RESEARCH ARTICLE

EFFECT OF WATER STRESS ON CHLOROPHYLL, NITRATE REDUCTASE ACTIVITY AND CANE YIELD IN SUGARCANE (*SACCHARUM OFFICINARUM* L.)

R.M Garkar*, R.W. Bharud and S.N. Mate

Abstract

The physiological response of sugarcane genotypes to water stress was investigated in a field experiment comprising 10 sugarcane genotypes and two water regimes laid out in factorial randomized block design with three replications. The reduction in net-photosynthetic rate (Pn), leaf chlorophyll, nitrate reductase activity (NRA), cane yield and CCS yield was 43.05, 18.20, 41.31, 26.99 and 28.50 % respectively due to 60 days water stress in the formative growth stage. The genotypes CoM 0265, Co 86032, CoM 0254 and CoM 08085 were found to be drought tolerant. Significant positive correlation was observed between cane yield under water stress condition and Pn leaf chlorophyll content and NRA.

Key words: *Water stress, chlorophyll, NRA and net-photosynthetic rate*

Introduction

Drought is one of the most important environmental stresses limiting sugarcane *(Saccharum officinarum L.)* productivity worldwide. The immediate effects of drought are stunting and poor growth resulting in drastic reduction in cane and sugar productivity and yield instability (Hemaprabha et al. 2004). Water stress reduces photosynthesis which may be due to reduction in photosynthetic area, inhibition of photosynthetic reactions and increased stomatal resistance to diffusion of carbon dioxide (Kramer and Boyer 1995).

Among the physiological processes, photosynthesis (Pn) is the basic determinant of plant growth and productivity and the ability to maintain the rate of carbon assimilation under environmental stress is of fundamental importance to plant production (Lawlor 1995). Chlorophyll is the universal pigment in leaves which absorbs the radiant energy required for plant metabolic processes. Green plant pigments are thermo-sensitive and degradation occurs when they are subjected to higher temperature and water stress. Analysis of chlorophyll content is important for evaluating the health or integrity of the internal apparatus during photosynthetic process within a leaf which provides a rapid and accurate technique of detecting and quantifying plants tolerant to drought stress (Rong hua et al. 2006). Lu Jian Lin et al. (1998) observed significant positive correlation between net photosynthesis and chlorophyll contents in sugarcane.

With a view to understand the traits which can be used for assessing drought tolerance, comparative response of 10 sugarcane genotypes was studied in terms of their response to water stress for 60 days during the formative growth stage. Determinations were made for changes in chlorophyll content, rate of photosynthesis, *nitrate reductase activity (NRA)*, cane yield, CCS yield and correlation between yield and physiological parameters.

Materials and methods

A field experiment was carried out during 2009 - 2010 at Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar dt., Maharashtra, India, in factorial

R.M.Garkar
Central Sugarcane Research Station, Padegaon- 415 521, Satara dt, Maharashtra, India
R.W. Bharud and S.N. Mate,
Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, Ahmednagar dt, Mahaharashtra, India
e-mail: rm_garkar@rediffmail.com

randomized block design with three replications. The treatments consisted of 10 sugarcane genotypes including seven promising clones, *viz*. CoM 08004, CoM 08011, CoM 08044, CoM 08065, CoM 08073, CoM 08085 and CoM 0254, and three released varieties *viz*. CoM 0265, Co 86032 and Co 740, and two water regimes, namely well-watered control (S_1) and water stress (S_0) at early growth stage.

The soil of the experimental field was medium black with 7.0 - 7.5 pH range and fairly well leveled with good drainage. The available N, P and K was 182, 23.3 and 220 kg/ha respectively.

The two eye-bud setts were planted in 1.0 m wide furrows after applying irrigation. The gross plot size was 4 rows of 4.20 m length each. Regular irrigations were applied to the experimental field up to 75 days after planting (DAP). After 75 DAP, regular irrigations were given throughout the experiment for well-watered control. For drought treatment, water was withheld from 75 to 135 DAP. Irrigation was resumed after 135 DAP and the stressed plots were irrigated in the same manner as control plot up to harvest. The recommended fertilizer dose (340 N:170 P:170 K kg/ha) was applied in four recommended splits. Soil moisture percentage was recorded at 20 days interval during the stress period in five places randomly at 0-20, 21-40, 41-60, 61-80 and 81-100 cm soil depth with soil moisture meter (neutron probes).

