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Varietal variation in physiological and biochemical attributes of sugarcane varieties under different soil moisture regimes

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Physiological and biochemical changes in response to deficit irrigation (drought stress) were studied at three moisture regimes based on available soil moisture (ASM) and four commercial sugarcane varieties differing in their maturity i.e. CoS 767 (Mid late), CoH128 (Mid late), CoJ 64 (Early) and Co 0238 (Early) Plant water status was affected significantly with duration and severity of stress with maximum reduction at 30% ASM level at 90 DAP. The water potential (from −0.62 to −1.16 MPa), osmotic potential (from −0.88 to −1.77 MPa) and relative water content (from 87.59 to 65.51%) decreased significantly at 30% ASM level than at 50% ASM in all the varieties. After stress revival, a remarkable recovery was recorded in all the varieties at all the ASM levels with maximum recovery in varieties Co 0238 and CoS 767. Higher membrane injury was recorded in CoJ 64 followed by CoH 128, Co 0238 and CoS 767at 30% ASM at 60 and 90 DAP. Remarkable decrease were observed in gaseous exchange parameters in leaves *viz*. photosynthetic rate, transpiration rate and stomatal conductance at 30 and 40% ASM levels in all the varieties. Significant reduction was also recorded in chlorophyll fluorescence (Fv/Fm). Severe stress conditions of 30% ASM led to approx. two fold increase in total soluble carbohydrates, four folds in proline and two fold increase in lipid peroxidation. ASM levels of 40% and 30% also significantly reduced total chlorophyll content. From the results, it can be concluded that varieties Co 0238 and CoS 767 are relatively more tolerant at moderate stress to severe stress than CoH 128 and CoJ 64.

Keywords: Abiotic stress, Available soil moisture (ASM), Drought stress, Gas exchange attributes, Osmoprotectants, *Saccharum* spp., Sugarcane, Water relations

Sugarcane (*Saccharum* spp.) is the world's largest crop in terms of production[1]. In many countries it plays an important role in improving rural livelihoods. Sugarcane, an important source of sugar and ethanol, is a relatively high water-demanding crop and its growth is highly sensitive to water deficit[2-4]. Drought is the most important constraint to sugarcane production in many areas. Drought, a period of abnormally dry weather, results in soil-water deficit and subsequently plant-water deficit. Water deficit is the single largest abiotic stress affecting sugarcane productivity and the development of water use efficient and drought tolerant cultivars is an imperative for all major sugarcane producing countries[5]. Scarcity of irrigation water is one of the major constraint of low cane yield and it is mostly restricted the sugarcane growing areas in the world. In sugarcane, four distinct growth stages (i.e., germination, tillering, grand growth and maturity) have been characterized. The tillering and grand growth stages, known as the sugarcane formative phase, have been identified as the critical water demand period. Water stress during formative phase (tillering phase) has negative impact on growth and yield. This is mainly because 70-80% of cane yield is produced during this phase[6].

Plants have evolved various drought tolerance strategies, such as changes in life cycle, modulation of growth and development to match with water supply, regulation of whole plant functions to balance resource allocation for growth and stress adaptation, and evolution of stress signal perception for rapid and long term expression of stress tolerance[7-9]. To achieve that, a better understanding of the stress induced responses and the interrelationships of physiological and biochemical traits can prove to be useful[10]. The increasing incidence, duration and intensity of severe water deficit, has prompted many large sugarcane

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crop improvement programs to invest in water use-efficient and water stress tolerant varieties and water use-efficient crop productions systems. In this context, quantifying plant water status, leaf photosynthetic components and accumulation of osmolytes during the formative phase may be useful, and here, we studied sugarcane plant response to water-deficit stress as well as suitable sugarcane genotype which can tolerate the drought conditions with minimum yield and sugar losses.

Materials and Methods

Experimental details

Present study was carried out on four sugarcane varieties of different maturity group. Two budded setts of four sugarcane varieties were planted during *Spring* season of the year 2014-15 and 2015-16 in the field conditions at Regional Research Station, Chaudhary Charan Singh, Haryana Agricultural University, Uchani, Karnal-132001, Haryana, India. Average rainfall is 600 mm and 70-80 per cent of it is received from July to September. To study the effect of irrigations at different available soil moisture (ASM) levels on four sugarcane varieties, an experiment was conducted in split plot design with 3 replications. Two budded setts of four sugarcane varieties, two under mid late group viz., CoH 128, CoS 767 and two under early group viz., Co 0238 and CoJ 64 were planted by half ridge irrigation method in Spring season. After complete germination (40 days after planting) three levels of available soil moisture (ASM) regimes were created i.e. irrigation at 50% ASM level (control), irrigation at 40% ASM level (mild stress) and irrigation at 30% ASM level (severe stress). These ASM levels were created only during pre-monsoon (in the month of April, May and June) period by withholding irrigation and later on i.e. post monsoon period (in the month of July), the crop was irrigated for stress revival as per requirement.

Plant material and growth conditions

Planting was done in Spring season during the year 2014-15 and 2015-16 by half ridge irrigation method of planting i.e. planting of two budded setts (seed rate 87.5 q ha^{-1}) in dry furrows followed by irrigation upto half of the ridge and then planking after 3-4 days of planting. All necessary managements i.e. fertilizer, irrigation, weed and insect pest were done at proper timing. Different physico-chemical properties of the experimental field soil before sowing of the crop are given in Table 1.

Physiological parameters

Physiological and biochemical parameters were studied after 60, 90 and 120 days after the imposition of stress treatments. Relative water content (RWC %)[11], membrane stability[12], osmotic potential (ψ_s) using 5100-B Vapour Pressure Osmometer and water potential (ψ_w) with the help of pressure chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), between 7:00 AM to 9:00 AM were measured of first TVD leaf.

Photosynthetic rate, stomatal conductance and transpiration rate

Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}) of TVD leaf were measured with an Infrared Open Gas Exchange System (LI-6400, LICOR Inc., Lincoln, NE, USA) between 9:00 AM to 10:00 AM.

Chlorophyll fluorescence

The photochemical efficiency of plants was obtained from the fluorescent analysis of chlorophyll between 9:00 AM to 10:00 AM. The measurements were made on the same leaves that were evaluated for gas exchange. The maximum photochemical efficiency (*Fv/Fm*) of photosystem II was determined using a Portable Pulse Modulated Fluorescence Measurer (Junior PAM Chlorophyll Fluorometer, Germany) after adapting the leaves to the dark for 5 min via special leaf clips. The readings were made after saturating one second light pulses to promote the closing of the photosystem II reaction centers.

Biochemical parameters

Chlorophyll content[13], total soluble carbohydrates[14], proline content[15] and lipid peroxidation[16] were analyzed from fresh TVD leaves.

Statistical analysis

All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary,

Table 1—Soil characteristics of the experimental field

	2014-15	2015-16
1. Mechanical Analysis		
(i) Sand	55.3%	54.6%
(ii) Fine Sand	15.4%	15.8%
(iii) Silt	15.50%	16.2%
(iv) Clay	13.7%	13.4%
2. Texture	Sandy loam	Sandy loam
3. Saturation capacity	34.2%	35.5%
4. pH (1: 2)	7.7	7.9
5. EC_2 (at 25°C)	0.36 dSm^{-1}	0.32 dSm^{-1}
6. Available nutrients (kg ha^{-1})		
(i) N	125.44	129.36
(ii) P	11.8	11.5
(iii) K	172.1	168.5
7. Organic carbon	0.42%	0.46%

NC, USA). Least significant difference test was applied at 5 per cent probability level to compare the mean differences.

