$ and at 240 DAP, $p < 0.05$, $r > 0.43$) (Figure 2). In the presented study, NMC emerged as the main cane yield-contributing trait with $R^2 > 0.63$, while the role of SCW was unclear, especially under the ENV1 (Figure 2). Singh *et al.* (2022) also reported that the main quality traits deciding CCS% were Brix %, pol %, and purity % because all showed major contributions with the R^2 value of >0.81%, >0.98%, and >0.39%, respectively. Here in this study, cane yield (t ha^{-1}) appeared as a more pronounced trait, comparatively, playing a major role ($R^2 > 0.58$) in determining the CCS (t ha^{-1}) as compared with CCS% ($R^2 > 0.01$).

Eberhart and Russell's regression coefficient analysis

The observed significant ($p \leq 0.05$) higher value of the "environmental (linear) effect" than the "G × E (linear) effect" revealed the role of the "environmental (linear) effect" for the adaptation of sugarcane genotypes concerning cane juice quality and cane yield (Singh *et al.*, 2022). Pooled deviation values differed substantially. Eleven sugarcane genotypes out of 33 manifested stability as they deviated non-significantly from zero (~0) for cane yield along with high per se mean performance (>45.66 t ha^{-1}, Table 1). Among these genotypes, four average-responsive (βi~1) genotypes (CoPb 18181, CoPb 13181, CoPb 1418, and CoPb 15212), four low-responsive (βi<1) genotypes (CoJ 88, CoPb 92, CoPb 94, and F 391/14 [CoPb 19182]), and two high-responsive (βi>1) genotypes (F301/11, F660/14) reached distinction to water deficit stress.

The CCS% comprised three average-responsive (βi~1) genotypes, i.e., CoJ88, CoPb18181, and CoPb15214. Moreover, it gave 11 highly responsive (βi>1) genotypes, viz., Co0118, CoJ64, CoPb91, CoPb92, CoPb13181, CoPb14181, CoPb14185, CoPb16181, CoPb18182, F 6/14, and F 362/14. Additionally, four genotypes—F 391/14 (CoPb 19182), F3/14, Co98014, and Co0238—were reportedly low responsive with high per se stable performance (>10.49 CCS%, ~0) (Table 1). For the trait of CCS t ha^{-1} (Table 1), five average-responsive (βi~1) genotypes included CoJ88, CoPb 92, CoPb 94, CoPb13181, and CoPb18181. The six high-responsive (βi>1) genotypes were CoPb91, CoPb14185, CoPb18182, CoPb15212, F404/13, and F660/14, while one low-responsive genotype, F391/14 (CoPb 19182), prevailed.

GGE biplot analysis

Based on the mean performance across replications, all three environments grouped into two mega-environments (Yan *et al.*, 2000)—one with the ENV1 (normal) and another with the ENV2 and ENV3 (Figures 2a, 2b, and 2c). In the 'what-won-where' view of mega-environments' analysis (Yan *et al*., 2000), the higher-yielding winning genotypes were AS 04-1687 (30) and F 391/14 (26) for cane yield (t ha^{-1}); Co 0118 (2), CoJ 88 (5), and SA 04-409 (31) for CCS (%); and CoPb 91 (6), CoPb 18182 (17), and F 391/14 (26) for the trait of CCS (t ha^{-1}) than others. The corner genotypes (most responsive to soil water