Total Chlorophyll content was determined following the method of Dhopte and Phadnawis (1989).The net-photosynthetic rate (Pn) was measured using Infra-red Gas Analyser (IRGA; Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). Measurements were made at mid day, between 11:30 and 12:00 eastern day time on top fully expanded third leaf blades. The flow rate of air in the sample line was adjusted to 500 ìmol s^{-1}.The NRA activity in vivo was estimated as per the procedure given by Klepper et al. (1971).

Cane yield and CCS yield were recorded at harvest. The cane yield (t ha^{-1}) was calculated from the total weight of all millable canes per plot. The CCS% was computed using the formulae CCS %= (1.022 x sucrose %) - (0.292 x Brix %) as per Sundara (1998). The CCS yield ha^{-1} was estimated as CCS yield (t ha^{-1}) = Cane yield (t ha^{-1}) X CCS% in cane at harvest.

Data analysis was carried out as per the method suggested by Panse and Sukhatme (1967). The level of significance used in 'F' test was P= 0.05. Critical difference (CD) values were calculated at 5 per cent probability level wherever 'F' test was significant.

Results

Soil moisture content

At the start of water stress treatment, soil moisture contents of non - stress and stress treatment were rather similar (Fig.1). At 60 days after imposing stress, the mean soil moisture content of stressed treatment was 19.10% whereas soil moisture content of non-stressed treatment (32.70%) was much higher. The soil moisture content of stressed treatment decreased linearly with advancement of drought period.

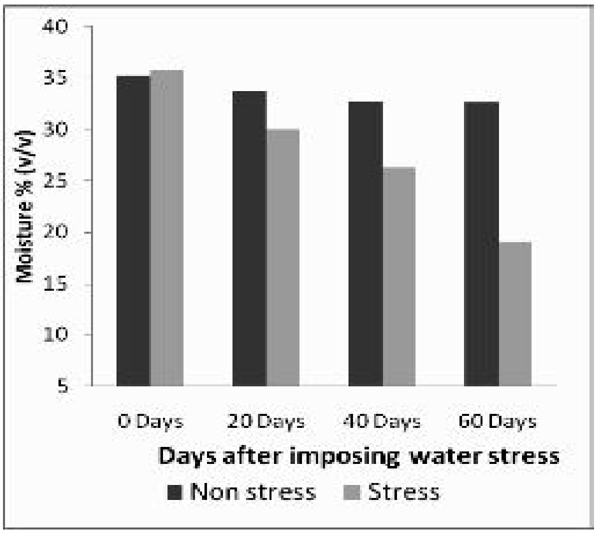


Fig.1 Soil moisture levels under water stress and non-stress conditions (v/v)

Chlorophyll content

Significant differences were observed among the sugarcane genotypes and also between the water regimes for total chlorophyll. The interaction effects were also significant (Table1). Total chlorophyll content was reduced by 18.20 % due to 60 days water stress irrespective of the varieties. The lowest reduction was found in the variety CoM 0265 (10.87 %) followed by the genotypes, Co 740 (16.78 %), Co 86032 (18.00 %), CoM 08065 (18.00 %)

Table 1. Leaf chlorophyll content and rate of photosynthesis of sugarcane genotypes as influenced by water stress for 60 days

