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Original Article

Evaluation of Genetic Variability, Phenotypic Stability and Interrelationships among the Quantitative Traits of Sugarcane under Drought Stress

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Abstract

Introduction: Drought is a serious abiotic stress that leads to low sugarcane productivity. The complex polyploidy and lack of heritability information for drought tolerance make breeding more difficult. Yield is a polygenic trait influenced by several phenological and physiological factors. Assessing the variability, correlation and heritability of various traits under water deficit conditions is imperative. In the present study, we measured 14 traits contributing to drought resistance and yield under control (well-watered) and drought (water-stressed) conditions.

Materials and Methods: Around 14 quantitative traits for a large population of 119 progenies cross BO 91 x Co 775 were evaluated. The agronomic traits, which included 6 morphological traits, germination%, tillers, cane height, internodal length, stalk diameter, and internodes in a stalk; 2 physiological traits, such as total chlorophyll content and chlorophyll fluorescence; and 6 yield-contributing traits, including single cane weight (SCW), number of millable canes (NMC), cane yield, Brix%, Pol%, and commercial cane sugar (CCS)%, were measured for each clone.

Results: Our multivariate analysis revealed a significant difference among the treatments, genotypes, and G × E interactions. Path analysis revealed that SCW had a positive direct effect on cane yield. Here, we suggest that the traits of tiller number, total chlorophyll content, SCW, and NMC could effectively evaluate drought-tolerant genotypes.

Conclusions: This formulated study with significant findings of phenotypic stability serves as a potential parameter for breeders in selecting genotypes for arid/semiarid regions.

Keywords: Sugarcane, Trait, Heritability, Genotypic and Phenotypic Correlation, Path Analysis

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Introduction

Sugarcane (*Saccharum spp*. hybrids) is a chief agro-economical crop contributing 70% of the world's mass sugar production and numerous value-added products such as ethanol, chewing cane, jaggery, cane top as fodder and paper making. In addition to Brazil, India is the second largest sugar producer worldwide. *Saccharum* is a complex genus comprising two wild species (*S. spontaneum* and *S. robustum*) and four cultivated species (*S. officinarum, S. barberi, S. sinense*, and *S. edule*).[1] Sugarcane hybrids were produced from interspecies hybridization between parental species of *S. spontaneum* (2n = 40-128, x = 8) and *S. officinarum* (2n = 80, x = 10) followed by several backcrosses with *S. officinarum*.[2] Owing to the large genome size (~10 Gbp) and high polyploidy level (aneuploid gametes), genetic studies of sugarcane have been extremely challenging compared to those of other crops. Despite these challenges, it is obligatory to breed sugarcane genotypes that suit varied growing conditions with better performance.[3]

Drought is a severe abiotic stress factor that affects sugarcane crop growth and yields in many water-scarce areas worldwide. Phenotypic stability under such stress conditions reflects the genotype's ability to withstand aridity and opens new opportunities for exploring hidden genetic variation.[4] Any biotic or abiotic stress occurring during the rapid growth phase can impact the regrowth and longevity of the sugarcane crop, leading to a drastic reduction in yield.[5] This condition often affects the growth of leaves and roots, disrupting processes such as photosynthesis and stomatal conductance. Since yield is considered a polygenic trait, precise selection for yield under drought stress is necessary. Family selection in a breeding program is crucial due to its role in increasing genetic gain and estimating the additive

and nonadditive variances of the crop.[6] An effective breeding program aims to develop stable genotypes with enhanced productivity.

Genotypes that possess high cane yield, sucrose percentage, disease resistance and greater ratooning ability are targeted for release as commercial varieties. The evaluation of morphological traits for their distinctiveness, uniformity and stability of performance under various agroclimatic conditions is considered for use in breeding.[7] Raza et al. (2017)[8] suggested that the weight of the individual cane, leaf area index, cane height, internodal length, total biomass and number of internodes per stalk strongly impact cane yield. Leanasawat et al. (2021)[9] reported that early drought stress on sugarcane crops lowered the net photosynthetic rate, the maximum quantum yield of PSII efficiency, stomatal conductance, and transpiration rate. Devi et.al (2018)[10] conducted an experiment and statistically analysed the physiological data obtained from cultivars under drought stress and after recovery and reported that chlorophyll fluorescence (*Fv/Fm*) and SPAD values are essential for combating drought conditions. The wild species naturally exhibit biotic/abiotic stress tolerance, and the stress tolerance of cultivated varieties is imparted by introgression breeding with wild types of *Saccharum spontaneum* and *Erianthus arundinaceus*.[11-14] Genotypes exhibiting higher transpiration rates, greater stomatal conductance, lower leaf and canopy temperatures and greater canopy cover under water deficit conditions are considered tolerant.[15,16]

In the present study, we measured 14 traits contributing to drought resistance and yield under control (well-watered) and drought (water-stressed) conditions. Selection for these polygenic traits is critical. We employed statistical methods to i) test the yield and heritability of the traits of the experimental clones through a linear mixed model, (ii) identify the potential genetic components for trait selection, and (iii) investigate the interrelationship between cane yield and its attributes under drought conditions. In sugarcane hybrids, both nonadditive (dominance and epistasis) and additive genetic effects rely mostly on allele frequency (AF) and genetic divergence (GD).[17] Hence, all the estimated variables were elucidated by the additive genetic variance. Finally, the data were analysed by employing PCA to identify the chief trait for the phenotypic characterization of sugarcane and thereby to select superior clones for commercial release.

Materials and Methods

Genetic Material

The experimental material for the study consisted of 119 elite progenies raised from the BO 91 x Co 775 cross-seedling population (F1 hybrids). One of the parents, BO 91, is a mid-season and late-ripening variety of subtropical India with good jaggery quality. It is resistant to drought and red rot, whereas the parent Co 775 is susceptible to drought and red rot disease. The selected varieties BO 91 (female parent) and Co 775 (male parent) were cross-pollinated, and the fluff collected from biparental mating was used for raising the seedlings. Our statistical data analysis could help us identify drought tolerance traits inherited by their offspring, indicating phenotypic stability.

Study Site and Weather Conditions

The study was conducted in an experimental farmland located at the Sugarcane Breeding Institute-ICAR (Indian Council of Agricultural Research) in Coimbatore, Tamil Nadu, India. All 119 clones were planted during the growing season of January in a split-plot design in a randomized complete block with two replications using the recom mended agronomic package. The site was prepared by ploughing, harrowing, and planking. To ensure greater homogeneity of the seedlings, two-budded stem cuttings (setts) were placed in the furrows. The plot size was 15 m^2, and the row-to-row distance was 1.2 m. The soil of the sugarcane plantation field was clay with a slightly alkaline pH range averaging 8. Irrigation was performed periodically, and all agronomic management practices were carried out throughout the growth period. Phenotypic data of the individuals were collected from two different clumps in each plot.

Irrigation Regimes

Control and drought irrigation regimes were used as treatments (main plot) to evaluate the performance of the genotypes (subplot). The crop was uniformly irrigated up to 90 DAP (Days After Planting), after which 3rd MAP (Months After Planting) drought stress was imposed on the field by withholding irrigation. The control blocks were irrigated at regular time intervals. The soil moisture content was checked periodically to determine the moisture conditions of the drought field. A low available moisture content of 20% was considered an indicator of the imposition of drought.

Phenotypic Data Collection

Sugarcane has four typical developmental phases: the germination phase, tillering phase, grand growth phase, and maturity phase. In the process of phenotypic characterization, several traits were simultaneously evaluated. Parameters such as germination percentage (45 DAP) and number of tillers (120 DAP) were recorded. Photosynthetic parameters such as chlorophyll fluorescence (*Fv/Fm* ratio) and total chlorophyll content (SPAD index) at the early and peak stages of drought, and the stay-green phenotype were recorded. The "stay-green phenotype" scoring for drought was based on visual observation of leaf greenness, tip drying, leaf rolling, and vigor of the plant. The score was indexed from 1-5 (greenness to drying of the plant).

Chlorophyll fluorescence (*Fv/Fm* ratio) from photosystem II was measured with a portable chlorophyll fluorometer OS-30p. Leaves were dark-adapted using plastic leaf clips for half an hour, after which the emission of leaf fluorescence was recorded to indicate drought-induced photoinhibition. The total chlorophyll content was recorded using a SPAD-502 chlorophyll meter, which is a simple hand-held instrument that measures the relative amount of leaf chlorophyll. Photosynthetic measurements were made between 9 to 12 h, which is the most critical time, reflecting the instantaneous status of PSII.

Stalk height, stalk diameter, internode length, and number of internodes were noted for all genotypes. All the ripened sugarcanes were manually harvested. The cane height was measured from the stalk base to the top visible dewlap leaf. The stalk diameter was measured from the middle portion of the cane by a Vernier caliper in centimeters without reference to the bud. The morphological characteristics are influenced by environmental conditions. Juice quality in terms of brix and pol was analysed at the maturity phase. All the millable canes were counted at the time of harvest. The yield was calculated in tonnes per hectare. Commercial cane sugar (CCS%) and cane yield were estimated for all the genotypes.

