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Effect of water deficit on physiological and biochemical responses in cocoa (*Theobroma cacao* L.) clones

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Abstract
The present pot culture study on the effect of water stress on physiological and biochemical characteristics of twenty cocoa genotypes was taken up with two irrigation regimes (100% FC and 50% FC) under glasshouse condition. The results revealed that irrigation at 50 % field capacity showed an inhibitory effect on overall growth characteristics of all genotypes and the changes in leaf epicuticular wax, chlorophyll content and NRase, phenol, and proline accumulations. Based on the biochemical changes observed between the genotypes in two irrigation regimes revealed that VTLCH 3 and VTLCH 4 were grouped as tolerant genotypes.

Keywords: Cocoa, drought, antioxidant enzymes, chlorophyll content

Introduction
Cocoa (*Theobroma cacao* L.) is an important plantation in the current horticulture scenario and it is grown in tropical regions of the world to produce beans which are used for the manufacture of chocolate and other confectioneries. Cocoa is an introduced crop in India during 1798 (Ratnam, 1961) [29], and it is cultivated as a rainfed mixed crop in palm based cropping systems in traditional areas of Kerala and Karnataka (Balasimha, 1987) [5]. Cocoa is widely cultivated as an irrigated intercrop under coconut and oil palm in non- traditional areas of Tamil Nadu and Andhra Pradesh. Several environmental factors control the growth of cocoa. Flowering, flushing and expansion of the leaves were influenced by the temperature (Sale, 1968 and 1969) [32, 33] humidity and soil moisture (Sale, 1970) [34]. The cocoa tree needs a high, well-distributed rain, possibly with a short dry period to stimulate flowering. In recent decades, cocoa productivity is very low due to high climate variability such as higher temperatures, decrease in rainfall and shorter rainy seasons (Brou, 2005) [9]. Cocoa is intolerant to drought (Mohdrazi *et al*., 1992; Wood and Loss, 2001) [23, 36] and this situation warrants the identification of cocoa varieties/clones for drought tolerance along with high yield. Cultivation of drought tolerant genotypes is one of the most sustainable ways to reduce the impacts of marginal rainfall and prolonged dry periods on cocoa production and productivity.
Physiological response to drought stress is characterized by a reduction in water content, diminished leaf water potential, loss of turgor, closure of stomata and cessation of cell enlargement and growth. Farooq *et al* (2009) [12] reported that drought stress inhibits the growth of the plant and causes oxidative and cellular damage. Various morphological adaptations for drought tolerance include reduction of leaf area, leaf rolling, presence of hairiness, epicuticular wax deposition, vigorous root system *etc*. Deposition of epicuticular wax on the leaf has often been suggested as valid criteria for the selection of drought tolerant genotypes. In cocoa, water stress damages the photosynthetic apparatus, thereby influencing chlorophyll content and photosynthesis. Therefore chlorophyll content and chlorophyll stability index were used to detect drought tolerance (Ravindran and Menon, 1982) [30]. The accumulation of compatible osmolytes such as proline, which acts as enzyme protectant and free radical scavenger, is considered an important parameter for selection of crop varieties for drought tolerance (Sharma and Aravind Kumar, 1991) [35]. Faghani Elham *et al*. (2012) [11] suggested that the deposition of epicuticular wax on the leaf has often used as valid criteria for the selection of drought tolerant genotypes. Phenolic compounds such as phenolic acid, anthocyanin, and flavonoids reduce ROS and prevent cell damage (Hatier and Gould, 2008) [16]. The activity of nitrate reductase is associated with a metabolic and physiological status of plants and can be used as a biomarker of plant stress including drought. Therefore, the aim of the present study was to identify drought tolerant genotypes on the basis of physiological and Biochemical factors and to evaluate the existence of genotypic variability at the initial growth phase.

Materials and Methods

Vegetative multiplied five months old cocoa clones (10 high yielding plus trees TNAUCC 1 to TNAUCC 10 besides the 7 KAU clones CCRP 1 to CCRP 5 and 5 Vittal clones VTLCC1, VTLCH-1 to VTLCH-4) were planted in pots under the protected condition at Tamil Nadu Agricultural University, Coimbatore. The treatments were imposed with two irrigation levels 100 % field capacity and 50 % field capacity (gravimetric method), when the first flush started growing at 15 days after transplanting. The experiment was laid out in a completely factorial randomized block design with four replications. All the analyses were performed 30 days after stress imposition. Chlorophyll content in the leaf was determined using a chlorophyll meter (SPAD 502) and the data were recorded as described by Peng *et al*. (1996) [28]. The chlorophyll stability index (CSI) was determined using the Koloyereas method (1958) [19]. The wax content was determined by a modification of the colorimetric method described by Ebercon *et al*. (1977) [10]. The nitrate reductase activity in the leaves was determined according to the method of Nicholas *et al*. (1976) [26]. Estimation of total phenols content by the method of Folin ciocalteau reagent described by Bray and Thrope, 1954 [8]. The proline content in the leaves was estimated by the method of Bates *et al*. (1973) [6]. The collected data were subjected to statistical analysis of their significance (Panse and Sukthme, 1961) [27].

