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Research Article

Evaluation of cocoa (*Theobroma cacao* L.) clones under natural rainfed conditions for drought tolerance

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Abstract

Seven cocoa clones (CCRP 1 to CCRP 7) were evaluated to study for their morpho-physiological and biochemical parameters under the rainfed situation at HRS, Thadiyankudisai. The results showed that significant differences between stress and non-stress periods as well as among the varieties. The rank sums of these parameters showed that the clone CCRP 4 followed by CCRP 3 showed promising drought-tolerant nature with high yielding potential.

Keywords: Cocoa, water stress, morphological, physiological and biochemical parameters

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Introduction

Cocoa (*Theobroma cacao* L.) is a cross-pollinated, perennial and diploid plant belonging to the family of Malvaceae [1]. It is considered an important plantation crop indigenous to South America- Amazon river basin and the seeds are extensively used in cocoa powder and chocolate industry. Cocoa, introduced in India during1798 [2] is now being cultivated predominantly in four states *viz*., Kerala, Andhra Pradesh, Tamil Nadu and Karnataka traditionally as mixed cropping in arecanut and coconut plantations. The crop prefers a warm humid tropical condition with an average rainfall of 1250 to 3000 mm per annum, preferably between 1500 to 2000 mm with a dry season of not more than three months with less than100 mm rainfall per month. An ideal mean temperature varying between 30°C to 32°C and a mean minimum between 18°C to 21°C with an absolute minimum of 10°C is desirable for cocoa cultivation [3]. Cocoa tree needs a high and well-distributed rainfall, possibly with a short dry spell to stimulate flowering. Unfortunately, with the recent impact of global warming, such an ideal climate is far from prevailing and rain in these areas is either irregular or low often causing water stress which affects not only the yield but also its contributory factors such as canopy architecture, photosynthesis and partitioning of assimilates *etc*. Therefore, it is of paramount importance to identify clones, which can withstand moisture stress conditions in the field, and to evolve management strategies for conserving soil water in order to mitigate the adverse effect of drought. The present investigation was undertaken under field conditions to study the physiological and biochemical mechanisms of water stress tolerance in cocoa clones.

Materials and Methods

The plant material consists of seven cocoa varieties from Kerala Agricultural University *viz*., CCRP 1 to CCRP 7were used in this study. These budded clones were planted in the field at a spacing of 3 m x 3 m during 2010 at Horticultural Research Station (HRS), Thadiyankudisai located at Lower Pulney hills (1098 MSL), Tamil Nadu, India. The experiment was laid out in Randomized Complete Block Design (RCBD)with three replications. The packages of practices recommended by Tamil Nadu Agricultural University were followed throughout the growing season. The relative humidity, maximum and minimum temperature and rainfall during the study period were presented in Figure **1**. The physiological parameters and biochemical parameters were observed in February 2014 following a severe dry month period *i.e.* from November 2013 to February 2014 (pre-monsoon period) and also in September 2014 following rainy period *i.e.* from March to September 2014 (monsoon period). Similar field screening in cocoa was followed in CPCRI, Regional Station, Vittal for taking advantage of the natural dry months as stress period and rainy months as a non-stress period [4, 5].

Morphological parameters

The plant height was taken from ground level up to the tip of the canopy and expressed in meter and the diameter of scion was measured at 2 cm above the bud union with vernier calipers and results were expressed in centimeter. The first branching or the jorquette height was measured on the main stem from ground level and expressed in centimeter. The fan branches arising from the first jorquette was counted and expressed in numbers. Spread of the canopy was measured in both East-West and North-South directions and expressed in meter. The canopy area was calculated from the mean growth parameters considering the canopy as cone-shaped using the formula πr land expressed in metre^2. Whereas $r = (EW+NS)/ 4$ and $l = \sqrt{r^2 + h^2}$, h = canopy height [6]. The pod harvested in each harvest was numbered in the field itself and the total number of pods was recorded.