Statistical Analysis

The observed data were analysed using R version 4.0.0, JMP Pro 15.0, META-R V 6.0.4 and SPSS 25.0.0. Heritability and variance components were estimated by the restricted maximum likelihood (REML) method using R software. The REML algorithm is employed for every estimation for all software because of the highly unbalanced nature of the data.[18] Likelihood-based methods mainly use a combination of residual maximum likelihood estimation of covariance components and generalized least squares (GLS) estimation of means, making them unbiased.[19] The mixed models were fitted with random effects and fixed effects:

$$y = Xb+Za+e,$$

Where y is the vector of the observed phenotypic trait values of the genotypes and vectors b = fixed effects, a = additive genetic effects (random), and e = residual effects.

The total phenotypic variance (Vp), additive genotypic variance (Vg) and residual variance (Vr) were calculated to understand genotype-by-environment (G × E) interactions. For the comparison between treatments, the phenotypic, genotypic and environmental variances (residuals) of every trait were computed by calculating coefficients of variation (CV) using variance components and the phenotypic mean of every trait.

Variability Parameters

The data collected across the treatments were subjected to analysis of variance (ANOVA), and the mean sum of squares (MSS) was computed with the least significant difference at 95% and 99%. Two-way ANOVA was performed between two independent variables called factors (replication and genotype). Genetic variable components such as genotypic variance, phenotypic variance, environmental variance, genotypic and phenotypic coefficient of variation, broad-sense heritability, genetic advance and genetic advance as a percent of the mean (genetic gain) were calculated. Genotypic and phenotypic correlations were employed as a matrix in path coefficient analysis to determine the direct and indirect effects on cane yield. Multivariate analysis was performed using JMP Pro 15.0 software to identify the principal components that influence sugarcane yield traits.

Estimation of Genotypic and Phenotypic Variability

To understand the genetic potential of this plant in terms of wider adaptation to water-scarce conditions, statistical para meters such as genetic variability, heritability (broad sense), pairwise Pearson correlation, path coefficient analysis, and PCA among the yield components were evaluated.

The genotypic and phenotypic variance were calculated using the formula suggested by Johnson et al.[20]

Environmental variance (Ve) $\sigma_e^2 = EMS$

Genotypic variance, (Vg) $\sigma_g^2 = \frac{GMS - EMS}{r}$

Where

GMS = genotypic mean square; *EMS* = error mean square, and *r* = number of replications.

Phenotypic variance, (Vp) $\sigma_p^2 = \sigma_g^2 + EMS$

Estimation of Heritability

Broad-sense heritability is defined as the proportion of traits affected by all genetic factors, including dominance and gene-gene interactions. Broad-sense heritability (H_b^2) was also calculated using the formula suggested by Johnson et al.[20]

Heritability$(H_b^2) = \frac{\sigma_g^2}{\sigma_p^2} x\ 100$

Estimation of Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV)

The genotypic and phenotypic coefficient of variation was calculated by the following formula,

$$GCV\% = \frac{\sqrt{\sigma_g^2}}{\bar{X}} x\ 100$$

$$PCV\% = \frac{\sqrt{\sigma_p^2}}{\bar{X}} x\ 100$$

Estimation of Genetic Advancement

The expected genetic advance represents a shift in the population mean towards the superior side under selection pressure after a single generation of selection. It is estimated by the following formula.[20]

$$GA = H_b^2 \text{ x K x } \sigma p$$

Where H_b^2 = Heritability in a broad sense, σp = Phenotypic standard deviation, K = Constant (Selection differential at 5% selection intensity in a large sample from a normally distributed population = 2.06).

Estimation of Genetic Advance

The expected genetic advance (GA) in terms of percent of the mean (genetic gain) was calculated by the following formula,[21]

$$G.A.\ as\ \%\ of\ mean = \frac{G.A\ x\ 100}{\bar{x}}$$

Path Coefficient Analysis

Path analysis was performed using the agricolae package in R version 4.0.0. Path analysis is a standardized partial regression coefficient that splits genotypic and phenotypic coefficients into direct and indirect effects. It measures the real cause and effect between two explanatory variables.
Total effect (r) = direct effect + indirect effect

Multivariate Analysis

To determine the total variation contributed by yield-contributing traits in clones, we employed PCA using JMP Pro 15.0, and plots were constructed for the traits. The eigenvalues and eigenvectors are computed from the correlation matrix (or covariance matrix). The loading matrix is computed by the product of the eigenvector matrix and the square root of the eigenvalues. For the traits, the principal components with eigenvalues above 1 (Kaiser criterion) were chosen for further analysis.

$$Loading\ matrix = Eigen\ vectors\ x\ \sqrt{Eigen\ values}$$

PCA was applied in this work as a statistical approach to identify major variance components, their contributions, and correlated traits. This dimensionality reduction method assists in reducing the number of traits involved in the data collection and selection process. Principal component analysis uses the correlation values of the traits. The eigenvalues, variance percentages, and cumulative percentages were also obtained to estimate the number of principal components that are sufficient to explain the relationship. The scree plot shows the principal components used to describe the association among the characteristics.

Results and Discussion

Phenotyping for Drought Tolerance

The physio-biochemical responses of the segregating population of 119 individuals in the control, drought stress and recovery phases were studied. The phenotypic characteristics of the clones under the control and drought treatments varied greatly. Observations of the “stay-green” phenotype revealed that the plants exhibited photosynthetic efficiency even under water deficit conditions. Excessive hydrological imbalance conditions modify the biochemical/ physiological behaviour of plants. After rewatering, the plants were allowed to recover. The drought-resistant/ tolerant genotypes of BO 91 x Co 775 resulted in better recovery relatively higher cane yield and better juice quality. This finding revealed the adaptive responses of these cultivars to drought. The REML methodology is the most noteworthy method for family selection.[22] ANOVA revealed highly significant differences among the genotypes for 14 quantitative traits.[8,23-25] Genetic variability is a prerequisite in the selection of germplasm.[26] The analysis of variance, population means and standard errors, genetic variability and heritability of 119 progenies of BO 91 x Co 775 showed a significant level of genetic variance among them (Table 2a and 2b). Drought greatly lowered the cane height ($\bar{x}$ = 171.7) compared with the control ($\bar{x}$ = 227.8). The weights of the cane ($\bar{x}$ = 0.71) and NMC[26] had a reduction with control SCW ($\bar{x}$ = 0.99) and NMC ($\bar{x}$ = 31). Therefore, the cane yield ($\bar{x}$ = 71.15) was 35.68% lower than that of the control. The changes in the behaviour of physiological variables in sugarcane might be due to differences in the genetic constitution or differences in the environment.[27] The variation due to environmental factors or uncontrolled factors is called environmental error variance. The relative CV% of the clones under drought conditions for some of the traits, such as *Fv/Fm*, total chlorophyll content, cane height, internodal length, SCW, and NMC, were greater than those of the control, which is due to their exceptional phenotypic responsiveness to water deficit. Hence, greater environmental care is needed to minimize the influence of these factors on the process of genotype discrimination. There was no reduction in the sucrose-related traits of drought-induced plants. This observation is consistent with the results reported by Terzi et al.[28]

Heritability, and Genetic Advancement of Traits under Control and Drought Conditions

Heritability is used as the key indicator and provides insights into the random inheritance of the parent’s traits to progenies.[29] Heritability estimates along with genetic gains, are more efficient at predicting the outcome of selection. High heritability estimates indicate that these characteristics are less influenced by the environment, so direct selection for these components could be exploited for yield development.

Table 1. ReML Results and Coefficient Variation (CV) of BO 91 x Co 775 Population for each Trait under Control and Drought Regimes

Variation Source	*df*	Mean Sum of Squares (MSS)																
		Germination % at 45 DAP	Tillers at 120 DAP	*Fv/Fm* _early	*Fv/Fm* _peak	Tchl_ early	Tchl_ peak	Cane height (7th month)	Cane height (12th month)	Stalk diameter	Internodes at 7th month	Internodes at 12th month	Internodal length at 7thmonth	SCW	NMC	Brix%	Pol%	CCS%	Cane yield (t/ha)
Treatment	1	590.48	659.10	0.1000	0.1180	0.0020	0.0010	141279.97	313507.79	1.56	77.38	0.99	371.40	7.49	1269.05	14.70	26.83	17.40	152701.20
Genotype	118	298.13	415.28	0.0020	0.0030	0.0001	0.0001	500.12	1791.16	0.13	2.81	25.81	16.49	0.15	163.36	6.25	8.42	5.05	2206.28
Treatment * Genotype	118	131.60	130.79	0.0020	0.0010	0.0001	0.0001	548.04	850.83	0.04	2.99	10.15	20.44	0.04	57.08	1.04	1.35	0.84	961.57
Error	236	106.61	110.76	0.0010	0.0010	0.0000	0.0000	361.24	705.39	0.05	2.23	8.47	14.56	0.04	48.56	1.40	1.89	1.16	1104.17
CV % Control		21.89	27.00	2.5000	3.7300	20.0600	16.3200	16.45	15.09	10.63	15.27	18.23	16.84	28.15	28.43	9.11	12.04	13.66	36.19
CV % Drought		20.88	29.73	5.6500	6.9700	20.4400	21.8000	16.29	17.08	10.80	16.84	15.79	21.62	31.91	34.65	8.98	11.87	13.37	46.93

