

EFFECT OF CCC AND FAP ON LEAF DIFFUSIVE RESISTANCE, PHOTOSYNTHESIS AND RESPIRATION IN PEARL MILLET UNDER STRESS CONDITIONS

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ABSTRACT

Studies conducted in sand culture conditions in screen house on pearl millet (*Pennisetum glaucum*) cv. HHB-45 revealed that photosynthesis declined and diffusive resistance increased at low leaf water potential. Assimilation ceased at -3.12 MPa at anthesis and at -3.52 MPa at grain filling stages. CCC and FAP increased rate of photosynthesis as compared to control at all stages of samplings. Increase in photosynthesis closely followed the levels of chlorophyll, soluble protein and moisture contents of the plants. The lowest figures (20.36, 25 and 40.35 sec/cm) for diffusive resistance of adaxial surface were obtained with FAP (0.50 mM) under water stress at vegetative, anthesis and grain-filling stages. Plants treated with CCC and FAP maintained higher levels of starch and respiration as compared to control plants at three stages of samplings.

INTRODUCTION

Moderate or severe water stress is known to inhibit photosynthesis both through stomatal control and at the level of mesophyll cell or chloroplast. In general, dark respiration declines moderately as water potential falls when stress is not too severe (Hsiao 1973). It has been further suggested that variations in endogenous concentrations of different growth regulators may act to modify plant water balance and change the physiological responses, under stress conditions. Lockhart (1965) suggested that CCC appears to involve in an alteration in metabolism of the plant such that it responds differently to lowered water potential. Exogenous application of CCC and cytokinin have been reported to delay senescence, thus aid in enhanced photosynthesis and mobilization of photosynthates to the reproductive sink (Osborne 1967; Skene 1968; Dong and Artica 1982).

The present study is aimed at evaluation of the efficiency of cytokinin (FAP) and chlorocholine-chloride (CCC), on diffusive resistance, photosynthesis and respiration in pearl millet (*Pennisetum glaucum*) under simulated drought conditions.

MATERIAL AND METHODS

The plants were raised during kharif season of 1984 in sand culture in earthen pots (12 x 12 cm). Ten surface sterilized seeds of pearl millet (*Pennisetum glaucum*)

cv. HHB-45 were sown in each pot and after thinning, one seedling was retained. These plants were supplied with 250 ml of complete nutrient solution per pot (Arnon and Hoagland 1940) at weekly intervals. The plants were sprayed with 6-furfuryl Aminopurine (0.25 mM and 0.50 mM) and chlorocholine-chloride (30 mM and 60 mM) at 20 days after sowing. A group of thirty plants was subjected to water stress (ST) by withholding irrigation till the first sign of wilting appeared in the leaves at three different stages of growth viz., vegetative (35 DAS), anthesis (70 DAS) and grain-filling (105 DAS). Half of the stressed plants were watered to recover (REV). A group of plants, considered as the control (UST) received the normal irrigation schedule during the entire life of the plant. The observations were recorded four days after each treatment of stress and recovery. Water potential of 3rd fully expanded leaf at vegetative stage and that of flag leaf at anthesis and grain-filling stages was determined by Pressure bomb technique using a model 3005, plant water status console (Soil Moisture Equipment Corporation, USA), at 10 a.m. Diffusive resistance of both upper and lower surfaces of the same leaf was measured with diffusive resistance meter. Rates of respiration and photosynthesis were monitored using the differential mode of Infra Red Gas Analyser (ADC 225/2K, England). The photosynthesis of individual leaf was measured with a perspex leaf chamber of known volume that could be placed over a single leaf. Leaf area was recorded with Leaf Area Meter (Li-3003). Starch, soluble protein and chlorophyll was estimated following Hassid et al. (1964) and Arnon (1949) respectively.

RESULTS AND DISCUSSION

Leaf water potential (ψ_w) was lower (-2.34, -3.12, -3.52 MPa) in pearl millet plants subjected to water stress (ST) conditions than those with normal supply of water (-1.00, -1.38, -1.56 MPa) at vegetative anthesis and grain-filling stages, respectively. Leaf diffusive resistance increased as a consequence of fall in water potential (Table 1). Data have been presented for the adaxial surface as the trend was same for abaxial surface of leaf. Highest values of ψ_w (-1.98, -2.84 MPa) and lowest values for diffusive resistance (25, 40.35 Sec cm⁻¹) were recorded in plants sprayed with 30 mM CCC and 0.50 mM FAP under ST conditions at 70 and 105 days after sowing respectively. On revival of moisture conditions (REV), plants were not able to recover to their prestressed levels (UST) of ψ_w and diffusive resistance. Plants treated with growth regulators appeared more turgid than control at same leaf ψ_w .

Water stress significantly decreased the rate of photosynthesis at 35, 70 and 105 days after sowing. Assimilation ceased in control plants when ψ_w dropped to -3.12 MPa and -3.5 MPa at anthesis and grain-filling stages, respectively under moisture stress. Plants sprayed with 30 mM CCC and 0.50 mM FAP maintained the rate of photosynthesis under ST conditions at par with those of control under UST conditions at later stage of growth. Statistical analysis reveals that interaction of stress x

Table 1. Effect of CCC and FAP on water potential (-M Pa) and leaf diffusive resistance (sec. cm⁻¹) in pearl millet.