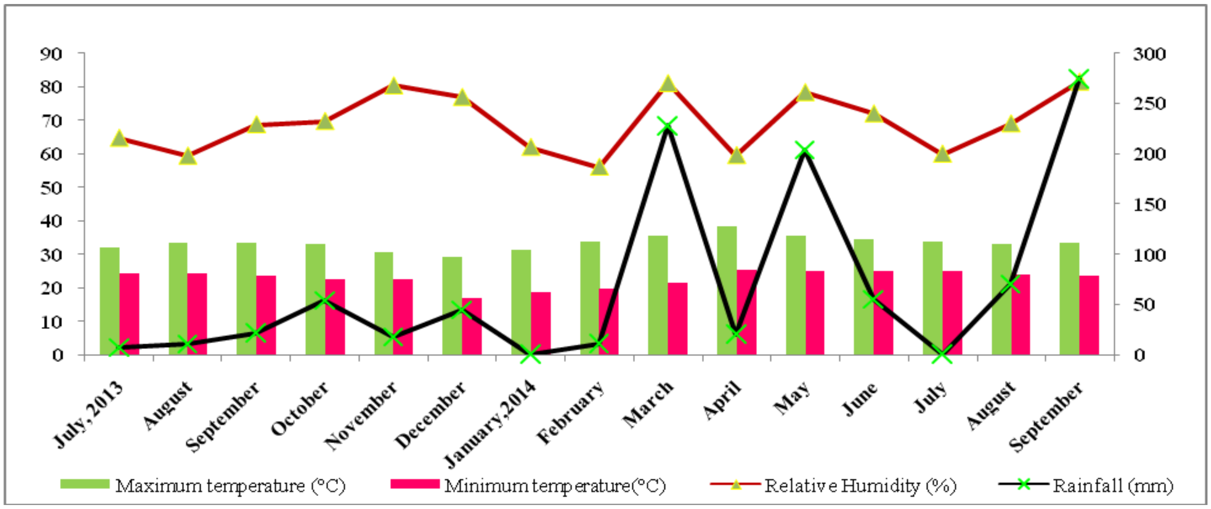

Figure 1 Monthly maximum and minimum mean temperature, total rainfall and relative humidity from July 2013 to September 2014

Physiological parameters

Specific Leaf Weight (SLW) of the leaf sample was measured by the method suggested by Pearce *et al.* [7]. The Membrane Stability Index (MSI) of the leaf sample was determined as per the method of Premachandra *et al.* [8]. The Chlorophyll stability index (CSI) was determined by adopting the method of Leopold *et al.* [9]. The Relative Water Content (RWC) was estimated according to Barrs and Weatherly [10].

Biochemical parameters

Nitrate reductase activity in leaves was estimated according to the method of Nicholas *et al.* [11]. The folin ciocalteau reagent method was followed for estimating the total phenols [12]. The proline content was measured as described by Bates *et al.* [13]. The catalase activity of the leaf sample was determined as per the method of Gopalachari [14]. The data collected were subjected to statistical analysis for their significance [15].

Results and Discussions

Morphological parameters

The present investigation, growth performance of seven clones was recorded during the end of the drought period and the results obtained are presented in **Table 1**.Clonal variability for plant height was significantly observed and CCRP 3 (2.64 m) followed by CCRP 4 (2.41 m) had the maximum height under rainfed condition. The girth of the plant also exhibited significant differences among the clones and CCRP 4 (18.71 cm) followed by CCRP 3 (16.38 cm) had higher girth in their plants. Jorquette height and number of fan branches among the clones, however, did not exhibit significant variation among them. The plants spread in both the direction (EW- NS) also exhibited significant differences among the clones and the maximum spread was observed in clones CCRP 3 (2.88 m and 2.73 m) and CCRP 4 (2.73 m and 2.78 m). The canopy area observed also manifested significant differences among the clones and among them, CCRP 3 (13.18 m^2) and CCRP 4 (12.01 m^2) produced maximum canopy area. The number of pods produced per season also showed significant variation among the clones and CCRP 4 (11.67) followed by CCRP 3 (9.33) produced the maximum number of pods. The height at first branching and number of branches did not show significant variation among the clones as primary training and pruning measures were undertaken almost uniformly to all the clones in the initial years of clones. Among the seven clones evaluated, CCRP 3 and CCRP 4 relatively performed better under the drought situation prevailed in the rainfed area of Thadiyankudisai condition, which is in line with the previous findings [4].

