DROUGHT STRESS IMPACT ON SUGAR YIELD RELATED TO PHYSIO-BIOCHEMICAL TRAITS OF SUGARCANE *(SACCHARUM* SPP*)*

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Sugarcane is one of the most important commercial crops in Egypt which has a long-life cycle and a high-water requirement in general. Sugarcane production and growth are negatively impacted by water constraint, especially during the key water requirement period. This study compared nine sugarcane genotypes to the commercial genotype GT.54-9 (*Saccharum* spp.) in terms of cane yield, sugar yield, and juice quality related to physio-biochemical attributes including chlorophyll (Chl) Soil Plant Analysis Development (SPAD index), Relative Water Content (RWC), and proline content as a biochemical constituent. Under three levels of drought stress (87.5, 75, and 62.5% of reference crop evapotranspiration (ET0), 2 Way Randomized Complete Block Design (RCBD) with two factors and three replications was used in this study. In the severe water scarcity situations (62.5% of ET0). The results showed that the most promising genotypes (G.2004-27 and G.99-103) had good values for the investigated parameters. The work adds to our knowledge of genotype-screening for drought resistance as an effective technique for choosing materials for advanced breeding programs, especially in controlled drought environments. More genomic and molecular research is required to comprehend the precise processes behind sugarcane drought resilience.

Keywords: *Saccharum*, drought, chlorophyll, relative water content, proline

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is a C4 grass with the distinctive ability to accumulate high sucrose concentrations. Because it produces 65% of the world's sugar and other byproducts, sugarcane is very significant economically (Misra et al., 2020). Sugarcane has a long-life cycle of about a year or more, this implies that it faces all the climate changes that occur over the year (Shrivastava et al., 2016). Sugarcane tillering and grand growth stage are the critical phases of water sensitivity due to the higher water demand for sustainable plant growth and development (Dinh et al., 2017). Changes in global climate pattern have similarly escalated the duration and frequency of various environmental stresses such as water deficit. The most serious environmental stress that contributes to poor agricultural productivity and yield decline is drought stress (Zougmoré, 2018).

Drought is a meteorological term and is commonly defined as a period without considerable rainfall that limits plant productivity, Drought tolerance is the result of coordinated physiological and biochemical alternation at the cellular and molecular levels (Ansari et al., 2019). The productivity of sugarcane has, however, drastically decreased as a result of the increasing drought that the crop is experiencing globally, particularly during the active growing season (tillering/grand growth stage) due to frequent climatic aberrations (Zhao and Li, 2015 and Yadav et al., 2020). Drought affects crop quality and output in addition to slowing down plant development and metabolism at various stages (Basu et al., 2016). Crop plants must have defenses in place to endure drought stress and retain agricultural output (Basu et al., 2016). This is especially important for crops that have been selected for their economic production. The capacity of plants to retain water during desiccation is an important tactic for plant tolerance to stress brought on by water deprivation stress (Mukami et al., 2019). In an effort to develop cultivars that can withstand drought, the inherent tolerance mechanism in agricultural plants has been researched (Tripathi et al., 2022).

Stress breeding is challenging for sugarcane due to its genetic complexity, polyploidy nature, and high levels of chromosomal mosaicism (Yadav et al., 2020). It may be beneficial to have a greater understanding of how the body responds to stress and the linkages between physiological and biochemical traits (Kumari and Kulshrestha, 2017). When choosing drought-tolerant genotypes in breeding programs, the features associated with drought tolerance might be a beneficial guide, reducing the negative effects of a water shortage on agricultural production. Examination of relative water content (RWC) change is the best representation and a simple way for evaluating genetic changes in cellular hydration, plant water deficit, and physiological water status following treatments for water deficit stress (Sánchez-Rodríguez et al., 2010). The most crucial indicator of dehydration tolerance is RWC, which assesses the state of plant water and reflects metabolic activity in tissues

(Tyagi and Pandey, 2022). A reduction in RWC has been observed as a response to drought stress in a variety of plants (Allahverdiyev et al., 2015).

Soil Plant Analytical Development (SPAD) Chlorophyll Meter Reading (SCMR), a physiological characteristic associated with photosynthesis, is a fast method for determining the density of chlorophyll (Chl) in various crops (Darkwa et al., 2016). In order to find characteristics in different plant species that are drought tolerance, SCMR can be employed as a screening strategy (Ruttanaprasert et al., 2016). The nitrogen status of the leaf is determined by SPAD SCMR value, and specific leaf nitrogen is a possible marker that can be used to rectify discrepancies in plant water usage efficiency, according to Kumar et al. (2021), SPAD SCMR can be used to effectively screen large samples (Jangpromma et al., 2010a). According to Zhang et al. (2020), one common reaction of plants to environmental challenges, such as drought, is the accumulation of osmolytes, such as free amino acids. Numerous sugars (such as sucrose, fructose, and trehalose), amino acids (such as proline, glycine, and alanine), and other nitrogen- and sulfur-containing substances have been discovered to play crucial roles in reducing free radicals, protecting membranes and enzymes, and maintaining osmotic balance (Handa et al., 2018). In order to associate an increase in concentration with greater drought tolerance, the amino acid proline accumulation has frequently been used as a physio-biochemical biomarker of water stress (Shao et al., 2009). and a rise in concentration has been linked to a higher tolerance for drought (Molinari et al., 2007).

Proline accumulation has been hypothesized to have a number of functions, such as osmotic regulation, carbon and nitrogen storage for usage during stress recovery, stability of proteins and membranes, and scavenging of reactive oxygen species (ROS) (Kishor et al., 2005). Furthermore, the positive activities of proline and proline accumulation is one of the indicators of drought stress (Cia et al., 2012 and Haghighi et al., 2022). Proline accumulation is one of the indicators of drought stress. Proline, betaines, and sugar alcohols are among the appropriate solutes whose concentrations rise and build up in cells during stressful situations (Chen and Murata, 2002).