Table 2a. Variance, Genetic Advance and Heritability of Measured Traits under Well-watered Condition

Trait	Range	Mean ± S.E	Genotypic Variance	Phenotypic Variance	Environmental Variance	GCV%	PCV%	GA	GA (as % of mean)	Heritability%
Germination % at 45 DAP	22.5 - 95	58.61 ± 0.84	43.96	163.99	120.02	11.31	21.85	11.17	19.06	42.28
Tillers at 120 DAP	16 - 90	48.12 ± 0.85	78.09	165.99	87.90	18.37	26.78	17.01	**35.34**	**63.98**
*Fv/Fm*_early	0.69 - 0.80	0.76 ± 0.00124	0.00023	0.00036	0.00012	2.01	2.50	0.03	4.06	78.77
*Fv/Fm*_peak	0.62 - 0.79	0.72 ± 0.00177	0.00041	0.00066	0.00025	2.81	3.56	0.04	5.64	76.68
Tchl_early	0.0115 - 0.036	0.0191836 ± 0.00025	0.0000104	0.0000123	0.0000018	16.85	18.27	0.01	**34.65**	**91.93**
Tchl_peak	0.0065 - 0.0303	0.0144879 ± 0.00016	0.0000030	0.0000036	0.0000005	12.03	13.01	0.00	**24.75**	**92.25**
CHt_7th month	90 - 220	145.20 ± 1.56	224.30	510.11	285.81	10.31	15.55	28.46	19.60	61.08
CHt_12th month	140 - 315	227.77 ± 2.26	494.47	1054.02	559.55	9.76	14.25	42.77	18.78	63.86
Stalk Diameter (cm)	1.83 - 3.34	2.55 ± 0.017	0.02	0.06	0.04	5.86	9.77	0.27	10.66	52.93
Internode count _7th month	6.5 - 15	10.56 ± 0.105	0.62	2.49	1.87	7.44	14.94	1.29	12.26	39.77
Internode count_12th month	13 - 33	21.57 ± 0.259	5.15	14.94	9.79	10.52	17.92	4.09	18.95	51.27
Internodal length_7th month	12.50 - 34.50	22.07 ± 0.24	1.61	9.36	7.75	5.75	13.86	1.85	8.40	29.38
SCW (kg)	0.49 - 1.99	0.99 ± 0.018	0.04	0.07	0.03	19.56	26.37	0.38	**38.62**	**70.98**
NMC	8 - 52	30.49 ± 0.570	31.90	71.75	39.85	18.52	27.78	10.76	**35.28**	**61.55**
Brix%	13.27 - 22.04	18.01 ± 0.108	1.40	2.66	1.26	6.57	9.05	2.32	12.88	68.98
Pol%	10.13 - 20.44	15.87 ± 0.126	1.96	3.61	1.65	8.83	11.98	2.76	17.41	70.44
CCS%	6 - 14.47	10.96 ± 0.098	1.19	2.21	1.02	9.97	13.57	2.15	**19.63**	**70.13**
Cane yield (t/ha)	24.20 - 228.58	110.62 ± 2.63	384.09	1329.46	945.38	17.72	32.96	33.72	**30.48**	**44.83**

The results revealed high heritability and a high genetic advance percentage for the following traits: number of tillers (H^2(b)% = 63.98, GAM% = 35.34), total chlorophyll content at the early stage (H^2(b)% = 91.93, GAM% = 34.65), total chlorophyll content at the peak stage (H^2(b)% = 92.25, GAM% = 24.75), single cane weight (H^2(b)% = 70.98, GAM% = 38.62), NMC (H^2(b)% = 61.55, GAM% = 35.28) and CCS% (H^2(b)% = 70.13, GAM% = 19.63). Cane height (H^2(b)% = 92.25, GAM% = 24.75), Pol% (H^2(b)% = 70.44, GAM% = 17.41), and Brix% (H^2(b)% = 68.98, GAM% = 12.88) exhibited moderate GAMs with high heritable values (Table 2a).

The population under drought treatment also exhibited high heritability and GA mean for traits such as total chlorophyll at early stress (H^2(b)% = 69.89, GAM % = 29.57), total chlorophyll at peak phase (H^2(b)% = 69.05, GAM% = 30.80), single cane weight (H^2(b)% = 61.53, GAM% = 40.58), and NMC (H^2(b)% = 60.93, GAM% = 42.08) (Table 2b). Moderate heritability and GAM estimates were observed for the number of internodes, Brix%, Pol%, CCS%, cane height and cane yield. Low heritability was not found for any traits. These characteristics were the primary constraints in the improvement of sugarcane yield. The results of the heritability studies indicated that traits such as the number of tillers, total chlorophyll content, weight of the individual cane, number of millable canes, cane yield, stalk height and CCS% can be considered for the selection of clones under drought conditions. This statistical approach of a simple selection procedure based on phenotypic performance would eventually lead to the selection of superior genotypes in limited selection cycles.

The selection/hybridization procedure relies mainly on genetic differences and high and moderate heritability percentage estimates. High heritability (broad sense) coupled with high genetic advance in percentage of the mean was recorded for traits such as total chlorophyll content, SCW, Brix, Pol%, cane height, number of tillers, NMC and CCS% which had high heritability (>60%), and medium heritability was observed for stalk diameter and number of internodes under control conditions. However, under drought treatment, the number of tillers, germination%, SCW and NMC were high (>60%), and traits such as the number of internodes, Brix%, Pol%, CCS% and cane height exhibited moderate heritability. Behou and Pene (2020)[30] reported heritability values ranging from 61 to 80.5% for traits such as sugar yield, sucrose content (62.6%), recoverable sucrose (60.6%), fibre content (72%), and internodes/stalk (67.7%). Thus, our study indicated that drought influenced the heritability of these traits.

Heritability and phenotypic variance are inversely proportional, i.e., as heritability increases, the phenotypic variance decreases. Hence, the greater the reduction in environmental variance, the greater the increase in the stability of heritability. High broad-sense heritability indicates a negligible environmental influence on the development of cane yield. High genetic advance indicates the probability of an additive gene effect for that trait. High heritability (>60%) coupled with high expected genetic advance (>20%) for the trait indicates that additive gene action effects and selection might be useful for improvement. The number of tillers, total chlorophyll content, SCW, and NMC satisfy these criteria for both the control and drought treatments. Low heritability (<30%) with low genetic advance (<10%) indicates that the traits are highly influenced by environ mental factors, and selection requires precise care for improving the traits. In our study, no such low heritable traits were observed in the control or drought treatments. Sanghera et al. (2022)[31] reported high broad-sense heritability for the number of internodes (92.89%), followed by total chlorophyll (90.38%), stalk length (84.92%), cane yield (73.55%), and commercial cane sugar (t/ha) (69.65%) under waterlogged conditions.

Phenotypic selection based on high GCV and PCV values (>20) would be a good indicator of genetic potential. If the phenotypic variance is larger than the genotypic variance, it could be concluded that phenotypic selection would be better. The genotypes in both treatments had greater GCVs and PCVs for the number of tillers, SCW, NMC, and cane yield (t/ha) traits. Similarly, Tena et al. (2016)[32] also reported high GCV and PCV for SCW and NMC. The genotypic coefficient of variation (GCV) is another measure of genetic variation of a trait in a population. A higher GCV% for a trait above 20% is favorable for selection. Nair et al. (1999)[24] reported greater PCV than GCV for NMC, cane height, cane diameter, SCW, Pol%, Brix%, and cane yield. In the control plants, higher GCV and PCV were estimated for the number of tillers (GCV% = 18.37, PCV% = 26.78), SCW (GCV% = 19.56, PCV% = 26.37), NMC (GCV% = 18.52, PCV% = 27.78), and cane yield (GCV% = 17.72, PCV% = 32.96).