Genotypes	Total Chlorophyll (mg g^{-1} f. wt.)				Rate of photosynthesis (µmol CO_2 $m^{-2}sec^{-1}$)				Nitrate reductase activity (µmoles of NO_2^- formed g^{-1} f. wt. h^{-1})			
	S_1	S_0	Mean	% Reduction	S_1	S_0	Mean	% Reduction	S_1	S_0	Mean	% Reduction
CoM 08004	1.314	1.150	1.232	12.48	21.34	9.51	15.43	55.44	0.839	0.483	0.661	42.38
CoM 08011	1.488	1.090	1.289	26.75	16.93	9.34	13.14	44.80	0.703	0.371	0.537	47.24
CoM 08044	1.507	1.170	1.338	22.36	20.42	11.28	15.85	44.74	0.725	0.389	0.557	46.40
CoM 08065	1.500	1.230	1.365	18.00	19.54	10.93	15.23	44.06	0.846	0.400	0.623	52.76
CoM 08073	1.546	1.209	1.377	21.80	18.69	10.41	14.55	44.30	0.726	0.398	0.562	45.26
CoM 08085	1.818	1.490	1.654	18.04	21.36	13.43	17.40	37.11	0.750	0.450	0.600	40.00
CoM 0254	1.853	1.500	1.676	19.05	19.74	11.05	15.39	44.05	0.764	0.483	0.624	36.77
CoM 0265	2.098	1.870	1.984	10.87	23.15	14.32	18.74	38.13	0.979	0.661	0.820	32.46
Co 86032	1.789	1.467	1.628	18.00	22.11	13.15	17.63	40.55	1.009	0.684	0.846	32.16
Co 740	1.477	1.260	1.368	14.69	18.70	11.73	15.22	37.30	0.873	0.502	0.688	42.49
Mean	1.639	1.344	1.491	18.20	20.20	11.52	15.86	43.05	0.821	0.482	0.652	41.31
	SE±	CD 5%	CV %		SE±	CD 5%	CV %		SE+	CD 5%	CV %	
Water stress (W)	0.035	0.103	8.81		0.24	0.68	8.32		0.012	0.032	9.73	
Genotypes (G)	0.074	0.220			0.54	1.53			0.026	0.072		
WxG	0.050	0.147			0.760	2.18			0.036	0.102		

S_1- Non-stress, S_0- Water stress

and CoM 08085 (18.05 %). The genotypes CoM 0265, CoM 0254, CoM 08085 and Co 86032 showed high total chlorophyll content under both well-watered and drought conditions.

Rate of photosynthesis

The genotypes, CoM 0265, Co 86032, CoM 08085 and Co 740 recorded above average net photosynthetic rate (Pn) under water stress condition (Table 1). Under non-stress condition the variety, CoM 0265 (23.15 µmol CO_2 m^{-2} sec^{-1}), recorded the highest rate of photosynthesis followed by Co 086032 (22.11 µmol m^{-2} sec^{-1}) and CoM 08085 (21.36 µmol m^{-2} sec^{-1}). The net-photosynthetic rate was significantly reduced in sugarcane by 43.05 % due to 60 days water stress. The minimum reduction in Pn was found in CoM 08085 (37.11%). The genotypes CoM 08085 (37.11 %), Co 740 (37.30 %) and CoM 086032 (40.55 %) recorded below average reduction in net-photosynthetic rate.

Nitrate reductase activity (NRA)

The NRA decreased by 41.31 %. The varieties Co 86032 (0.684 µmoles of NO_2^- formed g^{-1} f.wt.h^{-1}) and CoM 0265 (0.661 µmoles of NO_2^- formed g^{-1} f.wt.h^{-1}) recorded significantly higher NRA under moisture stress condition. Considering the mean NRA of stress and non-stress conditions, the variety Co 86032 (0.846 µmoles of NO_2^- formed g^{-1} f.wt.h^{-1}) recorded significantly higher NRA and was on par with, CoM 0265 (0.820 µmoles of NO_2^- formed g^{-1} f.wt.h^{-1}).

Cane and commercial cane sugar (CCS) yield

The data on cane and CCS yield as influenced by water stress are presented in Table 2. The cane yield under water stress condition (106.34 t ha^{-1}) was lower than non-stress condition (145.32 t ha^{-1}). The variety CoM 0265 produced significantly highest cane yield under non-stress (177.78 t ha^{-1}) and stress conditions (139.61 t ha^{-1}) respectively. The variety CoM 0265 (139.61 t ha-1) was found significantly superior for cane yield under water stress condition followed by Co 86032 (125.00 t ha^{-1}), CoM 0254 (113.49 t ha^{-1}) and CoM 08085 (112.00 t ha^{-1}). Considering the mean cane yield over stress and non-stress conditions, the variety CoM 0265 (158.69 t ha^{-1}) recorded the highest cane yield. The varieties / genotypes Co 86032 (139.02 t ha^{-1}), CoM 08085 (132.68 t ha^{-1}) and CoM 0254 (127.59 t ha^{-1}) also recorded significantly higher cane yield than the others and were on par. In general, the reduction in cane yield due to water stress was to the extent of 26.99% and it ranged from 18.33 (Co 86032) to 35.61 % (CoM 08011).