Table 1 Effect of drought on morphological characters of CCRP varieties at HRS, Thadiyankudisai

S. No.	Clones	Height (m)	Girth (cm)	Jorquette Height (cm)	Fan Branches (No.)	Canopy Spread		Canopy Area (m^2)	Pods (No.)
						EW (m)	NS (m)		
1	CCRP 1	2.34	15.26	16.33	4.00	2.49	2.53	10.53	7.67
2	CCRP 2	2.15	14.05	20.87	3.00	2.42	2.24	9.02	7.00
3	CCRP 3	2.64	16.38	16.77	3.33	2.88	2.73	13.18	9.33
4	CCRP 4	2.41	18.71	17.13	3.00	2.73	2.78	12.01	11.67
5	CCRP 5	1.85	14.14	16.70	3.00	2.26	1.84	6.83	6.33
6	CCRP 6	2.23	13.46	17.07	2.67	2.39	1.91	8.08	6.67
7	CCRP 7	1.81	14.07	19.50	2.33	2.15	1.80	6.42	5.33
Mean		**2.20**	**15.15**	**17.77**	**3.05**	**2.47**	**2.26**	**9.44**	**7.71**
SE(d)		0.23	1.01	2.32	0.65	0.08	0.33	1.17	1.19
CD (P=0.05)		0.50*	2.21**	NS	NS	0.17**	0.73*	2.55**	2.60**

NS- Non-Significant, * Significant, ** Highly SignificantEW: East-West spread, NS: North-South spread

Physiological parameters

The seven cocoa clones evaluated for drought tolerance under natural conditions exhibited significant differences among them for physiological and biochemical parameters during the pre-monsoon period. However, during monsoon month they failed to show significant variation among the clones (**Table 2** and **Table 3**). Among the physiological traits, clones did not exhibit significant differences for specific leaf weight under both pre-monsoon and monsoon seasons. The water potential of the leaf is a major quantitative characteristic used to assess water stress. Photosynthetic efficiency is a primary determinant of cocoa productivity and in cocoa high photosynthetic rate associated with thick leaves or high specific leaf weight which is characteristic of vigorous trees [16]. However, Balasimha [17] observed while screening under pot condition for drought tolerance, he observed certain cocoa clones exhibited significant differences for SLW but lamented not due to stress. CCRP 4 (64.33 percent) followed by CCRP 3 (61.79 percent) had higher relative water content during this period while CCRP 5 had the least relative water content (51.69 percent). Cocoa plants show changes in water relations when soil moisture drops to 60 to 70 percent of the available range [18]. The onset of drought decreased water potential and relative water content [19, 20]. During the drought period, a plant with higher RWC tends to overcome the drought impact. [17] Also observed higher RWC in tolerant clones than susceptible clones.

Table 2 Effect of drought on physiological parameters of CCRP varieties at HRS, Thadiyankudisai

S. No.	Clones	Specific Leaf Weight (mg/cm^2)		Membrane Stability Index (%)		Relative Water Content (%)		Chlorophyll Stability Index (%)	
		Pre-monsoon	Mon soon	Pre-monsoon	Monsoon	Pre-monsoon	Monsoon	Pre-monsoon	Monsoon
1	CCRP 1	7.20	7.28	53.13 (46.79)	79.65 (64.67)	57.90 (49.55)	85.25 (67.83)	66.71 (54.76)	87.73 (70.17)
2	CCRP 2	7.58	7.59	59.27 (50.34)	71.78 (58.03)	59.99 (50.77)	87.42 (70.14)	71.60 (57.80)	88.6 (71.45)
3	CCRP 3	7.04	7.17	59.06 (50.22)	70.28 (57.60)	61.79 (51.82)	86.43 (68.73)	70.38 (57.03)	86.17 (69.64)
4	CCRP 4	7.20	7.25	57.46 (49.30)	75.14 (60.79)	64.33 (53.34)	87.14 (69.66)	73.20 (58.85)	87.16 (69.67)
5	CCRP 5	7.13	7.20	54.28 (47.46)	73.71 (59.82)	51.69 (45.97)	85.61 (68.15)	67.29 (55.12)	84.36 (67.05)
6	CCRP 6	7.33	7.25	44.86 (42.05)	75.84 (60.75)	56.96 (49.00)	82.14 (65.40)	62.08 (51.99)	85.29 (68.08)
7	CCRP 7	7.52	7.59	50.22 (45.13)	74.96 (61.06)	55.84 (48.36)	83.683 (66.60)	65.61 (54.10)	86.42 (69.05)
Mean		7.29	7.33	54.04	74.48	58.36	85.38	68.12	86.53