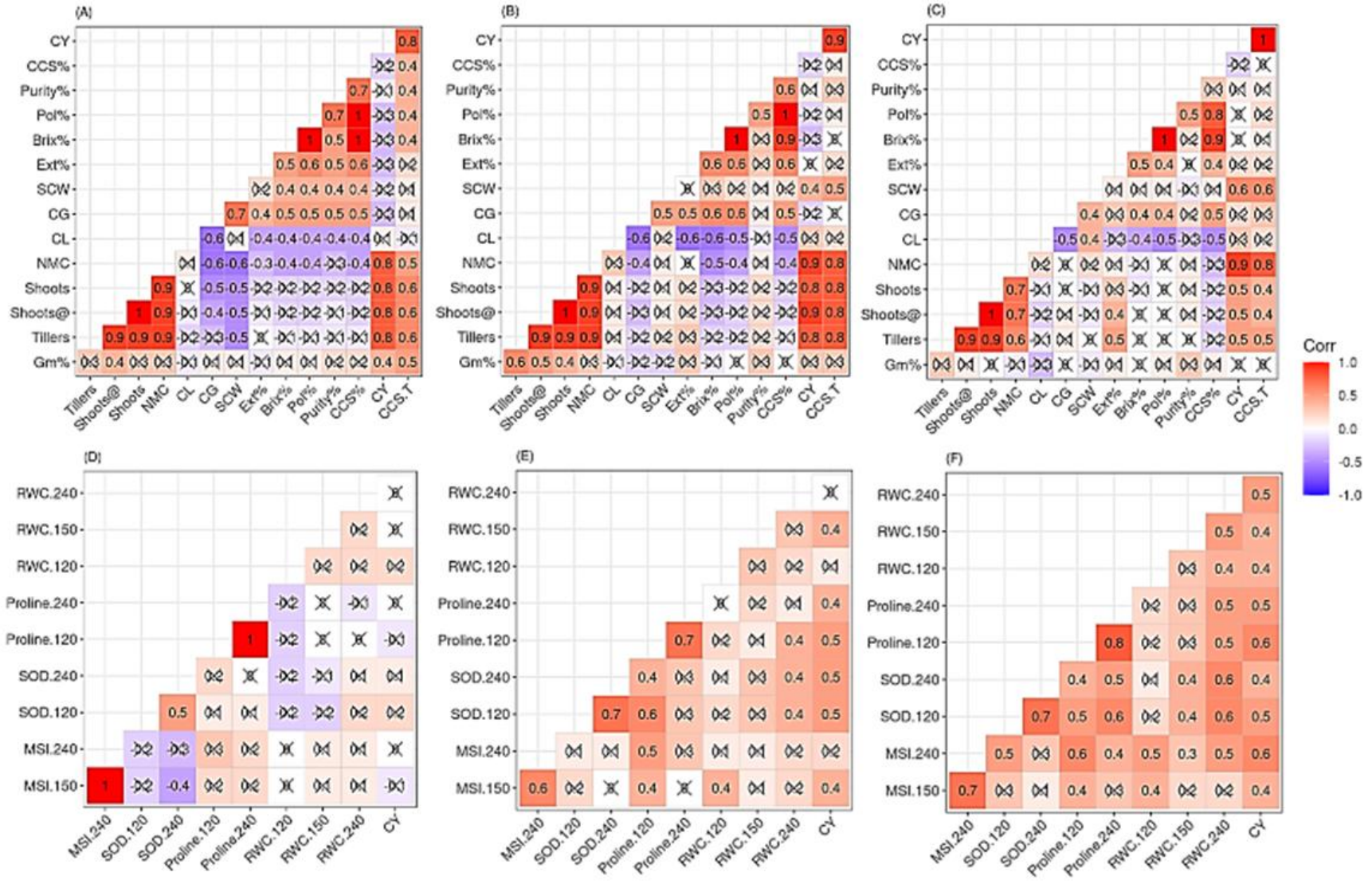

Figure 2. Correlation coefficients among different quantitative and qualitative traits (A–C) and physiological and biochemical traits (D–F) with cane yield (t ha^{-1}) under normal (A and D), mild water stressed (B and E), and rainfed (C and F) environments within the set of 33 sugarcane genotypes.