This study's goal was to determine how the ten sugarcane genotypes responded to various drought stress conditions by examining the impact of drought on cane yield, estimating sugarcane genotype performance in terms of juice quality and sugar yield, and assessing physio-biochemical traits such as the capacity for relatively quick parameters like SPAD index, RWC, and proline content.

MATERIALS AND METHODS

Ten sugarcane genotypes (GT.54-9, G.2009-11, K 81113, M.35-157, G.2003-49, G.84-47, G.2000-3, G.99-103, G.2004-27 and G.2003-47) were

planted in month of April (spring planting), obtained from Sugar Crops Research Institute (SCRI) (Giza, Egypt). The experiment was conducted in greenhouse at Sugar Crops Institute, Sabahia Agricultural Research Station (30.06263 'N latitude and 31.24967 'E longitude), with an altitude of 10 meters above sea level), in 2020 -2021and 2021-2022 growing seasons

1. Environment Setup

Crop has been grown under all approved practices up to 120 days after planting (DAP). The stress condition was artificially imposed when crop naturally faces such condition, in drought experiment, from June to March (2020- 2021 and 2021-2022) which corresponds to the crop's tillering phase up to the grand growth period.

The soil analysis for the experimental was performed at the Soil Analysis Lab at Agricultural Research Center (ARC), Giza, Egypt, as shown in Table (1 and 2). To maximize the potential production of the genotypes, all suggested cultural practices, such as fertilizers, plant protection, and irrigation, were has been applied.

Table (1). Physical and chemical properties of the experiment's soil before cultivation.

Chemical analysis		
Anions (mEq/L)	$CO_3^{=}$	-
	HCO_3^{-}	1.00
	Cl^{-}	35.40
	$SO_4^{=}$	2.60
Cations (mEq/L)	Ca^{++}	10.70
	Mg^{++}	6.10
	Na^{++}	21.50
	K^{+}	0.70
	pH	7.00
	EC (ds/m)	3.90
	Sp	25.00
Ground moisture constants		
	F.C.%	14.9
	P.W.P.%	8.7
	A.W.%	7.2

Field capacity (F.C.%), permanent wilting point (P.W.P.%), Available water (A.W.%).

Table (2). Mechanical properties of the experiment's soil texture.

Soil grain size distribution (%)	Smooth sand	42.3
	Rough sand	38.5
	Silt	14.2
	Clay	5.00
Soil texture	Sandy	

2. Drought Conditions

In general, sugarcane consumption of water was computed daily as the sum of water loss through transpiration and soil evaporation based on crop water requirement (ETcrop) equation as described by Doorenbos and Pruitt (1977) and Jangpromma et al. (2010b).

Every seven days, watering was provided to the plants. The total amount of irrigation water was determined using the Food and Agricultural Organization's Penman Monteith (PM) method (Koudahe et al., 2018). According to the approach (Allen et al., 1998), potential evapotranspiration (ET0) was determined up to the maturation stage (12 month).

3. Assessment of Parameters

3.1. Productivity traits

Data on cane yield and sugar yield were collected.

(i) Cane yield (ton/fed) was determined from cane weight of each pot (g), which was converted into ton/fed.

(ii) Sugar yield (ton/fed) was calculated according to the following equation as described by Mathur (1975): Sugar yield/ fed (ton) = cane yield/fad (ton) x sugar recovery %

3.2. Juice quality analysis

To determine quality features, all stalks were crushed, and juice was analyzed.

(i) Sugar recovery % (SR) was computed using Yadav and Sharma (1980)`s formula: SR = [Sucrose % - 0.4 (Brix % - Sucrose %) x 0.73].

(ii) Brix (total soluble solids) a refract meter was used to determine percentage of total soluble solids in cane juice.

(iii) Purity (percentage of pure sucrose in cane juice) was calculated according to the following equation: Purity (%)= (Sucrose %/ Brix) x 100 as described by Singh et al. (2016).

(iv) Sucrose percentage of clarified juice was calculated using an automated Saccharimeter as described by Motohashi et al. (1996).

3.3. Physiological parameters

3.3.1. Chlorophyll

It was determined the relative Chl content. A measurement of Chl was made between the hours of 9:00 and 12:00 am using a SPAD (Soil Plant Analytical Development) chlorophyll meter reading (SCMR) SPAD502-Plus (Konica Minolta, Tokyo, Japan), according to Namwongsa et al. (2018). After calibrating, SPAD meter was shut by squeezing it without inserting a leaf, measurements were taken for each control and treated on the second or third fully developed leaf from the top of the main stem. The top head of the Chl meter was selected, and the completely developed leaf was pressed. Three measurements were made, with the average value being recorded (Kumar et al., 2021).

3.3.2. Relative water content

To evaluate the severity of the drought, RWC was calculated. RWC was determined for both the control and treatment samples using the methodology reported by Barrs and Weatherley (1962) and Kumar et al. (2021) with a few minor modifications. With the aid of scissors, the collected leaf samples were removed from the region between the midvein and the margin at approximately 1*6 cm. When leaf discs were first weighed, they were immediately hydrated to full turgidity by floating in closed petri plates of deionized water for 24 h at 4°C in the refrigerator. After hydration, samples were removed from the water, rapidly and gently dried and weighed right away to get turgid weight. After that, samples were heated in an oven. After 48 h of oven drying at 80°C, samples were weighed (dry weight as mg).
Using the following formula, RWC of leaves was calculated:

RWC = (FW - DW/ TW – DW) x 100, where:

