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Evolution, identification, evaluation, and characterization of a stable salinity tolerant sugarcane variety CoG 7

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From the fluff generated during 2005, after the preliminary experiments (2005–2007), a promising clone G2005047 has been identified. It showed moderate resistance to red rot (3.6 on a 9-scale scoring system), less susceptibility to shoot borer (13.25%) and internode borers (25.35%), and resistance to woolly aphid (0%). In the Advanced Yield Trials (2008–2011), it showed advantages over check for cane yield (CY) (11.79%), commercial cane sugar percent (CCSP) (0.35%), and sugar yield (SY) (20.33%). To ascertain its large-scale cultivation suitability, it has experimented under adaptive research trials (2012–2014) at farmers' fields. It exhibited 18.04%, 1.27%, and 19.55% supremacy over the check Co 86032 for CY, CCSP, and SY respectively. The stability of G2005047 under salinity was ascertained through a multi-environment-based experiment (2015–2017). AMMI (Additive Main-effects and Multiplicative Interactions) and GGE (Genotype × Genotype-Environment interaction) biplots were utilized. ANOVA revealed that the genotypic variation exerted the most significant effect followed by genotype × environment interaction and environment. G2005047 had the highest mean values for yield and quality traits with minimal ASV (AMMI stability value) (2.38:CY; 0.57: CCSP; & 0.58:SY) indicating its good-yielding ability and stability. AMMI I, AMMI II, and GGE biplots confirmed the stability of G2005047. In the jaggery quality assessment trials (2018 and 2019), it yielded 37.1% increased jaggery over the check. Also, the clone G2005047, exhibited moderate resistance to red rot disease, less susceptibility to shoot borer (13.25%) and internode borer (25.35%), and resistance against sugarcane woolly aphid (SWA). Due to supremacy for yield, quality, better performance under salinized situations, and tolerance to disease and pests, the clone G2005047 was released as a variety CoG 7 in 2022.

Globally sugarcane (*Saccharum* spp.) is cultivated in 110 (approx.) countries and supplies raw materials to food and other industries[1]. It caters to 80% input requirements of the sugar industries and also satisfies 35% of the bioethanol requirement[2]. The ever-increasing demand for sugar and petrol-ethanol blending programs warrants adoptable, high-yielding, and quality varieties. Though breeding efforts are continuous, simultaneous yield and quality improvement in sugarcane is a difficult phenomenon that necessitates the identification of location-specific parents and targeted plant breeding programs[3]. In India, sugarcane is an important C4 agro-industrial crop cultivated from arid to semi-arid areas of India where it is exposed to various environmental situations. Frequently varied stress factors are affecting the yield potential. Red rot (RR) disease caused by *Colletotrichum falcatum* Went. alarmingly reduces sugarcane yield and quality. Its grave infection is reported in 77 countries including India. Its epidemics are reported every decade causing crucial losses and facing out a few mega varieties

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from cultivation[4]. The varied antagonistic potential of fungicides against this pathogen and environmental concerns necessitating the evolution of RR-resistant varieties[5]. In India, RR resistance is mandatory for a sugarcane variety release. *C. falcatum* mutates rapidly which challenges profitable sugarcane farming[6]. Further, sugarcane yield is reduced by many insects like borer complex, and sugarcane woolly aphid and these pests cause significant yield and quality loss[7].

By the year 2050, the researchers are to face two challenges (i) Intense salinization of agricultural areas (> 50%)[8], and (ii) a 50% increase is to be achieved in food production to cater world's food demand[9]. Furthermore, a 10% annual increase in the salinized areas is witnessed due to low precipitation combined with greater surface evaporation, faulty agricultural practices, and rock weathering. Soil salinity reduces the cultivable land area, and affects crop productivity, and quality[10]. Globally 20% of the total cultivable area and 33% of irrigated farmlands are highly salinized. The severity of soil salinity is significant in the farming systems supported by supplementary irrigation. The number of supplementary irrigations and frequency are higher in agricultural soils of arid and semi-arid situations like India and hence the problem of salinity is grave. Worldwide irrigation mismanagement affects 20% of supplementary irrigated lands due to secondary salinization[11]. In India, seventy-five percent of tanning industries are situated in Tamil Nadu and the majority are located presently in Vellore, Tirupattur, and Ranipet districts[12]. Disposal of untreated tannery industrial wastes renders the groundwater unsuitable for potting and agriculture. Continuous usage of this polluted water for agriculture over the years increases salinity[13]. Salinity stress reduces economic returns and modifies the agricultural properties of soil, the ecological equilibrium, and erosion properties[14]. Salinity affects almost all phases of plant development[15]. Salinity reduces nutrient and water uptake and induces osmotic and oxidative stresses[16,17]. Salinity-induced yield loss in sugarcane was reported[18].

Variable results are witnessed between the greenhouse-based salinity tolerant screening experiments and the real-time field conditions because (i) *in-house* screening techniques use nutrient media while in salt-affected soils all major nutrients are never present in adequate quantities, (ii) in field situations, plants also experience the negative stress effects of other sodic salts, and (iii) salt-affected soils have different complex combinations of NaCl, CaCl$_2$, CaSO$_4$, and Na$_2$SO$_4$[19] which cannot be created precisely under in vitro conditions, and (iv) further, in the field conditions, the salt concentrations are dynamic and fully season-based. These facts necessitate field experiments to understand the real salinity-tolerance capacity of genotypes by quantifying the interaction effects between genotypes and environments[20].

Stable and good-yielding genotypes can be identified through multiple-environment-based experiments (MEE) using AMMI and GGE biplot techniques. AMMI detects GxE interactions (GEI) through a biplot while GGE quantifies the interaction of G (genotype) + GxE (Genotype by environment)[21]. In the AMMI model, the genotypes are ranked based on stability using the AMMI stability value[22]. However, stability and yield performance should together be considered to achieve success[23] necessitating the use of different selection criteria[24]. Therefore, the present study aimed to evolve and test various fluff-grown sugarcane genotypes under several plant breeding trials, identify superior genotype(s) with good-yielding potential, test their tolerance to red rot and major sugarcane pests, check for stability for yield performance in the salinity stress environments, ascertain jaggery yielding potential, and release it or them for commercial cultivation.

Results and discussion

To sustain the yield, plant breeders are evolving continuously various good-yielding sugarcane varieties. SRS, Melalathur, is involved in evolving good-yielding and stable sugarcane clones suited for normal and salinity-affected soils. It evaluated many clones developed from the fluff of the 2005 hybridization season, in the ideal soil conditions for two cropping years (2006 and 2007) and identified clone G2005047 as promising and therefore further considered for pest and disease screening and evaluation under other advanced breeding trials like AYT, ART, jaggery quality trial, and OFT from the cropping years 2008 to 2019[3].

Screening for resistance to red rot and major pests

Upon visualizing the yield and quality potential of clone G2005047, it was tested for (i) red rot resistance using the plug method and (ii) tolerance to borer complex and sugarcane woolly aphid (SWA).

The clone G2005047 was inoculated with red rot pathotype Cf 06 obtained from the ICAR-Sugarcane Breeding Institute, Coimbatore, India which was isolated from the variety CoC 671 which was once an elite variety that revolutionized sugarcane cultivation and ruled the sugar economy for more than two decades but succumbed to red rot due to monoculture and fast mutating ability of *C. falcatum*. G2005047 exhibited moderate resistance to red rot with a pooled score of 3.6. The red rot inoculum did not modify the top leaf condition. The pooled scores for nodal transgression, lesion width, and white spot were 1.2, 1.1, and 1.3 respectively (Supplementary Table 1). Earlier the plug method was used to ascertain the red rot resistance levels of sugarcane clones[6].

