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# Dynamics of gas exchange and chlorophyll fluorescence parameters of cocoa genotypes in response to water deficit

**Janani P, N Kumar and V Jegadeeswari**

**Abstract**
The effect of drought stress on the photosynthetic gas exchange parameters of twenty cocoa genotypes (*Theobroma cacao* L.) was examined under glasshouse conditions. Photosynthesis, chlorophyll fluorescence and stomatal conductance evaluated in 100% and 50% of the field capacity revealed significant differences between non-stressed and stressed plants and different genotypes. Among the genotypes, the percentage reduction in terms of photosynthetic rate (29.22 and 27.68% respectively), chlorophyll fluorescence (4.2 and 4.01% respectively) was low in VTLCH 3 and VTLCH 4 while the highest reduction in stomatal conductance (48.70 and 50%) and transpiration rate (57.81 and 56.80%) under stress regimes was also recorded in the same genotypes. Therefore, VTLCH 3 and VTLCH 4 were grouped as water stress tolerant genotypes based on the dynamics of gas exchange parameters. Thus, these two genotypes could be utilized for cultivation in Tamil Nadu condition and in further breeding programs.

**Keywords:** Cocoa, water stress, photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll fluorescence

## Introduction
Cocoa (*Theobroma cacao* L.) is considered a drought sensitive crop (Wood and Lass, 1957) [27] and soil water limitation can have a negative impact on plant growth, bean yield and quality (Daymond *et al*. 2011; Carr and Lockwood 2011) [13, 10]. Water stressed plants show variations in morpho-physiological and biochemical responses that include reduction in cell expansion and elongation, closure of stomata followed by diminished mesophyll conductance, photosynthesis, respiration, nutrient metabolism, leaf chlorophyll a and b content, photosystem efficiency (Fv/Fm) and reduction of the accumulation of dry matter *etc*. (Wahid and Rasul 2005; Flexas *et al*. 2006) [26, 14]. Morpho-physiological, biochemical and photosynthetic changes activated by drought in cocoa have been studied by various researchers like Balasimha (1992) [7], Daymond and Hadley (2004) [12], Balasimha *et al*. (2013) [6] and Apshara *et al*. (2013) [1]. These studies have confirmed that gas exchange parameters based on photosynthesis and chlorophyll fluorescence (maximum quantum yield Fv/Fm) have been used as an indicator to study the response of plants under water deficit stress (Balasimha and Rajagopal 1988 [3]; Balasimha, 1992 [7]; Daymond and Hadley 2004 [12]).
In southern India, cocoa is cultivated as a mixed crop under palm based cropping system. Production of Indian cocoa is highly limited due to a long drought period which may extend up to six months (Apshara *et al*. 2013 [1]). Moreover, cocoa production in rainfed regions exposes the crop to abiotic stresses such as drought and high temperatures. Currently, area expansion in non- traditional areas of Tamil Nadu and Andhra Pradesh necessitates for the identification of high yielding genotypes with tolerance to stress due to water deficit (Balasimha *et al*. 2013) [6]. In this study, we evaluated the changes in photosynthetic gas exchange parameters of twenty cocoa clones under different irrigation regimes to screen the drought tolerant genotypes.

## Materials and methods
Screening of cocoa clones suited for water deficit condition was carried out as a pot culture experiment using gravimetric method. The experiment was conducted in a glasshouse of the University Orchard, TNAU, Coimbatore (77º E, 11º N, altitude 412 m above MSL) during 2014. The experiment was conducted with natural light and the minimum and maximum temperatures of 23˚C and 34˚C, respectively. In this study, five months old twenty cocoa clones *viz*. TNAUCC 1 to TNAUCC 10, CCRP 1 to CCRP 5 and VTLCC1, VTLCH 1 to VTLCH 4 were used for drought screening.

The gravimetric method of drought imposition has been adopted from studies conducted by Shivakumar (2013) [22] and Shilpa (2013) [21]. Two levels of water stress were imposed *viz*., 50% field capacity (FC) (moderate deficit) and 100% field capacity (FC) (Control). Five months old cocoa clones were planted in earthen pots and treatments were imposed after the first flush.

