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EARLY AND RAPID SCREENING OF SUGARCANE CLONES FOR DROUGHT STRESS TOLERANCE USING HYDROPONICS

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ABSTRACT:
Background: Drought is a major abiotic stress severely affecting sugarcane production in India, causing yield reductions of up to 49%. To address the lack of rapid screening methods at an early stage, present study evaluated 40 sugarcane genotypes for drought stress tolerance using a hydroponic platform. The seedlings were subjected to control and drought conditions. The drought stress treatment was induced by alternate wetting and drying for 30 days. Systematic measurements of morphological and physiological parameters such as root volume, relative water content (RWC), and canopy temperature were performed. Hydroponic screening revealed a very high genetic diversity among the genotypes. In the presence of water scarcity, plants have shown a drought prevention strategy by maintaining root length and volume, while reducing shoot biomass significantly to reduce transpirational water loss. Physiologically, drought stress decreased RWC and SPAD values while increasing canopy temperature. Genetic analyses revealed minimal variations in phenotypic and genotypic coefficients of variation, as well as strong broad-sense heritability (more than 85%) and significant genetic advance for stress-related characteristics such as shoot length and fresh root weight, indicating additive gene action. Correlation study revealed a substantial positive association between RWC and root volume, confirming that non-destructive measurements such as canopy temperature are accurate markers of hidden root vigor. The genotypes Co 14005 and Co 12005 were found to be drought-tolerant due to their better RWC and root length, whereas Co 775, Co 06002 showed high vulnerability. The study also demonstrates that hydroponic phenotyping is a rapid and effective method for identifying drought-tolerant sugarcane clones, making it a valuable tool for early-generation selection in breeding programs.

KEYWORDS: Sugarcane, Drought Tolerance, Hydroponic Screening, Root Phenotyping, Genetic Variability.

INTRODUCTION

Sugarcane (Saccharam spp.) is considered the most economically cultivated crop in both tropical and subtropical regions of India. It plays a significant role in the country with the crop occupying around 5.0 million hectares, corresponding to roughly 2.57% of the nation's gross cropped area (Solomon and Swapna, 2022). It is a major driver of the economy, employing approximately 500,000 workers in sugar mills alone, ranking second only to the cotton sector. Further, sugarcane significantly contributes to the national GDP, accounting for 1.1 %, while its contribution to the agricultural GDP is 10 % (Dinesh Babu et al., 2022).

Despite its immense agricultural and economic significance, sugarcane production in India is continuously affected by several biotech and abiotech stresses. Among the abiotic stresses, drought is the most important abiotic stress affecting the crop across major producing states like Tamil Nadu, Maharashtra, Andhra Pradesh, Bihar, Haryana, Rajasthan, and Uttar Pradesh (Kumar et al., 2023). As a high-water-demanding crop with a long-life cycle, drought conditions severely affect its growth, especially during the highly sensitive tillering and grand growth phases (Singh et al., 2013). Multi-locational studies have shown that moisture stress during these phases causes significant reductions in overall cane yield—by up to 49 %—through the negative impact on attributing traits such as stalk length, single cane weight, and the number of millable canes (Mahadevaiah et al., 2021). Further, drought tolerance evaluation across different climates has confirmed that the genotype by environment (G × E) interaction plays a major role in determining the yield stability of sugarcane (Mahadevaiah et al., 2021).

