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Amitha Kunikullaya G
Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

J Suresh
Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Balakrishnan
Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Kumar
Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Jeyakumar
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

N Kumaravadivel
Department of Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural, University, Coimbatore, Tamil Nadu, India

V Jegadeeswari
Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Amitha Kunikullaya G
Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Effect of water stress on photosynthetic parameters of cocoa (*Theobroma cacao* L.) genotypes

Amitha Kunikullaya G, J Suresh, S Balakrishnan, M Kumar, P Jeyakumar, N Kumaravadivel and V Jegadeeswari

Abstract
Cocoa (*Theobroma cacao*), crop of humid tropics is a drought sensitive crop. Evaluation of plant’s reaction for drought, screening for drought tolerant genotypes and to develop variety suited for regions with prolonged dry period has been given utmost importance to sustain the yield potential of cocoa under changing climatic scenario. A pot culture study was carried out with 16 Nigerian cocoa collections and two irrigation regimes (50% and 100%) to study the effect of water- deficit condition on growth and development of cocoa. Photosynthetic rate, stomatal conductance and transpiration rate in cocoa are highly influenced by environmental variables. Hence, a study on effect of drought on these physiological parameters revealed that the genotypes NC-23, NC-25 and NC-42 were drought tolerant as they recorded high photosynthetic rate, stomatal conductance coupled with low transpiration rate and thereby showed higher water use efficiency. These genotypes can be further evaluated for yield potential and can be used as one of the parent in future crop improvement program.

Keywords: cocoa- photosynthetic parameters- water deficit condition- response of genotypes

Introduction

Cocoa (*Theobroma cacao* L.) is a plantation crop of humid tropics with a rainfall requirement of 1500mm and 2000mm per year for its optimum growth and development. Climate change and limitations in availability of water in several regions of the world, pose a serious threat to plantation industry, especially cocoa as the crop is drought sensitive and productivity is greatly affected in the areas receiving erratic rainfall (Bae *et al.*, 2008) [1]. In India, cocoa, both in traditional belts of Kerala and Karnataka and non- traditional areas of Tamil Nadu and Andhra Pradesh undergo a dry period of four to six months a year. Moreover, Tamil Nadu experiences recurrent drought as 80% of the annual rainfall is received during North-East monsoon, and severe water-deficit prevails during summer months of February to May (Nathan, 1998) [2]. A physiological production model compiling data from 10 cocoa growing countries concluded that water limitation was responsible for 50% of yield loss (Zuidema *et al.*, 2005) [3]. Sustainability in crop production can only be achieved by understanding the complex phenomenon of global climate change and developing scientific approaches to face the weather uncertainities. Evaluation of plant’s reaction to stress is an essential step to screen and develop tolerant varieties for biotic and abiotic stresses. Abiotic stress like drought, temperature, salinity and elevated CO_2 have adverse impact on physiology and productivity of cocoa (Balasimha, 1983) [4].

Cocoa plants show several morphological, physiological and biochemical adaptive mechanisms for survival and growth under limited water supply. Sensitivity or tolerance to water stress can be determined by physiological characteristics as these mechanisms are not superficial and are dependent on genetic constitution of a plant (Raja and Hardwick, 1988) [5]. Photosynthetic rate, stomatal conductance and transpiration rate in cocoa are highly influenced by environmental variables. The present study was carried out with an objective to understand the changes in photosynthesis and stomatal behavior of cocoa genotypes under water stress and to screen the drought tolerant cocoa genotypes at an early growth phase.

Materials and Methods

A pot culture study was conducted at Tamil Nadu Agricultural University, Coimbatore to study the effect of imposed drought condition don gas exchange parameters of cocoa genotypes. Five months old seedlings of sixteen Nigerian cocoa collections (NC) were transplanted and one plant was maintained in each pot. The treatments were imposed when first flush started growing at 30 days after transplanting. 100 per cent field capacity for control pots and 50 per cent field capacity for treatment pots were maintained by weighing and watering each pot at regular interval. Photosynthetic parameters were recorded using an advanced Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) as open type principle after 30 days of stress imposition. The experiment was laid out in completely factorial randomized block design with three replications. The readings were recorded during 11.00 am to 12.30 pm (clear sunny day) when the photo synthetically active radiation was more than 1000 µmol photons m^{-2} s^{-1}. Fully expanded leaf from the top was clamped inside the leaf chamber and held perpendicular to incident light and computed values were recorded. Relative humidity was maintained at a steady level equal to the ambient RH to stimulate a condition very similar to that of ambient air. Using PPS system, the following gas exchange parameters were recorded and the values expressed as in parentheses.