FW= Fresh weight
DW= Dry weight
TW= Turgid weight

4. Biochemical Constituents

4.1. Free Proline Content

Proline was extracted and estimated by the colorimetric method (Bates et al., 1973 and Tripathi et al., 2022) with some modifications, where level of proline was expressed in µmol proline/g dry weight, 0.25 g of dry leaves were ground and homogenized in 10 ml of 3% sulfosalicylic acid and centrifuged for 15 min at 10,000 rpm, the extract was filtered with Whatman filter paper. The reaction mixtures in the test tubes were vigorously shaken and contain (2 ml of the filtrate, 2 ml of the ninhydrin reagent, and 2 ml of glacial acetic acid) and vigorously shacked. The reactions were terminated on ice for 10 min after an hour of heating in a water bath (65°C). Four ml of toluene were added to the tubes after the content had cooled and was swirled for 20–30 s. The toluene layer was separated using a separating funnel. Thermo Scientific UV-Vis spectrophotometer was used to determine the intensity of the red colour at 520 nm. Proline concentration was expressed as mg of proline per gram of dry weight (D.W.) for the leaf sample.
The following formula was used to determine proline content:

$$\frac{\text{µg proline} \times \text{ml toluene} / 115}{\text{g sample dry weight} / 5}$$

5. Statistical Analysis

Combined analysis of the two seasons was carried out and homogeneity of variance as well was detected for the studied characters. Statistical analysis using 2 Way Randomized Complete Block Design (RCBD) with three replications. The data of two seasons were combined and

analyzed using the computer "CoStat" statistical analysis version 6.311 (CoHort software, Berkeley, CA 94701).

The principal component analysis (PCA) was used to display the correlation between various physio-biochemicals and sugar yield and their relationship with ten sugarcane genotypes. The PCA analysis was carried out using the JMP genomics version 17.1.0.

RESULTS

At maturity (i.e. 12 months after planting), the ten sugarcane genotypes were screened for drought tolerance depending on yield attributes and juice quality related to physio-biochemical traits.

1. Yield Attributes

Data in Table (3) indicate that using the stressed irrigation level of 87.5% of ET0 revealed an appreciable decrease in cane yield amounting to 3.8%, in M.35-157 genotype, corresponds to 18.5 and 46.3% in genotypes M.35-157 and G.2004-27, respectively. In the stressed level of 75% of ET0, as compared with that irrigated at the stressed level (62.5%) of ET0, cane yield decreased by 43.45 and 82.004% in genotypes G.99-103 and K.81113, respectively. The evaluated sugarcane genotypes varied markedly in cane yield/fed in combined seasons. Sugarcane G.2000-3 occupied the 1st order in cane production over the other genotypes in the stressed level irrigation of 87.5 and 75% of ET0, respectively. Moreover, the lowest cane yield/fed was recorded by G. 2003-47 and K.81113 genotypes at the two stressed levels of 87.5 and 75%, respectively.

2. Sugar Yield

Data in Table (3) reveal that most of sugar yield/fed were significantly affected by the highest stressed irrigation level of 62.5% of ET0. Applying at the stressed irrigation level of 87.5% of ET0 to sugarcane showed insignificantly effect in the genotypes M.35-157, G.2003-49, G.84-47 and G.2003-47, respectively, which recorded simple significant at the stressed level of 75% of ET0. On the other hand, at the highest stressed irrigation level of 62.5% of ET0, the genotypes M.35-157 and G.2003-49 showed high significant differences. On the other hand, the two genotypes G.84-47 and G.2003-47 had simple significant differences. These results are probably attributed to the decrease in cane yield/fed and sugar recovery as mentioned before.

Sugar yield varied from 4.9 to 8.63 ton/fed, for GT.54-9 and G.2000-3 at the stressed irrigation level of 87.5% of ET0, and varied from 4.608 to 6.825 ton/fed for K.81113 and G.2009-11 at the stressed irrigation level of 75% of ET0, while, it varied from 2.975 to 5.716 ton/fed for K.81113 and G.84-47 at the stressed irrigation level of 62.5% of ET0. The poorest sugar

Table (3). Effects of drought cycle on cane yield (ton/fed), sugar yield (ton/fed) and sugar recovery (%) of sugarcane genotypes.

ETC	Cane yield (ton/fed)					Sugar yield (ton/fed)					Sugar recovery (%)				
	Control	87.5%	75%	62.5%	M.D	RED	Control	87.5%	75%	62.5%	M.D	RED	Control	87.5%	75%	62.5%	M.D	RED
GT.54-9	77.070	57.600^{c}	45.500^{b}	33.700^{ab}	45.60	77.50	5.629	4.900^{c}	4.750^{c}	4.106^{b}	4.580	144	13.960	11.760 ^{b}	9.570^{ab}	8.206^{a}	9.800	111.5
G.2009-11	110.300	74.960^{b}	70.490^{ab}	34.905^{a}	60.12	63.50	7.900	7.180^{c}	6.825^{c}	3.790^{a}	5.900	125	13.916	10.439^{ab}	10.338^{ab}	9.196^{ab}	9.990	115.0
K81113	99.970	66.570^{b}	41.590^{a}	17.990^{a}	42.05	26.10	7.230	5.360^{c}	4.608^{b}	2.975^{a}	4.300	79	13.820	12.401^{bc}	9.025^{ab}	6.038^{a}	9.150	98.7
M.35-157	72.460	69.700^{ns}	59.026^{c}	23.580^{ab}	50.76	110.00	6.350	6.180^{ns}	5.890^{c}	3.470^{b}	5.180	144	11.420	11.250^{ns}	10.010^{c}	6.790^{ab}	9.350	124.9
G.2003-49	89.119	72.690^{c}	54.330^{b}	34.630^{a}	53.88	81.30	6.679	6.650^{ns}	5.160^{c}	4.375^{b}	5.395	124	13.310	10.915^{b}	10.520^{b}	7.920^{a}	9.785	120.5
G.84-47	83.890	67.680^{c}	51.730^{b}	37.710^{ab}	52.37	87.29	7.525	7.350^{ns}	5.950^{c}	5.716^{c}	6.300	150	11.117	9.190^{bc}	8.690^{b}	6.600^{a}	8.160	120.0
G.2000-3	127.890	109.446^{c}	81.040^{ab}	32.760^{a}	74.40	74.50	9.420	8.630^{c}	6.530^{b}	5.130^{a}	6.760	115	13.570	12.670^{c}	12.405^{bc}	6.380^{a}	10.480	131.7
G.99-103	84.715	67.480^{c}	62.590^{c}	47.900^{b}	59.30	110.00	7.580	6.734^{c}	6.240^{c}	5.016^{b}	5.970	137	11.160	10.019^{bc}	10.030^{bc}	9.548^{bc}	9.860	165.0
G.2004-27	91.390	60.186^{b}	49.060^{ab}	30.429^{a}	46.55	52.80	8.225	5.860^{b}	5.020^{a}	4.345^{a}	5.075	85	11.100	10.220^{c}	9.780^{bc}	7.013^{ab}	9.000	143.0
G.2003-47	69.240	49.029^{c}	44.860^{bc}	34.690^{b}	42.85	85.700	5.775	5.513^{ns}	5.160^{c}	4.140^{c}	4.900	156	11.940	8.890^{ab}	8.720^{ab}	8.370^{ab}	8.660	217.5