One of the major challenges in phenotyping a large number of sugarcane clones for drought tolerance is the absence of an efficient phenotyping system that emphasizes root traits, which are critical for accurately assessing the drought tolerance of sugarcane genotypes under controlled conditions (Shoaib et al., 2022). The root is the first plant organ to sense drought and respond by morphological, anatomical and genetic changes that explore and conserve resources at minimal metabolic cost, making the assessment of root traits a key part of the drought tolerance screening (Shoaib et al., 2022). Root length, root biomass, root density are the key drought-adaptive structural traits that are easy to phenotype and applied to screening genotypes and provide rapid results for the identification of drought tolerance germplasm (Tang et al., 2024). A common response to drought stress in crop is adaptation, including changes in root systems to increase water and nutrient uptake from deeper soil layers, and plants have developed mechanisms for drought tolerance by increasing the root length and root biomass that can access water by penetrating deeper soil layers (Shoaib et al., 2022). Hydroponic based phenotyping offers accurate control of the root environment, reduced labour and almost no damage to phenotyping compared to field phenotyping and making it a preferred platform for assessing the plasticity of root systems under drought stress in a variety of crop genotypes (Rajaseger, 2023). Studies have shown that hydroponic systems have a higher maximum root length and root-to-shoot ratio than field conditions, which confirm that hydroponics provide a highly sensitive and discriminating environment for the detection of genotypic differences in the response of root to water stress (Anandan et al., 2021). Several methods, including hydroponic phenotyping, have been suggested for the characterization of root traits in crops, as root structure traits, including root length and root biomass at the early seedling stages have been shown to be informative for the differentiation between drought tolerant and drought sensitive genotypes (Tang et al., 2024). At present, sugarcane lacks rapid early-stage screening methodologies to screen large number of genotypes for drought. In present study, efforts were made to screen the sugarcane genotypes for drought using hydroponic-based evaluation.

MATERIALS AND METHODS

Plant Materials

The 40 genotypes viz., ISH 100, Co 14011, Co 11015, Co 12005, Co 06022, Co 13034, Co 12029, Co 09022, Co 11012, Co 98017, Co 15027, Co 10024, Co 09004, Co 98008, Co 86032, Co 18024, Co 15023, Co 18009, Co 112, CYM 08 922, Co 2000-2, CoM 0265, Co 14005, Co 94005, Co 05001, Co 06015, Co 92002, Co 06002, Co 90003, Co 14034, Co 07010, Co 05007, Co 8368, Co 775, Co 95020, CYMA 101690, Co 92020, Co 10017, Co 99004, and Co 93009 were were evaluated under hydroponic condition. The study was conducted at ICAR-Sugarcane Breeding Institute, Coimbatore, during 2025-26 cropping season.

Hydroponic Screening for Drought Tolerance

Forty sugarcane genotypes were evaluated for drought tolerance under hydroponic conditions using alternative wetting and drying methods (Figure 1). Healthy seedlings of each genotype were raised from single buds in protrays for 25 days to ensure uniform growth before transferring to hydroponic conditions containing half MS nutrient solution. The seedlings were allowed to adjust to hydroponic conditions for a week.

After a week, the seedlings were subjected to two treatments, a control treatment and a drought stress treatment. In the control treatment, the seedlings were continuously maintained in half MS nutrient solution throughout the experiment. The nutrient media is changed for every two days to ensure healthy roots and supply of nutrients. In the drought treatment, an alternative wetting and drying method was imposed, where the seedlings were taken out daily, and the root system was allowed to dry for three hours in the morning and three hours in the evening. After each drying period, the seedlings were returned to nutrient solution. Three replications were maintained for each genotype and maintained for 30 days.

At the end of the stress period, various morphological and physiological traits related to drought tolerance were recorded. The morphological parameters included shoot length (cm), root length (cm), shoot weight (g), fresh shoot(g) and root weight (g), dry shoot (g) and root weight (g), and volume of the root (ml). The average root length was measured from tip of the root to crown of the root, while the root volume was measured by water displacement method, using a measuring cylinder wherein the cleaned roots were placed in a measuring cylinder containing water and the rise in the level of water was recorded. Root volume was calculated as the difference between the final and initial water volumes and expressed in milliliters (mL) (Swamy et al. 2019). Physiological parameters such as relative water content (RWC), Chlorophyll content, and canopy temperature were measured during the peak period of stress. The recorded data were used to evaluate the response of sugarcane genotypes to drought stress to identify drought tolerant genotypes.