Similarly, under drought treatment, the magnitude of genetic variance was greater than the magnitude of environmental variance for the traits. Higher GCV and PCV were estimated for the number of tillers at 120 DAP (GCV% = 20.36, PCV% = 26.95), total chlorophyll content (GCV% = 15.70, PCV% = 21.63), single cane weight (GCV% = 21.31, PCV% = 31.97), number of millable canes (GCV% = 22.16, PCV% = 33.47) and cane yield (GCV% = 25.60, PCV% = 47.14) (Table 2b). The drought tolerance trait total chlorophyll fluorescence showed moderate heritability coupled with low GCV%, PCV% and GAM under control and drought conditions. Hence, chlorophyll fluorescence is highly influenced by environmental factors. Moderate GCV and PCV percentages were reported for germination % at 45 DAP and the number of internodes. Low GCV and PCV were recorded for stalk height, CCS%, Pol%, Brix%, and

Table 2b. Variance, Genetic Advance and Heritability of Measured Traits under Drought Treatment

Trait	Range	Mean ± S.E	Genotypic Variance	Phenotypic Variance	Environmental Variance	GCV%	PCV%	GA	GA (as % of mean)	Heritability%
Germination % at 45 DAP	22.5 - 87.5	61.5 ± 0.829	75.25	165.18	89.93	14.11	20.90	16.60	26.99	62.59
Tillers at 120 DAP	14 - 96	50.821 ± 0.975	107.06	187.61	80.55	20.36	26.95	20.53	40.40	72.67
*Fv/Fm*_early	0.222 - 0.78	0.727 ± 0.00265	0.00076	0.000825	0.0000649	3.79	3.95	0.06	7.82	95.91
*Fv/Fm*_peak	0.503 - 0.784	0.686 ± 0.00309	0.000707	0.001332	0.0006251	3.88	5.32	0.05	7.61	69.34
Tchl_early	0.0066 - 0.0249	0.015 ± 0.0002	0.0000053	0.0000099	0.0000046	15.03	20.51	0.00	29.57	69.89
Tchl_peak	0.0042 - 0.0219	0.012 ± 0.00016	0.0000033	0.0000063	0.0000030	15.70	21.63	0.00	30.80	69.05
CHt_7th month	50.75 - 159	108.388 ± 1.14	11.92	216.23	204.30	3.19	13.57	3.17	2.93	10.45
CHt_12th month	80 - 235	171.752 ± 1.917	254.91	862.42	607.51	9.30	17.10	27.64	16.10	45.63
Stalk Diameter (cm)	1.74 - 3.21	2.423 ± 0.017	0.02	0.07	0.05	5.32	10.77	0.21	8.72	39.24
Internode count _7th month	6 - 15	9.696 ± 0.105	0.18	1.60	1.42	4.41	13.06	0.53	5.52	20.47
Internode count _12th month	13 - 31	21.44 ± 0.221	4.68	11.29	6.61	10.09	15.67	4.06	18.96	58.64
Internodal length_7th month	10.25 - 48.5	20.222 ± 0.282	2.69	17.60	14.92	8.11	20.75	2.29	11.34	26.49
SCW (kg)	0.3 - 1.67	0.714 ± 0.015	0.02	0.05	0.03	21.31	31.97	0.29	40.58	61.53
NMC	6 - 52	26.761 ± 0.606	35.16	80.24	45.08	22.16	33.47	11.26	42.08	60.93
Brix%	12.04 - 22.34	17.688 ± 0.104	1.02	2.51	1.49	5.72	8.96	1.89	10.69	57.82
Pol%	8.93 - 20.64	15.409 ± 0.12	1.28	3.31	2.03	7.33	11.81	2.09	13.56	55.68
CCS%	5.61 - 14.57	10.583 ± 0.093	0.75	1.98	1.23	8.16	13.29	1.59	15.00	54.71
Cane yield (t/ha)	8.21 - 213.14	71.148 ± 2.183	331.76	1124.92	793.17	25.60	47.14	31.52	44.30	45.55

Table 3a. Genotypic and Phenotypic Correlation Coefficients of Cane Yield with Various Quality Components of BO 91 x Co 775 Clones under Control

Trait		Germination % at 45 DAP	Tillers at 120 DAP	*Fv/Fm*_early	*Fv/Fm*_peak	Tchl_early	Tchl_peak	Cane height_7th month	Cane height_7th month	Stalk diameter	Internode count_7th month	Internode count_12th month	Internodal length_7th month	SCW	NMC	Brix%	Pol%	CCS%	Cane hectare yield
Germination % at 45 DAP	rg	1.00																	
	rp	1.00																	
Tillers at 120 DAP	rg	0.91	1.00																
	rp	0.80	1.00																
*Fv/Fm*_early	rg	-0.03	0.14	1.00															
	rp	0.07	0.12	1.00															
*Fv/Fm*_	rg	0.21	0.19	0.14	1.00														

Peak	rp	0.16	0.12	0.17	1.00														
Tchl_early	rg	0.46	0.26	0.04	-0.04	1.00													
	rp	0.27	0.18	0.04	-0.06	1.00													
Tchl_peak	rg	-0.05	-0.00	0.06	0.00	0.45	1.00												
	rp	0.02	0.02	0.06	0.05	0.32	1.00												
CHt_7th MAP	rg	0.21	0.22	0.00	0.11	0.07	0.17	1.00											
	rp	0.14	0.15	0.02	0.05	0.12	0.08	1.00											
CHt_12th MAP	rg	0.18	0.07	-0.02	0.20	0.02	0.17	0.79	1.00										
	rp	0.17	0.08	0.01	0.12	0.02	0.10	0.63	1.00										
Stalk diameter	rg	0.01	-0.20	-0.38	-0.18	-0.05	0.05	-0.20	-0.04	1.00									
	rp	-0.08	-0.11	-0.17	-0.11	0.02	0.07	-0.06	0.02	1.00									
Internode count 7MAP	rg	0.05	-0.04	0.06	0.27	-0.21	0.45	0.97	0.78	-0.02	1.00								
	rp	0.05	0.04	0.03	0.10	-0.04	0.22	0.61	0.42	0.04	1.00								
Internode count 12MAP	rg	-0.20	-0.26	0.06	0.30	-0.26	-0.03	0.31	0.58	0.06	0.62	1.00							
	rp	-0.02	-0.10	0.08	0.15	-0.15	-0.03	0.26	0.48	0.04	0.30	1.00							
Internodal length 7MAP	rg	0.83	0.48	-0.18	-0.03	0.35	0.19	0.58	0.58	0.29	0.70	-0.34	1.00						
	rp	0.26	0.17	-0.11	-0.04	0.16	0.07	0.48	0.41	0.12	0.14	-0.04	1.00						
SCW	rg	-0.15	-0.19	-0.17	0.24	-0.02	0.09	0.48	0.65	0.79	0.60	0.58	0.38	1.00					
	rp	-0.02	-0.08	-0.09	0.12	0.01	0.05	0.42	0.64	0.59	0.40	0.46	0.27	1.00					
NMC	rg	0.73	0.77	0.30	-0.09	0.29	-0.05	0.11	0.06	-0.68	-0.20	-0.25	0.45	-0.54	1.00				
	rp	0.48	0.56	0.21	-0.03	0.21	-0.00	0.08	0.05	-0.40	-0.01	-0.14	0.15	-0.30	1.00				
Brix%	rg	-0.33	-0.20	-0.20	0.01	-0.04	0.07	0.29	0.22	0.20	0.28	0.09	0.33	0.50	-0.26	1.00			
	rp	-0.15	-0.11	-0.10	0.01	-0.04	0.02	0.15	0.21	0.08	0.11	0.01	0.06	0.30	-0.16	1.00			
Pol%	rg	-0.38	-0.20	-0.19	0.03	-0.03	0.06	0.29	0.22	0.22	0.28	0.06	0.40	0.52	-0.27	0.99	1.00		
	rp	-0.15	-0.09	-0.10	0.02	-0.02	0.01	0.16	0.19	0.11	0.13	0.01	0.08	0.32	-0.16	0.97	1.00		
CCS%	rg	-0.39	-0.20	-0.18	0.04	-0.03	0.06	0.30	0.22	0.23	0.28	0.05	0.42	0.53	-0.28	0.98	0.99	1.00	
	rp	-0.15	-0.08	-0.11	0.02	-0.02	0.01	0.16	0.18	0.11	0.13	0.01	0.08	0.31	-0.16	0.95	0.99	1.00	
Cane hectare yield	rg	0.63	0.62	0.14	0.15	0.30	0.08	0.61	0.66	0.15	0.48	0.36	0.81	0.49	0.47	0.15	0.19	0.20	1.00
	rp	0.43	0.44	0.13	0.09	0.21	0.05	0.41	0.53	0.12	0.31	0.24	0.37	0.53	0.63	0.06	0.07	0.08	1.00

Table 3b. Genotypic and Phenotypic Correlation Coefficients of Cane Yield with Various Quality Components of BO 91 x Co 775 Clones under Drought