Photosynthetic rate (pn: µmol CO_2 $m^{-2}s^{-1}$)
Stomatal Conductance (gs: mol H_2O m^{-2} s^{-1})
Transpiration rate (E: mmol H_2O $m^{-2}s^{-1}$)

The ratio of photosynthetic rate: stomatal conductance (pn/gs ratio) and Water Use Efficiency as ratio of photosynthetic rate: transpiration rate (pn/ E ratio) was calculated.

Results and Discussion

The results of photosynthetic rate of cocoa genotypes at different irrigation regimes are given in Table 1. Significant variations were observed among the genotypes for photosynthetic rate at both irrigation regimes of 50% and 100% field capacity. The highest photosynthetic rate was recorded in the genotype NC-23 (5.24 µmol CO_2 $m^{-2}s^{-1}$) closely followed by NC-25 (5.16 µmol CO_2 $m^{-2}s^{-1}$), while the photosynthetic rate was lowest in the genotype NC-20 (3 µmol CO_2 $m^{-2}s^{-1}$). Among the irrigation regimes, photosynthetic rate was higher in all the genotypes at 100% field capacity (4.75 µmol CO_2 $m^{-2}s^{-1}$) when compared with plants of the same genotypes subjected to water stress (50% field capacity). The reduction observed in the photosynthetic rate at 50% field capacity was lower in NC-23 with 27.97 per cent over control. Drought tolerant genotypes showed the least reduction in photosynthesis under water stress as they maintain high leaf water potential. Joly (1988) [6] suggested that net photosynthesis of cocoa seedlings declined when the leaf water potential fell below -0.8 to -1.0 MPa. Balasimha *et al*. (1988) [7] identified five accessions *viz.*, NC-23, NC-29, NC-31, NC-39 and NC-42 which were drought tolerant. The stressed plants showed 25% reduction in daily photosynthesis when compared to non- stress plants (Balasimha *et al.*, 1991) [8].

Table 1: Effect of different irrigation regimes on photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) of cocoa genotypes

Genotypes (G)	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) Irrigation regime (I)		Mean	Percentage over control
	50% FC	100% FC		
NC-15	2.15	4.27	3.21	49.65
NC-20	2.32	3.68	3.00	36.96
NC-21	2.41	3.51	2.96	31.24
NC-23	4.39	6.10	5.24	27.97
NC-25	4.29	6.03	5.16	28.94
NC-26	2.53	3.81	3.17	33.60
NC-27	2.57	3.90	3.24	34.10
NC-29	2.63	4.66	3.64	43.61
NC-30	2.99	4.89	3.94	38.96
NC-31	3.07	5.11	4.09	39.92
NC-36	3.50	5.20	4.35	32.79
NC-37	3.48	5.63	4.55	38.28
NC-42	3.02	5.35	4.18	43.50
NC-49	2.99	5.09	4.04	41.30
NC-50	2.91	4.25	3.58	31.57
NC-51	2.80	4.50	3.65	37.82
Mean	3.00	4.75	3.87	
	G	I	GxI	
SE(d)	0.13	0.36	NS	
CD (P=0.05)	0.26**	0.73**		

Significant differences were observed among cocoa genotypes subjected to 100% FC and 50% FC for stomatal conductance (Table 2.). Highest stomatal conductance was recorded in NC-36 (0.096 mol H_2O m^{-2} s^{-1}) followed by NC-25 (0.090 mol H_2O m^{-2} s^{-1}) while the lowest was observed in NC-42 and NC-49 (0.079 mol H_2O m^{-2} s^{-1}). However, no significant difference was recorded in stomatal conductance between 100% and 50% water regimes. The leaf conductance of stressed plant declined and decline in photosynthesis rate was also found to be closely related to stomatal conductance than with leaf water potential. The decline in stomatal conductance began well in advance to reduction in leaf water potential and it could be the reason for maintenance of high leaf water potential by reduction in transpiration rate (Joly and Hanh, 1989) [9]. Hutcheon (1977) [10] reported a close relationship between photosynthesis and stomatal conductance as the response of stomata to dry condition plays an important role in net assimilation rate of any crop.