Results and Discussion

Physiological parameters

Plant water relations (*RWC, OP and WP*)

Plants under water deficit conditions show modifications in their metabolism to tolerate water loss. Our results showed that when all the four studied varieties were exposed to decreasing level of available soil moisture (30 and 40%), a significant decline in leaf RWC (Table 2), leaf osmotic potential and leaf water potential was observed as compared to 50% ASM level at 60 and 90 DAP (Figure 1). Substantial variations of plant water status of leaves were observed in four varieties. It is suggested that under identical situations, change in ψ_w of leaf may reflect change in ψ_s and can be used in screening of sugarcane genotypes for difference in osmotic adjustment. In Co 0238 and CoS 767 less negative values of ψ_w of leaf and ψ_s of leaf resulted in better water status (RWC%) as compared to varieties CoH 128 and CoJ 64. The proposed mechanism for decreasing ψ_s potential might be that plants adjust under low available soil moisture condition to maintain the turgor. Decrease in ψ_s may be due to accumulation of osmolytes *viz.*, proline and total soluble carbohydrates content.

RWC significantly decreased with average values of 67.8 and 65.51% at 30% ASM level and 71.15 and 71.49% at 40% ASM level as compared to at 50% ASM level (87.52 and 87.59%) at 60 and 90 DAP, respectively (Table 2). Varieties CoS 767 (78.24 and 79.18%) and Co 0238 (77.42 and 77.12%) were at par and significantly maintained higher RWC as compared to varieties CoH 128 (73.18 and 70.97%) and CoJ 64 (73.09 and 72.18%) at 60 and 90 DAP, respectively. On stress revival (at 120 DAP), a significant increase in RWC was recorded by 19.2 and 23.37% at 30% ASM level and 16.79 and 15.93% at 40% ASM level as compared to their values at 60 and 90 DAP, respectively whereas among varieties no significant differences were observed after 120 DAP (stress revival).

Osmotic potential (ψ_s) of leaves declined progressively with the advancement of stage of sampling and also with the decrease in ASM levels (Fig. 1). A significant reductions in ψ_s was recorded at 30% ASM level (–1.45 and –1.77 MPa) and (–1.24 and –1.53 MPa) at 40% ASM level as compared to 50% ASM level (–0.78 and –0.88 MPa) at 60 DAP and 90 DAP, respectively. On average values, varieties CoS 767 (–0.97 and –1.24 MPa) and Co 0238 (–1.06 and –1.26 MPa) showed lowest negative values of ψ_s as compared to varieties CoH 128 (–1.27 and -1.6 MPa) and CoJ 64 (–1.34 and –1. 84 MPa) at 60 and 90 DAP, respectively. On stress revival (120 DAP), a recovery of the plant water status was observed. An increase in ψ_s by 43.45 and 53.67% at 30% ASM level and 33.87 and 46.41% at 40% ASM level was observed over their values at 60 and 90 DAP, respectively

Water potential (ψ_w) of leaves become more negative with decrease in available soil moisture levels (Fig. 1). The more negative values of ψ_w was recorded at 90 DAP than 60 DAP. Reduction percentage was significantly higher at 30% ASM level (–1.16 MPa) than 40% ASM level (–1.01 MPa) as compared to 50% ASM level (–0.62 MPa) at 90 DAP. Among the varieties, more negative values of ψ_w were noticed in varieties CoH 128

Table 2—Effect of different soil moisture regimes on relative water content (%) in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	RWC after 60 DAP					RWC after 90 DAP					RWC after 120 DAP (stress revival)				
Irrigation at 50% ASM (Control)	85.03	88.98	87.78	88.27	87.52^{A}	83.30	89.75	88.38	88.92	87.59^{A}	86.10	88.55	88.75	88.15	87.89^{A}
Irrigation at 40% ASM (Mild stress)	68.65	73.91	74.70	67.32	71.15^{B}	67.35	77.03	74.30	67.27	71.49^{B}	80.60	85.00	83.10	82.80	82.88^{B}
Irrigation at 30% ASM (Severe stress)	65.87	71.83	69.78	63.70	67.8^{C}	62.27	70.75	68.68	60.35	65.51^{C}	78.32	81.42	82.39	81.13	80.82^{C}
Mean	73.18^{B}	78.24^{A}	77.42^{A}	73.09^{B}		70.97^{C}	79.18^{A}	77.12^{B}	72.18^{C}		81.67^{B}	84.99^{A}	84.75^{A}	84.03^{A}	
CV	Varieties, 2.488; Treatments, 2.241					Varieties, 2.814; Treatments, 2.101					Varieties, 2.123; Treatments, 2.021				
LSD	V, 1.78	T, 1.92	T×V, 3.25	V×T, 3.07		V, 1.99	T, 1.78	T×V, 3.45	V×T, 3.45		V, 1.68	T, 1.92	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

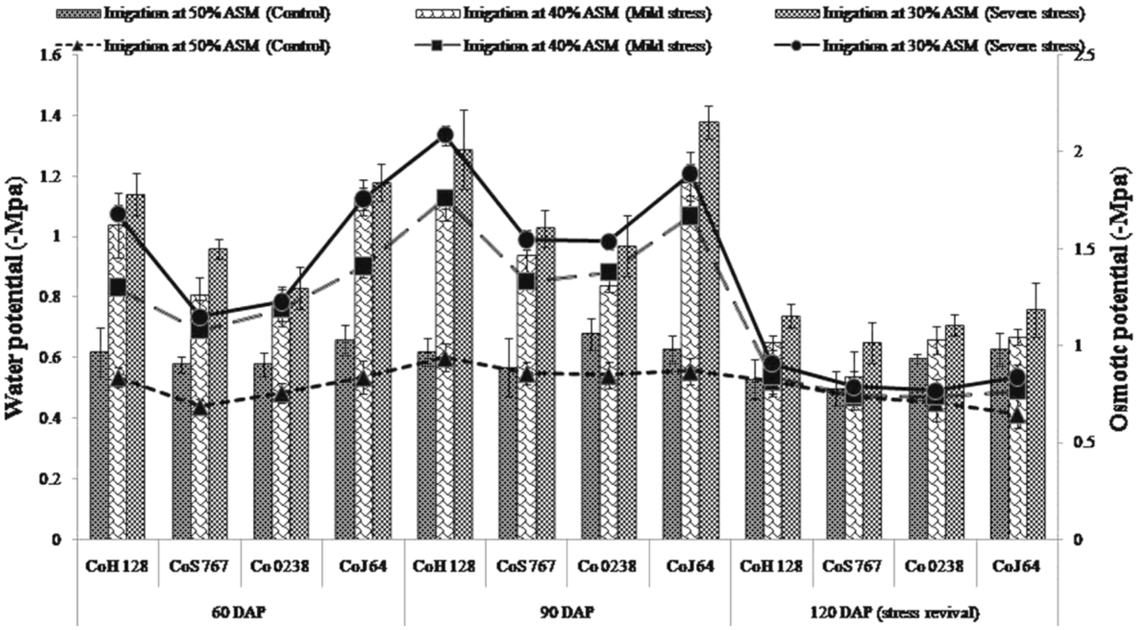

Fig. 1 — Effect of different soil moisture regimes on water potential (-MPa) and osmotic potential (-MPa) in sugarcane varieties differing in their maturity group.

(–0.93 and –1.0 MPa) and CoJ 64 (–0.99 and –1.06 MPa) than Co 0238 (–0.73 and –0.83 MPa) and CoS 767 (–0.78 and -0.84 MPa) at 60 DAP and 90 DAP, respectively. On stress revival (at 120 DAP), values of ψ_w become less negative at 30% (–0.72 MPa) and 40% ASM level (–0.63 MPa), respectively.