Treatments	Water Potential						Diffusive Resistance					
	CCC			FAP			CCC			FAP		
	Control	30 mM	60 mM	0.25 mM	0.50 mM	0.50 mM	Control	30 mM	60 mM	0.25 mM	0.50 mM	
DAS	UST	-1.00	0.88	-1.00	-0.96	-0.84	8.80	8.32	7.99	8.29	8.09	
35	ST	-2.34	-2.18	-2.30	-2.22	-2.10	25.82	22.86	23.64	22.76	20.36	
	REV	-1.12	-0.96	-1.10	-1.20	-0.94	14.98	14.59	14.02	13.28	12.99	
	UST	-1.38	-1.28	-1.34	-1.32	-1.30	10.99	9.80	9.55	9.83	8.66	
70	ST	-1.12	-1.98	-0.04	-3.08	-3.02	35.31	35.56	34.22	33.03	25.00	
	REV	-1.52	-1.44	-1.33	-1.48	-1.36	14.98	14.28	13.99	14.59	12.02	
	UST	-1.56	-1.38	-1.42	-1.44	-1.32	12.51	11.85	9.72	10.23	9.12	
105	ST	-3.52	-2.84	-3.34	-3.36	-2.92	51.22	51.05	50.22	46.56	40.35	
	REV	-1.62	-1.39	-1.52	-1.54	-1.42	18.58	18.38	17.92	17.13	16.05	
CD at 5 %												
			35 DAS	70 DAS	105 DAS				35 DAS	70 DAS	105 DAS	
ST			0.02	0.01	0.02				0.39	0.34	0.61	
Chem			0.02	0.02	0.02				0.50	0.43	0.79	
ST x Chem			0.16	0.03	0.05				0.87	0.76	1.37	

chemical was significant. Data (Table 2) indicate that total soluble protein decreased as leaf ψ_w decreased. Decrease in total soluble protein was greater at anthesis irrespective of the treatments. Growth regulators decreased the loss of total soluble protein at all stages of growth and development.

Water stress also reduced chlorophyll content and leaf area, irrespective of the treatments, at all stages of growth and development (Table 3). These results are in accordance with those of Hsiao (1973), who showed that decrease in chlorophyll content may be due to the decreased synthesis or degradation of chlorophyll. Foliar spray of growth regulators helped in better retention of green leaf area and chlorophyll thus aid in enhanced photosynthesis.

Photosynthetic depression caused by soil water deficit has been usually considered as the result of stomatal closure (Brown et al. 1976; Hall and Hoffman 1976). Diffusive resistance of cycocel treated plants was at par with control under ST conditions at anthesis and grain-filling stage. Water potential and rate of photosynthesis were higher in CCC treated plants under stress at 70 and 105 days after sowing. Therefore, correlation of assimilation with leaf ψ_w is slightly better than with diffusive resistance. An increase in the rate of photosynthesis in CCC and FAP treated plants could be primarily related to hydration and permeability of chloroplast and its membranes (Richmond et al. 1971). Since RuBP carboxylase is the most abundant leaf soluble protein, the decrease in total soluble protein indirectly indicates that reduced rate of photosynthesis during water stress was partially caused by a reduction in the level of the enzyme protein. Hence, water stress also caused non-stomatal mediated photosynthetic depression (Xu et al. 1990). Thus, high levels of total soluble protein appear to be the reason for high rates of photosynthesis in CCC and FAP treated plants. High photosynthetic activity has been related to the regulation of the activity of Ribulose, 1-5 bisphosphate carboxylase by CCC in grape leaves (Niimi 1979) and with FAP in matured bean leaves (Treharne et al. 1970).

Rate of respiration and starch content declined in plants subjected to water stress conditions (Table 4). Application of CCC and FAP increased the rates of respiration under ST conditions at all stages of growth. The reduction in rates of respiration has been attributed to the inability of mitochondria to utilise available substrates. Data indicate that substrate supply was not limiting under stress conditions. Treatments with CCC and FAP further increased starch content at 35, 70 and 105 days after sowing. The accumulation of starch may be a result of arrested extension growth associated with comparatively undisturbed photosynthesis in regulators treated plants under ST conditions. Increased rate of respiration under ST condition in CCC and FAP treated plants are in confirmity with those of Goswami and Srivastava (1985).

Table 2. Effect of CCC and FAP on photosynthesis ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) and total soluble proteins (mg/g fr. wt.)