Table 2: Effect of different irrigation regimes on stomatal conductance (mol H_2O m^{-2} s^{-1}) of cocoa genotypes

Genotypes (G)	Stomatal conductance (µmol CO_2 $m^{-2}s^{-1}$) Irrigation regime (I)		Mean	Percentage over control
	50% FC	100% FC		
NC-15	0.067	0.102	0.084	34.84
NC-20	0.079	0.098	0.088	20.05
NC-21	0.061	0.111	0.086	44.64
NC-23	0.062	0.104	0.083	40.89
NC-25	0.071	0.110	0.090	35.32
NC-26	0.068	0.107	0.087	36.21
NC-27	0.066	0.095	0.080	31.09
NC-29	0.055	0.102	0.079	46.32
NC-30	0.075	0.104	0.090	28.02
NC-31	0.071	0.091	0.081	21.40
NC-36	0.087	0.104	0.096	16.55
NC-37	0.074	0.102	0.088	27.87
NC-42	0.061	0.105	0.083	42.63
NC-49	0.062	0.097	0.079	36.28
NC-50	0.065	0.106	0.085	38.38
NC-51	0.069	0.098	0.083	29.28
Mean	0.068	0.102	0.085	
	G	I	GxI	
SE(d)	0.001	NS	NS	
CD (P=0.05)	0.003**			

Transpiration rate in cocoa genotypes showed marked differences as presented in Table 3. Highest transpiration rate was recorded in NC-20 (2.38 mmol H_2O $m^{-2}s^{-1}$) closely followed by NC-15 (2.30 mmol H_2O $m^{-2}s^{-1}$), while the lowest transpiration rate was observed in NC-23 (1.49 mmol H_2O $m^{-2}s^{-1}$). Irrigation regime of 50% and 100% field capacity also significantly influenced the transpiration rate with reduction of transpiration at 50% (1.45 and 2.53 mmol H_2O $m^{-2}s^{-1}$ at 50% and 100% FC respectively) in all the genotypes suggesting the efficient stomatal closure in cocoa during the drought period. The percentage of reduction of transpiration at 50% FC over control ranged from 25.78 per cent in NC-29, 28.19 per cent in NC-30 and 29.81 per cent in NC-15 implying the susceptibility of these genotypes due to in efficient control of water loss and loss of leaf water potential during stress period to 54.43 per cent in NC-42 and 52.44 per cent in NC-25. The results suggests that NC-42, NC-25, NC-27 and NC- 23 which showed high reduction in transpiration rate without much reduction in photosynthetic rate under 50% FC can be considered as tolerant genotypes. Baligar *et al.* (2008) [11] suggested that cocoa was ineffective in limiting transpiration loss under drought condition when compared to other rain forest tree species.

Table 3: Effect of different irrigation regimes on transpiration rate (mmol H_2O $m^{-2}s^{-1}$) of cocoa genotypes

Genotypes (G)	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$) Irrigation regime (I)		Mean	Percentage over control
	50% FC	100% FC		
NC-15	1.90	2.7	2.30	29.81
NC-20	1.73	3.035	2.38	43.16
NC-21	1.74	2.76	2.25	36.96
NC-23	0.99	1.99	1.49	50.50
NC-25	0.98	2.05	1.51	52.44
NC-26	1.59	2.44	2.01	35.04
NC-27	1.26	2.635	1.95	52.18
NC-29	1.90	2.56	2.23	25.78
NC-30	1.89	2.625	2.26	28.19
NC-31	1.32	2.415	1.87	45.34
NC-36	1.30	2.445	1.87	47.03
NC-37	1.55	2.695	2.12	42.49
NC-42	1.06	2.315	1.69	54.43
NC-49	1.14	2.26	1.70	49.56
NC-50	1.45	2.52	1.98	42.66
NC-51	1.52	3.02	2.27	49.67
Mean	1.45	2.53	1.99	
	G	I	GxI	
SE(d)	0.031	0.087	0.123	
CD (P=0.05)	0.063	0.177	0.250	