Our results are accordance with the earlier findings of Borretto[17] that in tolerant sugarcane varieties, an active accumulation of solutes (osmoregulation) was occurring. The accumulation of osmoregulators in response to drought is an important mechanism for maintaining cell turgor, contributing to alleviate the reduction of the Ψ_w. Nevertheless, only solute accumulation does not favour the tolerance to drought per se, but the pathway that leads to the mechanism of drought tolerance is strongly influenced by this factor[18]. Previous studies have observed an increase in solutes in sugarcane and other species under water-deficient conditions. Osmotic adjustment also protects the photosynthetic apparatus against photoinhibition and hence confers dehydration tolerance[19]. The Ψ_w predawn is known as the most sensitive variable when evaluating water stress in plants, because transpiration does not occur at predawn. Thus, the accumulation of organic solutes, although necessary to maintain the turgor, could be a result of the reduction in the relative water content on the tissue, which tends to concentrate the cell contents[19,20].

Membrane stability (% *injury*)

Measurement of membrane stability (% injury) indicates the stress damage to assess of existing stress. In our investigations, MI increased at 30% and 40% ASM levels at 60 and 90 DAP in all the varieties (Table 3). The MI was least in leaves of varieties CoS 767 (17.91 and 20.79%) followed by Co 0238 (19.32 and 21.88%), CoH 128 (21.84 and 25.97%) and CoJ 64 (24.29 and 27.69%) at 60 DAP and 90 DAP. Lower MI in varieties CoS 767 and Co 0238 might be due to the lower accumulation of MDA content and ROS content as compared to varieties CoJ 64 and CoH 128. An increased leakage from tissue is usually an expression of modification in physical properties of cell membrane. The maximum MI was recorded at 90 DAP than 60 DAP (Table 3), and MI was significantly higher at 30% ASM level (31.54%) followed by 40% ASM level (25.89%) as compared to 50% ASM level (14.81%). Since a decreased in electrolyte can be related to increase membrane stability. This shows the importance of this test in discriminating among tolerant and sensitive varieties. This is in agreement with the conclusion of Martin[21] that electrolyte leakage correlated with drought tolerance. Changes in plasma membrane permeability (electrolyte leakage) is controlled by the membrane transport proteins[22] and linked with the modifications in protein, lipid matrix of the plasma membrane accumulation of reactive oxygen species content under stress conditions[23].

Photosynthetic rate, stomatal conductance and transpiration rate

Our results reveal that gas exchange parameters *viz.*, photosynthetic rate (Table 4), stomatal conductance (Table 5) and transpiration rate (Table 6) reduced significantly at 30% and 40% ASM levels. The performance of sugarcane varieties regarding gas exchange parameters was in the order of CoS 767 > Co 0238 > CoH 128 > CoJ 64. At 30% and 40% ASM levels gas exchange parameters were inhibited mainly by the reduction in RWC, ψ_w, chlorophyll content, MI and leaf area.

Photosynthetic rate significantly decreased by 49.93% at 30% ASM level and 34.73% at 40% ASM level as compared to 50% ASM level, at 60 DAP. However, at 90 DAP reduction percentage was more and average values decreased by 51.86% at 30% ASM level and 39.53% at 40% ASM level as compared to 50% ASM level (Table 4). Significantly highest photosynthetic rate was recorded in variety Co 0238 (18.84 and 17.59 µmol CO$_2$ m^{-2}s^{-1}) and CoS 767 (17.95 and 16.72 µmol CO$_2$ m^{-2}s^{-1}) than varieties CoH 128 (14.85 and 13.31 µmol CO$_2$ m^{-2}s^{-1}) and CoJ 64

Table 3—Effect of different soil moisture regimes on membrane injury (%) in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	MI after 60 DAP					MI after 90 DAP					MI after 120 DAP (stress revival)				
Irrigation at 50 % ASM (Control)	15.17	13.03	12.60	14.10	13.73^{C}	16.88	14.58	13.99	13.81	14.81^{C}	14.80	12.60	13.20	13.92	13.63
Irrigation at 40 % ASM (Mild stress)	22.63	19.69	20.53	25.38	22.06^{B}	26.75	21.90	23.65	31.25	25.89^{B}	15.20	13.52	13.51	14.16	14.10
Irrigation at 30 % ASM (Severe stress)	27.71	21.02	24.82	33.40	26.74^{A}	34.29	25.88	27.99	38.01	31.54^{A}	15.63	13.30	14.15	14.88	14.49
Mean	21.84^{B}	17.91^{D}	19.32^{C}	24.29^{A}		25.97^{B}	20.79^{D}	21.88^{C}	27.69^{A}		15.21^{A}	13.14^{C}	13.62^{C}	14.32^{B}	
CV	Varieties, 3.831; Treatments, 5.781					Varieties, 3.228; Treatments, 3.987					Varieties, 5.588; Treatments, 8.991				
LSD	V, 0.75	T, 1.37	T×V, 1.75	V×T,1.31		V, 0.73	T, 1.09	T×V, 1.53	V×T, 1.27		V, 0.74	T, NS	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

Table 4—Effect of different soil moisture regimes on photosynthetic rate (µmol CO$_2$ m^{-2} s^{-1}) in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	Pn after 60 DAP					Pn after 90 DAP					Pn after 120 DAP (stress revival)				
Irrigation at 50% ASM (Control)	21.82	22.77	24.63	22.57	22.95^{A}	20.47	21.85	22.86	20.76	21.48^{A}	21.95	24.19	24.87	23.43	23.61^{A}
Irrigation at 40% ASM (Mild stress)	12.81	17.59	18.12	11.39	14.98^{B}	11.22	15.38	16.22	9.16	12.99^{B}	19.97	23.55	23.88	20.73	22.03^{B}
Irrigation at 30% ASM (Severe stress)	9.91	13.48	13.78	8.80	11.49^{C}	8.24	12.93	13.69	6.50	10.34^{C}	18.95	22.33	22.74	19.44	20.87^{C}
Mean	14.85^{C}	17.95^{B}	18.84^{A}	14.25^{D}		13.31^{C}	16.72^{B}	17.59^{A}	12.14^{D}		20.29^{C}	23.36^{A}	23.83^{A}	21.2^{B}	
CV	Varieties, 5.239; Treatments, 3.13					Varieties, 6.936; Treatments, 6.074					Varieties, 5.098; Treatments, 3.424				
LSD	V, 0.82	T, 0.58	T×V, 1.35	V×T, 1.41		V, 0.98	T, 1.03	T×V, 3.45	V×T, 1.7		V, 1.07	T, 0.86	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

Table 5—Effect of different soil moisture regimes on stomatal conductance (mmol H$_2$O m^{-2} s^{-1}) in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	gS after 60 DAP					gS after 90 DAP					gS after 120 DAP (stress revival)				
Irrigation at 50% ASM (Control)	0.30	0.33	0.35	0.33	0.32^{A}	0.32	0.33	0.37	0.34	0.34^{A}	0.38	0.45	0.44	0.38	0.41^{A}
Irrigation at 40% ASM (Mild stress)	0.14	0.18	0.19	0.13	0.16^{B}	0.11	0.14	0.16	0.11	0.13^{B}	0.31	0.40	0.42	0.32	0.36^{B}
Irrigation at 30% ASM (Severe stress)	0.11	0.12	0.12	0.11	0.11^{C}	0.10	0.11	0.14	0.09	0.11^{C}	0.29	0.38	0.38	0.29	0.33^{C}
Mean	0.18^{C}	0.21^{B}	0.22^{A}	0.19^{C}		0.18^{C}	0.19^{B}	0.22^{A}	0.18^{C}		0.33^{B}	0.41^{A}	0.41^{A}	0.33^{B}	
CV	Varieties, 5.804; Treatments, 6.402					Varieties, 7.201; Treatments, 7.996					Varieties, 5.401; Treatments, 11.441				
LSD	V, 0.01	T, 0.01	T×V, 0.02	V×T, 0.02		V, 0.01	T, 0.02	T×V, 0.03	V×T, 0.02		V, 0.04	T, 0.02	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