Figure. 1. Hydroponic experimental setup of both Control and Drought (a) Measurement of shoot length (left) and root length (right) in sugarcane genotypes (b)

RESULTS AND DISCUSSION

Hydroponics Screening for Drought Tolerance

The hydroponic screening of the 40 sugarcane genotypes indicated substantial morphological variation across treatments (Table 1,2), under control conditions, shoot length exhibited a general mean (GM) of 68.92 cm (minimum 39.50 cm and maximum 105 cm). Under drought conditions, the shoot length general mean (GM) was 69.08cm (minimum 40.5cm and maximum 102.33cm). Root length exhibited notable adaptability, with the GM decreasing only marginally from 23.99cm (minimum 18 cm and maximum 24.67 cm) under control conditions to 23.35cm (minimum 35 cm and maximum 21.67 cm) under drought conditions. Similarly, the fresh shoot weight general mean (GM) decreased from 14.93 g (minimum 5 g and maximum 22 g) in the control conditions to 11.86 g (minimum 3.5 g and maximum 18.67 g) under drought conditions. The fresh root weight GM also saw a reduction from 32.62 g (minimum 26.5 g and maximum 32.67 g) in the control conditions to 29.97 g (minimum 12 g and maximum 37.33 g) under drought conditions. This relative retention of root biomass and length, along with a notable drop in aerial biomass, indicates a fundamental drought-avoidance strategy in which plants prioritize photosynthetic allocation to root development in order to search for depleted moisture while limiting transpirational surface area. Recent studies validate that sugarcane cultivars exhibiting minimal root growth penalty under hydroponic drought screening translate into highly resilient field crops capable of avoiding severe dehydration (Kaur et al. 2026; Mohanraj et al. 2021). Genotypes displaying this architectural plasticity are critical for maintaining yield under prolonged water deficits (Zahid et al. 2025).

Table 1. Analysis of variance for hydroponic root phenotyping traits under control conditions (CRD, 40 genotypes, 3 replications)

Source of variation	Treatments (df = 39)	Error (df = 80)	CD	CV	Min	Max	GM	GV	PV	EV	GCV	PCV	ECV	H^2	GA	GAM
Shoot Length (cm)	709.51**	94.13	15.79	14.08	39.50	105.00	68.92	307.70	401.80	94.13	25.45	29.08	14.08	76.57	31.62	45.88
Root Length (cm)	86.40**	16.73	6.66	17.05	18.00	24.67	23.99	34.80	51.60	16.73	24.60	29.93	17.05	67.55	9.99	41.65
Fresh shoot Weight. (g)	229.49**	57.24	12.32	50.66	5.00	22.00	14.93	86.10	143.40	57.24	62.15	80.18	50.66	60.07	14.82	99.22
Dry Shoot Weight (g)	3.77**	0.22	0.76	18.78	1.00	4.33	2.48	1.80	2.00	0.22	53.73	56.92	18.79	89.10	2.59	104.49
Fresh Root weight	769.20**	161.78	20.71	38.99	26.50	32.67	32.62	303.70	465.50	161.78	53.42	66.13	38.99	65.24	29.00	88.88

(g)																
Dry Root Weight (g)	6.24**	0.32	0.91	13.12	3.00	5.67	4.28	3.00	3.30	0.32	40.25	42.34	13.13	90.38	3.37	78.83
Root Volume	220.43**	20.00	7.28	14.58	25.00	36.67	30.67	100.20	120.20	20.00	32.64	35.75	14.58	83.36	18.83	61.40
RWC	189.59ns	133.57	-	12.62	31.00	30.43	93.95	28.00	161.60	133.57	5.63	13.52	12.30	17.33	4.54	4.83
SPAD value	120.28**	22.11	7.65	12.04	35.77	34.43	39.06	49.10	71.20	22.11	17.94	21.60	12.04	68.95	11.98	30.68
Canopy Temperature	9.55ns	8.68	-	9.52	91.30	95.10	30.95	0.40	9.10	8.68	2.14	9.76	9.52	4.79	0.30	0.96

** significant at ≤ 0.001alpha; ns- Non significant

Note:-RWC: Relative water content; SPAD value: Soil Plant Analysis Development meter value (SPAD Chlorophyll meter readings were used as a non-destructive indicator of relative leaf chlorophyll content and chlorophyll retention under drought stress).