The resistance levels of G2005047 against the borer pest tolerance and woolly aphid were assessed between the 2008 and 2011 cropping years. The levels were compared with the mega variety Co 86032 (Supplementary Table 2). The clone exhibited less susceptibility to shoot borer (13.25%) and internode borer (25.35%). It possessed a complete resistance against SWA under the infector row technique which assumes importance in the resistance breeding standpoint. It can be used as a donor parent in the targeted resistance breeding program. From a sugarcane plant resistance to major pests' perspective, G2005047 possesses the required level of resistance and therefore offers scope for its commercial cultivation.

Advanced yield trials (2008–2011 cropping years: three trials)

Farmers, to reduce the cost of production, are practicing sugarcane farming on a perennial pattern owing to its ratoonability. Therefore, though the yield performances of the genotypes were checked under IYT and PYT

earlier, the trials that are inclusive of ratoon performance starting from advanced yield trials are considered for assessing the yield performance for a perennial cropping pattern. In the AYT, the performances of G2005047 were compared with well-adopted local (CoG 94077) and commercial check (Co 86032) (Table 1). Among the tested genotypes, G2005047 ranked first for yield and quality traits. By considering the mean of plant and ratoon performances, the clone G2005047 had a CY advantage of 11.79% and 19.87% over the checks CoG 94077 and Co 86032 respectively. The clone's advantage for CCSP is implicated in its sugar yield.

Adaptive research trials (2012–2014 cropping seasons: 36 trials)

Though many high-yielding sugarcane varieties are developed in India, a single potential variety is grown in larger areas leading to monoculture farming. Ecological theory suggests that growing a variety in a larger area may not maximize regional productivity because monoculture invites the emergence of several yield-challenging factors. Also, in this climate change era, a single variety cannot perform well in all locations. A mixture of adapted varieties would maximize crop productivity. It is proven that a plant or organism has an adaptation advantage in areas of its origin or evolution and may fail to perform in low-intensive agriculture situations. It is necessary to evaluate the newly bred genotypes over a variety of agro-environments to select consistent performer(s). Therefore, for testing the large-scale performance of G2005047, the sugarcane growing areas of Tamil Nadu were divided into four environmental heterogeneity regions viz., Coimbatore, Cuddalore, Vellore, and Trichy (Table 2). A total of 36 ARTs were laid out in the farmers' holdings with the support of the sugar mills operating in the respective regions. For one trial, the performance of two plant crops and one ratoon was considered, and thereon mean for a region and the overall mean for all the regions arrived. The performance of the clone G2005047 was compared with two checks CoC 24 and Co 86032. It has out-yielded the checks for CY, CCSP, and SY. The advantage for CY is 13.3% and 18.04 respectively. G2005047 exhibited 4.0% and 1.27% supremacy for CCSP. Eventually, it portrayed a better SY of 17.77 t/ha which is the most preferred trait by the sugar mills.

Trait/entry	The mean of two plant crops	Ratoon yield	Mean of plant and ratoon crops	% increase over checks
CY (t/ha)*				
CoG 94077	122.34 ± 12.36	118.13 ± 14.51	120.24	11.79
Co 86032	111.94 ± 9.84	112.32 ± 13.48	112.13	19.87
G2005047	138.37 ± 7.98	130.46 ± 8.26	134.42	
CCSP*				
CoG 94077	12.83 ± 0.75	12.85 ± 0.89	12.84	1.52
Co 86032	12.98 ± 0.42	13.00 ± 0.27	12.99	0.35
G2005047	13.03 ± 0.69	13.04 ± 0.44	13.04	
SY (t/ha)*				
CoG 94077	15.69 ± 6.74	15.18 ± 7.49	15.44	13.51
Co 86032	14.52 ± 8.21	14.60 ± 4.28	14.56	20.33
G2005047	18.03 ± 5.59	17.01 ± 2.75	17.52	

Table 1. Mean performance of the sugarcane clone G2005047 under AYT. *The values are of a mean of three replications with SE.

Traits/entry	Region				Mean of four regions	% increase over check
	Coimbatore (Mean of 12 trials)	Cuddalore (Mean of 12 trials)	Vellore (Mean of six trials)	Trichy (Mean of six trials)		
CY (t/ha)*						
CoC 24	123.52 ± 8.6	119.33 ± 11.3	113.52 ± 10.73	120.03 ± 12.2	119.10	13.30
Co 86032	116.54 ± 10.4	117.32 ± 9.4	107.81 ± 12.41	115.58 ± 7.94	114.31	18.04
G2005047	139.16 ± 7.8	133.53 ± 6.9	131.43 ± 10.24	135.63 ± 8.76	134.94	
CCSP*						
CoC 24	12.59 ± 0.94	12.51 ± 0.71	12.96 ± 0.84	12.33 ± 0.82	12.60	4.50
Co 86032	13.35 ± 0.28	12.74 ± 0.54	12.98 ± 0.52	12.93 ± 0.51	13.00	1.27
G2005047	13.67 ± 0.76	12.93 ± 0.48	13.04 ± 0.63	13.02 ± 0.74	13.17	
SY (t/ha)*						
CoC 24	15.53 ± 8.2	14.94 ± 9.8	14.71 ± 7.9	14.81 ± 6.9	15.00	18.47
Co 86032	15.56 ± 6.3	14.95 ± 7.2	14.00 ± 4.8	14.94 ± 4.8	14.86	19.55
G2005047	19.01 ± 7.4	17.26 ± 4.5	17.14 ± 5.2	17.66 ± 6.2	17.77	

Table 2. Mean performance of the sugarcane clone G2005047 under ART. *The values are of a mean of three replications with SE.

On-farm trials under salinity stress conditions (2015–2017 cropping seasons: 60 trials)

Further, from a global and Indian perspective, the percentage of agricultural land affected by salinity is increasing. Generally, sugarcane is moderately sensitive to salinity[25], it can withstand a salinity level of 1.7 dSm^{-1}[22] beyond which changes in the essential metabolic processes associated with yield were reported[26]. Rao et al.[18] reported a 5.9% yield reduction with a per-unit increase in salinity. Tolerance for any stress with a yield compromise is not a practical solution warranting precise assessment of the sustainability and/or yield compromise of genotypes. Therefore, the ability of G2005047 to withstand salinity stress and yielding ability was assessed in the OFTs since the sugarcane cropping areas situated around the SRS, Melalathur are predominantly affected by the salinity stress.

The stability and yield potential of a genotype can be ascertained by MEE[27,28]. Since sugarcane is an annual crop, the environment greatly influences its trait expression[23]. To estimate the GEI, the AMMI biplot, and the GGE biplot are widely used. AMMI detects GEI and displays the interactive effects through biplots. However, in AMMI, (i) stability level is not quantified and hence ranking of the genotypes is not possible[29] and (ii) ASV alone is used to decide the suitability of the genotype without considering the yielding potential[22] which is not considered effective in the plant breeding standpoint because a stable genotype may not yield better[23], and (iii) the scientific inferences lacking in the AMMI II are non-feasibility of detection of genotypic effects since the GEI is decomposed in the PCA. These facts necessitate, the utilization of other methods which can utilize both yield and stability for ranking of genotypes[24]. The GGE biplots generated from the MEE greatly helped in the perfect evaluation of genotype(s), and environmental ranking or valuation. It is considered effective and preferentially used (both in ideal and stressed situations) because it can classify the study environments into mega environment(s), rank the genotypes, and identify the ideal environments[21,30,31]. Therefore, in the present stability assessment experiment, both AMMI and GGE techniques were utilized to study the suitability of G2005047 over different salinity stress environments (Supplementary Table 3).