			(47.33)	(60.39)	(49.83)	(68.07)	(55.67)	(69.30)
SE(d)	0.90	1.01	0.54	7.74	0.62	5.43	0.77	6.37
CD (P=0.05)	NS	NS	1.19**	NS	1.36**	NS	1.68**	NS

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

Table 3 Effect of drought on biochemical parameters of CCRP varieties at HRS, Thadiyankudisai

S. No.	**Clones**	**Nitrate Reductase (μmol NO_2 $g^{-1}h^{-1}$)**		**Phenol (mg g^{-1})**		**Proline (μg g^{-1})**		**Catalase (μg H_2O_2 g^{-1} min^{-1})**	
		Pre-monsoon	**Monsoon**	**Pre-monsoon**	**Monsoon**	**Pre-monsoon**	**Monsoon**	**Pre-monsoon**	**Monsoon**
1	CCRP 1	5.85	8.36	3.25	1.68	884.78	136.1	7.05	9.26
2	CCRP 2	6.27	9.25	3.69	1.85	901.45	129.2	6.85	9.18
3	CCRP 3	6.00	8.91	3.73	1.96	914.69	136.4	6.95	9.23
4	CCRP 4	6.31	9.38	3.76	1.76	913.56	126.5	7.23	9.44
5	CCRP 5	5.64	8.52	3.38	1.64	845.60	137.7	6.19	9.21
6	CCRP 6	5.39	9.40	3.20	1.61	849.86	132.1	6.27	9.25
7	CCRP 7	5.02	8.88	2.87	1.76	836.15	139.3	6.42	9.65
Mean		5.78	8.96	3.41	1.75	878.01	133.89	6.71	9.32
SE(d)		0.10	1.01	0.06	0.26	15.31	12.55	0.11	1.39
CD (P=0.05)		0.22**	NS	0.13**	NS	33.37**	NS	0.25**	NS

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

CCRP 2 (59.27 percent) and CCRP 3 (59.06 percent) exhibited the maximum membrane stability index; however, they remained on par with each other. The least membrane stability index was observed with CCRP 6 (44.86 percent).CCRP 4 (73.20 percent) followed by CCRP 2 (71.60 percent) and CCRP 3 (70.38 percent) relatively exhibited higher chlorophyll stability index whereas CCRP 6 recorded least chlorophyll stability index (62.08 percent) during pre-monsoon month. Chlorophyll Stability Index (CSI) is an indicator of the stress tolerance capacity of the plants and is a measure of the integrity of the membrane [21]. The reduction in CSI and membrane stability during the drought period (pre-monsoon) due to the primary effect of drought at the cellular level to affect the integrity of membrane which in turn leads to disruption of cellular compartment ultimately destruction of chlorophyll contents. However, studies by some researchers [22-24] and revealed that the ability of the genotype maintaining the higher CSI under drought is a desirable character for tolerance and this might be due to high membrane stability.