Table 2. Analysis of variance for hydroponic root phenotyping traits under Drought conditions (CRD, 40 genotypes, 3 replications)

Source of variation	MSS Treatments (df = 39)	Error (df = 80)	CD	CV	Min	Max	GM	GV	PV	EV	GCV	PCV	ECV	H^2	GA	GAM
Shoot Length (cm)	621.26**	40.88	10.41	9.26	40.50	102.33	69.08	290.20	331.10	40.88	24.66	26.34	9.26	87.65	32.85	47.56
Root Length (cm)	94.54**	14.66	6.23	16.39	35.00	21.67	23.35	39.90	54.60	14.66	27.06	31.64	16.39	73.15	11.14	47.68
Fresh shoot Weight (g)	89.94**	5.35	3.77	19.51	3.50	18.67	11.86	42.30	47.60	5.35	54.84	58.21	19.51	88.76	12.62	106.44
Dry Shoot Weight (g)	4.32**	0.20	0.72	18.04	1.00	3.67	2.47	2.10	2.30	0.20	58.21	60.94	18.04	91.24	2.83	114.53
Fresh Root Weight (g)	163.28**	12.63	5.79	11.86	12.00	37.33	29.97	75.30	88.00	12.63	28.96	31.30	11.86	85.64	16.55	55.21
Dry Root Weight (g)	5.28**	0.34	0.95	13.59	1.00	5.33	4.27	2.50	2.80	0.34	36.77	39.21	13.60	87.97	3.04	71.04
Root Volume	171.66**	15.31	6.37	13.51	15.00	30.00	28.96	78.20	93.50	15.31	30.53	33.39	13.51	83.62	16.66	57.51
RWC	84.07**	14.15	7.63	4.25	35.26	36.47	86.23	35	49.10	14.15	6.85	8.12	4.36	71.18	10.27	11.91
SPAD value	73.64**	9.63	5.05	9.18	27.47	37.73	33.81	32.00	41.60	9.63	16.73	19.09	9.18	76.87	10.22	30.22
Canopy Temperature	5.32**	0.19	0.70	1.25	65.55	91.10	34.62	2.60	2.80	0.19	4.63	4.8	1.25	93.20	3.18	9.20

** significant at ≤ 0.001alpha; ns- Non significant

Note:-RWC: Relative water content; SPAD value: Soil Plant Analysis Development meter value (SPAD Chlorophyll meter readings were used as a non-destructive indicator of relative leaf chlorophyll content and chlorophyll retention under drought stress).

Physiological Response to Drought Stress

Physiological parameters showed stark contrasts between the two environments; for example, the general mean for SPAD (Chlorophyll content) decreased from 39.06 to 33.80 under stress, and the relative water content decreased from 91.59% to 88.59% (Figure 2). On the contrary, the average canopy temperature increased from a mean of 30.94ºC in optimal conditions to 34.61º in drought conditions (Figure 3). This physiological disturbance causes rapid stomatal closure, which stops the flow of air, which in turn increases the temperature of the leaf surface. In addition, the parallel decrease in RWC and SPAD indicates a degraded cell surface and photosynthetic apparatus degradation under water stress conditions. The maintenance of high RWC and cooler canopy temperature under stress are reliable physiological markers for the selection of drought-tolerant sugarcane, as these characteristics directly determine the continued assimilation of carbon and the stability of the membrane in the event of severe stress events (Dhansu et al. 2022; Khera et al. 1986).