The individual and combined effects of G, E, and GEI were significant for the experimented traits. Among the sources of variation, the genotypic variation exerted the most significant effect followed by GEI and E. The genotypic main effect explained 94.49%, 94.87%, and 59.13% for the traits CY, SY, and CCSP respectively. The components G (59.13%), and GEI (28.17%) influenced the CCSP more significantly than the GxE source of variation. The total variation is represented by four various principal components (PCs). Of them, PC1 and PC2 accounted for 77.33% for CY, 77.85% for SY, and 94.43% for CCSP (Table 3). While the effect of PC 4 was zero.

Identifying a stable sugarcane genotype is tough because of its significant environmental effect and GEI. The AMMI analysis incorporates the results of PCA and ANOVA in a single model and visualizes the GEI. Based on similarity responses of characteristics over targeted environments AMMI clusters the test genotypes and identifies potential trends [63–68]. In the present investigation, it is observed that (i) the sum of the squares due to genotype was large, indicating the existence of genetic dissimilarity among the tested genotypes, (ii) the presence of significant variation between the environmental means, which has influenced the trait expression, (iii) the significant GEI implies the difference in genotypic ability for yielding potential in differential environments. Earlier, a significant environmental influence on trait expression in sugarcane was reported[32].

The clone G2005047 exhibited its supremacy for yield and quality traits in the MEE. It produced the highest CY, CCSP, and SY (128.78 t/ha, 13.02%, and 16.77 t/ha respectively). Based on the mean performance over environments, the genotypes were ranked (rY). The ASV indicates the stability of a genotype. The environmental scores of PCA 1 and PCA 2 are considered for calculating the ASV, and therefore the variation due to GxE is justified. A stable genotype is identified with its minimal ASV and high yield[33]. For all three traits, G2005047 had a minimal value indicating its stability for these traits over environments. The genotypes were ranked based on ASV to arrive at rASV. The sum of rY and rASV was utilized to attain YSI or genotypic stability index[24]. It is inferred from YSI and rYSI that clone G2005047 has emerged as the potential accession for its further utilization owing to its yield and stability (Table 4). Earlier, using YSI stable sugarcane entries were identified[20].

To understand the simultaneous effects of genotype and environment AMMI-I and AMMI-II biplots were produced. In the biplot, the sugarcane genotype(s) aligned on the vertical line indicate higher genotype or

Source	DF	CY		SY		CCSP	
		MS	VE %	MS	VE %	MS	VE %
Environment	19	40.03829**	1.59	0.71684**	1.50	0.04606 ***	12.77
Genotype	3	15050.49438***	94.49	288.11424***	94.87	1.35064***	59.13
Interaction (Env x Genotype)	57	32.80823**	3.91	0.5815**	3.64	0.03379***	28.10
PC1	21	40.51585**	45.50	0.64378*	40.79	0.06358***	69.32
PC2	19	31.32749**	31.83	0.64646*	37.06	0.02545***	25.11
PC3	17	24.94202^{NS}	22.67	0.43197^{NS}	22.16	0.00631***	5.57
PC4	15	0^{NS}	0	0^{NS}	0	0^{NS}	0
Residuals	160	19.39025	0	0.33283	0	0.00224	0

Table 3. Analysis of variance of main effects and interactions for yield and quality attributing traits (AMMI). *CY* Cane Yield, *SY* Sugar Yield, *CCSP* Commercial cane sugar percent, *df* degrees of freedom, *MS* Mean sum of square, *VE%* variability explained in percentage, *NS* non-significance. *, ** and ***indicate 5%, 1% and 0.1% levels of significance respectively.

Genotype	CY (t/ha)						CCSP						SY (t/ha)					
	Mean	rY	ASV	rASV	YSI	rYSI	Mean	rY	ASV	rASV	YSI	rYSI	Mean	rY	ASV	rASV	YSI	rYSI
CoG 95076	96.05	3	4.94	4	7	3	12.66	4	1.89	4	8	4	12.16	4	1.07	2	6	4
Co 86032	100.97	2	2.54	2	4	2	12.84	2	0.76	2	4	2	12.96	2	1.37	4	5	2
CoG 94077	95.46	4	3.45	3	7	4	12.82	3	1.00	3	6	3	12.24	3	1.11	3	6	3
G2005047	128.78	1	2.38	1	2	1	13.02	1	0.57	1	2	1	16.77	1	0.58	1	2	1

Table 4. The details of the mean of the trait, rank of yield (rY), AMMI stability values (ASV), rank of AMMI stability values (rASV), yield stability index (YSI), and rank of the YSI (rYSI).

environment main effects otherwise similar interaction patterns are inferred from the genotypes aligned horizontally. The contribution of factor 1 (PCA 1) for CY, SY, and CCSP are 44.1%, 39.70%, and 70.2% respectively (Supplementary Figs. 1 and 2). The CY vs. PC1 biplot shows that E2, E5, E7, E6, E1, E13, E19, E15, and E17 expressed the highest main effect. The environments E15, E11, E19, E18, E5, E7, and E2 and E3, E8, E4, E6, E12, E7, E2, E18, E20, E19, and E16 expressed significant main effects for the traits SY and CCSP respectively. While considering all three traits together, the environments E2, E7, and E19 exhibited a greater effect. The environments displaying PCA scores close to the origin indicate minimal or negligible interaction. In this experiment, depending on the trait, the list of environments with minimal interaction is varied. For CY and SY, environments E1, E4, and E13 are located near zero indicating their minimal interaction on trait expression. While for CCSP, E18, E19, and E20 are close to the origin. The genotypes G 2005047, CoG 95076, and CoG 94077 expressed higher main effects for CY, SY, and CCSP. In the AMMI II biplots, for CY, G2005047 is inferred as the best performer. For SY, G2005047 and Co86032 emerged as the good performers.

GGE biplot analyses
Plant breeders worldwide release several varieties with multiple yield-sustaining attributes however the yield potential is not realized due to greater GEI[21,34]. It is widely used in sugarcane[35]. From the GGE biplot analyses, four important components can be derived (i) representativeness and discriminating ability for assessing the experimented environments, (ii) stability vs mean performance of genotypes over the environment which can be utilized for genotype evaluation; (iii) ranking of genotypes; and (iv) 'which-won-where/what' (www) pattern[36] which provides GEI information by utilizing the G and E correlation values.

Discriminativeness versus representativeness biplots (DRB)
The graphical output of DRB provides information about the best environment(s) that can effectively differentiate the tested genotypes. The 'average-environment coordinates' (AEC) classify the experimental environments into (i) type-1: where vectors are short and exhibit the mean yielding potential of genotypes, (ii) type-2: where the vectors are long and have maximum differentiating power, and are capable of distinguishing the performance of the genotypes, and (iii) type-3: where the vectors have great angles, and appropriate to depict the severe effects of environments[36]. In the current experiment, environments E8, E11, and E15 were identified as suitable environments for testing the performance of CY and SY because they had the maximum and narrow angled environmental vectors with the AEC. These environments also have better-differentiating power to assess the performance of the genotypes (Fig. 1). Among the twenty environments, for CY and SY, environments E2 and E3 have the lesser differentiation power due to the shortest environmental vectors, indicating that all the genotypes exhibited either an average or analogous performance in these environments. While for the trait CCSP, E9, E17, and E13 are tagged as ideal environments.