The CCS yield under water stress condition (13.03 t ha^{-1}) was significantly lower than non stress condition (18.14 t ha^{-1}). The varieties CoM 0265 (17.03 t ha^{-1}), Co 86032 (16.00 t ha^{-1}) and CoM 0254 (15.33 t ha^{-1}) also recorded significantly higher CCS yield under water stress condition than the others and were on par. The variety, CoM 0265 (19.70 t ha^{-1}) recorded the highest mean CCS yield and it was on par with Co 86032 (17.91 t ha^{-1}) and CoM 0254 (17.26 t ha^{-1}). The reduction in CCS yield due to water stress was 28.50 % and it ranged from 19.31% (Co 86032) to 35.51% (CoM 08004). The varieties, Co 86032, CoM 0254, CoM 0265 and Co 740 recorded less reduction than the mean value (28.50 %).

The cane yield in response to water stress was highly significant and positively correlated with total chlorophyll (Fig. 2a) and net-photosynthetic rate (Fig. 2b).

Table 2. Cane yield and CCS yield of sugarcane genotypes as influenced by water stress.

Genotypes	Cane yield (t ha^{-1})				CCS yield (t ha^{-1})			
	S_1	S_0	Mean	% reduction	S_1	S_0	Mean	% reduction
CoM 08004	146.27	97.56	121.92	33.30	18.74	12.09	15.41	35.51
CoM 08011	136.15	87.67	111.91	35.61	17.10	11.07	14.09	35.27
CoM 08044	122.67	88.00	105.33	28.26	14.39	10.13	12.26	29.62
CoM 08065	147.62	97.62	122.62	33.87	15.96	10.31	13.14	35.43
CoM 08073	143.65	100.80	122.22	29.83	18.38	12.39	15.38	32.58
CoM 08085	153.35	112.00	132.68	26.96	19.64	14.50	17.07	26.18
CoM 0254	141.68	113.49	127.59	19.90	19.19	15.33	17.26	20.09
CoM 0265	177.78	139.61	158.69	21.47	22.37	17.03	19.70	23.84
Co 86032	153.05	125.00	139.02	18.33	19.83	16.00	17.91	19.31
Co 740	131.00	101.67	116.33	22.39	15.77	11.49	13.63	27.12
Mean	145.32	106.34	125.83	26.99	18.14	13.03	15.59	28.50
	SE±	CD 5%	CV%		SE±	CD 5%	CV%	
Water stress (W)	2.528	7.238	12.09		0.284	0.816	9.67	
Genotypes (G)	5.64	16.18			0.638	1.824		
W x G	8.00	22.9			0.900	2.58		

S_1- Non-stress, S_0- Water stress

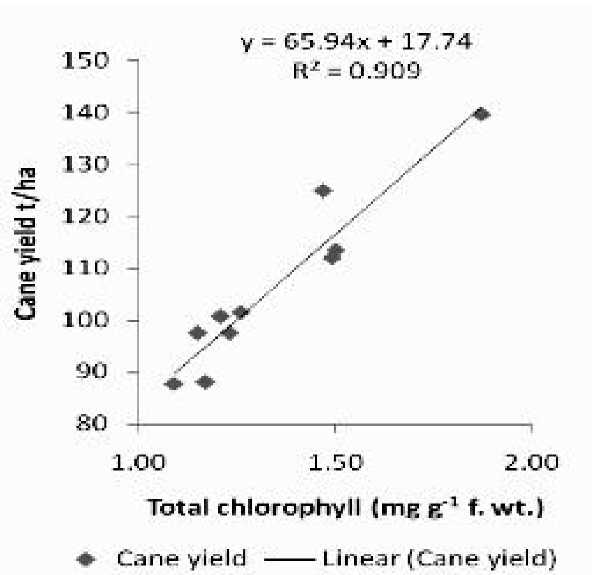
Discussion

In the present study, water stress during formative stage significantly decreased rate of photosynthesis as was also observed by Koonjah et al. (2006) and Kramer and Boyer (1995). The process of photosynthesis takes place in the chloroplasts, using chlorophyll, the green pigment involved in photosynthesis. As water is removed from cell, the water potential and water content decrease, and the cell shrinks. Enzyme mediated reactions requiring water as a substrate encounter less water in the cell.