Treatments	Photosynthesis						Total soluble protein					
	CCC			FAP			CCC			FAP		
	Control	30 mM	60 mM	0.25 mM	0.50 mM	Control	30 mM	60 mM	0.25 mM	0.50 mM	0.50 mM	
DAS	UST	12.40	14.52	14.03	12.76	14.49	11.56	13.88	23.75	13.70	22.05	
35	ST	5.30	5.70	7.30	5.60	8.20	7.27	10.55	13.66	9.37	12.44	
	REV	6.90	8.90	8.81	6.70	8.60	10.40	12.50	23.19	12.63	21.75	
	UST	15.00	16.40	15.68	15.20	16.40	18.64	27.32	36.96	26.53	33.97	
70	ST	0.00	5.30	5.70	5.07	5.60	12.09	16.79	23.46	15.38	22.08	
	REV	3.60	9.92	9.20	8.64	9.10	15.71	19.96	27.82	19.09	27.60	
	UST	6.90	9.35	11.50	8.30	12.60	9.74	10.43	15.80	10.83	15.34	
105	ST	0.00	7.67	5.30	3.62	5.90	6.28	8.16	11.52	7.86	10.34	
	REV	3.19	8.14	6.67	4.90	7.62	8.12	9.72	12.90	9.18	12.55	
	CD at 5 %		35 DAS	70 DAS	105 DAS		35 DAS	70 DAS	105 DAS			
ST		0.03	0.09	0.02		0.34	0.45	0.37				
Chem		0.04	0.12	0.02		0.44	0.59	0.48				
ST x Chem		0.08	0.22	0.04		0.75	1.02	0.81				

Table 3. Effect of CCC and FAP on chlorophyll (mg/g. fr. wt.) and leaf area (cm²) of pearl millet.

Treatments	Chlorophyll						Leaf Area				
	CCC			FAP			CCC				
	Control	30 mM	60 mM	0.25 mM	0.50 mM	Control	30 mM	60 mM	0.25 mM	0.50 mM	
DAS	UST	2.03	3.19	3.28	2.96	3.84	407	500	498	498	506
35	ST	1.48	1.60	1.91	1.56	2.07	471	488	475	480	493
	REV	1.78	3.40	4.48	4.96	5.89	475	490	479	483	499
	UST	3.31	3.78	4.48	4.29	9.07	599	618	609	603	641
70	ST	0.83	0.84	0.93	0.82	1.11	383	400	392	388	400
	REV	4.37	4.39	8.50	5.26	9.81	387	405	400	390	409
	UST	2.71	3.19	3.45	2.88	3.13	221	277	265	253	287
105	ST	1.28	1.47	2.14	2.11	4.48	120	160	159	159	176
	REV	1.93	2.73	3.46	3.01	5.22	120	162	162	160	181
	CD at 5%		35 DAS	70 DAS	105 DAS		35 DAS	70 DAS	105 DAS		
ST		0.15	0.29	0.21		1.80	0.98	0.50			
Chem		0.19	0.38	0.27		2.30	1.20	0.70			
ST x Chem		0.34	0.66	0.47		3.90	2.16	1.20			

Table 4 Effect of CCC and FVP on respiration ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) and starch contents (mg/g. d. wt.)

Treatments	Respiration						Starch Contents				
	CCC			FAP			CCC		FAP		
	Control	30 mM	60 mM	0.25 mM	0.50 mM	1.00 mM	Control	30 mM	60 mM	0.25 mM	0.50 mM
DAS	UST	1.17	1.33	1.38	1.38	1.59	5.94	6.89	7.25	7.91	9.24
35	ST	0.29	0.35	0.36	0.34	0.32	3.80	5.13	5.46	4.32	5.77
	REV	0.53	0.84	0.88	0.73	0.83	5.27	7.21	7.09	6.52	8.55
70	UST	1.57	1.50	1.63	1.57	1.64	33.22	33.09	35.32	30.51	30.79
	ST	0.30	0.30	0.46	0.49	0.40	17.06	24.31	21.98	19.60	20.12
105	REV	1.32	1.38	1.44	1.40	1.43	20.53	25.72	24.02	23.02	25.59
	UST	1.57	1.72	1.97	2.38	3.29	22.90	27.71	27.26	24.28	27.58
CD at 5%	ST	0.60	0.64	1.54	0.92	1.83	16.77	25.08	24.20	18.57	20.45
	REV	2.33	0.78	2.24	1.58	0.96	20.63	23.97	24.53	20.07	23.37
ST		35 DAS	70 DAS	105 DAS			35 DAS	70 DAS	105 DAS		
		0.01	0.02	0.02	0.02		0.50	1.53	1.00		
	Chem	0.02	0.02	0.02	0.02		0.63	1.99	1.29		
ST x Chem		0.03	0.04	0.04			1.11	3.45	1.58		

Our results indicate that water stress reduces net photosynthesis by reducing green leaf area, chlorophyll, total soluble protein and increasing diffusive resistance. CCC and FAP treated plants may resist drought stress by their ability to maintain a high internal water content, total soluble protein i.e. RuBP carboxylase, chlorophyll and by decreasing diffusive resistance to some extent. Revival capacity of the components determining photosynthesis would depend on the intensity of stress and stage at which stress occurs. Revival in photosynthesis was earlier than compensation in leaf area. However, growth regulators appear to affect the functional sites of chloroplasts and mitochondria which need to be studied.

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