### Mean versus stability biplots (MSB)
Using MSB in an MEE, the mean performance of the genotypes can be ascertained. The line passing through the origin is considered as the AECs under the condition of SVP (single value portioning) = 1. The vertical AEC line passing through the origin is referred to as abscissa and the horizontal line as AEC ordinate. The MSB in this experiment explained 98.99%, 99.14%, and 92.90% of G + GEI for the traits CY, SY, and CCSP respectively (Fig. 2). The arrow in the abscissa line indicates the greater performance of genotypes. A stable genotype falls on the horizontal axis i.e. AEC abscissa and is not projected from the AEC ordinate[21]. By considering these two stipulations, the clone G2005047 is therefore marked as a highly productive and stable genotype for CY, SY, and CCSP.

Ranking of genotype biplots (RGB)
In an MEE, using RGB, genotype(s) with a better yielding ability and stability are identified. The ring placed at the head of the arrow of AEC abscissa which is otherwise the smallest circle is considered for raking of genotype(s)[21]. The genotype(s) placed in this circle are considered ideal or stable. Additionally, the genotype(s) placed on the left side of the vertical line are earmarked as better-performer(s). The pictorial depiction of genotype ranking biplots (Fig. 3) revealed that G2005047 is the most productive and stable genotype for CY, SY, and CCSP.

Which won where/what GGE biplots (WWWB)
Based on the GEI between the highest yielders, the WWWB-derived polygon divides the study environments[37] and is presented in Fig. 4. The polygon sides are equal to the number of lines originating from the biplot's origin
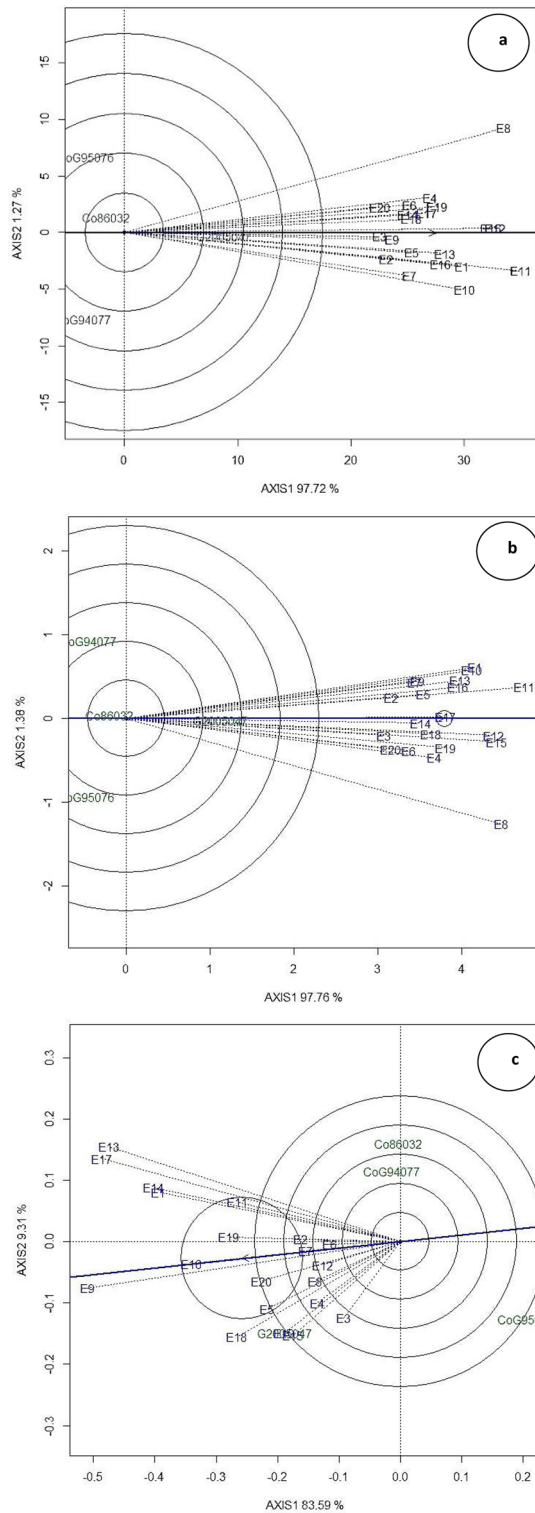
Fig. 1. Discriminativeness VS representativeness biplots for (**a**) CY; (**b**) SY; and (**c**) CCSP where E1, E2… represents environments 1–20.

and intercepting the polygon vertically. The lines initiating from the origin are used to divide the biplot into different mega environments. In this MME, the study environments were grouped as one mega environment (ME) for all three traits. Positioning all environments onto one ME indicates that a single among the tested genotypes outperformed in all the studied environments. The genotype(s) positioned on the vertex of a polygon and placed in an ME is marked as the best performer(s) otherwise called vertex genotype[37] of that particular ME. The genotypes that are positioned on the vertex of a polygon where no environments are placed are the poorest
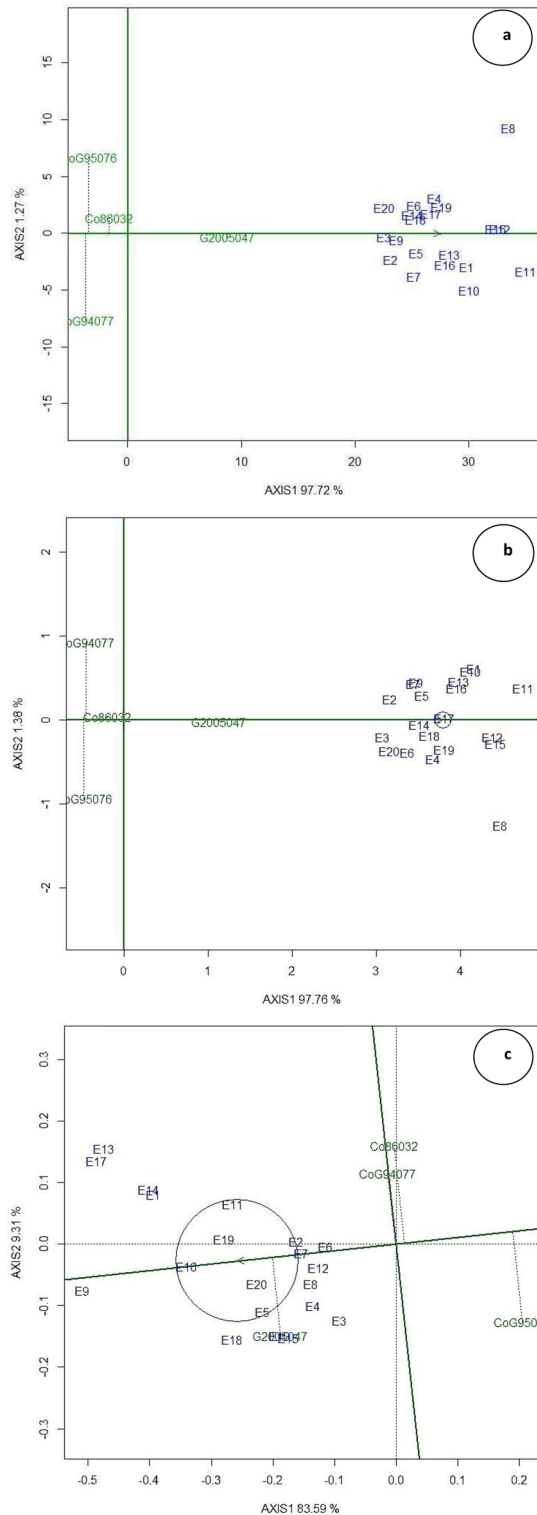
Fig. 2. Mean vs stability biplots for (**a**) CY; (**b**) SY; and (**c**) CCSP where E1, E2… represents environments 1–20.