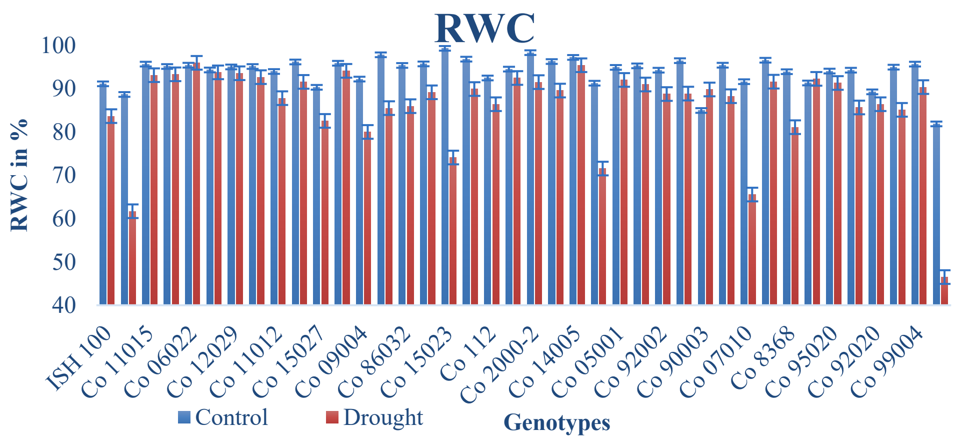


Figure. 2. Comparison of RWC under control and drought conditions

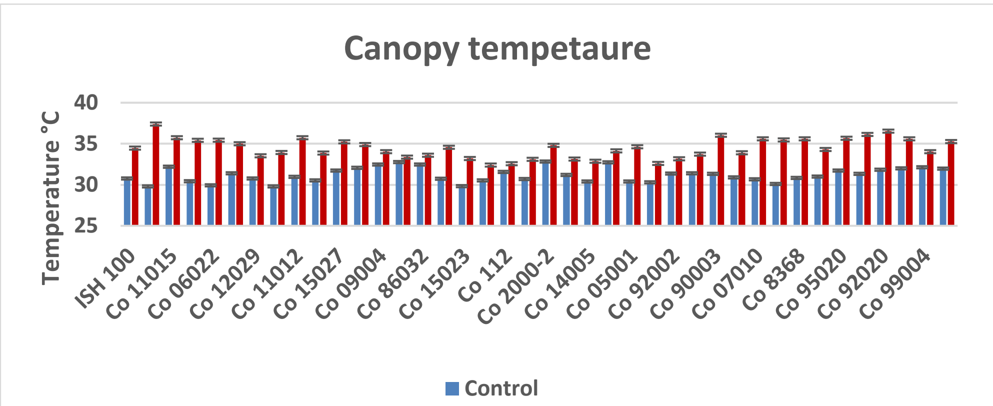


Figure 3. Canopy Temperature readings of plants under control and drought stress conditions

Analysis of variance

The Analysis of Variance (ANOVA) for the Completely Randomized Design (CRD) with 40 genotypes revealed highly significant differences ($p < 0.01$) among the genotypes in all the morphological and physiological traits observed under both conditions except for RCW and Canopy temperature under normal condition (Table 1,2). The highly significant differences confirm the existence of a high genetic diversity between the genotypes assessed, which is a basic requirement for any successful breeding programme aimed at selecting better stress-resistant clones (Liu et al. 2016). The hydroponic experimental setup effectively allowed for the expression and quantification of this genetic variation without the confounding variables typical of field soil. The results clearly show the variation for RWC and Canopy temperature among genotypes only in stress condition and not in control, which validate the hydroponic screening can create an artificial discriminating environment for drought screening in the early stages of the crop.