The ratio of photosynthetic rate and stomatal conductance (Pn:gs) showed significant difference among the genotypes and was high in NC-23 (65.09) followed by NC-25 (57.89). However, there was no significant difference in Pn: gs between either the two irrigation regime treatments or in the interaction of genotype and irrigation regime (Table 4.). Non-linear, highly significant relationship between Pn and gs indicated that net assimilation rate approached maximal values when high stomatal conductance of 55-65 mmol H_2O $m^{-2}s^{-1}$ (Joly and Hahn, 1989) [9].

The ratio of photosynthetic rate and transpiration rate (Pn: E) showed significant differences among the genotypes (Table 5.). The Pn: E ratio was high in NC-23 (3.77) and NC-25 (3.67) representing high water use efficiency in these genotypes. Low ratio of Pn: E was observed in NC-20 (1.28), NC-21 (1.33) and NC-15 (1.36) suggesting that these are susceptible genotypes. It can also be noted that the average Pn:E ratio was higher in stressed plants subjected to 50% field capacity, implying that during stress, cocoa genotypes show higher water use efficiency. Similar results were reported by Rada *et al*. (2005) [12] that drought increased Water Use Efficiency (WUE) in genotypes TARS-14 and Amelonado from Brazil. This may be due to efficient closure of stomatal aperture by the genotypes when subjected to drought to reduce loss of water through transpiration, thereby increasing WUE.

Table 4: Effect of different irrigation regimes on photosynthetic rate: stomatal conductance of cocoa genotypes

Genotypes (G)	Photosynthetic rate: Stomatal conductance		Mean
	Irrigation regime (I)		
	50% FC	100% FC	
NC-15	32.44	42.30	37.37
NC-20	29.54	37.43	33.48
NC-21	39.23	31.70	35.47
NC-23	71.25	58.94	65.09
NC-25	60.33	55.46	57.89
NC-26	37.22	35.43	36.32
NC-27	39.09	41.08	40.09
NC-29	47.59	45.60	46.59
NC-30	39.79	46.78	43.29
NC-31	43.03	56.41	49.72
NC-36	40.28	49.89	45.09
NC-37	47.23	55.66	51.44
NC-42	49.92	51.11	50.51
NC-49	48.49	52.59	50.54
NC-50	44.68	40.14	42.41
NC-51	40.51	46.09	43.30
Mean	44.41	46.66	45.54
	G	I	GxI
SE(d)	4.34	NS	NS
CD (P=0.05)	8.85		

Table 5: Effect of different irrigation regimes on photosynthetic rate: transpiration rate of cocoa genotypes

Genotypes (G)	Photosynthetic rate: Transpiration rate		Mean
	Irrigation regime (I)		
	50% FC	100% FC	
NC-15	1.14	1.58	1.36
NC-20	1.35	1.21	1.28
NC-21	1.39	1.27	1.33
NC-23	4.46	3.07	3.77
NC-25	4.39	2.94	3.67
NC-26	1.59	1.55	1.57
NC-27	2.05	1.47	1.76
NC-29	1.38	1.82	1.60
NC-30	1.58	1.88	1.73
NC-31	2.34	2.12	2.23
NC-36	2.71	2.14	2.42
NC-37	2.24	2.11	2.17
NC-42	2.86	2.31	2.59
NC-49	2.62	2.25	2.44
NC-50	2.01	1.68	1.85
NC-51	1.84	1.49	1.66
Mean	2.25	1.93	2.09
	G	I	GxI
SE(d)	0.060	0.19	0.27
CD (P=0.05)	0.130**	0.39**	0.55**

High clonal diversity in cocoa for photosynthetic parameters was also reported by Elain Apshara *et al.* (2013) [13]. Water deficit leads to progressive suppression of photosynthesis and it is associated with alterations in C and N assimilation (Yordanov *et al.*, 2000) [14]. The ability to maintain functionality of photosynthetic apparatus under water stress is an important parameter for drought tolerance. Thus, knowledge on fluctuations in physiological parameters to environmental changes can be used to identify genotypes whsich are tolerant and can be utilized in further breeding programme.

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