In the present study, there was a significant reduction in total leaf chlorophyll content due to water stress. This finding derives support from previous observations that total chlorophyll in sugarcane leaves was reduced due to drought (Becker and Fock 1986; Silva et al. 2007 and Jangpromma et al. 2010). The reduction in chlorophyll may be due to less absorption of nutrients particularly nitrogen from soil due to shortage of water and low activity of nitrate reductase (Begum and Paul 1993). Green plant pigments are thermo-sensitive and degradation occurs when they are subjected to higher temperature and water stress. The genotypes CoM 0265, CoM 0254, CoM 08085 and Co 86032 showed high chlorophyll content under both well-watered and drought conditions which helped in better photosynthesis. Jangpromma et al. (2010) observed that drought tolerant sugarcane cultivars have higher level of chlorophyll than drought susceptible cultivars.

In the present study, NRA decreased due to water stress. In sugarcane, nitrate (NO_3^-) is the principal form of nitrogen available to the plant from soil that is reduced to ammonia via the process of nitrate assimilation, which involves sequential participation of two enzymes *viz.* nitrate reductase (NR) and nitrite reductase (NiR). Ammonia so produced is assimilated via glutamine synthetase (GS)/GOGAT cycle or via glutamate dehydrogenase (GDH). Nitrate reductase, the rate limiting enzyme in the process of nitrate assimilation, is known to be associated with water stress in many crop plants (Balasubramanian et al. 1974). Ammonia so formed is used for synthesis of chlorophyll. Reduction in NR activity decreased the supply of ammonia for the formation of chlorophyll.

Sugarcane varieties Co 86032, CoM 0265, CoM 0254, CoM 08085, and Co 740 showed comparatively less reduction in the *in vivo* NR activity than other genotypes. Therefore, it appears that sugarcane genotypes with minimum reduction in *in vivo* NR activity under water stress, besides other physiological parameters, can be considered



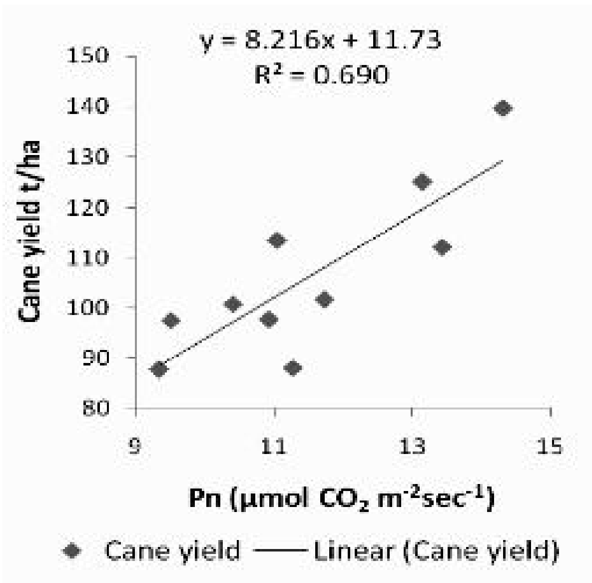


Fig. 2. Relationship between cane yield and (a) total chlorophyll and (b) photosynthetic rate (Pn) of sugarcane under water stress

as a selection criterion for evolving drought tolerant varieties.

In this study, the mean cane and CCS yield were significantly reduced due to water stress. The varieties CoM 0265 and Co 86032 were found superior for cane and CCS yield even under stress condition. Subramanian et al. (1992) recorded 11.7 to 19.2 % reduction in cane yield due to stress imposed at tillering and grand growth stages. Vasantha et al. (2005), Pawar et al. (2006) and Singh and Reddy (1980) recorded considerable reduction in cane and CCS yield under water stress condition compared to non-stress condition. According to Kumar (2005) a plant or a group of plants showing better growth and productivity with limited soil moisture than other plants in a given set of similar environments is understood to be tolerant to drought. Bearing this definition in mind and based on the yield performance under drought conditions, the genotypes CoM 0265, Co 86032, CoM 0254, and CoM 08085 were found to be drought tolerant as they have recorded above average cane yield under water stress condition, whereas the genotypes CoM 08011, CoM 08044, CoM 08004, CoM 08065 and Co 740 recorded below average cane yield under stress condition.

Conclusion

In the present study, water stress during the formative stage significantly decreased rate of photosynthesis, leaf chlorophyll content, NRA, cane yield and CCS yield. The cane yield under water stress condition was significantly and positively correlated to leaf chlorophyll content and net photosynthetic rate and NRA. These parameters can be used as indices to screen drought tolerant sugarcane genotypes.

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