Table 6—Effect of different soil moisture regimes on transpiration rate (mmol H_2O m^{-2} s^{-1}) in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	E after 60 DAP					Pn after 90 DAP					Pn after 120 DAP (stress revival)				
Irrigation at 50% ASM (Control)	6.79	7.13	7.31	7.03	7.06^A	6.52	6.93	7.05	6.47	6.74^A	6.50	7.05	7.11	6.69	6.84^A
Irrigation at 40% ASM (Mild stress)	4.24	4.84	4.75	3.70	4.38^B	3.43	3.99	3.86	3.18	3.62^B	6.02	6.65	6.80	6.09	6.39^B
Irrigation at 30% ASM (Severe stress)	3.17	3.69	3.15	2.93	3.23^C	2.76	3.07	3.02	2.32	2.79^C	5.93	6.57	6.71	5.86	6.26^B
Mean	4.73^B	5.22^A	5.07^A	4.55^B		4.24^B	4.66^A	4.64^A	3.99^C		6.15^B	6.76^A	6.87^A	6.21^B	
CV	Varieties, 6.308; Treatments, 4.627					Varieties, 7.043; Treatments, 8.926					Varieties, 5.377; Treatments, 3.635				
LSD	V, 0.29	T, 0.26	T×V, 0.50	V×T, 0.51		V, 0.29	T, 0.44	T×V, NS	V×T, NS		V, 0.33	T, 0.27	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

(14.25 and 12.14 µmol CO_2 m^{-2} s^{-1}) at 60 and 90 DAP, respectively (Table 4). Interactive effect of varieties and ASM levels was found significant at 60 and 90 DAP. At 120 DAP (on stress revival), plant exhibited increase in photosynthetic rate from 10.34 to 20.87 µmol CO_2 m^{-2} s^{-1} at 30% ASM level, 12.99 to 22.03 µmol CO_2 m^{-2} s^{-1} at 40% ASM level and 21.48 to 23.61 µmol CO_2 m^{-2} s^{-1} at 50% ASM level over their values at 90 DAP. After rehydration, significantly highest photosynthetic rate was recorded in all the varieties. Sage and Kubien[24] have also reported that damages in photosynthetic apparatus are more closely related to changes in membrane properties and with the decoupling of the mechanisms of energy transfer in chloroplasts than to protein denaturation. Decrease in photosynthesis under low relative water content is caused by impaired metabolism (shortage of ATP, limiting RuBP synthesis without or with less inhibition of photosynthetic enzyme) including Rubisco. Photosynthesis is particularly sensitive to water deficit because the stomata tend to close to conserve water under deficit conditions, reducing CO_2 diffusion to the fixation sites in the leaf mesophyll in the vicinity of the enzyme Rubisco, which causes diminished photosynthesis and consequently reduced productivity[24-26].

Monitoring gas exchange in plants is a common approach, with stomatal conductance (gs) reported as one of the most sensitive indicators of stress. Data presented in Table 5 showed that the rate of stomatal conductance decreased significantly with average value 0.11 and 0.11 mmol H_2O m^{-2} s^{-1} at 30% ASM level and 0.16 and 0.13 mmol H_2O m^{-2} s^{-1} at 40% ASM level as compared to 50% ASM level (0.32 and 0.34 mmol H_2O m^{-2} s^{-1}) at 60 and 90 DAP, respectively. Varieties Co 0238 (0.22 and 0.22 mmol H_2O m^{-2} s^{-1}) and CoS 767 (0.21 and 0.19 mmol H_2O m^{-2} s^{-1}) maintained higher stomatal conductance as compared to varieties CoH 128 (0.18 and 0.18 mmol H_2O m^{-2} s^{-1}) and CoJ 64 (0.19 and 0.18 mmol H_2O m^{-2} s^{-1}) at 60 and 90 DAP, respectively. Interactive effect of ASM levels and varieties was found significant (Table 5). On stress revival (at 120 DAP), stomatal conductance was at par at all the ASM levels, respectively over their values at 90 DAP. The maximum values of stomatal conductance were recorded in variety CoS 767 (0.41 mmol H_2O m^{-2} s^{-1}) followed by Co 0238 (0.41 mmol H_2O m^{-2} s^{-1}) and both were at par and the lowest in CoH 128 (0.33 mmol H_2O m^{-2} s^{-1}) and CoJ 64 (0.33 mmol H_2O m^{-2} s^{-1}) and latter two were also at par with each other (Table 5). Stomatal closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses[28]. Possible reasons for decrease in gas exchange parameters include stomatal closure, feedback inhibition due to reduced sink activity, decreased efficiency of Rubisco, displacement of essential cations from the endomembrane structure (leading to changes in permeability), and swelling and disorganization of the grana, or due to the direct effects of salt on stomatal conductance via a reduction in guard cell turgor and intercellular CO_2 partial pressure[29].

Transpiration rate in leaves of sugarcane varieties were significantly affected at 30% and 40% ASM levels as compared to 50% ASM level. Transpiration rate significantly decreased by 2.1 and 2.42 fold at 30% ASM level and 1.62 and 1.86 fold at 40% ASM level as compared to 50% ASM level, at 60 and 90DAP, respectively (Table 6). Among the varieties, significantly highest transpiration rate was recorded in varieties CoS 767 (5.22 and 4.66 mmol H_2O m^{-2} s^{-1}) and Co 0238 (5.07 and 4.64 mmol H_2O m^{-2} s^{-1}) and both were at par while lowest in varieties CoH 128 (4.73 and 4.24 mmol H_2O m^{-2} s^{-1}) and CoJ 64 (4.49 and 3.95 mmol H_2O m^{-2} s^{-1}) at 60 and 90 DAP, respectively. Interactive effect of varieties and ASM levels was found significant (Table 6). At 120 DAP (on stress revival), plant showed a significant increase in transpiration rate and effect of different ASM levels and interactive effect of varieties and ASM level were found non-significant. Results are also confirmatory with the findings of Medeiros[30] that stomatal closure may be the first response to drought in sugarcane variety RB 867515 to minimize water losses, once this variety reduced stomatal conductance and transpiration rate faster than RB 962962. In other words, such results could demonstrate the sensitivity of RB 867515 to water deficit. The control of physiological functions is related to plant water content and changes in RWC seem to directly affect the photosynthetic apparatus in sugarcane plants[24]. When plants under water deficit start to lose water, RWC decreases and triggers a significant reduction in the CO_2 uptake rate due to the stomatal closure[31].