Different letter indicates significance difference as per LSD at 5% level.

yield among the test genotypes was GT.54-9 under 87.5% of ET0; in the contrary, the highest values observed in G.2000-3 was 8.63 at ton/fed, compared to the control.

3. Juice Quality

3.1 Sugar recovery

Data in Table (3) from the combined analysis, show that sugar recovery % ranged from 11.1 for G.2004-27 to 13.96 for the commercial genotype GT.54-9. The highest sugar recovery % was recorded for the genotype GT.54-9 (13.96%), followed by the genotypes; G.2009-11 (13.916%) and K.81113 (13.82%), respectively. All the previous genotypes gave significantly sugar recovery % as compared to the commecial genotype GT54-9 (11.76%) in the stressed level of 87.5% of ET0, the genotypes; G.2000-3 (12.405%), G.2003-49 (10.52%) and G.2009-11 (10.338%), respectively insignificantly surpassed the commercial genotype GT54-9 (9.57%). At the stressed level of 75% of ET0, the genotypes G.99-103 (9.548%) and G.2009-11 (9.196%) insignificantly surpassed the commercial genotype GT54-9 (8.206%) at the stressed level of 62.5% of ET0. Sugarcane G.99-103 promising genotype gave the highest sugar recovery (9.548%), while K.81113 recorded the lowest value of this trait (6.038%), in the high stressed level of 62.5%. Such varietal differences can be referred to the same trend of both sucrose % and juice purity % (Table 4) recorded by the previously mentioned varieties.

3.2. Brix

Brix values in normal conditions ranged from 21.75% in genotype GT.54-9 to 23.66% in G.2009-11, followed by G.2003-47 and K.81113 (23%), respectively (Table 4). The interacting effects of drought to genotypes on brix were varied insignificantly in the stressed level of 87.5% of ET0, while varied significantly in both the two stressed level of 75 and 62.5% of ET0, values in genotypes grown in the stressed irrigation level of 87.5% of ET0 ranged from 21.5% in GT.54-9 and G.84-47 to 23% in G.2009-11, while, in the stressed irrigation level of 75% of ET0 it ranged from 21.16% in G.84-47 to 22.66% in G.2009-11. At the stressed irrigation level of 62.5% of ET0, the highest brix was obtained in G.2003-47 (21.75%) followed by G.2004-27 which was 21.66%. The mean reduction in brix due to drought stress was 3.38% showing a marginal increase in the total sugars under drought. The minimum reduction observed was 179% in M.35-157 and the maximum was 190% in GT.54-9, G.2003-49, G.84-47 and G.99-103 genotypes, respectively.

3.3. Purity

Data in Table (4) show that at the stressed irrigation levels of 87.5 and 75% of ET0 the genotype G.2000-3 recorded the highest mean value of 67.028 and 66.86%, respectively and the highest mean at the stressed level of 62.5% of ET0 was in the genotype G.99-103.

3.4. Sucrose

Table (4) shows that some genotypes significantly recorded higher mean values of sucrose % in the cane plant as compared to commercial genotype GT54-9. Sucrose % varied from 13.57% for G.84-47 to 16.12% for G.2009-11, where it attained 4.13%, compared with the commercial genotype GT54-9. Despite this, sucrose % ranged from 13.57% in control genotype G.84-47 to 9.55% at the least stressed irrigation levels (62.5%) of ET0 for K.81113 that gave significantly the highest mean value of sucrose % in the three stressed irrigational levels as compared to other genotypes, which was 13.65% of the mean of the commercial genotype GT.54-9. However, sucrose content in normal conditions ranged from 13.57% in G.84-47 to 16.12% in G.2009-11, under the stressed irrigation levels of 87.5% of ET0 ranged between 11.95% in G.2003-47 to 14.8% in G.2000-3. While at the stressed irrigation levels of 75% of ET0, the range was 11.51% in G.84-47 to 14.5% in G.2000-3 and under the least stressed irrigation levels of 62.5% of ET0, the range was 9.55% in K.81113 to 12.15% in G.99-103. Mean sucrose % under normal and drought conditions was 14.77 and 12.17%, respectively. The maximum reduction being 175.77% in G.99-103, as compared to 127.5% in K.81113.

4. Physiological Parameters

4.1. Chlorophyll

The SCMR suggests an efficient screening and determines nitrogen status of leaf. The SCMR was affected by genotypes, water regime and the interaction between these factors at all the stressed irrigation levels of 87.5, 75 and 62.5% of ET0. At 90 days after stress, the largest decrease in the SCMR occurred in G.99-103 followed by K.81113 genotype at both the stressed irrigation levels of 87.5 and 75% of ET0. At the stressed irrigation level of 62.5% of ET0, only the tolerant genotypes (M.35-157 and G.2000-3) had its high SCMR index values. The SCMR was maintained at an average of 21.33% for G.2000-3, which suggested a higher capacity to keep the leaf area greener to conserve the photosynthetic pigments during drought conditions. Table (5) showed that the reduction was ranged from 1.13 to 22.017% in G.2003-49 and G.99-103 genotypes at the stressed irrigation levels of 87.5% of ET0, while at the stressed irrigation levels of 75% of ET0 it was noticed that the reduction was ranged from 4.49 to 25.5% in G.2003-49 and K.81113 genotypes and at the stressed irrigation level of 62.5% of ET0 the reduction was ranged from 5.78 to 48.9% in G.2003-49 and G.99-103 genotypes, respectively. So, the genotype G.99-103 was the most stressed at the highest irrigation level of 62.5% of ET0 and the least stressed was the genotype G.2003-49 at the three stress levels.