Results and Discussion

Performance of twenty cocoa genotypes for physiological and biochemical parameters under 50% and 100% Field capacity are presented in Table 1 and 2. High significant differences were recorded for all the parameters among the genotypes, water regimes and their interactions except for chlorophyll index which recorded non-significant differences among the genotype and irrigation regime interaction. ures 1, 2, 3 and 4 illustrate the relationships between the various physiological and biochemical parameters under stress and optimal conditions. Under optimum conditions, five clones VTLCH 4, VTLCH 3, CCRP 1, CCRP 2 and VTLCC 1 recorded a higher rate of chlorophyll index (40.74, 39.56, 38.05, 36.86 and 36.32) and remained on par with each other. When subjected to 50% field capacity chlorophyll index significantly decreased (32.42) as compared to 100 % field capacity (37.36). Reduction in SPAD values under drought shows that the drought stress blemishes the chlorophyll content through an internal modification in the thylakoid membrane. Similar to this finding, Ghaffari *et al*. (2012) [13] stated that the tolerant sunflower line had a higher SPAD value than the susceptible line in drought condition. Among the 20 different clones screened for drought tolerance, the higher chlorophyll index was found in VTLCH 4 and VTLCH 3 under stress condition. Due to imposed drought stress a reduction in chlorophyll stability index from 81.82 to 70.35 was observed. The genotypes, VTLCH 4 and VTLCH 3 showed more than 80 percent of the chlorophyll stability index and TNAUCC 2 and CCRP 5 recorded the lowest percentage of chlorophyll stability indices (73.13 and 73.07). Under water stress condition, VTLCH 4 and VTLCH 3 relatively experienced the highest chlorophyll stability index (79.80 and 78.67) indicating their drought tolerant nature. The chlorophyll stability index is considered as an important physiological factor to screen drought tolerance (Murthy and Majumder, 1962) [24].

Cocoa genotypes responded differentially under water deficit conditions for biochemical parameters. Estimation of NRase activity in the present investigation revealed that NRase activity was significantly reduced under stress condition. Genotypes, VTLCH 4 showed higher enzyme activity (10.91 μmol NO_2 g^{-1} h^{-1}) followed by VTLCH 3 (10.77 μmol NO_2 g^{-1} h^{-1}) while the least nitrate reductase activity was observed in TNAUCC 2 (8.26 μmol NO_2 g^{-1} h^{-1}) followed by TNAUCC 7 (8.46 μmol NO_2 g^{-1} h^{-1}) and VTLCH 1 (8.59 μmol NO_2 g^{-1} h^{-1}) which were on par with each other. Among treatments, the plants imposed with 50 % field capacity registered lower enzyme activity (6.11) than 100 per cent field capacity (13.08). Under drought condition, VTLCH 3, VTLCH 4, VTLCH 2 and VTLCC 1 recorded a significantly higher nitrate reductase activity (7.23, 7.19, 6.63 and 6.63 μmol NO_2 g^{-1} h^{-1} respectively). The reduction of NRsae values under drought is associated with a high proline accumulation in drought tolerant genotypes (Matt and Pauli, 1965) [22]. Differential performance of genotypes for enzyme activity could be realized under water deficit condition. Among the 20 different clones screened for drought tolerance, it was found that NRase is higher with VTLCH 3 and VTLCH 4 under stress condition. Genotypic differences in nitrate reductase activity also reported in several other crops (Sinha *et al*., 1974; Reed and Hageman, 1980) [37, 31].