performers. Additionally, the genotype(s) that are located inside the polygon are also referred to as the poor performers. From the WWWB, the clone G2005047 is figured as the better-performing vertex genotype for CY, SY, and CCSP and offers scope for further exploitation. The environments E3, E12, & E15, E14, & E17, and E2, E6, & E19 are aligned along the margin for CY, SY, and CCSP respectively indicating the tested genotypes performed equally in these environments. While other environments due to their interactive effects are placed at different positions of the ME. The usage of WWWB for selecting stable and high-yielding sugarcane genotypes is proven[38].
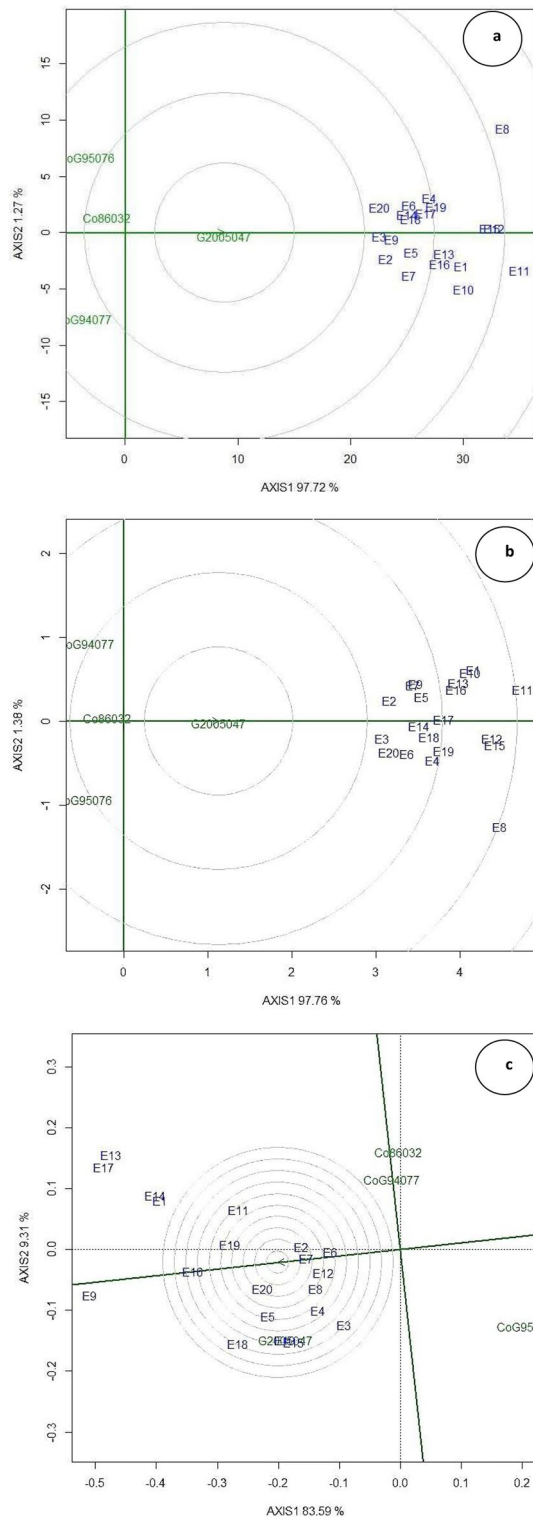
Fig. 3. Ranking of genotypes—biplots for (**a**) CY; (**b**) SY; and (**c**) CCSP where E1, E2… represents environments (1–20).

Estimation of jaggery quality parameters (2018 and 2019 cropping seasons: three trials)

Jaggery is a highly preferred natural sweetener due to its nutritional, and pharma-values[39]. The emerging demand for organic products in the international markets creates increasing demands on jaggery production. It is estimated that of the total sugarcane production, 53% is used for white sugar, 36% is used for jaggery making, 3% is directly consumed as cane juice, and 8% is used for seeding[40]. To find the suitability of the clone G2005047, for jaggery making it was tested for the quality parameters like juice recovery percentage, brix percent, purity
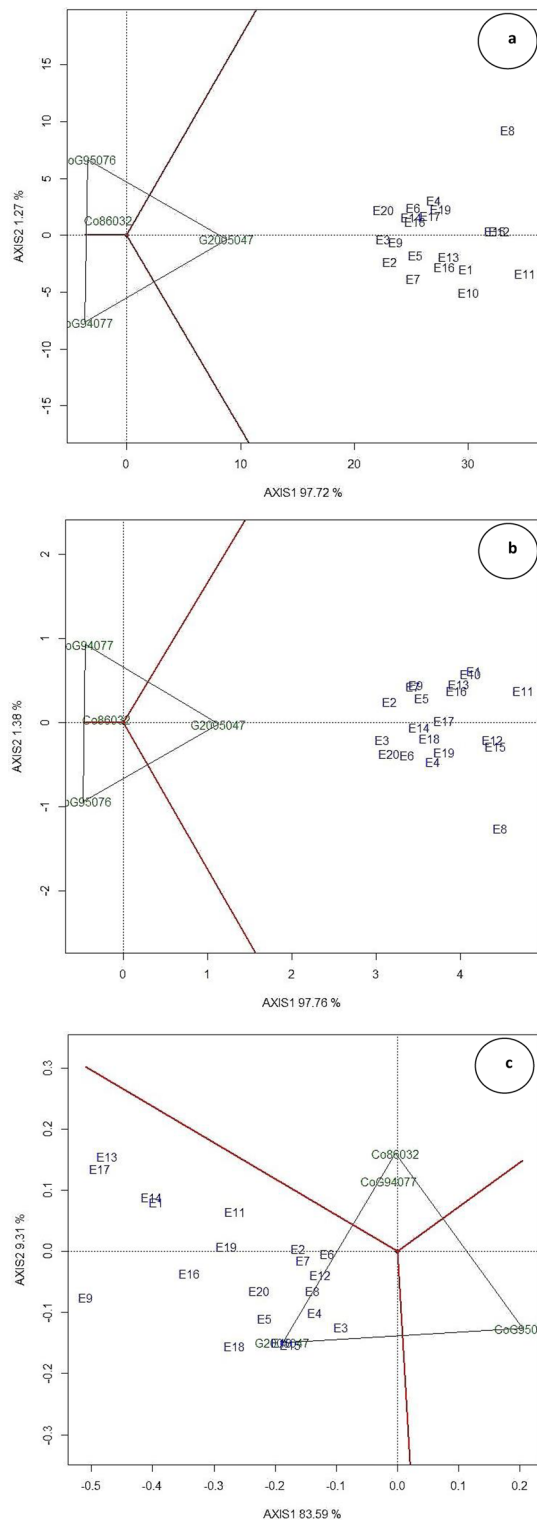
Fig. 4. Which won where/what polygon biplots for (**a**) CY; (**b**) SY; and (**c**) CCSP where E1, E2… represents environments 1–20.

percent, jaggery recovery percentage, and jaggery yield (Supplementary Table 4) and compared with the mega variety Co 86032 and two other jaggery varieties (CoG 94077 and CoG 95076). In G2005047, though the brix percent (21.00 ± 0.52) is a bit lower than Co 86032 (21.60 ± 0.76), its supremacy for other jaggery traits made it promising. It has 37.1%, 44.94%, and 45.09% increased jaggery yield over the checks Co 86032, CoG 94077, and CoG 95076 respectively. A sugarcane genotype with a good purity percentage would reduce the dependence on chemical clarificants which otherwise increases the taste and shelf-life of jaggery. G2005047 has the highest

purity percentage of 90.87 ± 1.21 and therefore offers the scope of producing good quality jaggery. Thereon, the seeds of G2005047 were multiplied during the 2020 and 2021 cropping seasons.