4.2. Relative water content

Drought stress and the genotypes showed significant effects on RWC (Table 5). Genotype G.2003-47 produced the highest RWC (96.72), followed

Table (4). Effects of drought cycle on brix (%), sucrose and purity (%) of sugarcane genotypes.

ETC	Brix (%)					Sucrose (%)					Purity (%)				
	Control	87.50%	75%	62.50%	M.D	RED	Control	87.5%	75%	62.5%	M.D	RED	Control	87.50%	75%	62.50%	M.D	RED
GT.54-9	21.750	21.50^{ns}	21.25^{ns}	20.83^{c}	21.2	190	15.480	13.96^{bc}	12.21^{b}	11.06^{a}	12.41	140.00	71.130	64.940^{c}	57.535^{b}	53.240^{b}	58.570	147.039
G.2009-11	23.660	23.00^{ns}	22.66^{c}	20.83^{b}	22.2	180	16.120	13.27^{b}	13.13^{b}	11.83^{a}	12.70	137.00	68.120	57.700^{b}	57.970^{b}	56.910^{b}	57.530	153.350
K81113	23.000	22.33^{ns}	22.08^{c}	21.58^{c}	21.9	186	15.896	14.65^{bc}	11.98^{b}	9.55^{a}	12.06	127.50	69.127	65.630^{c}	54.260^{b}	44.320^{a}	54.800	137.500
M.35-157	22.290	21.66^{ns}	21.25^{c}	19.50^{b}	20.8	179	13.870	13.65^{ns}	12.55^{bc}	9.66^{a}	11.90	158.20	62.290	62.820^{ns}	59.070^{c}	49.560^{b}	57.150	175.235
G.2003-49	22.660	21.83^{c}	21.78^{c}	21.25^{c}	21.6	190	15.425	13.38^{b}	13.06^{b}	10.95^{a}	12.45	142.30	68.100	61.410^{c}	60.015^{c}	51.470^{b}	57.600	153.880
G.84-47	22.000	21.50^{ns}	21.16^{c}	20.66^{c}	21.1	190	13.570	11.98^{bc}	11.51^{b}	9.78^{ab}	11.09	145.14	61.690	55.680^{c}	54.570^{c}	47.360^{b}	52.540	155.490
G.2000-3	22.616	22.17^{ns}	21.80^{c}	21.50^{c}	21.8	189	15.616	14.80^{c}	14.5^{b}	9.80^{a}	13.04	150.50	69.127	67.028^{ns}	66.860^{ns}	45.590^{a}	59.830	159.600
G.99-103	22.166	22.42^{ns}	21.66^{ns}	21.00^{c}	21.7	190	13.640	12.80^{c}	12.66^{c}	12.15^{bc}	12.50	175.77	61.620	58.450^{c}	57.850^{c}	57.235^{c}	57.850	181.600
G.2004-27	22.660	21.87^{c}	21.66^{c}	21.66^{c}	21.7	187	13.716	12.86^{c}	12.47^{bc}	10.33^{b}	11.88	159.88	60.520	58.780^{ns}	57.680^{c}	47.770^{b}	54.740	171.360
G.2003-47	23.000	22.42^{ns}	22.00^{c}	21.75^{c}	22.1	188	14.440	11.95^{b}	11.72^{b}	11.46^{b}	11.69	142.80	62.830	53.310^{c}	53.530^{c}	52.427^{bc}	53.088	153.600

Different letter indicates significance difference as per LSD at 5% level.

Table (5). Effects of drought cycle on chlorophyll index (SPAD) and RWC of sugarcane genotypes.

ETC	SPAD chlorophyll meter reading value						Relative water content (RWC %)					
genotypes	Control	87.50%	75%	62.50%	M.D	RED	Control	87.50%	75%	62.50%	M.D	RED
GT.54-9	21.93	19.840^{c}	18.87^{bc}	18.40^{bc}	19.037	160.45	83.39	82.58^{ns}	80.57^{ns}	68.31^{b}	77.15	177.56
G.2009-11	21.27	19.580^{c}	19.13^{c}	14.16^{a}	17.630	148.50	87.41	71.12^{b}	70.47^{b}	50.73^{a}	64.15	120.02
K81113	20.34	19.190^{c}	15.15^{ab}	14.69^{ab}	16.340	141.05	86.02	73.42^{b}	62.45^{ab}	61.18^{a}	65.70	129.12
M.35-157	28.83	24.780^{b}	23.05^{ab}	21.08^{a}	22.970	139.05	86.75	79.43^{c}	64.73^{ab}	64.38^{a}	69.51	140.39
G.2003-49	20.24	20.011^{ns}	19.33^{ns}	19.07^{ns}	19.470	188.58	88.47	80.9^{c}	65.86^{ab}	57.95^{a}	68.26	131.38
G.84-47	22.34	20.116^{c}	19.06^{bc}	18.12^{b}	19.097	156.47	81.31	71.93^{c}	64.41^{b}	61.47^{ab}	65.95	143.28
G.2000-3	25.11	22.750^{c}	21.76^{bc}	21.33^{bc}	21.950	162.21	93.90	84.43^{c}	73.90^{ab}	57.09^{a}	71.81	129.40
G.99-103	23.39	18.240^{ab}	17.89^{ab}	11.95^{a}	16.027	105.55	85.25	62.68^{ab}	62.45^{ab}	28.07^{a}	51.06	79.70
G.2004-27	24.66	22.104^{c}	19.19^{ab}	18.90^{ab}	20.065	144.08	85.98	81.45^{c}	80.25^{c}	68.40^{c}	76.70	167.60
G.2003-47	22.47	22.110^{ns}	18.83^{bc}	17.14^{ab}	19.360	158.46	96.72	78.13^{b}	65.18^{a}	55.73^{a}	66.35	105.78