The phenol content varied significantly between genotypes, water regimes and genotype water regime interactions. Genotypes, VTLCH 4 showed significantly higher phenol content (2.81mg g^{-1}) followed by VTLCC 1 (2.68mg g^{-1}) and VTLCH 3 (2.68mg g^{-1}) compared to the other genotypes. TNAUCC 5 (1.98mg g^{-1}) followed by TNAUCC 10 (2.06mg g^{-1}), TNAUCC 2 (2.07mg g^{-1}) and CCRP 5 (2.08mg g^{-1}) registered the least phenol content. With respect to water regimes, the stress condition enhanced significantly higher phenol content (2.94mg g^{-1}) than 100 per cent field capacity (1.58mg g^{-1}). Under 50% FC, VTLCH 4 showed its supremacy by recording higher phenol content (3.87mg g^{-1}) followed by VTLCH 3 (3.75mg g^{-1}) and VTLCC1 (3.5mg g^{-1}). The Accumulation of phenol content in water stress is a mechanism of drought adaptation (Hernández *et al*., 2000; Koskeroglu and Tuna, 2008; Venkatesan and Sridevi, 2009) [17, 20, 38].

The highest proline content was observed in VTLCH 4 (514.63μg g^{-1}) and VTLCH 3 (510.61μg $g^{-1)}$ while the lowest value was observed in TNAUCC 2 (441.24μg g^{-1}). Under water stress condition, proline content substantially increased to 703.78 versus 247. 31 under 100 % field capacity. At 100 percent field capacity, CCRP 2 and VTLCH 2 recorded the highest proline content (259.61 and 259.60μg g^{-1}) compared to all the genotypes. Performance of the genotypes under drought indicated that VTLCH 4 and VTLCH 3 were significantly superior as expressed higher proline accumulation of 781.39μg $g^{-1}$and 776.46μg g^{-1} respectively than any other genotypes. Proline accumulation during water stress is a drought adaptive mechanism (Kramer, 1983) [21]. An increase in the proline content by water stress has been suggested as evidence of resistance to water stress (Gupta and Gupta, 1997) [14]. Similar results were obtained in cocoa Balasimha (1982) [3], pepper (Nath *et al*., 2005) [25], coconut (Kasturi Bai and Rajagopal, 2000) [18], wheat (Hamada, 2000) [15] and sorghum (Yadav *et al*., 2005) [40]. Abdellah *et al*. (2011) [1] reported that the increased proline content was in observed tolerant wheat cultivar by water stress (30 per cent FC) over control.

Epicuticular wax plays an important role in plant's ability to withstand water deficits and is known to increase due to stress (Baker, 1974; Bengston *et al*., 1978) [2, 7]. A higher

epicuticular wax content was associated with drought tolerant accessions of cocoa (Balasimha, 1984) [4]. The drought tolerant clones i.e. VTLCH 4, VTLCH 3 had a higher wax content (0.37µg/cm^2 and 0.34µg/cm^2) TNAUCC 5 clone sensitive to drought (0.21µg/cm^2), which confirms that a higher wax content is associated with drought tolerance. Shepherd and Wynne Griffiths (2006) [36] reported that increased cuticular wax content minimize the adverse effects of drought stress by reducing leaf transpiration and maintained stomatal conductance.

Table 1: Effect of irrigation regime on chlorophyll index (SPAD value) and Chlorophyll Stability Index (%) of cocoa genotypes