Chlorophyll fluorescence

Chlorophyll florescence (Fv/Fm ratio) has been documented a reliable indicator for stress and also correlated with the quantum yield of net photosynthesis[23,32,33]. Fv/Fm values reduced by 23.94 and 24.62% at 30% ASM level and 12.68 and 15.39% at 40% ASM level as compared to 50% ASM level at 60 and 90 DAP, respectively (Fig. 2). A decrease in the Fv/Fm suggests loss in photosynthesis due to damage to the photosynthetic apparatus. It causes disturbances in adequate electron translocation from PSII to electron acceptor, needed for regeneration of RuBP under stress situations[34]. Colom and Vazzana[35] have reported similar correlations between Fv/Fm and drought tolerance in *Erasgrostis curvula* cultivars, with high Fv/Fm values being associated with drought tolerance and low Fv/Fm values being associated with susceptibility to drought stress. Among the varieties, significantly higher Fv/Fm was recorded in varieties Co 0238 and CoS 767 as compared to varieties CoH 128 and CoJ 64 at 60 as well as 90 DAP. Interactive effect of ASM levels and varieties was found non-significant. At 120 DAP (on stress revival), a significant recovery in chlorophyll fluorescence was observed and maximum values of Fv/Fm were recorded at 50% ASM level (0.71) followed by 40% ASM level (0.67) and least at 30% ASM level (0.66). Goncalves[36] reported reduction of the photochemical efficiency of photosystem II in sugarcane varieties

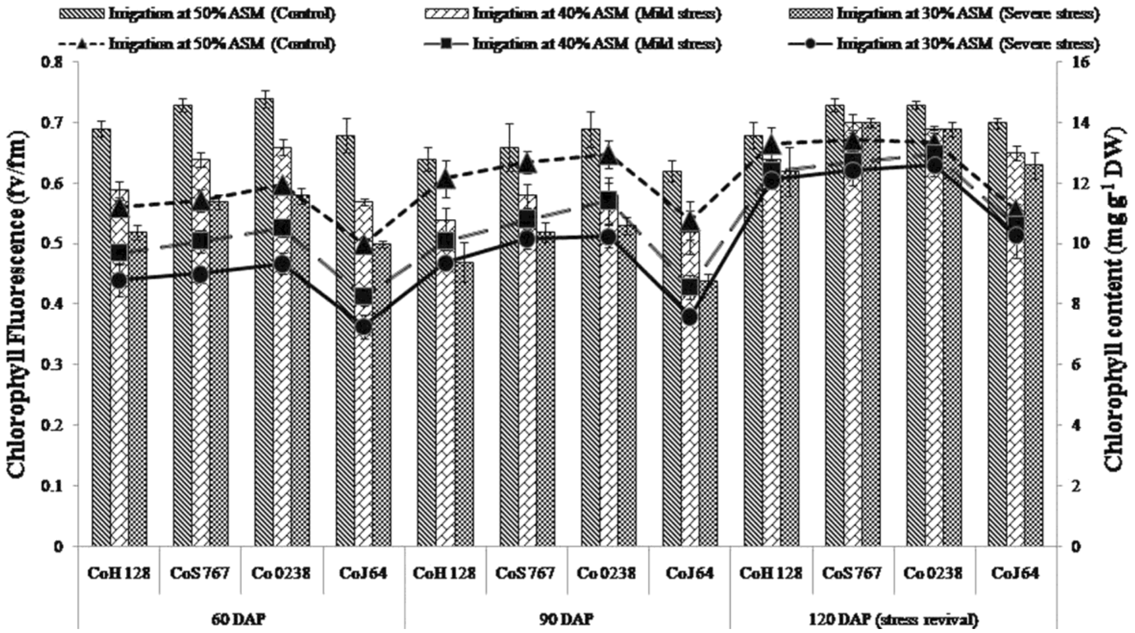

Fig. 2—Effect of different soil moisture regimes on chlorophyll fluorescence (Fv/Fm) and chlorophyll content (mg g^{-1} DW) in sugarcane varieties differing in their maturity group.

when plants were submitted to 20% of field capacity. This reduction was expressive for SP79- 1011, RB72454, and RB98710, except for RB92579, showing that the radiation intercepted by this variety was used in the photochemical phase of photosynthesis, with no damage of photosystem II under drought stress. Keeping Fv/Fm values under water stress similar to those values in sugarcane plants under suitable water conditions indicates high efficiency on the use of radiation, possibly by the reactions of carbon assimilation[30].

Water use efficiency

Results shows that maximum water use efficiency was recorded at 30% ASM level as compared to 40% and 50% ASM levels at 60 and 90 DAP (Table 7). Among the varieties, maximum water use efficiency was recorded in Co 0238 and CoS 767 than CoH 128 and CoJ 64. The higher water use efficiency in varieties Co 0238 and CoS 767 might be due to lower transpiration rate and higher photosynthetic rate under low available soil moisture. The present findings are confirmatory with the conclusion of Farooq[37] that maximum water use efficiency was observed under 60% irrigation coefficient as compared to 80% and 100% irrigation coefficient and under 60% irrigation coefficient maximum water use efficiency was recorded in sugarcane variety NSG than HSF-240. Jangpromma[38] reported high water use efficiency in sugarcane cultivar 03-4-425 and phill66-07 and it was due to higher root system to capture soil water. As water use efficiency was well-associated with root traits for transpiration as indicated by high and significant correlation. Crop that maintain high water use efficiency under drought or well–irrigated conditions are considered to be drought tolerance in term of total dry matter production and higher yield[39,40].

Biochemical parameters

Total chlorophyll content

Chlorophyll content of leaf reduced significantly at 30% ASM level (22.85 and 22.96%) followed by 40% ASM level (13.53 and 15.72%) as compared to 50% ASM level at 60 and 90 DAP (Fig. 2) in all the varieties. The maximum reduction was recorded in varieties CoJ 64 and CoH 128 than varieties Co 0238 and CoS 767. The corresponding decrease in chlorophyll content with increasing stress conditions implies a lower capacity of leaf tissues for light harvesting and production of reactive oxygen species which is mainly driven by excess energy absorption in the photosynthetic apparatus; this might be avoided by degrading the absorbing pigments[41]. At 30% ASM level, variety CoJ 64 showed lowest chlorophyll content (8.99 mg g^{-1} DW) followed by CoH 128 (10.55 mg g^{-1} DW) and highest in CoS 767 (10.23 mg g^{-1} DW) and Co 0238 (11.55 mg g^{-1} DW) at 90 DAP. After stress revival (at 120 DAP), chlorophyll content increased at 30, 40 and 50% ASM level, respectively over their values recorded at 90 DAP. The present results are in accordance with the earlier findings in sugarcane[30,42]. The deleterious effect on total chlorophyll content due to drought has been ascribed to its adverse effect on photosynthetic apparatus like suppression of chloroplast development and changes in its lamellar structure due to instability of bonds between chlorophyll, protein lipid complex and destruction of pigment due to oxidative damage[43].

Total soluble carbohydrates content

Total soluble carbohydrates (TSCs) content in leaves of sugarcane varieties showed significant increase with increase in stress intensity and sampling time. Among the varieties, significantly higher value

Table 7—Effect of different soil moisture regimes on water use efficiency in sugarcane varieties differing in their maturity group

Varieties/ Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	Chl content after 60 DAP					Chl after 90 DAP					Chl after 120 DAP (stress revival)				
Irrigation at 50% ASM (Control)	3.22	3.20	3.37	3.21	3.25^{C}	2.92	3.07	3.15	2.84	3.0^{B}	3.39	3.44	3.50	3.51	3.46
Irrigation at 40% ASM (Mild stress)	3.05	3.66	3.85	3.11	3.42^{B}	3.25	3.86	4.23	3.01	3.59^{A}	3.32	3.56	3.52	3.41	3.45
Irrigation at 30% ASM (Severe stress)	3.14	3.54	4.38	3.01	3.52^{A}	3.01	4.25	4.64	2.73	3.66^{A}	3.21	3.42	3.40	3.33	3.34
Mean	3.14^{C}	3.46^{B}	3.87^{A}	3.11^{C}		3.06^{C}	3.73^{B}	4.01^{A}	2.86^{C}		3.31	3.47	3.47	3.41	
CV	Varieties, 11.026; Treatments, 1.866					Varieties, 9.222; Treatments, 7.314					Varieties, 7.636; Treatments, 6.774				
LSD	V, 0.07	T, 0.35	T×V, 0.54	V×T,0.61		V, 0.30	T, 0.28	T×V, 0.52	V×T, 0.52		V, NS	T, NS	T×V, NS	V×T, NS	