Different letter indicates significance difference as per LSD at 5% level.

by G.2000-3 (93.9) at the control condition. The interacting effects of drought to genotypes on RWC were varied significantly at different levels of water stress. The highest RWC obtained by genotype G.2000-3, GT.54-9 and G.2004-27 were 84.43, 80.57 and 68.4 at 87.5, 75 and 62.5% water stress of ET0, respectively. The results estimated the average reduction in RWC by 79.7% in genotype G.99-103 to 177.56% in genotype GT.54-9 with an average of 132.4% at the stressed irrigation levels of ET0. All genotypes had a reduction at the three stressed irrigation levels, at the level 87.5% of ET0, the reduction ranged from 0.97 to 26.47 in GT.54-9 and G.99-103 genotypes, at the stressed irrigation level of 75% of ET0, the reduction ranged from 3.38 to 32.6 in GT.54-9 and G.2003-47 genotypes. At the stressed irrigation levels 62.5% of ET0, the reduction ranged from 18.08 to 67.07 in GT.54-9 and G.99-103 genotypes. The high RWC reduction in genotypes G.99-103 and G.2003-47 at the highest stressed level (62.5%) of ET0 is a strong indicator of these plants' sensitivity to drought. A progressive decline in RWC varyied between 67.07 and 42.38%.

5. Proline Content

Proline accumulation was induced in all genotypes at the stressed levels of ET0, at the stressed level 87.5% of ET0 (Fig.1) there was a clear decline in all genotypes except the genotype G.99-103, there was a slight increase. In contrast, at the stressed level 75% of ET0, the proline content of all genotypes was declined and there was evidence of a reduction, and from the stressed level of 75% to the highest stressed level of 62.5% of ET0, the most genotypes slightly increased. The maximum proline content released in G.84-47 (0.6284 μg/g) and the minimum proline content released by K.81113 (0.0985 μg/g) in Fig. (1).

6. The Physio-biochemical Parameters

Table (6) summarizes the results from the combined analysis of variance for productivity, juice quality and physio-biochemical traits, hence, separate analysis of variance showed highly significant differences among the main effects of genotypes, water stress and their interactions.

7. Principal Component Analysis

The principal component analysis (PCA) of the control and the three stressed treatments (87.5, 75 and 62.5%) of ET0 and their correlation with physio-biochemical, yield attributes and juice quality parameters are shown in Fig. (2). In the present study, PC1 represented 87.7, 76.5, 66.6 and 74.8% of the variability in control, treatment I, II and III, respectively indicating varietal variability for productivity, juice quality and physio-biochemical traits. Maximum variability was observed at treatment I (87.5%) of ET0 showing the differential behavior of genotypes under stress compared to the control condition, thus revealing the impact of drought. PCA also revealed that, the stress level of 87.5% of ET0 had moderately less variability in yield between

genotypes compared to control, which can be attributed to the near ambient condition. Sugarcane genotypes occupied on both right and left side of the bi-plot and among the parameters RWC, Chl, purity, SUC, SR, sugar yield, cane yield and brix were observed on the left side of the bi-plot, while proline was observed in the down of right side of the bi-plot in the stress level of 87.5% of ET0. At the stress level of 75% of ET0, the parameters RWC and Chl were observed on the left side of the bi-plot, while the parameters cane yield, purity, SUC, SR and sugar yield were among proline and brix at the right side of the bi-plot having positive correlation among themselves, and at the stress level of 62.5% of ET0. Similarly, a positive correlation was observed between proline, SUC, sugar yield and cane yield also between RWC and Chl. On the other hand, there was a negative correlation between cane yield, brix and proline at the treatment I. At the treatment II the positive correlation was observed between Chl & sugar recovery, sugar yield & cane yield and SUC & purity. At the treatment III, similarity was observed between purity & cane yield, sugar yield & sugar recovery and sugar recovery & SUC, these traits can be considered as potential physiological and biochemical traits for screening sugarcane genotypes. The PCA indicated that under the highly stressed level proline, brix, Chl and RWC have much influence during water stress followed by GT.54-9 (negative side of bi-plot under drought conditions). Similar multivariate comparison of varieties and traits association in response to drought (Queiroz et al., 2011) and thermo tolerance (Gomathi et al., 2016) selection and can be selected together. The genotypes, G.84-47 and G.2003-49 were found to be susceptible sugarcane genotypes.

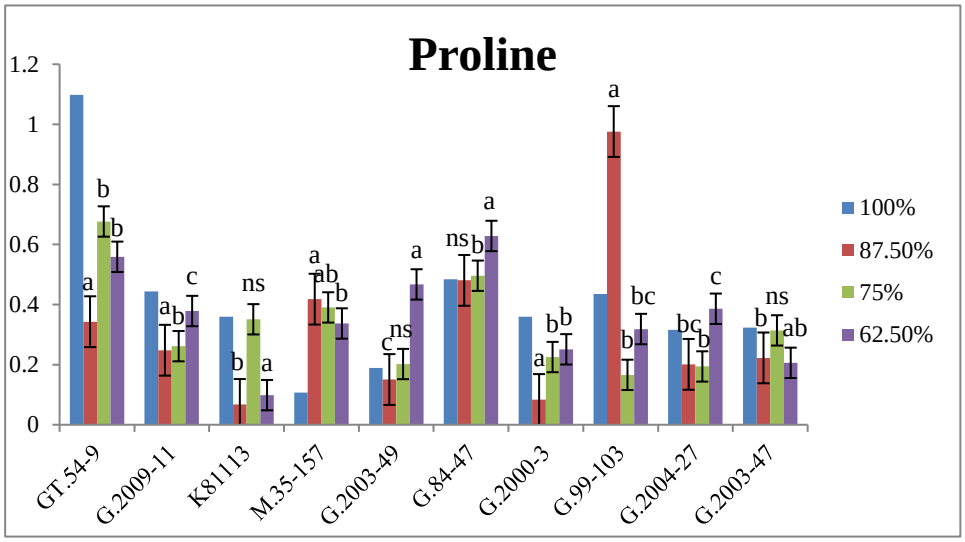

Fig (1). Proline content in ten sugarcane genotypes under drought stress.