Genotypes (G)	Chlorophyll index (SPAD value)				Chlorophyll Stability Index (%)		
	Irrigation Regime (I)		Mean	Percentage over Control	Irrigation Regime (I)		Mean
	100 %FC	50 %FC			100%FC	50 %FC	
TNAUCC 1	37.69	33.09	35.39	12.20	88.42 (70.12)	70.62 (57.17)	79.52 (63.65)
TNAUCC 2	36.52	29.90	33.21	18.13	79.58 (63.14)	66.67 (54.75)	73.13 (58.94)
TNAUCC 3	35.72	30.20	32.96	15.45	87.34 (69.19)	68.69 (55.98)	78.02 (62.59)
TNAUCC 4	38.20	33.03	35.62	13.53	82.34 (65.22)	66.68 (54.80)	74.51 (60.01)
TNAUCC 5	36.41	31.21	33.81	14.28	81.47 (64.51)	65.47 (54.01)	73.47 (59.26)
TNAUCC 6	35.35	28.80	32.08	18.53	78.74 (62.54)	69.94 (56.75)	74.34 (59.64)
TNAUCC 7	37.51	31.51	34.51	16.00	78.67 (62.50)	68.10 (55.61)	73.39 (59.05)
TNAUCC 8	34.69	29.59	32.14	14.70	79.56 (63.12)	68.13 (55.63)	73.85 (59.37)
TNAUCC 9	33.81	28.59	31.20	15.44	80.42 (63.74)	67.68 (55.35)	74.05 (59.55)
TNAUCC 10	37.31	31.33	34.32	16.03	76.01 (60.68)	69.29 (56.35)	72.65 (58.51)
CCRP 1	40.31	35.78	38.05	11.24	83.85 (66.43)	74.04 (59.40)	78.95 (62.92)
CCRP 2	38.91	34.81	36.86	10.54	80.45 (63.76)	72.65 (58.47)	76.55 (61.12)
CCRP 3	38.77	32.63	35.70	15.84	82.22 (65.06)	69.41 (56.42)	75.82 (60.74)
CCRP 4	36.11	31.61	33.86	12.46	79.67 (63.20)	70.27 (56.96)	74.97 (60.08)
CCRP 5	36.79	31.49	34.14	14.41	78.43 (62.32)	67.70 (55.37)	73.07 (58.84)
VTLCC 1	38.33	34.31	36.32	10.49	80.92 (64.10)	72.69 (58.49)	76.81 (61.29)
VTLCH 1	34.52	30.23	32.38	12.43	79.50 (63.09)	69.68 (56.59)	74.69 (59.84)
VTLCH 2	37.47	32.40	34.94	13.53	82.47 (65.32)	70.85 (57.34)	76.66 (61.33)
VTLCH 3	40.91	38.21	39.56	6.60	87.67 (69.46)	78.67 (62.49)	83.17 (65.98)
VTLCH 4	41.79	39.68	40.74	5.05	88.76 (70.41)	79.80 (63.28)	84.28 (66.85)
Mean	37.36	32.42	34.89		81.82 (64.89)	70.35 (57.06)	76.09 (60.98)
	G	I	G X I		G	I	G X I
SE(d)	2.25	0.71	3.18		0.758	0.239	1.072
CD (P=0.05)	4.48**	1.41**	NS		1.509**	0.477**	2.135**

NS- Non Significant, * Significant, ** Highly Significant Figures in parentheses are arcsine transformed values

Table 2: Effect of irrigation regime on Nitrate Reductase activity (µmol NO_2 g^{-1} h^{-1}), Phenol (mg g^{-1}) and Proline (µg g^{-1}) of cocoa genotypes

No types (G)	Nitrate Reductase activity (µmol NO_2 g^{-1} h^{-1})			Phenol (mg g^{-1})			Proline (µg g^{-1})		
	Irrigation Regime (I)			Irrigation Regime (I)			Irrigation Regime (I)		
	100 %FC	50 %FC	Mean	100 %FC	50 %FC	Mean	100 %FC	50 %FC	Mean
TNAUCC 1	12.64	6.12	9.38	1.63	2.98	2.30	248.56	664.25	456.41
TNAUCC 2	11.42	5.10	8.26	1.45	2.68	2.07	232.72	649.75	441.24
TNAUCC 3	14.11	6.53	10.32	1.49	2.71	2.10	237.53	661.12	449.33
TNAUCC 4	12.61	5.73	9.17	1.67	2.59	2.13	246.00	689.78	467.89
TNAUCC 5	14.60	5.64	10.12	1.34	2.62	1.98	254.03	700.74	477.39
TNAUCC 6	13.72	5.63	9.67	1.57	2.93	2.25	241.82	721.33	481.58
TNAUCC 7	10.75	6.16	8.46	1.48	2.84	2.16	253.07	668.31	460.69
TNAUCC 8	13.24	6.34	9.79	1.65	2.96	2.31	238.94	679.70	459.32
TNAUCC 9	12.90	5.22	9.06	1.61	2.76	2.19	246.04	736.88	491.46
TNAUCC 10	13.91	5.36	9.64	1.49	2.63	2.06	248.94	693.66	471.30
CCRP 1	13.96	6.43	10.20	1.61	2.60	2.11	248.63	666.67	457.65
CCRP 2	14.46	6.44	10.45	1.81	3.14	2.48	259.61	744.93	502.27
CCRP 3	13.69	5.88	9.79	1.75	3.03	2.39	240.08	742.64	491.36
CCRP 4	13.25	6.16	9.71	1.45	2.96	2.21	248.60	703.22	475.91
CCRP 5	12.23	5.85	9.04	1.34	2.81	2.08	233.53	685.03	459.28
VTLCC 1	12.14	6.63	9.39	1.77	3.59	2.68	257.20	723.17	490.19
VTLCH 1	11.24	5.93	8.59	1.47	2.77	2.12	258.75	716.18	487.47
VTLCH 2	11.74	6.63	9.19	1.65	2.64	2.15	259.60	670.42	465.01
VTLCH 3	14.30	7.23	10.77	1.60	3.75	2.68	244.76	776.46	510.61
VTLCH 4	14.62	7.19	10.91	1.75	3.87	2.81	247.86	781.39	514.63
Mean	13.08	6.11	9.59	1.58	2.94	2.26	247.31	703.78	475.55
	G	I	G X I	G	I	G X I	G	I	G X I
SE(d)	0.170	0.053	0.241	0.027	0.008	0.0390	9.676	3.050	13.68
CD (P=0.05)	0.339**	0.107**	0.479**	0.0549**	0.0173**	0.077**	19.25**	6.08**	27.22**