[Least significant difference test was applied at 5 per cent probability level to compare the mean differences. ASM, Available Soil Moisture; V, Varieties; T, Treatments; T × V, Treatments at the same level of varieties; and V × T, Varieties at the same level of treatments]

of TSCs content were recorded in Co 0238 (19.18 mg g^{-1} DW) as compared to CoS 767 (18.22 mg g^{-1} DW), CoH 128 (17.23 mg g^{-1} DW) and CoJ 64 (16.7 mg g^{-1} DW) during 60 DAP (Fig. 3). However, at 90 DAP, more amount of TSCs was accumulated i.e. Co 0238 (23.46 mg g^{-1} DW) and CoS 767 (23.12 mg g^{-1} DW) as compared to varieties CoH 128 (20.11 mg g^{-1} DW) and CoJ 64 (19.14 mg g^{-1} DW) that resulted into maintenance of higher RWC, ψ_s and thus better plant water status in these varieties by maintaining high turgor. Medeiros[30] have also reported that soluble carbohydrates content increased in sugarcane under drought treatment, and increase was higher in RB 86751 (51.2%) than RB 962962 (28%). Interactive effect of varieties and ASM levels was found significant. These changes could be related to activation of responses to cope with this adverse environmental condition, to assist in the maintenance of cell water relations. The accumulation of soluble carbohydrates during water deficient is considered a plant response to maintain hydration of the shoot and also protect enzyme and membrane system through the stabilization of proteins and lipids[43,44]. Increase in soluble carbohydrates may occur at the beginning of stress as a result of growth cessation and due to starch degradation[27].

Proline content

Proline is a strong source to store carbon, nitrogen and a purifier of free radicals. Proline also maintains the structure of cell membrane and proteins[20] and contributes to membrane stability[45]. It may also act as a signalling regulatory molecule able to activate multiple responses that are components of the adaptation process[43,46]. Similar to TSCs content, overall accumulation of proline content was more in leaves of Co 0238 (270.14 and 289.49 µg g^{-1} DW) and CoS 767 (258.24 and 291.61 µg g^{-1} DW) than varieties CoH 128 (222.94 and 247.97 µg g^{-1} DW) and CoJ 64 (182.53 and 217.35 µg g^{-1} DW) at 30% and 40% ASM levels as compared to 50% ASM level both at 60 and 90 DAP (Fig. 3). This increased proline content acts as an osmotic compatible solute and adjusts osmotic potential which resulted in avoidance of drought stress. The concentration of this metabolite usually increased in response to drought, which showed inverse relationship with ψ_w and ψ_s of leaf and maintained higher RWC. Present findings are confirmatory with the results of Farooq[37] that maximum proline concentration was observed at 60% irrigation co-efficient level, while minimum values was at 100% irrigation co-efficient level in sugarcane cultivar. The important role of proline is to assist in osmotic adjustment, stabilizing the membrane and eliminating oxygen radicals, and preventing damage to cell structures caused by environmental stresses in sugarcane[46].

Lipid peroxidation

Malondialdehyde (MDA) content was measured to determine the lipid peroxidation level because MDA

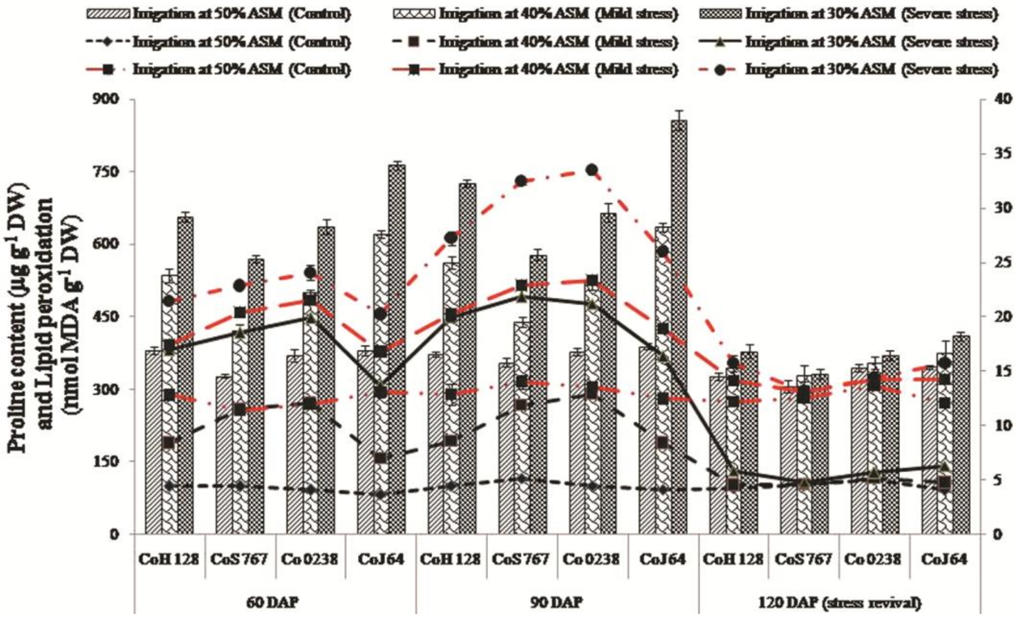

Fig. 3 — Effect of different soil moisture regimes on total soluble carbohydrates (mg g^{-1} DW), proline content (µg g^{-1} DW) and lipid peroxidation (nmol MDA g^{-1} DW) in sugarcane varieties differing in their maturity group.

is a by-product of lipid peroxidation. The content of MDA has been considered as an indicator of oxidative injury. MDA content showed increasing trend over the sampling stage as well as increasing stress intensity. Significantly higher values of MDA content were recorded at 30% ASM level (656.69 and 706.69 nmole MDA g^{-1} DW) followed by 40% ASM level (519.82 and 539.66 nmole MDA g^{-1} DW) and least at 50% ASM level (364.59 and 374.22 nmole MDA g^{-1} DW) at 60 and 90 DAP, respectively (Fig. 3). However, extent of increment was less in varieties CoS 767 and Co 0238 as compared to varieties CoJ 64 and CoH 128. It may be due to formation of reactive oxygen species and increased MI. After stress revival (at 120 DAP), the interactive effect of varieties and ASM levels was found non-significant and lower values were recorded in varieties CoS 767 and Co 0238 than varieties CoJ 64 and CoH 128 for MDA content. Our results are confirmatory with the earlier findings of Abbas[47] who reported that drought stress imposed at various stages of sugarcane crop growth resulted in an increase in lipid peroxidation and decrease in membrane stability. In the present study, lower level of lipid peroxidation in varieties CoS 767 and Co 0238 may be due to increased activity of antioxidative enzyme APX, POX and CAT which act as a damage control system and thus provide protection from oxidative stress. Sairam & Tyagi[48] have also reported that antioxidative enzymes provide protection from oxidative stress which would otherwise cause destruction of cell membranes and protein, DNA structure and inhibit the photosynthesis under water stress condition.