Biochemical parameters

Results pertaining to biochemical parameters revealed that the estimation of nitrate reductase (NRase) activity exhibited significant differences among the clones during the pre-monsoon period. The NRase activity was higher with CCRP 4 (6.31μmol NO_2 g^{-1} h^{-1}), CCRP 2 (6.27μmol NO_2 g^{-1} h^{-1}) followed by CCRP 3 (6.00μmol NO_2 g^{-1} h^{-1}) in the order during pre-monsoon month (Table 3). NRase is an important enzyme for nitrogen assimilation ultimately protein synthesis in a plant cell that is highly sensitive to the water stress condition. The NRase activity described as a marker of abiotic stress tolerance in plants, which reduced under moisture stress conditions [25]. However, [26] stated that maintenance of NRase activity is an imperative role by the tolerant genotypes for nitrogen assimilation and protein synthesis, which ultimately leads to improved productivity under drought. The phenol content was found to be relatively high in CCRP 4 (3.76mg/g) followed by CCRP 3 (3.73mg/g) and CCRP 2 (3.69mg/g) in order during pre-monsoon months (Table 3). The phenylpropanoid pathway is one of the important pathways of plant secondary metabolism, which yields a variety of phenolic compounds involved in various structural and defense-related functions [27]. Phenolic acids and flavonoids have been used as tolerance markers to screen the plants for biotic and abiotic stress. Plants under water stress conditions exhibit an increased synthesis of phenolic compounds compared to non-stressed plants [28]. The clones CCRP 3 and CCRP 4 had the maximum proline content of 914.69 µg/g and 913.56 µg/g respectively followed by CCRP 2 (901.45 µg/g). Proline is an osmoprotectant, is largely confined to the cytoplasm and is mostly absent from the vacuole [29]. Accumulation of proline is one of the strategies used by plants when exposed to water-deficit conditions to reduce injury to cells. In the present investigation also, during the pre-monsoon period, proline accumulation was 5-6 times higher than what was observed during the monsoon months in all the clones, confirming the role of proline accumulation in the stress condition. Balasimha [30] opined that in cocoa, proline accumulation indicates some adaptive significance to water deficit and higher proline accumulation associated with better growth and maintenance of leaf turgidity. The results obtained in this study on proline accumulation were also observed in other crops like Coffee [31], Sorghum [32], Rice [24] and Chickpea [33]. Among the seven clones, CCRP 4 (7.23µg H_2O_2 g^{-1} min^{-1}) followed by CCRP 1 (7.05µg H_2O_2 g^{-1} min^{-1}) and CCRP 3 (6.95µg H_2O_2 g^{-1} min^{-1}) exhibited relatively higher catalase activity, whereas CCRP 5 recorded the least catalase activity (6.19µg H_2O_2 g^{-1} min^{-1}) during the pre-monsoon month. Catalase is a powerful antioxidant enzyme under abiotic stress conditions to nullify the effect of H_2O_2 and protects the plants against oxidative stress. Catalase enzyme regarded as H_2O_2 scavenger [34] and H_2O_2reported to be involved in the enhancement of damage of cell oxidation function [35]. Higher accumulation of H_2O_2 coupled with a low rate of enzyme activity indicates the susceptible nature of the genotype to water stress conditions.

The rank sums of morphological, physiological and biochemical parameters were calculated based on the relationships to drought tolerance, which showed that clone CCRP 4 was tolerant of drought followed by CCRP 3 (**Table 4**).

Table 4 Ranking of cocoa varieties under rainfed condition of HRS, Thadiyankudisaiduring stress period (pre-monsoon)

Ranking of the clones for each trait											
Clones	**Canopy area**	**Pods**	**RWC**	**CSI**	**MSI**	**Phenol**	**Catalase**	**Proline**	**NRase**	**Total**	**Rank**
CCRP 1	3	3	4	5	5	5	2	4	4	35	4
CCRP 2	4	4	3	2	1	3	4	3	2	26	3
CCRP 3	1	1	2	3	2	2	3	1	3	18	2
CCRP 4	2	2	1	1	3	1	1	2	1	14	1
CCRP 5	6	6	7	4	4	4	7	6	5	49	5
CCRP 6	5	5	5	7	7	6	6	5	6	52	6
CCRP 7	7	7	6	6	6	7	5	7	7	58	7

RWC- Relative Water Content, CSI- Chlorophyll Stability Index, MSI- Membrane Stability Index, NRase- Nitrate Reductase

Conclusion

In the present study, totally seven varieties assessed for their growth and yield performance under natural drought conditions revealed that two clones CCRP 3 and CCRP 4 identified as drought tolerant as it expressed drought-tolerant characteristics like physiological (high chlorophyll stability index, membrane stability index, relative water content) and biochemical (high proline accumulation, catalase activity, NRase activity and phenol) traits. Further, these two clones need field study for another three to four years to assess their adaptability, stability and productivity. This may lead to the development of high yielding and drought-tolerant varieties in cocoa.

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