Seed multiplication (2020 and 2021 cropping seasons) and variety release

The genetically pure seeds of the clone G2005047 were multiplied through a three-tier seed nursery system to ensure sufficient seed availability for distribution. The clone G2005047 offered scope for commercial cultivation through a variety release, owing to the reasons that it performed better under ideal sugarcane cultivation regions, possessed moderate resistance to red rot, borer complex, and resistance to SWA, registered stability under salinity conditions, and exhibited significant jaggery making qualities. Therefore, the sugarcane clone G2005047 by the virtue of significant results obtained from several plant breeding trials (totaling 100) conducted from 2005 to 2021, was released for commercial cultivation during the year 2022 with the approval of the State Variety Release Committee, Government of Tamil Nadu, India.

Materials and methods
Evolution of genetic material

During the 2005 sugarcane flowering season, a total of seven bi-parental crosses and 43 general crosses were attempted involving genetically diverse parents at the national sugarcane hybridization facility of ICAR-Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India. The arrows of bi-parental crosses were collected approximately after thirty days of hybridization. While in the general crosses, arrows were harvested at an appropriate time. The arrows were dried sufficiently, and the arrows of a particular bi-parental/general cross were pooled together, debris and other inert matter were removed, and only matured fluff was sown in pots filled with the potting mixture, and maintained at a partial shade net house for a month. The seedlings were watered at an appropriate time interval. On the 31st day, the seedlings were transplanted in the main field (clonal nursery I) at a spacing pattern of 80 cm (between rows) and 45 cm (between seedlings). The recommended package of practices was adopted. At the age of six months, the clumps were harvested individually and planted under the clonal nursery II trial. Thereon the promising clones were promoted for further testing under various breeding trials.

Testing of breeding potency of clones in various plant breeding experiments

The breeding potency of the sugarcane clones evolved during 2005 was tested in the following experiments, initial yield trial (IYT: 2006 cropping season), preliminary yield trial (PYT: 2007 cropping season), and advanced yield trials (AYT: 2008 to 2011 cropping seasons) at Sugarcane Research Station (SRS), Melalathur, Tamil Nadu Agricultural University, Vellore, Tamil Nadu, India. In the IYT and PYT, the clones were checked for miller-preferable traits like more millable cane counts, good cane yield, high sucrose content, spineless leaves and stems, the self-detaching ability of leaves upon drying, and serration-less leaves. The potential clones identified for miller-preferable traits at PYT were concurrently tested for major pests and disease resistance. The best cane and sugar-yielding clone(s) with at least moderate resistance to red rot and less susceptibility to pests were forwarded to subsequent adaptive research trials (ARTs: 2012 to 2014 cropping seasons). The ARTs were conducted with the support of various sugar mills in Tamil Nadu. For conducting the ARTs, the sugarcane cropping area in the state of Tamil Nadu was divided into four regions Coimbatore, Trichy, Cuddalore, and Trichy. A total of 36 ARTs were laid out in the farmers' holdings across these regions in RBD with three replications. For one trial, the mean values for three replications for yield and quality traits were averaged. Likewise, for one region, the values for all the trails were averaged. The mean values for these four regions were utilized to ascertain the yielding potential of the test clone. After suitability confirmation in the ARTs, the potent clone was tested under on-farm trials (OFTs) under salinity conditions from 2015 to 2017 cropping seasons through an MEE. The jaggery quality parameters like juice recovery percentage, brix percent, purity percent, jaggery recovery percentage, and jaggery yield were assessed between 2018 and 2019. The juice recovery percentage is calculated from the quantum of cane crushed and juice recovered. To measure the total soluble solids (TSS or Brix) in the juice brix spindles ranging from 11 to 20 and/or 21 to 30 were used. The purity percentage was calculated using the formula: (Sucrose %/Corrected brix%) × 100.

The genetically pure seed materials of the promising clone were multiplied during the 2020 and 2021 cropping seasons. In all the above-mentioned trials the genetic materials were planted in randomized block design and replicated thrice. The row length was 6 m and the number of rows per entry was five. The seeding rate was 12 buds per meter. The recommended cultivation practices were followed to raise healthy crops.

Assessment of genotypic performance for yield and quality traits

In all the above-mentioned trials, the crops were harvested in 360 days. The trait cane yield per hectare (CY) was recorded at harvest. The quality parameters like pol%, brix%, commercial cane sugar percent (CCSP), and purity%, were estimated from the juice as per ICUMSA methods[41] immediately after harvest. The pol% and brix% values were utilized to calculate the CCSP as per Meade and Chen[42]. The CY and CCSP were utilized to calculate sugar yield per hectare (SY) ((CYxCCSP)/100).

Screening for disease and pest resistance
Disease: red rot

Red rot screening was done at the common screening facility of TNAU available at the sugarcane research station, Cuddalore, TNAU, at the beginning of the North East monsoon (Oct-Nov) to ensure sufficient humidity during the pathogen incubation period. A seven-month-aged crop of the sugarcane clones along with the susceptible (CoC 671), and resistant (Co 86249) checks were considered for the plug method of red rot screening. The ten-day-old culture of *C. falcatum* (grown on an oatmeal agar medium) was utilized for inoculation. The spore

suspension with 1×10^6 conidia/ml was prepared. Well-grown canes free from pests and other diseases were selected for inoculation. Using a cork borer, a borehole measuring 0.5 cm was made in the middle of the third internode from the cane bottom and the cane tissue with the rind was removed. After adding about 0.5 ml of spore suspension, the borehole was re-plugged by the rind tissues and sealed with plastic clay. The experiment was replicated thrice. In one replication, fifteen well-grown canes per genotype were inoculated. After the incubation period of 60 days, the 0–9 screening scale adopted by Srinivasan and Bhat[43] and Mohanraj et al.,[44] were utilized to categorize the red rot reaction of the clones. For scoring, the canes were longitudinally split open, the factors like the condition of the cane top, nodal transgression, lesion width, and white spots were considered. The scales adopted for these factors are 0–1, 1–3, 1–3, and 1–2 respectively (Supplementary Tables 5 and 6). The average of three replications for these factors was considered for ascertaining the red rot reaction.

Pest tolerance
The field experiments aimed at screening for borer pest tolerance were conducted from 2008 to 2011 cropping seasons at the sugarcane research station, Melalathur. The trials were planted during March's second week and harvested during the first fortnight of the preceding year. After PYT, the best-yielding sugarcane clones along with the commercial check (Co 86032) were grown in fifteen replications. For one replication, the plot size was 6 m × 5 × 0.8 m. The seedling rate was 12 buds per meter. All recommended agronomic practices were adopted to raise a healthy crop. No plant protection spray was given. The pooled mean incidence of three cropping years was utilized to assess the tolerance levels.