Genetic Variability (GCV & PCV)

Estimates of genetic variability revealed that the phenotypic variation coefficient (PCV) was consistently, albeit slightly, higher than the Genotypic Coefficient of Variation (GCV) for all traits in both screening assays. For example, shoot length recorded a PCV of 29.08% and a GCV of 25.45% under control conditions, which, in drought conditions, decreased slightly to 26.34% and 24.66%, respectively (Table 1,2). The small difference between PCV and GCV for these characteristics suggests that although environmental factors are necessarily involved, phenotypic expression is mainly driven by genetic factors. The close grouping of GCV and PCV values ultimately highlights the effectiveness of the controlled hydroponics method in reducing noise and in demonstrating that true genetic variation is present and that early phenotypic selection is highly reliable (Meena et al. 2023; Charumathi et al. 2024).

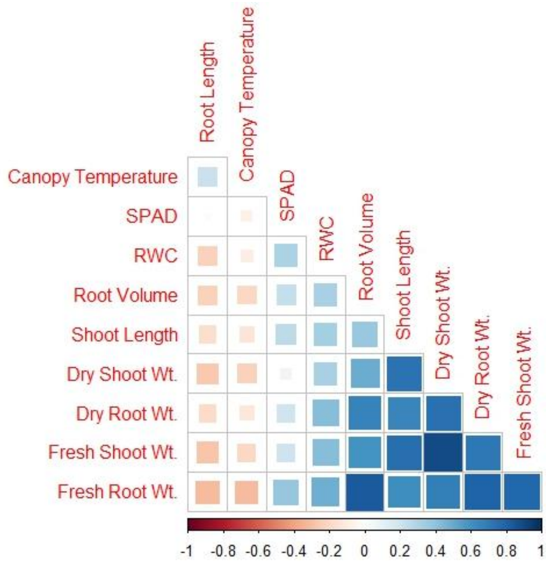
Heritability, Genetic advance and GAM

Broad-sense heritability estimates were extremely high throughout the studied characteristics, with shoot length having a heritability of 76.57% under control conditions and increasing significantly to 87.65% under drought stress. Similarly, fresh root weight heritability increased significantly, from 65.24% in the optimum treatment to 85.63% in the drought treatment (Table 1,2). High heritability (more than 70%) indicates that the observed phenotypes are accurate representations of the underlying genes, with low environmental masking. The fact that heritability for these key morphological traits increased under drought stress suggests that restrictive conditions promote the expression of genuine genetic differences, implying that direct selection based on raw phenotypic performance will consistently yield rapid genetic gains in crop improvement cycles (Zhao et al. 2017; Basnayake et al. 2012).

In addition to substantial heritability, the key morphological features showed significant Genetic Advance as a percentage of Mean (GAM). Under the control condition, fresh shoot weight had a GAM of 99.22%, which scaled to 106.43% under drought stress, whereas dry shoot weight GAM increased dramatically from 104.49% to 114.53% (Table 1,2). High heritability combined with high GAM is a classic quantitative genetics indicator that features are mostly governed by additive gene action, enabling early-generation selection both lucrative and genetically stable across contexts. These findings confirm that simple recurrent selection can effectively improve root architecture and shoot vigor in drought-prone environments, as opposed to targeting traits with low GAM, which are frequently heavily influenced by unpredictable non-additive genetic variance (Kumar et al. 2023a; Charumathi et al. 2025).

Correlation Analysis under Drought Condition

The correlation analysis (Fig. 4.) revealed different structural relationships driving the physiological stress response, including strong positive correlations between root volume, fresh biomass, and Relative water Content. In contrast, canopy temperature showed consistent and significant negative associations with all primary yield-contributing and physiological characteristics, including SPAD, RWC, and total biomass (Figure 4). The strong positive correlation between extensive root systems and RWC confirms that genotypes capable of foraging effectively can maintain superior correlation with canopy temperature, suggesting that cooler leaves are an excellent proxy for active transpiration and healthy metabolism. Similar behavior is observed in other crop grass like rice (Soumya et al. 2023) As a result, non-destructive, high-throughput techniques like canopy thermography and SPAD metrics can reliably be utilized as surrogate traits to quickly screen for concealed root vigor and overall drought resistance in large breeding populations (Kim et al. 2021; Wirojsirasak et al. 2024).