Trait		Germination % at 45 DAP	Tillers at 120 DAP	*Fv/Fm*_early	*Fv/Fm*_peak	Tchl_early	Tchl_peak	Cane height_7th month	Cane height_12th month	Stalk diameter	Internode count_7th month	Internode count_12th month	Internodal length_7th month	SCW	NMC	Brix%	Pol%	CCS%	Cane hectare yield
Germination % at 45 DAP	rg	1.00																	
	rp	1.00																	
Tillers at 120 DAP	rg	0.97	1.00																
	rp	0.86	1.00																
*Fv/Fm*_early	rg	-0.03	-0.10	1.00															
	rp	0.05	-0.01	1.00															
*Fv/Fm*_Peak	rg	-0.08	-0.08	-0.02	1.00														
	rp	-0.00	-0.04	0.13	1.00														
Tchl_early	rg	-0.04	-0.01	0.03	-0.23	1.00													
	rp	0.01	-0.04	0.03	-0.06	1.00													
Tchl_peak	rg	0.09	0.06	0.19	-0.15	0.70	1.00												
	rp	0.08	0.01	0.04	-0.02	0.48	1.00												
CHt_7th MAP	rg	-0.08	0.00	0.09	-0.07	0.48	-0.25	1.00											
	rp	-0.02	-0.02	0.10	0.06	0.21	-0.03	1.00											
CHt_12th MAP	rg	0.28	0.37	-0.02	-0.17	0.22	0.06	-0.24	1.00										
	rp	0.13	0.13	-0.03	0.05	0.05	0.04	0.14	1.00										
Stalk diameter	rg	-0.24	0.06	0.27	-0.00	0.52	0.47	-0.49	0.25	1.00									
	rp	-0.09	-0.01	0.11	0.04	0.18	0.23	-0.02	0.28	1.00									
Internode count 7MAP	rg	-0.29	-0.21	0.00	-0.37	0.27	0.06	0.67	0.04	0.12	1.00								
	rp	-0.17	-0.04	0.10	-0.04	0.08	-0.00	0.52	0.14	0.10	1.00								
Internode count 12MAP	rg	-0.30	-0.20	-0.04	0.14	0.27	0.16	-0.09	0.51	0.65	0.18	1.00							
	rp	-0.19	-0.16	0.06	0.12	0.11	0.03	0.07	0.53	0.37	0.17	1.00							
Internodal length 7MAP	rg	-0.07	-0.16	-0.03	0.13	0.80	-0.28	0.99	0.39	-0.36	0.41	0.48	1.00						
	rp	0.06	-0.06	0.03	0.11	0.34	-0.06	0.51	0.21	-0.09	0.08	0.13	1.00						
SCW	rg	0.07	0.17	0.22	-0.00	0.35	0.41	-0.06	0.68	0.75	0.40	0.68	-0.10	1.00					
	rp	0.03	0.05	0.13	0.10	0.13	0.28	0.10	0.71	0.68	0.23	0.59	-0.00	1.00					
NMC	rg	0.75	0.72	-0.09	-0.14	0.16	-0.14	-0.44	0.15	-0.70	-0.20	-0.21	-0.06	-0.24	1.00				
	rp	0.55	0.48	-0.04	0.05	-0.00	-0.03	-0.00	0.24	-0.22	-0.05	-0.06	0.13	0.00	1.00				
Brix%	rg	-0.22	-0.17	-0.19	0.13	0.35	0.28	-0.54	0.36	0.35	0.21	0.41	-0.26	0.41	-0.24	1.00			
	rp	-0.13	-0.11	-0.14	0.04	0.13	0.14	-0.14	0.20	0.19	0.11	0.19	-0.08	0.27	-0.16	1.00			
Pol%	rg	-0.21	-0.15	-0.18	0.12	0.31	0.23	-0.50	0.38	0.35	0.21	0.43	-0.26	0.41	-0.23	0.99	1.00		
	rp	-0.13	-0.11	-0.14	0.04	0.12	0.11	-0.13	0.21	0.20	0.12	0.19	-0.10	0.28	-0.15	0.99	1.00		
CCS%	rg	-0.21	-0.14	-0.18	0.12	0.30	0.22	-0.49	0.38	0.35	0.21	0.44	-0.27	0.41	-0.22	0.98	0.99	1.00	
	rp	-0.13	-0.11	-0.13	0.04	0.12	0.10	-0.13	0.22	0.20	0.12	0.19	-0.11	0.28	-0.14	0.97	0.99	1.00	
Cane hectare yield	rg	0.62	0.76	0.14	-0.08	0.35	0.21	-0.35	0.66	0.06	0.17	0.44	-0.11	0.65	0.58	0.17	0.19	0.20	1.00
	rp	0.40	0.39	0.06	0.09	0.07	0.15	0.06	0.65	0.31	0.12	0.37	0.09	0.69	0.70	0.08	0.09	0.10	1.00

stalk diameter. To corroborate our results, Singh and Sangwan (1980)[33] also evaluated high genotypic and phenotypic variance for the weight of individual cane and millable cane.

Sanghera et al. (2022)[31] reported greater phenotypic and genotypic coefficients of variation for the total chlorophyll content (32.86% and 31.24%, respectively), while the weight of the individual canes exhibited a high PCV (23.96%) under waterlogged conditions. Additionally, high genetic advance (percent mean) was observed for the total chlorophyll content (61.18%) under waterlogged conditions. The moderate heritability with low genetic advance indicated the existence of nonadditive gene action. Therefore, selection based on phenotype might not be effective.[34] In support of our studies, the results of Hiremath and Nagaraja (2016)[35] showed greater genotypic and phenotypic coefficients for the number of tillers, sugarcane yield, NMC and SCW.

Selection Indices based on Genetic Variability and Correlation Studies

We conducted a combined analysis of genotypic and phenotypic correlations to show the degree of proximity between traits and phenotypic stability. We found a positive correlation of cane yield among all the identified traits, both genotypically and phenotypically. According to Pearson's correlation coefficients, some pairs of qualitative traits showed a high level of correlation. Genotypic (rg) and phenotypic (rp) correlations are presented in Tables 3a and 3b.

Sugarcane yield (t/ha) was highly significantly correlated with germination percentage at 45 DAP (rg = 0.63, rp = 0.43), number of tillers (rg = 0.62, rp = 0.44), cane height at 12th MAP (rg = 0.66, rp = 0.53), internodal length (rg = 0.81, rp = 0.37), SCW (rg = 0.49, rp = 0.53) and NMC (rg = 0.47, rp = 0.63) under irrigated conditions (Table 3a). A moderate relationship was found for other physiological traits and juice attributes, and there was no negative relationship either phenotypically or genotypically. The germination percentage at 45 DAP had a strong positive correlation with the number of tillers, NMC, and cane yield (t/ha). Cane height had a strong positive correlation with the number of internodes and internodal length. The weight of the cane was highly positively correlated with cane height, stalk diameter and number of internodes. Overall, the correlation matrix of the control showed a strongly significant genotypic relationship compared with the phenotypic correlation.

Similarly, under drought treatment, the cane yield had a high positive correlation with the germination percentage at 45 DAP (rg = 0.62, rp = 0.40), number of tillers (rg = 0.76, rp = 0.39), cane height (rg = 0.66, rp = 0.65), SCW (rg = 0.65, rp = 0.69), and NMC (rg = 0.58, rp = 0.70), while the remaining traits showed a moderate positive genotypic correlation (Table 3b). The phenotypic correlation in the drought treatment was weak for some of the traits. The germination percentage had a strong positive correlation with the number of tillers at 120 DAP and under NMC. The number of tillers had a moderate correlation with NMC and a negative correlation with juice attributes. The total chlorophyll content at the early and peak stages showed positive and weak correlations with the other traits except for NMC, which had a negative correlation, and the number of internodes per cane had a moderate positive correlation. Cane diameter had a strong positive relationship with SCW and the number of internodes, whereas a moderately significant relationship existed with the juice attributes. The weight of the individual cane was highly correlated with stalk height, cane diameter and internodes per cane and moderately correlated with juice attributes.

In our present study, cane (t/ha) showed a very strong, highly significant relationship with the weight of the individual cane, NMC, cane height, and number of tillers.[36] Most of the yield-contributing traits had positive or moderate correlations with the other traits. However, some nonsignificant weak relationships also existed among the traits. Lower correlation estimates of the phenotypic matrix revealed that they were influenced by environmental factors. Even though the tiller is a primordial character of sugarcane and is a genetically controlled character, it is influenced by several environmental factors and cultural management.[37-38] Tiller dynamics are genotypic effects and are affected by many other environmental factors. Silva et al. (2007)[39] showed that physiological traits such as PSII maximum quantum yield (the ratio between the variable *Fv* and the maximum chlorophyll-fluorescence *Fm*), leaf total chlorophyll content (SPAD index), and leaf temperature can be employed as indirect and reliable tools for the selection of drought-tolerant sugarcane genotypes. Kohila and Gomathi (2018)[40] conducted an experimental study on the adaptive physiological and biochemical responses of sugarcane genotypes subjected to high-temperature stress. These variables were considered independent variables or predictor variables. Leilah and Al-khateeb (2005)[41] performed seven statistical methods to study the association between the yield and yield components of wheat under drought conditions.

Path Analysis

Path coefficient analysis reveals the interrelationship among the measured variables, which have direct and indirect effects. Path analysis was performed by assigning the cane yield (t/ha) as the primary variable. The direct (uni directional) and indirect (bidirectional) influences of each explanatory variable on cane yield under drought conditions are depicted in Table 4.