Cane yield and Sugar yield

Water deficits during formative phase significantly reduced cane yield and sugar yield in all the four varieties. Among the varieties, Co 0238 produced significantly highest cane yield (83.05 t ha^{-1}) followed by CoS 767 (68.23 t ha^{-1}), CoH 128 (66.59 t ha^{-1}) and lowest in CoJ 64 (60.43 t ha^{-1}). A significant decrease in cane yield at 30% ASM level (36.18%) and 40% ASM level (27.5%) was recorded as compared to 50% ASM level (Fig. 4). Sugar yield is the product of cane yield and sugar recovery. Sugar yield decreased significantly at 30 and 40% ASM levels as compared to 50% ASM level in all varieties. Among the varieties, Co 0238 and CoS 767 produced higher sugar yield as compared to CoH 128 and CoJ 64 (Fig. 4). It might be due to that reduction in sugar

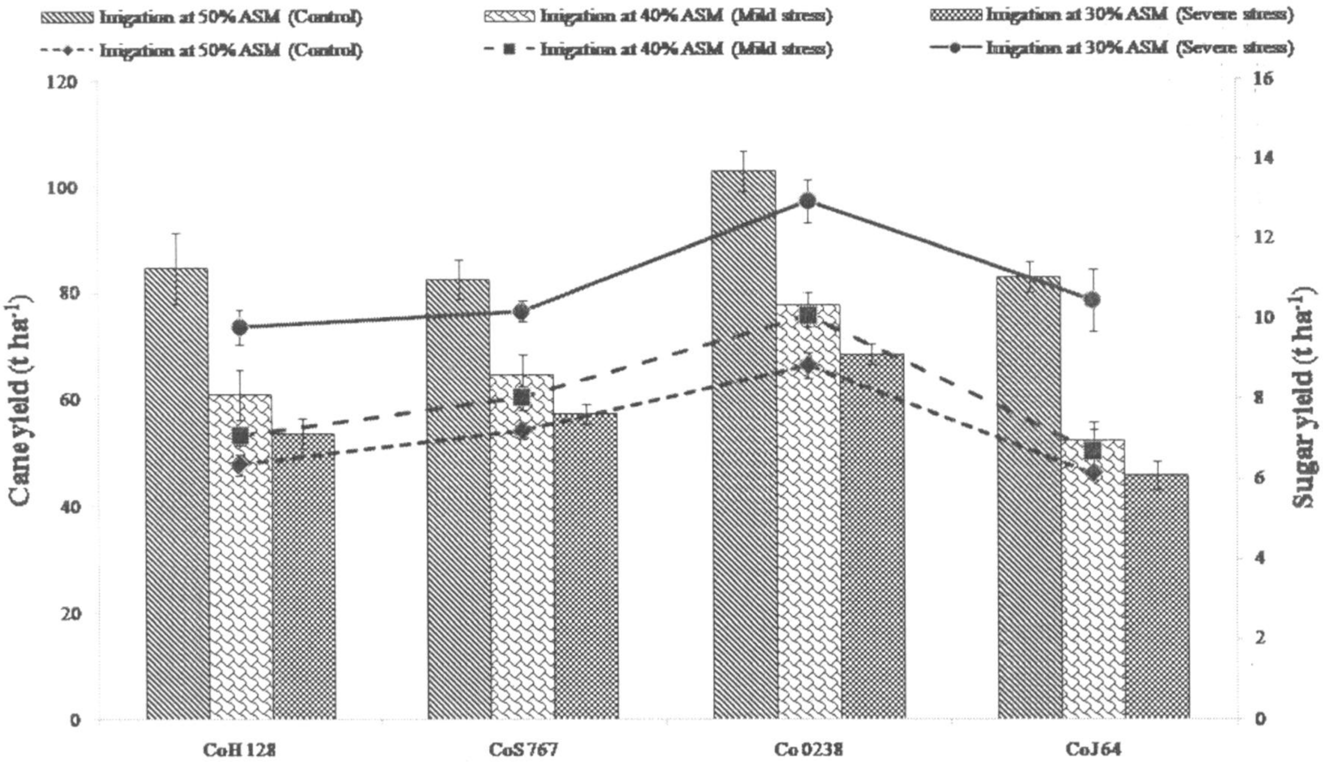

Fig. 4 — Effect of different soil moisture regimes on cane yield (t ha^{-1}) and Sugar yield (t ha^{-1}) in sugarcane varieties differing in their maturity group.

yield contributing factors *viz.,* cane length, single cane weight, NMC and cane yield were less affected in these varieties (Co 0238 and CoS 767). Similar findings of reduction in sugar yield of different sugarcane varieties under water stress conditions had been reported[49,50].

Conclusion

Based upon the physiological and biochemical analysis, it is concluded that varieties Co 0238 and CoS 767 are identified relatively more tolerant at 40% (moderate stress) and 30% (severe stress) ASM levels than CoH 128 and CoJ 64, because these varieties maintained better plant water status, higher amount of osmoptrotectant to maintain cell turgor, membrane integrity, canopy temperature, chlorophyll content and gas exchange parameters, which ultimately contributed towards higher dry matter production and yield in these varieties. Moreover, after stress revival, Co 0238 and CoS 767 was able to recover faster than CoJ 64 and CoH 128, a characteristic that qualifies these varieties to support short periods of drought without major losses in the initial phase of its development.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1 *FAOSTAT*, (The Statistics Division of the Food and Agriculture Organization of the United Nations), 2013.

2 Lakshmanan P & Robinson N, Stress physiology: Abiotic stresses in Sugarcane: Physiology, Biochemistry, and Functional Biology, (Ed. Moore PH & Botha FC; Publisher and place of publication?), 2014, 411.

3 Santillán-Fernández A, Santoyo-Cortés VH, García-Chávez LR, Covarrubias-Gutiérrez I & Merino A, Influence of drought and irrigation on sugarcane yields in different agroecoregions in Mexico. *Agric Syst*, 143 (2016) 126.

4 Pooja, Nandwal AS, Chand M, Kumari A, Rani B, Goel V & Singh S, Genotypic differences in growth behavior and quality parameters of sugarcane (*Saccharum officinarum*) varieties under moisture stress conditions. *Indian J Agric Sci*, 89 (2019) 65.

5 Ferreira THS, Tsunada MS, Bassi D, Araújo P, Mattiello L, Guidelli GV, Righetto GL, Gonçalves VR, Lakshmanan P & Menossi M, Sugarcane water stress tolerance mechanisms and its implications on developing biotechnology solutions. *Front Plant Sci*, 8 (2017) 1077. DOI: 10.3389/fpls.2017.01077.

6 Zingaretti SM, Rodrigues FA, da Graca JP, de Matos Pereira L & Lourenco MV, Sugarcane responses at water deficit conditions, water stress, (Ed. Ismail Md. Mofizur Rahman; Water Stress, IntechOpen) (2012), DOI: 10.5772/30986.

7 Hirayama T & Shinozaki K, Research on plant abiotic stress responses in the post-genome era: past, present and future. *Plant J*, 61 (2010) 1041. DOI: 10.1111/j. 1365-313X.2010.04124.x

8 Hu H & Xiong L, Genetic engineering and breeding of drought-resistant crops. *Annu Rev Plant Biol*, 65 (2014) 715. DOI: 10.1146/annurev-arplant-050213-040000

9 You J & Chan Z, ROS regulation during abiotic stress responses in crop plants. *Front Plant Sci*, 6 (2015) 1092. DOI: 10.3389/fpls.2015.01092

10 Pooja, Nandwal AS, Chand M, Kumar A, Rani B, Kumari A & Kulshrestha N, Comparative evaluation of changes in protein profile of sugarcane varieties under different soil moisture regimes. *Int J Curr Microbiol Appl Sci*, 6 (2017) 1203.

11 Weatherley PE, Studies in the water relation of cotton plants. The field measurement of water deficit in leaves. *New Phytol*, 49 (1950) 81.