Table (6). Results of one-way ANOVA for selected physio-biochemical parameters related to sugar yield of ten sugarcane genotypes under drought stress and control.

Source of Variation	df	F ratio and probability								
		Productivity traits			Juice quality			Physiological attributes		Biochemical traits
		C.Y	S.Y	S.R	Brix	Purity	SUC	Chl	RWC	Proline
Blocks	2	12.195***	2.725^{ns}	14.58***	2.9^{ns}	14.2***	9.58***	3.39*	1.106^{ns}	3.386*
Drought (D)	3	547.79***	446.29***	311.4***	14.85***	140.35***	352.99***	59.78***	179.89***	97.02***
Genotypes (G)	9	54.87***	76.445***	16.9***	3.01**	9.17***	18.42***	19.9***	14.5***	492.05***
G*D	27	9.55***	11.22***	8.95***	0.45^{ns}	5.49***	8.438***	1.96*	4.5***	190.29***
Error	78									
Total	119									
C.V. %		9.108	5.496	6.19	4.15	4.898	3.906	8.35	7.14	6.58

DF degrees of freedom, level of significance ***, **, * and NS indicate significant difference at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$ and non-significant, respectively.

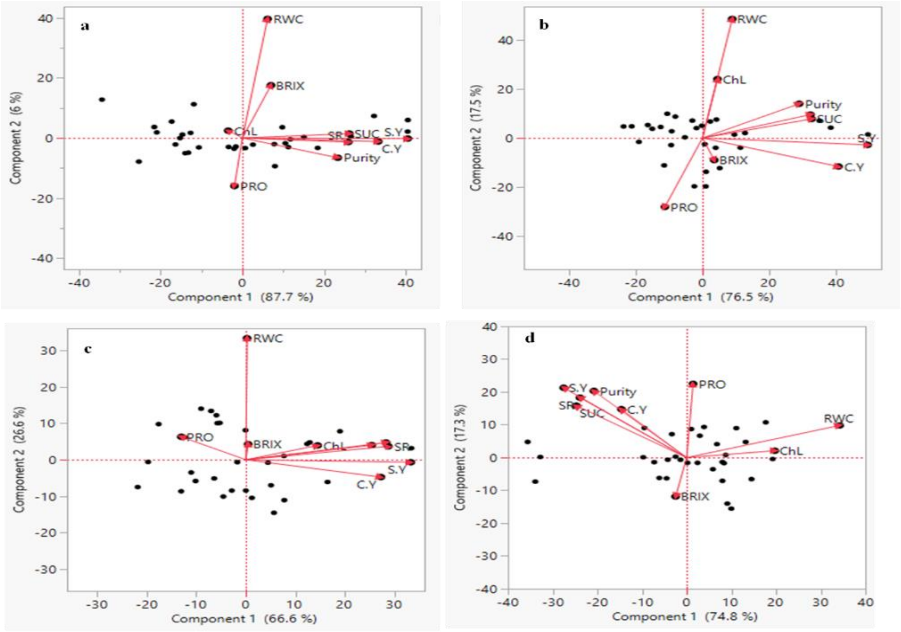

Fig (2). Biplot based on principal component analysis (PCA) for multivariate comparison of 10 genotypes of sugarcane for physio-biochemical parameters and sugar yield during drought stress and control with yield attributes. **a.** Control for drought; **b.** treatment I (87.5%) of ET0; **c.** treatment. II (75%) of ET0; and **d.** treatment III (62.5%) of ET0. Chl (Chlorophyll), RWC (Relative Water Content), PRO (Proline), C.Y (Cane Yield), S.R (Sugar Recovery), S.Y (Sugar Yield), Brix, PUR (Purity) and SUC (Sucrose).

DISCUSSION

According to Gentile et al. (2015), drought may result in yield reductions ranging from 46.2 to 50%. The results which showed that cane yield varied from treatment to treatment and was reduced in about 50% of genotypes, were consistent with the findings. According to Mehareb and Gadallah (2020), the tested sugarcane genotypes varied significantly in sugar recovery %. Sugarcane G.2003-47 promising genotype gave the highest sugar recovery %, while G.2004-27 recorded the lowest value of this trait. Contrary to the results of the present study, showed that the promising genotype G.99-103 recorded the highest sugar recovery %, while K.81113 recorded the lowest value of this trait at the highest stress level drought treatment III. According to Avivi et al. (2016), brix content of various sugarcane genotypes ranged from 21 to 23% under drought or flood alone or a combination of treatments, which was not significantly different from the control (22%) and these results did not differ most with the results that ranged from 19.5 to 23 under drought treatments. Additionally, the results of brix content at

controlled treatment that ranged from 21.75 to 23.66 are in line with Hemaprabha et al. (2013), who indicated that brix values in normal conditions ranged from 19.0 to 24.5%. The results of purity % are in agreement with those mentioned by Neana and Abd El Hak (2014) and Ahmed et al. (2013).

Mohamed et al. (2012) indicated that the variance among varieties in this trait may be due to their gene structure. Mehareb et al. (2018) found that the studied genotypes significantly differed in brix, sucrose, and purity percentage. As reported by Naidu and Venkataramana (1989), the percentage of sucrose in the canes was more adversely affected by drought than the amount of total sugars. This could be explained by the fact that sucrose inverts into glucose and fructose, two hexose sugars, and by variations in how sucrose accumulates and is transported to the storage sink. The results revealed a significant difference between those fed with both the stressed irrigation levels of 75% and 62.5% of ET0 and the highest sucrose % at the stressful irrigation levels of 87.5% of ET0. This results are in harmony with those obtained by Neana and Abd El Hak (2014)**,** Mehareb and Gadallah (2020), who noted that the number of irrigations had a substantial impact on sucrose percentage.