A relatively low residual effect (R^2) implies that the choice of the components for path analysis was appropriate.[24] If the correlation coefficient is positive but the direct effect is negative or negligible, then the indirect

Table 4. Genotypic (G) and Phenotypic (P) Path Analysis of Cane Yield and its Contributing Traits of BO 91 x Co 775 Cross under Drought Treatment

		Germination % at 45 DAP	Tillers at 120 DAP	Fv_fm_ early	Fv_fm_ peak	Tchl_ early	Tchl_ peak	CHt_7th month	CHt_12th month	Diameter (cm)	Internode count_7th month	Internode count_12th month	Internode length_7th month	SCW(kg/cane)	NMC	BRIX%	POL%	CCS%	Cane yield (t/ha)
Germination % at 45 DAP	rg	-1.5054	0.0676	0.0362	-0.0277	-0.1355	0.0571	-0.006	0.0382	0.005	0.2171	0.6725	0.0735	-0.6711	1.4759	-0.0438	0.3261	0.0704	0.6229
	rp	-0.3224	0.2096	0.0046	0.0182	0.0013	0.0171	0.0245	0.0287	0.1692	0.0731	0.0635	-0.0090	-0.5522	0.6172	0.5679	-0.6935	0.2360	0.4029
Tillers at 120 DAP	rg	-1.4753	0.0690	0.460	-0.0315	-0.1355	0.0471	-0.0090	0.0591	0.0038	0.2062	0.5934	0.0928	-0.4656	1.3611	-0.0394	0.2934	0.0634	0.7609
	rp	-0.3160	0.2139	0.0099	0.0228	0.0016	0.0233	0.0259	0.0209	0.1398	0.0585	0.0591	-0.0103	-0.4531	0.5874	0.5225	-0.6380	0.2124	0.3883
Fv_fm_early	rg	0.1656	-0.0097	-0.3288	-0.0151	-0.0174	-0.0672	0.0070	-0.0973	-0.0023	0.0036	0.1088	0.0193	0.1893	-0.3608	-0.0306	0.2348	0.0507	0.1373
	rp	0.0129	-0.0171	-0.1161	-0.0171	0.0006	0.0109	-0.0191	0.0678	-0.0147	-0.0073	0.0066	0.0000	-0.0850	-0.0818	0.5111	-0.6241	0.2124	0.0655
Fv_fm_peak	rg	0.3312	-0.0172	0.0395	0.1260	-0.1459	0.1008	-0.0090	-0.0730	0.0002	0.1592	-0.1483	0.0387	-0.2925	-0.3608	0.0123	-0.0913	-0.0197	-0.0840
	rp	0.0516	-0.0428	-0.0174	-0.1138	0.0016	0.0389	0.0054	0.0287	0.0294	0.0292	-0.0066	0.0037	-0.1133	-0.0521	0.1136	-0.1387	0.0425	0.0980
Tchl_early	rg	0.5871	-0.0269	0.0164	-0.0529	0.3475	-0.1549	0.0175	0.0278	-0.0024	-0.1845	-0.3461	-0.1586	0.2753	-0.5412	0.0114	-0.0717	-0.0141	0.3490
	rp	0.0838	-0.0685	0.0139	0.0953	-0.0051	-0.0964	-0.0245	0.0521	-0.0405	-0.0015	0.0022	0.0244	-0.0991	-0.2082	-0.0227	0.0000	0.0047	0.0661
Tchl_peak	rg	0.2559	-0.0097	-0.0658	-0.0378	0.1598	-0.3361	-0.0185	0.0417	-0.0075	0.0036	-0.2472	0.1586	0.9809	-0.3772	0.0403	-0.2804	-0.0606	0.2096
	rp	0.0355	-0.0321	0.0081	0.0284	-0.0032	-0.1555	0.0368	0.0365	-0.1177	0.0278	0.0033	-0.0199	0.2832	-0.1993	-0.1249	0.1248	-0.0378	0.1538
CHt_7th month	rg	0.1806	-0.0124	-0.0460	-0.0227	0.1216	0.1244	0.0501	-0.1181	0.0042	-0.2062	0.1780	-0.3365	-0.6367	-0.1476	-0.0543	0.4043	0.0873	-0.3476
	rp	0.0580	-0.0406	-0.0163	0.0046	-0.0009	0.0420	-0.1362	0.0130	0.0919	-0.0936	0.0044	0.0532	-0.2690	-0.0521	0.5225	-0.6380	0.2171	0.0635
CHt_12th month	rg	-0.1656	0.0117	0.0921	-0.0265	0.0278	-0.0409	-0.0170	0.3475	-0.0037	0.0181	-0.5538	0.0426	1.0925	0.1804	0.0368	-0.2804	-0.0620	0.6595
	rp	0.0355	-0.0171	0.0302	0.0125	0.0010	0.0218	0.0068	-0.2607	-0.1508	-0.0088	-0.0777	0.0007	1.1186	0.0223	-0.1477	0.1942	-0.0708	0.6474
Diameter(cm)	rg	0.7226	-0.0255	-0.0723	-0.0025	0.0799	-0.2454	-0.0205	0.1251	-0.0103	-0.0962	-0.6923	0.1470	1.5487	-0.9347	0.0560	-0.4108	-0.0888	0.0637
	rp	0.1483	-0.0813	-0.0046	0.0091	-0.0006	-0.0498	0.0941	-0.1069	-0.3678	-0.0117	-0.0690	-0.0281	1.2036	-0.4313	-0.3294	0.4022	-0.1369	0.3150
Internode count _7th month	rg	0.9092	-0.0393	0.0033	-0.0554	0.1772	0.0034	0.0285	-0.0174	-0.0010	-0.3618	-0.3066	-0.1818	0.4302	-0.9019	0.0088	-0.0652	-0.0141	0.1733
	rp	0.1612	-0.0856	-0.0058	0.0228	-0.0001	0.0295	-0.0872	-0.0156	-0.0294	-0.1462	-0.0230	0.0096	0.2124	-0.2751	-0.0341	0.0555	-0.0189	0.1162
Internode count _12th month	rg	1.0297	-0.0414	0.0362	0.0189	0.1216	-0.0840	-0.0090	0.1946	-0.0072	-0.1122	-0.9889	-0.0193	1.2906	-1.0331	0.0534	-0.3978	-0.0859	0.4411
	rp	0.1870	-0.1155	0.0070	-0.0068	0.0001	0.0047	0.0054	-0.1851	-0.2317	-0.0907	-0.1095	-0.0090	1.1328	-0.3272	-0.2953	0.3606	-0.1227	0.3679
Internode length _7th month	rg	0.2860	-0.0166	0.0164	-0.0126	0.1425	0.1378	0.0436	-0.0382	0.0039	-0.1701	-0.0494	-0.3868	-0.5162	-0.1148	-0.0403	0.3000	0.0648	-0.1128
	rp	0.0129	-0.0299	0.0000	-0.0057	-0.0017	0.0420	-0.0981	-0.0026	0.1398	-0.0190	0.0044	0.0738	-0.4531	0.0892	0.4998	-0.6241	0.2124	0.0919
SCW (kg/cane)	rg	0.5871	-0.0186	-0.0362	-0.0214	0.0556	-0.1916	-0.0185	0.2085	-0.0093	-0.0905	-0.7417	0.1160	1.7208	-0.8199	0.0534	-0.3978	-0.0859	0.6483
	rp	0.1257	-0.0685	0.0070	0.0091	0.0004	-0.0311	0.0259	-0.2060	-0.3126	-0.0219	-0.0876	-0.0236	1.4160	-0.2900	-0.3180	0.4022	-0.1369	0.6899
NMC	rg	-1.3549	0.0573	0.0723	-0.0277	-0.1147	0.0773	-0.0045	0.0382	0.0059	0.1990	0.6230	0.0271	-0.8604	1.6399	-0.0350	0.2608	0.0564	0.5832
	rp	-0.2676	0.1690	0.0128	0.0080	0.0014	0.0404	0.0095	-0.0078	0.2133	0.0641	0.0482	0.0089	-0.5522	0.7436	0.6134	-0.7351	0.2501	0.6990
BRIX%	rg	0.7527	-0.0310	0.1151	0.0176	0.0452	-0.1546	-0.0910	0.1459	-0.0066	-0.0962	-0.6032	0.1779	1.0497	-0.6559	0.0875	-0.6521	-0.1409	0.1737
	rp	0.1612	-0.0984	0.0523	0.0114	-0.0001	-0.0171	0.0627	-0.0339	-0.1067	-0.0044	-0.0285	-0.0325	0.3965	-0.4015	-1.1359	1.3869	-0.4720	0.0763
POL%	rg	0.7527	-0.0310	0.1184	0.0176	0.0382	-0.1445	-0.0810	0.1494	-0.0065	-0.0962	-0.6032	0.1779	1.0497	-0.6559	0.0875	-0.6521	-0.1409	0.1934
	rp	0.1612	-0.0984	0.0523	0.0114	0.0000	-0.0140	0.0627	-0.0365	-0.1067	-0.0058	-0.0285	-0.0332	0.4106	-0.3941	-1.1359	1.3869	-0.4720	0.0942
CCS%	rg	0.7527	-0.0310	0.1184	0.0176	0.0347	-0.1445	-0.0810	0.1529	-0.0065	-0.0962	-0.6032	0.1779	1.0497	-0.6559	0.0875	-0.6521	-0.1409	0.1991
	rp	0.1612	-0.0963	0.0523	0.0102	0.0001	-0.0124	0.0627	-0.0391	-0.1067	-0.0058	-0.0285	-0.0332	0.4106	-0.3941	-1.1359	1.3869	-0.4720	0.0994