NS- Non Significant, * Significant, ** Highly Significant

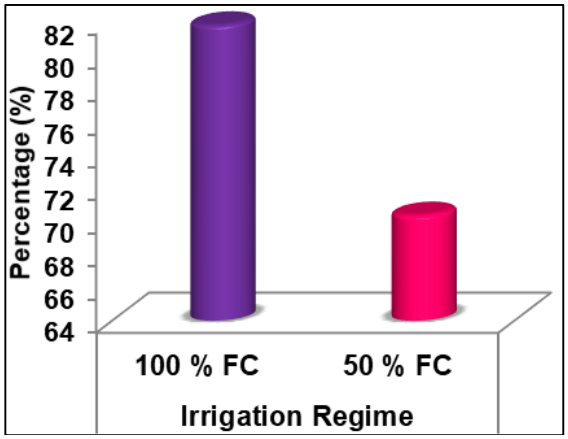

Fig 1: Effect of irrigation regime on Chlorophyll Stability Index (%) in cocoa

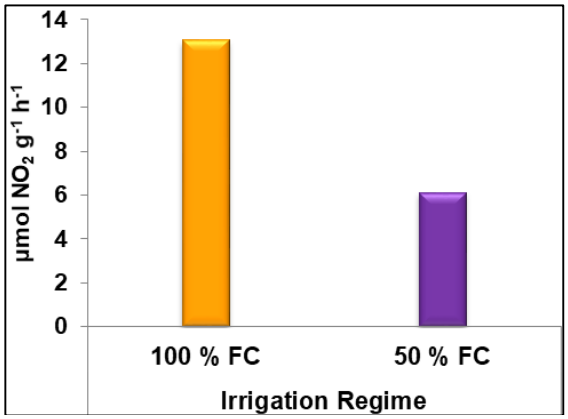

Fig 2: Effect of irrigation regime on Nitrate Reductase activity (µmol NO_2 g^{-1} h^{-1}) in cocoa

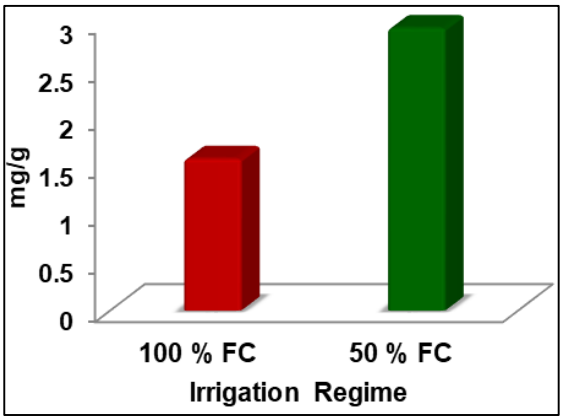

Fig 3: Effect of irrigation regime on phenol (mg g^{-1}) content in cocoa

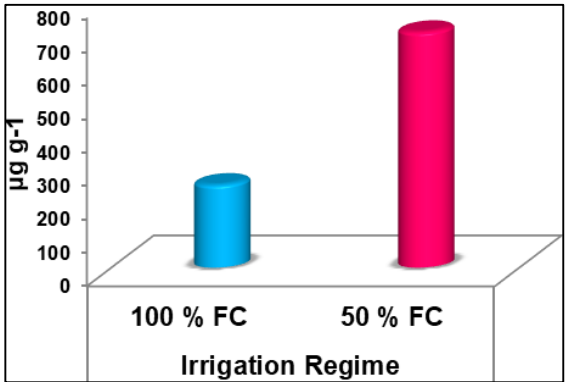

Fig 4: Effect of irrigation regime on proline (µg g^{-1}) content in cocoa

Conclusion

Physiological and biochemical parameters were used as a predictor or marker for the indirect selection of drought tolerant genotypes at drought stressed conditions. VTLCH 3 and VTLCH 4 showed tolerance to drought due to chlorophyll stability and a greater accumulation of proline, phenols, NRase and epicuticular wax. The genotype with drought tolerance characteristics can be used in the future breeding programme.

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