Early shoot borer
The appearance of a dead heart was considered an acute form of early shoot borer incidence. The number of dead hearts concerning the total number of shoots in one replication was counted on the 30 days after planting (DAP), 60 DAP, 90 DAP, and 120 DAP. The percentage of shoot borer incidence and pooled percent incidence were worked out. The clone was categorized into different resistant classes (Less Susceptible: Below 15, Moderately susceptible: 15.1–30, and highly susceptible: Above 30)[45].

Internode and top borer
Visual observations for bunchy and dead tops were made at maturity and the percent incidence ((Total number of infected canes/total number of canes observed) × 100)) was worked out. Based on the pooled percent incidence level the grading was done (Less Susceptible: Below 10, Moderately susceptible: 10.1–20, and highly susceptible: Above 20)[46].

Screening for sugarcane woolly aphid resistance
The mean population of SWA and leaf area infected were counted/measured in 1m² area in one replication on a monthly interval. The pooled value for 15 replications arrived. For recording SWA density, a scale of 0–4 grade was adopted. The clones were categorized as resistant (scale = 0), moderately resistant (scale = 1), moderately susceptible (scale = 2), susceptible (scale = 3), and highly susceptible (scale = 4). The percentage of leaf area infected by SWA for these scales are 1–25%, 26–50%, 51–75%, and 76–100% respectively[47].

Stability analysis under salinity situations
The SRS, Melalathur is located in the Vellore district of Tamil Nadu where salinity is one of the major threats to sugarcane cultivation, therefore the promising clone(s) identified after ART with appropriate checks (Co 86032, CoG 94077, and CoG 95076) were tested under 60 OFTs (20 plant crops 1 + 20 plant crops 2 + 20 ratoon crops) in the salt-affected soils from 2015 to 2017 cropping seasons. The soil and water characteristics of the salinity trial plots were assessed as per Mani et al.,[48] and presented in supplementary table 3. For performing AMMI analysis, the procedure advocated by Gauch and Zobel[29] was used. The procedure as suggested by Yan et al.[49] was utilized for GGE estimations.

AMMI stability value (ASV)
The ASV was calculated by using the formula advocated by Purchase et al.[22] and utilized to compare the genotypic stability.

$$ASV = \sqrt{\left[\left(\frac{SS_{IPCA1}}{SS_{IPCA2}} \right) \times (IPCA1_{Score}) \right] + (IPCA2_{Score})^2}$$

where SS$_{IPCA1}$ and SS$_{IPCA2}$ are the sum of squares for IPCA1 and IPCA2 respectively. A highly stable genotype across environments has smaller ASV scores. The genotypes suited for a specific location were identified by larger IPCA scores (either positive or negative).

Yield stability index (YSI)
The YSI is calculated using the yielding ability and stability of a genotype. The formula is YSI = RASV + RY[7]. Where RASV denotes the AMMI-derived stability value rank of a genotype; RY specifies the yield rank of a genotype. Low RASV and RY values earmark a stable and good-yielding genotype[50].

Statistical analyses

The data on yield and quality parameters of various plant breeding trials were analyzed by Microsoft Excel (Microsoft 365). In the stability analysis, R studio Metan package was utilized for the AMMI and GGE biplot-based graphical analysis[51,52]. The graphical outputs were utilized to illustrate the GEI.

Plant experiments

All experiments in this manuscript concerning plants were carried out following relevant institutional, and national guidelines and legislation.

Conclusion

The painstaking plant breeding efforts at SRS, Melalthur helped in the evolution of variability for economic traits in sugarcane. Thereon, various plant breeding cyclic experiments assisted in the identification of yielding potential of promising genotypes. The AMMI and GGE biplot analyses facilitated the identification of the better yielding ability and stability of the clone G2005047 under salinity-stressed situations. A total of 103 experiments conducted in 17 long cropping years helped in the release of the sugarcane clone G2005047 as a variety CoG 7 for commercial cultivation for ideal and salinity-affected areas.

Data availability

All data documented in these experiments are published in this article.

References

1. FAO. *Food and agriculture society of the United Nations* https://www.fao.org/faostat/en/#data/QC (2020).
2. O'Connell, A. *et al.* Combining genomic selection with genome-wide association analysis identified a large-effect QTL and improved selection for red rot resistance in sugarcane. *Front. Plant Sci.* **13**, 1021182 (2022).
3. Sudhagar, R., Rajkumar, S., Ramachandiran, K. & Saravanan, N. A. Identification of location-specific male and female sugarcane parents and formulation of breeding strategies. *Sugar Tech.* **25**(3), 670–680 (2023).
4. Viswanathan, R., Ramesh Sundar, A., Selvakumar, R. & Malathi, P. Progress in understanding fungal diseases affecting sugarcane: red rot. In *Achieving Sustainable Cultivation of Sugarcane* (ed. Rott, P.) 201–219 (Burleigh Dodds Science Publishing, 2018).
5. Jackson, P. Advances in conventional sugarcane breeding programmes. In *Achieving Sustainable Cultivation of Sugarcane* (ed. Rott, P.) 75–108 (Burleigh Dodds Science Publishing, 2018).
6. Viswanathan, R. Sustainable sugarcane cultivation in India through threats of red rot by varietal management. *Sugar Tech.* **23**(2), 239–253 (2021).
7. Bhatt, R. *et al.* Growth, yield, quality, and insect-pests in sugarcane (*Saccharum officinarum*) as affected by differential regimes of irrigation and potash under stressed conditions. *Agronomy* **12**(8), 1942 (2022).
8. Jamil, A., Riaz, S., Ashraf, M. & Foolad, M. R. Gene expression profiling of plants under salt stress. *Crit. Rev. Plant Sci.* **30**(5), 435–458 (2011).
9. Godfray, H. C. J. *et al.* Food security: the challenge of feeding 9 billion people. *Science* **327**(5967), 812–818 (2010).
10. Shahbaz, M. & Ashraf, M. Improving salinity tolerance in cereals. *Crit. Rev. Plant Sci.* **32**(4), 237–249 (2013).
11. Glick, B. R., Cheng, Z., Czarny, J. & Duan, J. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.* **119**, 329–339 (2007).
12. Williams, A. R. E. & Ahmed, M. P. P. Impact of leather industries on groundwater in Tamil Nadu with special reference to Vellore district. *Int. Rev. Bus. Econ.* **4**, 351–357 (2020).
13. Sinduja, M. *et al.* Groundwater quality assessment for agricultural purposes at Vellore District of Southern India: A geospatial based study. *Urban Clim.* **47**, 101368 (2023).
14. Hu, Y. & Schmidhalter, U. *Limitation of salt stress to plant growth* in (eds Hock, B. & Elstner, C. F.) 91–224 (New York: Marcel Dekker Inc., 2004).
15. Javid, M. G., Sorooshzadeh, A., Moradi, F., Sanavy, S. A. M. M. & Allahdadi, I. The role of phytohormones in alleviating salt stress in crop plants. *Aust. J. Crop Sci.* **5**(6), 726–734 (2011).
16. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* **25**(2), 239–250 (2002).
17. Bano, A. & Fatima, M. Salt tolerance in *Zea mays* (L.) following inoculation with rhizobium and pseudomonas. *Biol. Fertil. Soils.* **45**(4), 405–413 (2009).
18. Rao, V. P., Sengar, R. S., Singh, S. & Sharma, V. Molecular and metabolic perspectives of sugarcane under salinity stress pressure. *Prog. Agric.* **15**(1), 77–84 (2015).
19. Joseph, B. & Jini, D. Salinity induced programmed cell death in plants: Challenges and opportunities for salt-tolerant plants. *J. Plant Sci.* **5**(4), 376–390 (2010).
20. Kumar, R., Meena, M. R., Kulshreshtha, N., Kumar, A. & Ram, B. Genotypic response of recently evolved sugarcane "Co" clones under different levels of saline irrigation water. *J. Sugarcane Res.* **7**, 159–168 (2017).
21. Oladosu, Y. *et al.* Genotype × Environment interaction and stability analyses of yield and yield components of established and mutant rice genotypes tested in multiple locations in Malaysia. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **67**(7), 590–606 (2017).
22. Purchase, J. L., Hatting, H. & van Deventer, C. S. Genotype × environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. *S. Afr. J. Plant Soil* **17**(3), 101–107 (2013).
23. Alarmelu, S., Balakrishnan, R. & Hemaprabha, G. G. × E interaction studies in multi-location trials of sugarcane using GGE biplot and ANOM analysis. *J. Sugarcane Res.* **5**(1), 12–23 (2015).
24. Farshadfar, E., Mahmodi, N., Yaghotipoor, A. & Sability, A. Value and simultaneous estimation of yield and yield stability in bread wheat (*Triticum aestivum* L.). *Aust. J. Crop Sci.* **5**(13), 1837–1844 (2011).
25. Nelson, P. N. & Ham, G. J. Exploring the response of sugar cane to sodic and saline conditions through natural variation in the field. *Field Crops Res.* **66**(3), 245–255 (2000).
26. Rietz, D. N. & Haynes, R. J. Effect of irrigation-induced salinity and sodicity on sugarcane yield. *Proc. S. Afr. Surg. Technol. Assoc.* **76**, 173–185 (2002).
27. Kumar, A. Climate change and sugarcane productivity in India: An econometric analysis. *J. Soc. Dev. Sci.* **5**(2), 111–122 (2014).
28. Wolde, L., Keno, T., Tadesse, B., Bogale, G. & Abebe, B. Mega environment targeting of maize varieties using AMMI and GGE-biplot analysis in Ethiopia. *Ethiop. J. Agric. Sci.* **28**(2), 65–84 (2018).
29. Gauch, H. G. & Zobel, R. W. Imputing missing yield trial data. *Theor. Appl. Genet.* **79**, 753–761 (1990).