effects seem to be the cause of the correlation. These negative direct effects are due to the indirect negative effects of other influencing traits. Under drought conditions, the path coefficients for the number of tillers (rg = 0.0690, rp = 0.2139), single cane weight (rg = 1.7208, rp =1.4160), and NMC (rg = 1.6399, rp = 0.7436) traits had a positive direct influence on cane yield (Table 4). Traits such as total chlorophyll content at early stress and cane height had a very low negative phenotypic effect and a positive genotypic effect directly on cane yield. Track or path analysis of Silva et al. (2012)[42] suggested that the SPAD index, transpiration and stomatal conductance could be chiefly considered in conventional selection programs for sugarcane targeted for mass production and drought tolerance, and selection indices should be used with due consideration of these variables.

Sara and Balwant (2017)[43] conducted experiments to estimate the significant variance in cane yield and juice attributes for red rot-inoculated and uninoculated early-maturing sugarcane clones. They investigated traits such as the number of shoots in 240 days, the number of millable canes, and the weight of the individual cane, which are the true components contributing to the cane yield. Lal et al. (2018)[44] investigated the variability and heritability of soybean. The relative effect of the number of stalks on yield was confirmed via PCA by Khairwal and Babu (1975).[45] Tyagi and Lal (2007)[46] and Singh et al. (2005),[23] from their correlations and path analysis, suggested that NMC, SCW, and the number of tillers are the best agronomic traits in hybridization programs. Our present study suggests that genotypes from the control with SCW and NMC are the best candidate traits for developing breeding cultivars, and the number of tillers can also be considered for yield improve ment in sugarcane. Under drought conditions, genotypes with a high number of tillers can be selected. Cane height and total chlorophyll content at peak also showed positive genotypic effects at a considerable rate.

The present study demonstrates that effective selection for drought tolerance breeding relies on the number of tillers, total chlorophyll content, SCW, NMC, germination percentage, and cane height to increase higher cane yield (t/ha). Traits that strongly affect cane yield at the genotypic level must be selected for a breeding program to improve high-yielding varieties even under imbalanced hydrological conditions.

Principal Component Analysis

PCA classifies phenotypic traits based on similarity. PCA can be performed by eigenvalue decomposition of a correlation (or covariance matrix). The eigenvalues (scale) and eigenvectors (direction) of correlation or covariance matrices represent the "core" of a PCA and determine the direction of a new feature in a space. Loading matrices are the covariance or correlation between the original variables and the unit-scaled components. The eigenvalues and loading matrices estimated under control and drought conditions are presented in Tables 5 and 6. Through PCA, we were able to identify and segregate clones and determine the principal traits that must be considered when phenotyping sugarcane genotypes. This clustering method serves as a data reduction tool to eliminate the influence of noise/outliers.

Table 5. Eigen Values of the Examined Traits

PC Number	Control			Drought treatment		
	Eigen value	Variance percentage	Cumulative Percentage	Eigen value	Variance percentage	Cumulative percentage
PC1	4.07	22.62	22.62	5.92	32.87	32.87
PC2	2.74	15.22	37.85	2.40	13.35	46.21
PC3	2.16	12.03	49.87	2.01	11.19	57.40
PC4	1.31	7.28	57.15	1.26	6.98	64.38
PC5	1.27	7.07	64.23	1.18	6.55	70.93
PC6	1.13	6.30	70.52	1.10	6.12	77.05
PC7	0.99	5.52	76.04	0.94	5.23	82.28
PC8	0.93	5.14	81.19	0.77	4.30	86.57
PC9	0.78	4.34	85.53	0.60	3.31	89.88
PC10	0.70	3.87	89.39	0.53	2.95	92.83
PC11	0.66	3.66	93.06	0.35	1.95	94.78
PC12	0.41	2.30	95.36	0.33	1.86	96.63
PC13	0.30	1.69	97.05	0.27	1.50	98.13
PC14	0.26	1.43	98.48	0.17	0.93	99.07
PC15	0.20	1.13	99.62	0.13	0.74	99.81
PC16	0.05	0.28	99.89	0.02	0.12	99.93
PC17	0.02	0.11	100.00	0.01	0.07	100.00
PC18	0.00	0.00	100.00	0.00	0.00	100.00

PC 1 to PC 6 – Variance explained 70.52 % (control)
PC 1 to PC 6 – Variance explained 77.05 % (drought)

Table 6. Loading Matrix under Control and Drought

Variable	Control						Drought					
	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Germination % at 45 DAP	0.19	0.59	0.54	-0.09	-0.03	-0.15	0.27	0.76	-0.40	0.12	-0.09	0.22
Tillers at 120 DAP	0.12	0.56	0.61	-0.09	-0.01	-0.17	0.28	0.71	-0.43	0.09	-0.08	0.18
fv_fm_early	0.01	0.18	0.19	0.33	-0.46	0.42	0.05	0.16	0.17	-0.22	0.55	0.48
fv_fm_peak	0.04	0.14	0.08	0.46	-0.51	0.08	0.12	0.04	0.16	-0.41	0.39	0.47
Tchl_early	0.10	0.23	0.23	0.18	0.55	0.38	0.23	-0.04	0.32	0.69	0.06	0.31
Tchl_peak	-0.01	0.16	0.01	0.43	0.37	0.55	0.27	-0.04	0.04	0.71	0.39	-0.05
CHt_7th month	0.50	0.39	-0.37	0.36	0.17	-0.31	0.08	0.27	0.79	0.01	-0.29	0.02
CHt_12th month	0.75	0.24	-0.34	0.01	-0.12	0.00	0.86	0.06	0.12	-0.10	0.04	-0.22
Diameter (cm)	0.46	-0.22	-0.24	-0.39	0.10	0.38	0.84	-0.19	-0.01	-0.01	0.04	-0.04
Internode count _7th month	0.28	0.09	-0.13	0.58	0.16	-0.31	0.04	0.12	0.67	-0.06	-0.08	-0.04
Internode count _12th month	0.55	-0.02	-0.34	0.01	-0.38	0.04	0.81	-0.18	0.11	-0.12	0.11	-0.07
Internode length_7th month	0.34	0.36	-0.20	-0.10	0.34	-0.23	0.10	0.30	0.52	0.00	-0.37	0.26
SCW (kg)	0.76	0.05	-0.45	-0.13	-0.02	0.14	0.79	0.01	0.20	-0.05	0.33	-0.30
NMC	0.27	0.55	0.43	-0.22	-0.10	0.11	0.50	0.64	-0.09	-0.06	-0.11	-0.11
BRIX%	0.68	-0.56	0.43	0.06	0.03	-0.03	0.85	-0.32	-0.15	-0.01	-0.23	0.20
Pol%	0.68	-0.58	0.43	0.08	0.05	-0.06	0.85	-0.35	-0.15	-0.02	-0.23	0.21
CCS%	0.67	-0.57	0.42	0.09	0.05	-0.07	0.83	-0.36	-0.15	-0.02	-0.22	0.21
Cane yield (t/ha)	0.68	0.52	0.00	-0.21	-0.08	0.16	0.68	0.52	0.10	-0.07	0.20	-0.34