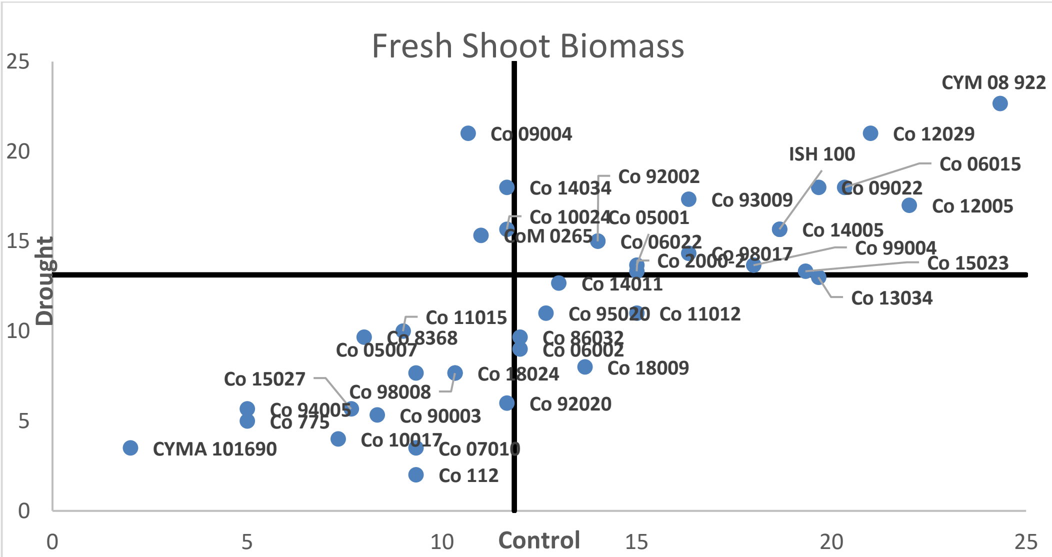


Blue and red colors represent positive and negative correlations, respectively, with color intensity indicating the strength of the correlation coefficient (r)

Figure 4. Pearson's correlation heatmap illustrating th traits under drought stress

Identification of drought tolerant clones

The relationship between control and drought conditions was visualized via a quadrant-segmented scatter plot (Figure 5). The genotypes ISH 100, Co 14005, Co 12005, Co 06015, Co 06022, Co 99004, Co 98017 CYM 08 922 were grouped in the elite group (top right) which indicated them as winning genotypes under both normal and stress conditions. ISH 100, 99004, Co 98017 and Co 06015 were known drought tolerant genotypes which were identified by several studies as drought tolerant (Hemaprabha et al. 2013; Devi et al. 2018; Devi et al. 2019; Mall et al. 2022; Mahadevaiah et al. 2021). Similarly, the genotypes in left bottom represents the poor performers, the genotypes viz., Co 775, Co 06002, Co 10017, Co 94005, C 93003 CYMA 10690 were found poor in the study. The clones Co 775 and Co 06002 were used as sensitive checks in most of the drought experiment (Simon and Hemaprabha 2010). The grouping pattern also indicates the hydroponic screening can be used to eliminate many sensitive genotypes in the early stage of selection.



The genotypes ISH 100, Co 99004, Co 06022 well known drought tolerant genotypes in the top right quadrant and Co 775 well known sensitive check in the left bottom quadrant