12 Dionisio-Sese ML & Tobita S, Antioxidant responses of rice seedlings to salinity stress. *Plant Sci*, 135 (1998) 1.

13 Hiscox JD & Israelstam GF, A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot*, 57 (1979) 1332.

14 Yemm EW & Willis AJ, The estimation of carbohydrates in plant extract by anthrone. *Biochem J*. 57 (1954) 508-514.

15 Bates LS, Waldren RP & Teare ID, Raid determination of free proline for water stress studies. *Plant Soil*, 39 (1973) 205.

16 Heath RL & Packer L, Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*, 125 (1968) 189.

17 Boaretto LF, Carvalho G, Borgo L, Creste S, Landell MGA, Mazzafera P & Azevedo RA, Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. *Plant Physiol Biochem*, 7 (2014) 165.

18 Medeiros DB, Silva EC, Santos HRB, Pacheco CM, Musser RS & Nogueira RJMC, Physiological and biochemical response to drought stress in the Barbados cherry. *Braz J Plant Physiol*, 24 (2012) 181.

19 Flower DJ & Ludlow MM, Contribution of osmotic adjustment to the dehydration tolerance of water stressed pigeon pea (*Cajanus cajan* L.) leaves. *Plant Cell Env*, 9 (1986) 33.

20 Silva MA, Jifon JL, dos Santos CM, Jadoski CJ & da Silva JAG, Photosynthetic capacity and water use efficiency in sugarcane genotypes subject to water deficit during early growth phase. *Braz Arch Biol Tech*, 56 (2013) 735.

21 Martin U, Alladru SG & Bahari ZA, Dehydration tolerance of leaf tissues of six woody angiosperm species. *Physiol Plant*, 69 (1987), 182.

22 Jacobs A, Ford K, Kretschmer J & Tester M, Rice plants expressing the moss sodium pumping *ATPase PpENA1* maintain greater biomass production under salt stress. *Plant Biotech J*, 9 (2011) 838.

23 Graca JP, Rodrigues FA, Farias JRB, Oliveira MCN, Hoffmann-Campo CB & Zingaretti SM, Physiological parameters in sugarcane cultivars submitted to water deficit. *Braz J Plant Physiol*, 22 (2010) 189.

24 Sage R & Kubien DS, The temperature response of C_3 and C_4 photosynthesis. *Plant Cell Env*, 30 (2007) 1086.

25 Lawlor DW & Tezara W, Causes of decreased photosynthetic rate and metabolic capacity in water deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann Bot*, 103 (2009) 561.

26 Kumar A, Kumar A, Kumar P, Lata C & Kumar S, Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of marvel grass. *Indian J Expt Biol*, 56 (2018a) 573.

27 Galmes J, Ribas-Carbo M, Medrano H & Flexas J, Rubisco activity in Mediterranean species is regulated by the chloroplastic CO_2 concentration under water stress. *J Expt Bot*, 62 (2011) 653.

28 Flexas J & Medrano H, Drought inhibition of photosynthesis in C_3 plants: Stomatal and non stomatal limitation revisited. *Ann Bot*, 89 (2002) 183.

29 Dionisio-Sese ML & Tobita S, Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *J Plant Physiol*, 157 (2000) 54.

30 Medeiros DB, da Silva EC, Nogueira RJMC, Teixeira MM & Buckeridge MS, Physiological limitations in two sugarcane varieties under water suppression and after recovering. *Theo Expt Plant Physiol*, 25 (2013) 213.

31 Buckley TN, The control of stomata by water balance. *New Phytol*, 168 (2005) 275.

32 Kumar A, Kumar A, Lata C & Kumar S, Eco-physiological responses of *Aeluropus lagopoides* (grass halophyte) and *Suaeda nudiflora* (non-grass halophyte) under individual and interactive sodic and salt stress. *South Afr J Bot*, 105 (2016) 36.

33 Silva MA, Jifon JL, Sharma V, da Silva JAG, Caputo MM & Damaj MB, Use of physiological parameters in screening drought tolerance in sugarcane genotypes. *Sugar Tech*, 13 (2011) 178.

34 Kafi M, The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.) cultivars. *J Agr Sci Tech*, 11 (2009) 535.

35 Colom MR & Vazzana C, Photosynthesis and PSII functionality of drought-resistant and drought sensitive weeping lovegrass plants. *Env Expt Bot*, 49 (2003) 135.

36 Goncalves ER, Ferreira VM, Silva JV, Endres L, Barbosa TP & Duarte WG, Gas exchange and chlorophyll a fluorescence of sugarcane varieties submitted to water stress. *Rev Bras Eng Agric*, 14 (2010) 378.

37 Farooq U, Mehmood S, Afghan S, Shahzad A & Asad M, Comparative study on agro-physiology of sugarcane (*Saccharum officinarum* L.) genotypes at different irrigation co-efficient values. *Pak J Bot*, 47 (2015) 527.

38 Jangpromma N, Thammasirirak S, Jaisil P & Songsri P, Effects of drought and recovery from drought stress on above ground and root growth, and water use efficiency in sugarcane (*Saccharum officinarum* L.). *Aust J Crop Sci*, 6 (2012) 1298.

39 Jongrunklang N, Toomsan B, Vorasoot N, Jogloy S, Kesmala T & Patanothai A, Identification of peanut genotypes with water use efficiency under drought stress conditions from peanut germplasm of diverse origins. *Asian J Plant Sci*, 7 (2008) 628.

40 Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C & Patanothai A, Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agr Water Manage*, 96 (2009) 790.

41 Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A & Grill D, Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiol Biochem*, 40 (2002) 691.

42 Cha-um S, Wangmoon S, Mongkolsiriwatana C, Ashraf M & Kirdmanee C, Evaluating sugarcane (*Saccharum* sp.) cultivars for water deficit tolerance using some key physiological markers. *Plant Biotech*, 29 (2012) 431.

43 Kumar A, Krishnamurthy SL, Lata C, Kumar P, Devi R, Kulshrestha N, Yadav RK & Sharma SK, Effect of dual stress (salinity and drought) on gas exchange attributes and chlorophyll fluorescence characteristics in rice. *Indian J Agric Sci*, 86 (2016) 19.

44 Lawlor DW, Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. *Ann Bot*, 89 (2002) 1.

45 Zhou Q & Yu B, Plant physiology and biochemistry changes in content of free, conjugated and bound polyamines and osmotic adjustment in adaptation of vetiver grass to water deficit. *Plant Physiol Biochem*, 48 (2010) 417.

46 Lata C, Kumar A, Sharma SK, Singh J, Sheokand S, Pooja, Mann A & Rani B, Tolerance to combined boron and salt stress in wheat varieties: Biochemical and molecular characterization. *Indian J Expt Biol*, 55 (2017) 321.

47 Abbas SR, Ahmad SD, Sabir SM, Wajid A, Aiya B, Abbas MR & Sabir HS, Screening of drought tolerant genotypes of sugarcane through biochemical markers against polyethylene glycol. *Int J Sci Eng Res*, 4 (2013) 980.

48 Sairam RK &Tyagi A, Physiology and molecular biology of salinity stress tolerance in plants. *Curr Sci*, 86 (2004) 407.

49 da Silva ALC & da Costa WAJM, Varietal variation in growth, physiology and yield of sugarcane under two contrasting water regimes. *Trop Agr Res*, 16 (2004) 1.

50 Khan IA, Bibi S, Yasmin S, Khatri A & Seema N, Phenotypic and genotypic diversity investigations in sugarcane for drought tolerance and sucrose content. *Pak J Bot*, 45 (2013) 359.