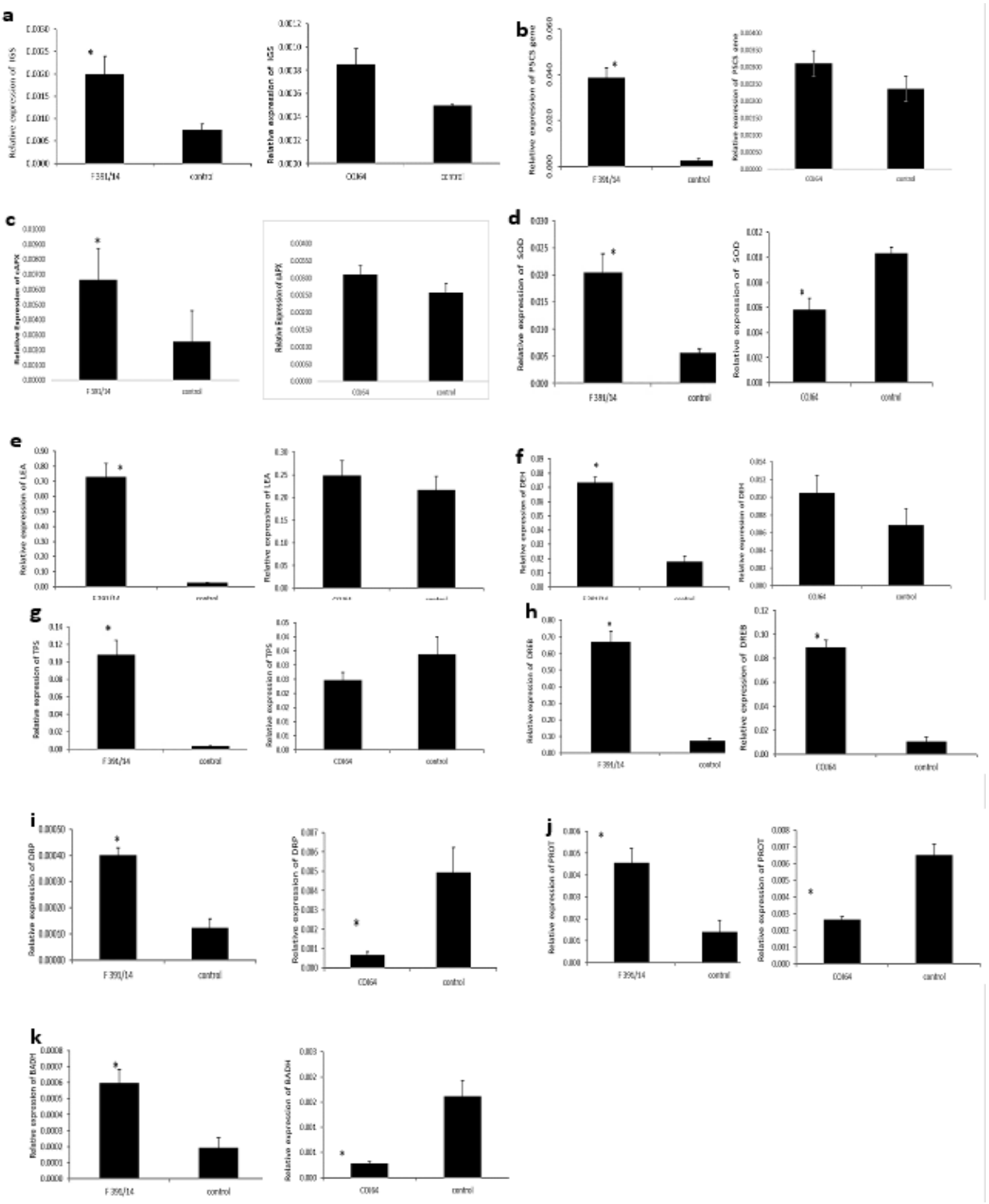

Figure 3. Relative expression of (a) *IGS* gene, (b) *P5CS* gene, (c) *cAPX* gene, (d) *SOD* gene, (e) *LEA* gene, (f) *DEH* gene, (g) *TPS* gene, (h) *DREB* gene, (i) *DRP* gene, (j) *PROT* gene, and (k) *BADH* gene in cultivar F 391/14 and CoJ 64 subjected to drought in comparison to the control. Here, the control is a well-irrigated plant. The error bars represent the standard deviation (n = 3), and * represents significant differences of the expression of genes in F 391/14 compared with the control ($P \leq 0.05$, Student's t-test).

availability, Singh *et al.*, 2022) can be visually evident as most favorable and lowest yielding, respectively (Figure 1). Here, the GGE represents the (G+ [G × E]), while 'AEC abscissa' approximates the genotype's contributions to the 'G × E.' It is the measure of their stability/instability (Singh *et al.*, 2022). Hence, AS 04-1687(30) for cane yield, F 391/14 (26) for CCS (t ha^{-1}), and Co 0118 (2) for CCS% emerged as ideal cultivars (Yan *et al.*, 2000; Singh *et al.*, 2022). Here, stress environments, i.e., ENV2 and ENV3, for cane yield, CCS%, and CCS t ha^{-1} were noticeable to be more representative of mega-environments with a high discriminating power of genotypes. This is due to these environments having long vectors and small angles with the AEC abscissa, making them useful for selecting resilient superior genotypes under drought conditions.

Expression of target genes in response to water deficit stress

Differential expression analyses of 11 candidate genes among 13 (Table 2, Figure 3) revealed that up-regulation of the genes under drought plays their positive role in adapting the genotype against stress. Expression patterns disclosed a high and significant expression of *IGS* (Figure 3a), *P5CS* (Figure 3b), *cAPX* (Figure 3c), *SOD* (Figure 3d), *LEA* (Figure 3e), *DEH* (Figure 3f), *TPS* (Figure 3g), *DRP* (Figure 3i), *ProT* (Figure 3j), and *BADH* (Figure 3k) genes in the F391/14 clone. In contrast, the susceptible cultivar CoJ 64 showed non-significant high expression of *IGS, P5CS, cAPX, LEA*, and *DEH*. Furthermore, significant low expressions of genes, i.e., *SOD*, *DRP*, *ProT*, and *BADH*, were evident in CoJ 64, but the expression of the *TPS* gene was non-significantly low. Interestingly, expression of the DREB (Figure 3h) was remarkably high in both genotypes, indicating its broader role in drought stress response. Among the identified genes, each may have an exclusive role in enhancing drought tolerance (Rentsch *et al.,* 1996; Ferreira *et al.*, 2017; Tripathi *et al*., 2019). They are highly expressed under drought conditions. In the presented study, maximum proline content attained accumulation in clone F 391/14. Similarly, the expression of the *P5CS* gene was significantly high in this clone under drought. This combined physiological and molecular evidence reinforces the superior drought tolerance of clone F 391/14 (CoPb 19182) compared with CoJ 64.

CONCLUSIONS

The promising study focused on identifying suitable genotypes and traits contributing to stable performance over different levels of drought. High genetic advance coupled with high heritability succeeded in expressing the traits of tillers (000/ha), shoots (000/ha), SCW (kg), NMC (000/ha), and CCS (t ha^{-1}). Yield and CCS displayed a positive association with SOD and proline under mild water stress as well as rainfed conditions. The identification of the most vulnerable CoJ 64 and highly tolerant F 391/14 (CoPb 19182) genotypes under droughts was successful. The expression of drought-responsive genes (*P5cs, SOD, DEH, BADH, IGS, cAPX, LEA, TPS, ProT,* and *DRP*) was significantly higher in the F 391/14 (CoPb 19182) clone than in their respective controls. These findings can be beneficial in cultivar selection to develop resilient, high-yielding sugarcane clones.

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