30. Kumar, A. *et al.* High-yielding, drought-tolerant, stable rice genotypes for the shallow rainfed lowland drought-prone ecosystem. *Field Crops Res.* **133**, 37–47 (2012).
31. Karimizadeh, R. *et al.* GGE biplot analysis of yield stability in multi-environment trials of lentil genotypes under rainfed condition. *Not. Sci. Biol.* **5**(2), 256–262 (2013).
32. Tena, E. *et al.* Genotype × environment interaction by AMMI and GGE-biplot analysis for sugar yield in three crop cycles of sugarcane (*Saccharum officinirum* L.) clones in Ethiopia. *Cogent Food Agric.* **5**(1), 1651925 (2019).
33. Kumar Bose, L., Namdeorao Jambhulkar, N., Pande, K. & Nath Singh, O. Use of AMMI, and other stability statistics in the simultaneous selection of rice genotypes for yield and stability under direct-seeded conditions. *Chil. J. Agric. Res.* **74**(1), 3–9 (2014).
34. Yan, W., Hunt, L. A., Sheng, Q. & Szlavnics, Z. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Sci.* **40**(3), 597–605 (2000).
35. Mahadevaiah, C. *et al.* Delineation of genotype × environment interaction for identification of stable genotypes for tillering phase drought stress tolerance in sugarcane. *Sci. Rep.* **11**(1), 18649 (2021).
36. Yan, W., Kang, M. S., Ma, B., Woods, S. & Cornelius, P. L. GGE biplot versus AMMI analysis of genotype-by-environment data. *Crop Sci.* **47**(2), 643–653 (2007).
37. Neisse, A. C., Kirch, J. L. & Hongyu, K. AMMI and GGE Biplot for genotype × environment interaction: A medoid–based hierarchical cluster analysis approach for high–dimensional data. *Biometrical Lett.* **55**(2), 97–121 (2018).
38. Yadawad, A., Patil, S. B., Kongawad, B. Y., Kadlag, A. D. & Hemaprabha, G. Multi environmental evaluation for selection of stable and high yielding sugarcane (*Saccharum officinarum* L.) clones based on AMMI and GGE biplot models. *Indian J. Genet. Plant Breed.* **83**(3), 389–397 (2023).
39. Rao, P. V. K. J., Das, M. & Das, S. K. Jaggery–a traditional Indian sweetener. *Indian J. Trad. Knowl.* **6**, 95–102 (2007).
40. Singh, J., Singh, R. D., Anwar, S. I. & Solomon, S. Alternative sweeteners production from sugarcane in India: Lump Sugar (jaggery). *Sugar Technol.* **13**, 366–371 (2011).
41. Chen, J. C. P. & Chi, C. C. *Cane Sugar Handbook: A Manual for Cane Sugar Manufacturers and Their Chemists* 1090 (John Wiley & Sons, 1993).
42. Meade, G. P. & Chen, J. C. P. *Cane Sugar Handbook 10th Wiley Inter Science* 947 (Wiley & Sons, 1977).
43. Srinivasan, K. & Bhat, N. Red rot of sugarcane – criteria for grading resistance. *J. Indian Bot. Soc.* **40**, 566–577 (1961).
44. Mohanraj, D., Padmanaban, P. & Viswanathan, R. Screening for red rot resistance in sugarcane. *Funct. Plant Sci. Biotechnol.* **6**(Special issue), 51–62 (2012).
45. Rao, S. & Krishnamurthy, R. M. M. Studies on loss in yield of sugarcane due to shoot borer incidence, *Chilo infuscatellus* snellen (Pyralidae: Lepiodoptera). *Indian Surg.* **22**, 867–868 (1973).
46. Paudel, K., Dangi, N., Ansari, A. R. & Regmi, R. Screening of sugarcane genotypes against Top Borer (*Scirpophaga exerptalis* Walker) infestation. *J. Agric. Nat. Res.* **4**(2), 148–154 (2021).
47. Patil, S. B. *et al.* Identification of sugarcane clones resistant to the sugarcane woolly aphid, (*Ceratovacuna lanigera*). *Curr. Sci.* **88**, 284–288 (2004).
48. Mani, A. K., Santhi, R. & Sellamuthu, K. M. *A Handbook of Laboratory Analysis* 156–167 (AE Publications, 2007).
49. Yan, W. & Kang, M. S. *GGE biplot analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists* 288 (CRC Press, 2002).
50. Dashiell, K. E., Ariyo, O. J. & Ojo, K. Genotype X environment interaction and simultaneous selection for high yield and stability in soybeans (Glycine max (L.) Merr.). *Ann. Appl. Biol.* **124**(1), 133–139 (1994).
51. Boston, M.A., *R Studio Team. RStudio: Integrated Development for R; RStudio* http://www.rstudio.com. (2020).
52. Olivoto, T. & Lúcio, A. D. Metan: An R package for multi-environment trial analysis. *Methods Ecol. Evol.* **11**(6), 783–789 (2020).

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Author contributions

R.S.; Conceptualization; Methodology; Conducted experiments; formal analyses; Writing—original draft; editing; N.A.S.; Conducted experiments; formal analyses; review; R.K.; Conducted experiments; M.S; Conducted experiments; S.G.; Conducted experiments; C.B.; review-editing; A.T.; Conducted experiments; V.R.; conducted experiments; C.A.; Conducted experiments; A.A.; Conducted experiments.

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