According to Bamrungrai et al. (2021), leaf Chl content is a reliable sign of plant disturbances brought on by environmental variables. These results were consistent with those of de Almeida Silva et al. (2011), who reported SPAD index values below 40 in sugarcane genotypes sensitive to water deficit. Most of the tested genotypes under varying water stress had SPAD indices below 40. Furthermore, the findings concurred with those of Silva et al. (2018)**,** who showed that the interaction between cultivar and water regime had an impact on SPAD index. On the other hand, the findings showed that for the M.35-157 genotype (14.05%), the greatest reduction was recorded at the stressed irrigation levels of ET0. In this regard, Li et al. (2006) mentioned that in most plant species, Chl is generally sensitive to drought, however drought can increase Chl content in some cases (Mensah et al., 2006) or has no detrimental effect on Chl content (Schlemmer et al., 2005). The findings are in agreement with Silva et al. (2007), who found that drought decreased the amount of Chl in sugarcane leaves, but this reduction differed among genotypes. In studies on drought, RWC has always been crucial. According to Dapanage and Bhat (2018), higher RWC values were observed in well-watered sugarcane plants as opposed to a decline in the RWC of plant leaves under drought stress. Nevertheless, sugarcane cultivars exposed to water deficit showed a difference in RWC, according to Graça et al. (2010). These findings indicated that all sugarcane genotypes were reduced at stressed irrigation levels, although, de Almeida Silva et al. (2012) observed no relationship between RWC and yield in various genotypes. However, Sato et al. (2010) reported that even watered plants (about 75%) had low RWC. These investigations have demonstrated that genotypes of sugarcane are strongly tolerant when RWC values are high during water shortages. Reyes et al.

(2021) found that plants with higher rates of RWC reduction under drought conditions exhibited wilting symptoms significantly earlier than varieties with relatively lower rates of RWC reduction, indicating its potential use in the selection and classification of tolerant and susceptible sugarcane lines. My results are consistent with earlier research. Shao et al. (2008) reported increased proline accumulation in water stressed sorghum. The accumulation of proline in stressed plants provides energy for growth and survival, enabling the plant to withstand the stress. Manivannan et al. (2007 and 2008) found that proline contents increased in response to abiotic stressors such ultraviolet light. Proline accumulation in plants might be a scavenger and acting as an osmolyte. The increased proline buildup may be caused by the diminished proline oxidase. According to Molinari et al. (2007), proline is a compatible solute/osmoprotectant that builds up to high concentrations in plant cells under osmotic stress. The results also indicated a significant increase in proline in leaves, which was more pronounced at the stress level of 62.5% of ET0 in most genotypes in comparison with the two stressed levels of 87.5 and 75% of ET0. PCA is one of the oldest and most widely used multivariate techniques used for plotting the data in the space. The two or three largest PCAs provide a quick way to see similarities or differences in the data set, possibly allowing for improved sample discrimination (Sumner et al., 2003). The findings support the claims made by Begum and Islam (2012) that sugarcane varieties that are susceptible to drought tend to have significant yield declines when drought stress is present.

CONCLUSION

Plant breeders want to create genotypes of sugarcane that can withstand drought. It is effective to choose materials for advanced breeding programmers by genotype-screening for drought tolerance, especially in managed drought situations. According to the current findings, there were a variety of mechanisms involved in how drought stress affected the physiology, biochemistry, and sugar yield in 10 sugarcane genotypes. The study found that the majority of physio-biochemical genotype traits, including Chl content, RWC, and proline, as well as sugar yield (cane yield, sugar yield, sugar recovery, brix, sucrose, and purity), were significantly impacted by drought. The results showed that the two genotypes (G.2004-27 and G.99-103) were the most drought tolerant sugarcane genotypes based on the superior physio-biochemical responses and best production under water-limited level (62.5%) of ET0 compared with the control level (100% of ET0).

ACKNOWLEDGEMENT

The authors thank The Sugar Crops Research Institute for providing the experimental materials.

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تأثير إجهاد الجفاف على محصول السكر مرتبطًا بالصفات الفسيولوجية والكيميائية لنبات قصب السكر (جنس سكارم)

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يعد قصب السكر من أكثر المحاصيل الهامة تجاريًا في مصر والتي تتميز بصفة عامة بدورة حياة طويلة واحتياجات مائية عالية. يتأثر إنتاج قصب السكر ونموه سلبًا بمحدودية المياه خاصة خلال فترة الاحتياج المائي الضرورية. في هذه الدراسة تم مقارنة تسعة تراكيب وراثية لنبات قصب السكر مقارنة بالصنف التجاري (GT.54-9) من حيث إنتاجية محصول القصب، محصول السكر وجودة العصير (البركس، السكروز، النقاوة واستخلاص السكر) وعلاقتها بالصفات الفسيولوجية والكيميائية مثل مؤشر تحليل كلوروفيل النبات بالتربة باستخدام جهاز SPAD، تقدير نسبة المحتوى الرطوبي بالأوراق وكذلك تقدير البرولين كمكون كيموحيوي، تحت ثلاث مستويات من الاجهاد (٨٧.٥، ٧٥ و٦٢.٥٪) تبعًا للنتح بخر من النبات باستخدام تصميم القطاعات كاملة العشوائية مع وجود عاملين وثلاث مكررات. وفقًا للنتائج، وجد تحت أقصى مستوى من الإجهاد (٦٢.٥٪) أن التراكيب الوراثية الواعدة (G.2004-27 & G.99-103) كانت الأعلى قيم تبعًا للصفات المدروسة. لذلك يعد فحص التراكيب الوراثية لمقاومة الجفاف طريقة فعالة لاختيار الطرق المناسبة لبرامج التربية المتقدمة خاصة في اعدادات الجفاف المتحكم فيه، ولفهم الآليات المحددة لمقاومة نبات قصب السكر للجفاف يجب إجراء المزيد من الأبحاث الجينومية والجزيئية.