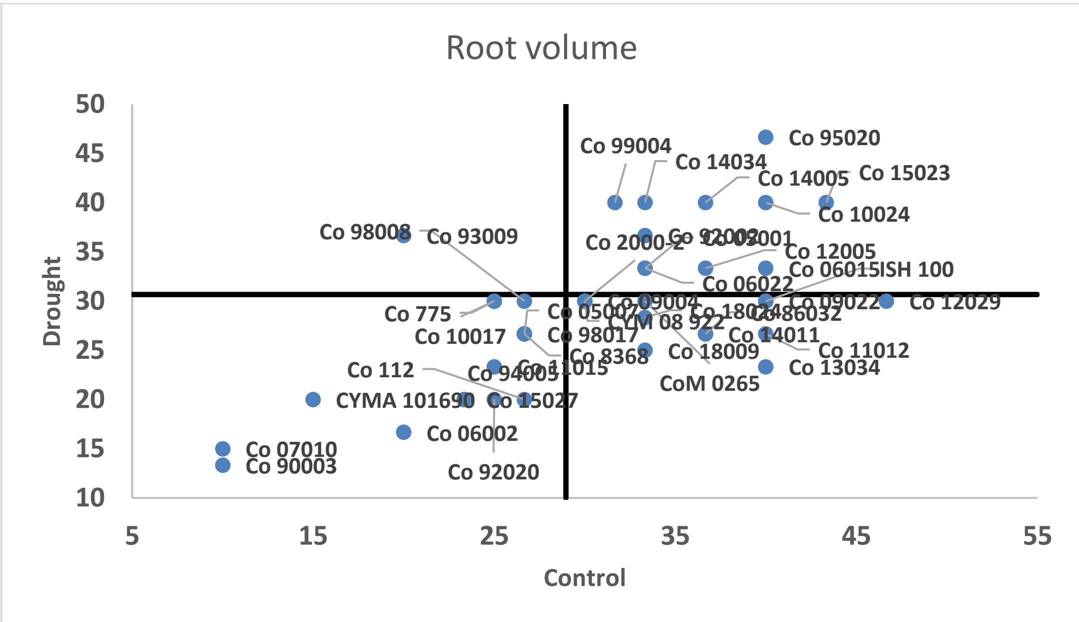


Fig 5. Quadrant scatter plot illustrating the drought tolerance performance of sugarcane genotypes. a. Fresh root biomass b. Root volume

The genotypes ISH 100, Co 99004, Co 06022 well known drought tolerant genotypes in the top right quadrant and Co 775 and 06002 well known sensitive check in the left bottom quadrant

CONCLUSION

The current work successfully demonstrates the efficacy of hydroponic screening as a fast and reliable method for assessing drought tolerance in sugarcane genotypes. Under ideal and water-deficit situations, all 40 genotypes exhibited highly substantial genetic diversity for both morphological and physiological parameters. The application of drought stress demonstrated that adaptive resistance is predominantly driven by root length and volume preservation, combined with reduced shoot biomass to reduce transpirational water loss. Crucially, drought tolerant and sensitive genotypes were identified by analysing their root architecture and physiological stability under stress. Tolerant genotypes capable of maintained high relative content (RWC), chlorophyll (SPAD), and root length. for example, Co 14005 maintained outstanding drought tolerance alongside Co 12005 maintained a root length of under drought stress. On the other hand, sensitive genotypes, such as Co 07010, Co 94005, and Co 775 struggled to manage water status, experiencing reduced RWC, lower SPAD, and higher canopy temperature. Notably, Co 07010 dropped RWC with a low SPAD. Genetic analysis revealed modest differences between PCV and GVC, as well as significant broad-sense heritability and substantial GAM for drought-related variables like as shoot length and fresh root weight. This suggests additive gene activity, making drought-adaptive characteristics especially susceptible to early-generation selection. Furthermore, correlation analysis revealed that non-destructive physiological metrics- specifically, the positive connection between RWC and root volume and the negative correlation with canopy temperature-are very accurate markers of hidden root vitality. The study identified many relevant information for identifying the drought tolerance traits and drought tolerant genotypes and the methodology followed in the present hypophonic study will definitely help the researchers to screen large number genotypes rapidly for drought tolerance.

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Statements and Declarations
Conflict of Interest: The authors declare that they have no competing interests

Author Contributions Statement
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