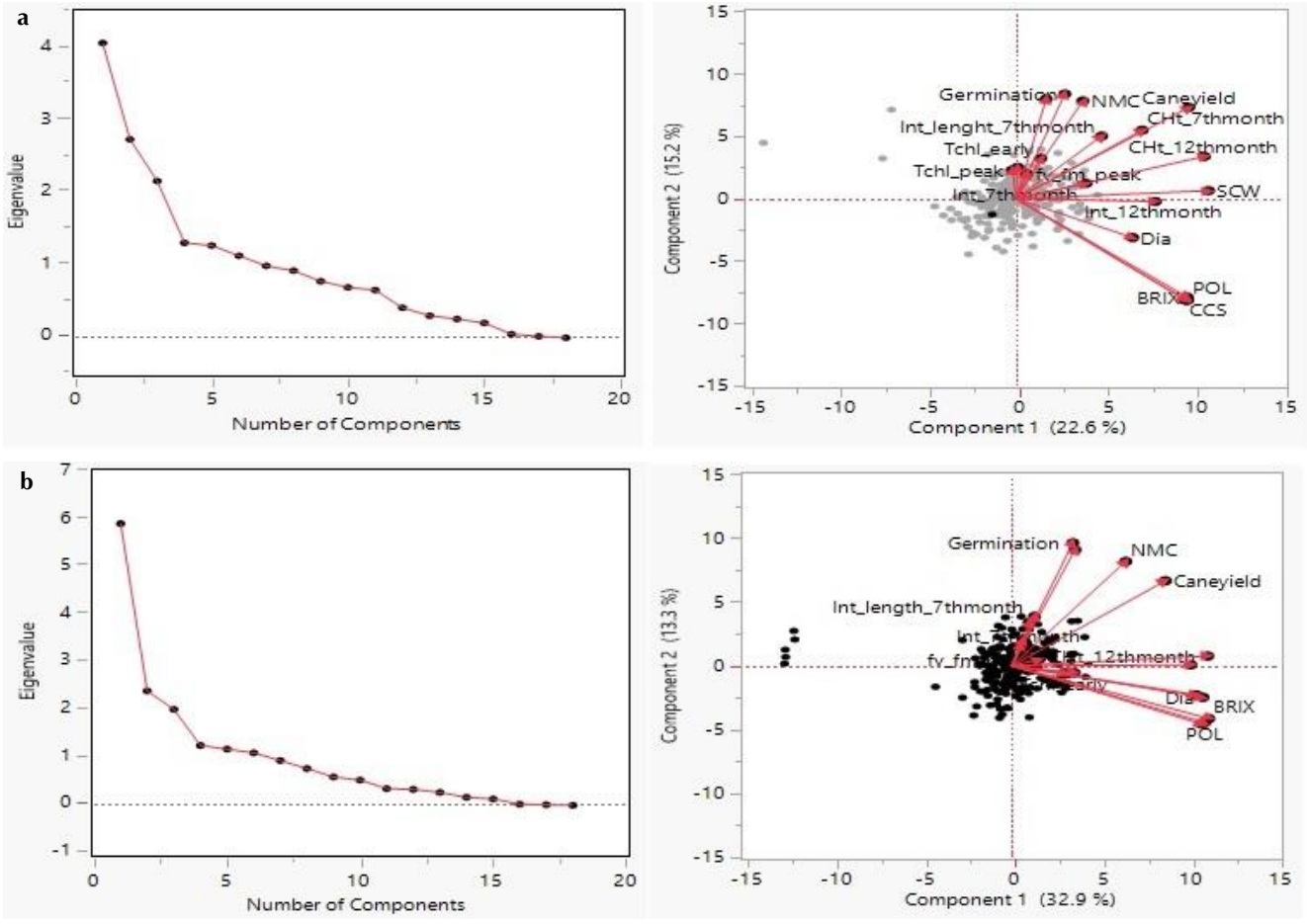

Figure 1. a) Scree plot and Biplot of Principal Components under control condition; **b)** Under drought condition.

Ong'ala et al. (2016)[47] performed PCA to identify the best genotypes for the breeding program. Zhou et al. (2015)[48] simplified four principal components from nine traits comprising cane diameter, millable canes, and cane height. Consistent with our results, Zhou et al. (2015)[48] also stated that the sugar factor was the principal component with the largest possible variance. Similarly, Herbert et al. (1965)[49] noted a strong positive association between cane yield and plant genotype. PCA offers visual differentiation among the entries and identifies possible associations among

the traits.

Figure 1a clearly shows that PC1 to PC4 are significant and Figure 1b shows that PC1 and PC2 are significant. Eigenvalues greater than 1 were used to assign the principal components. In the evaluation of diversity among the BO 91 x Co 775 clones using 14 important agronomic characteristics, the first six principal components explained up to 70.52% of the total variation among the traits in the control group, while in the drought group, PCs explained approximately 77.05% of the cumulative variance (Table 5). The loading matrix of PC1-PC6 is tabulated in Table 6. In the control group, PC1 was a basic component of the SCW due to its high and positive value. In the drought group, cane height was the basic component of PC1. The PCA results (Tables 5 and 6) revealed that the first principal component had the largest possible variance (%), which was farthest from zero, and highly positively correlated with cane yield under the control treatment. Similarly, PC2 explained that 15.22% of the variance had positive values for germination% (0.59), number of tillers (0.56), NMC (0.55), and cane yield (0.52). PC3 explained approximately 12.03% of the variance, with positive values for the number of tillers (0.61), germination% (0.54), NMC, Brix, and Pol% (0.43). PC4 explained only <10% of the variance but had positive values, so it can be considered less important for cane yield. The PCA variability gradually decreased and ended at a variance of 0.11% for CCS %.

Under drought treatment, the primary component PC1 is cane height (Table 6), which has the highest positive value of 0.86 and explains a total variance of about 32.87%, leading to Brix%, Pol%, cane diameter, internode count, CCS%, and SCW with positive values of 0.85, 0.85, 0.84, 0.83, 0.81, and 0.79, respectively. PC2 had a variance of 13.35% and revealed possible positive values for germination% at 45 DAP (0.76), number of tillers (0.71), NMC (0.64), cane yield (0.52), internode length (0.30), cane height at the 7th month (0.27), chlorophyll fluorescence early (0.16). PC3 in drought has explained 11.19% of the variance with high positive values for cane height in the 7th month (0.79), internode counts in the 7th month (0.67), and internode length in the 7th month (0.52). Therefore, it is of less importance to consider.

The scree plot and biplot (genotypes x traits) for control and drought treatments (Figures 1a and 1b) helped us determine the number of components to retain. The negative loadings in the PCs were not significant. The geometrical distances among the traits in the plot revealed the genetic distances and relatedness among them. Therefore, based on the PCA, it could be concluded that the number of tillers, stalk height, SCW, NMC, Brix%, Pol%, and CCS% had positive effects on cane yield under water stress. Additionally, the cane diameter and number of internodes contributed to the cane yield. The results of our investigation indicated that 9 out of 14 traits were significant for identifying superior genotypes and their contribution to drought stress.

Conclusion

We employed the REML statistical procedure due to its accuracy in the selection of sugarcane families in unbalanced datasets and in predicting potential values. REML is more precise than other least squares methods because it takes genetic covariance among treatments into consideration, weighing genotype imbalances within the adopted statistical design. It more precisely reveals information from genetic variability parameter estimates to breeders and leads to further stages of crop breeding programs. The present study evaluated the quality characteristics and juice attributes of water-logged and water-stressed plants in an experimental population. The grand mean average for all drought-related traits decreased compared with those of the control. A lower broad-sense heritability was observed for cane height (10.14%), number of internodes (20.47%) and internodal length (26.49%) under water stress. This suggested that these 3 traits were more sensitive to drought. We found a high heritability for total chlorophyll content (69%) and yield-related traits such as germination% (62.59%), tillers (72.67%), SCW (61.53%), and NMC (60.93%). These genes also exhibited a high percentage of genetic advancement (>30%), which was assumed to indicate additive gene action. There was a highly significant positive correlation between cane yield and germination, number of tillers, internode count, cane height, stalk diameter, SCW, and NMC. A cumulative of six PCs accounted for 77.05% of the cumulative variation in the traits of drought stress. The first component showed high positive loading for cane height, stalk diameter, internode count, internode length, NMC, Brix%, Pol%, and CCS% and there was no negative loading for the *Fv/Fm* or total chlorophyll content parameters. The cultivars derived from these commercial parental varieties exhibit better photosynthetic performance during early drought stress and after recovery. This showed their genetic adaptability and stability. For traits such as germination, tillers at 120 DAP and chlorophyll fluorescence at an early stage, the total chlorophyll content had a stronger influence on cane yield under drought conditions than under irrigated conditions. Then, we partitioned the correlations into direct and indirect effects to explore the relationships among those yield components which were consistent with the results of the overall correlation coefficients. In sugarcane breeding, several authors have quantified the interrelationship between yield and its attributing traits. Here, we suggest that the number of tillers, total chlorophyll content, SCW and NMC could be used as effective selection criteria in breeding programs to develop high-yielding water-resilient sugarcane hybrids under water deficit conditions. Thus, the feasibility

of genomic selection with a potential increase in the rate of genetic gain related to drought tolerance with high yield was explored.[50] Furthermore, we can proceed with genome-wide association studies (GWASs) to identify the genomic markers associated with these traits. The phenotypic stability of these traits favours genetic analysis to determine the QTLs responsible for trait stability. This study of the phenotypic stability of yield and its associated traits in different environments will likely lead to the identification of genes that could be used to develop drought-tolerant sugarcane clones. Therefore, genetic parameter prediction and sugarcane family selection via mixed models of the restricted-maximum likelihood method are deemed to be more effective in ensuring the selection of superior cultivars. The classification of these agronomic traits using multivariate analysis reduces the cost and time of hybridisation programs.

Authors' Contributions

RM: Writing, editing, and project management; AJM: Writing, MS editing, data analysis; JN, RV, KPM, and MG: Observation and recording data; RK and GRS: Data analysis, MS editing; AS and VS: Drought experiment, data analysis; GH: Field experiments, data analysis; KM: Field experiment; JS: Data recording and analysis.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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