Gas exchange parameters i.e. net photosynthetic rate (Pn: µmol $CO_2$ $m^{-2}$ $s^{-1}$), transpiration rate (TR: mmol $H_2O$ $m^{-2}$ $s^{-1}$), stomatal conductance ($g_s$: mol $H_2O$ $m^{-2}$ $s^{-1}$) and Photosynthetic rate: Stomatal Conductance (Pn/$g_s$ ratio) was recorded using a Portable Photosynthesis System LI-6400 (LICOR inc. Lincoln, Nebraska, USA). The readings were recorded between 11.00 to 12.30 (clear sunny day) when the photosynthetically active radiation was above 1000 µmol photons $m^{-2}$ $s^{-1}$. The Plant Efficiency Analyser (PEA) was used to measure chlorophyll fluorescence and method recommended by Lu and Zhang (1999) [16] was followed. Measurements were made on intact leaves, which were adjusted in dark for 30 minutes before measurement. The minimum fluorescence level (Fo) with all open PS II reaction centres was evaluated by measuring the modulated light, which was sufficiently low (< 0.1 µmol $m^{-2}$ $s^{-1}$). The maximum fluorescence level (Fm) with all PS II reaction was determined by a saturation pulse of 0.8 to 8000 µmol m-2 s-1 in dark (Lu *et al*. 2001). Using light and dark fluorescence parameters, the maximum efficiency of PS II photochemistry was calculated in the dark-adapted state, Fv/Fm = (Fm-Fo) / Fm (Van Kooten and Snell 1990).The data were analyzed as a Completely Randomized Factorial Design with 40 treatments (20 cocoa clones x 2 levels of irrigation) and three replications. The results of the experiment were statically analysed using the procedure given by Panse and Sukhatme (1961) [19] and Snedecor and Cochran (1967) [23].

**Results and discussion**

The stomatal conductance, rate of photosynthesis, transpiration rate and chlorophyll fluorescence (Fv/Fm) varied significantly between cocoa clones ($P < 0.05$). This study demonstrated that the genotypes showed variations in photosynthetic rate under water stress compared to control. Irrigation regime treatments could substantially reduce the rate of photosynthesis from 6.48 at 100% FC to 4.09 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 50% FC. The highest photosynthetic rate (Pn) 7.46 µmol $CO_2$ $m^{-2}$ $s^{-1}$ was recorded in VTLCH 3 at 100% field capacity, while the lowest photosynthetic rate value was 2.63 µmol $CO_2$ $m^{-2}$ $s^{-1}$ from CCRP3 at 50% field capacity (Table 1). Among G X I interactions, VTLCH 3, VTLCC 1, CCRP 2, VTLCH 4 and CCRP 4 relatively expressed high rate of photosynthesis (7.46, 7.42, 7.41, 7.37 and 7.35) under 100% field capacity. It was also noted that under imposed stress (50% FC) all the above mentioned genotypes except CCRP 4 had the highest photosynthetic rate (5.28, 5.11, 5.07 and 5.33) as an expression of their ability to tolerate water stress. In addition, these four genotypes showed the lowest percent reduction in the photosynthetic rate over control (less than 32%), while all the other clones registered reduction of over 31%. Balasimha *et al.* (1991) [4] also observed a higher photosynthetic rate for tolerant clones than susceptible clones. The genotypic variation in the rate of photosynthesis has been demonstrated in cotton (Pettigrew and Turle1998) [20] and grapes (Bota *et al*. 2001) [8].

Stomatal conductance in cocoa genotypes under different irrigation regime showed higher dispersion in normal irrigation condition (min= 0.115; max= 0.167) than under water stress 50% FC (min= 0.059; max= 0.098) condition. The average of stomatal conductance under control was 0.134 mmol $H_2O$ $m^{-2}$ $s^{-1}$which substantially decreased under water stress (0.078 mmol $H_2O$ $m^{-2}$ $s^{-1}$) (Table 1). Under 100% FC, high stomatal conductance was achieved by TNAUCC 5 with 0.167 mmol $H_2O$ $m^{-2}$ $s^{-1}$ followed by TNAUCC 9 with 0.158 mmol $H_2O$ $m^{-2}$ $s^{-1}$ and CCRP 2 with 0.118 mmol $H_2O$ m-2 $s^{-1}$. However, VTLCH 3 with 0.115 mmol $H_2O$ $m^{-2}$ $s^{-1}$ and CCRP3 with 0.118 mmol $H_2O$ $m^{-2}$ $s^{-1}$ recorded the lowest stomatal conductance. Similarly, under 50% FC, TNAUCC5 with 0.098 mmol $m^{-2}$ $s^{-1}$ and CCRP 5 with 0.089 mmol $H_2O$ $m^{-2}$ $s^{-1}$ achieved maximum stomatal conductance while VTLCH 3 with 0.059 mmol $m^{-2}$ $s^{-1}$ and VTLCH 4 with 0.062 mmol $H_2O$ $m^{-2}$ $s^{-1}$ showed the lowest levels of stomatal conductance (Table 1). VTLCH 3 and VTLCH 4 clones showed the highest percent reduction in stomatal conductance under hydric stress when compared to control (more than 48). The decrease in the rate of photosynthesis under stress conditions normally attributed to the suppression of mesophyll conductance and closure of stomata in situations of moderate and severe stress (Flexas *et al*. 2004) [14]. Thus the reduction in stomatal conductance is attributed to the reduction in transpiration water loss with efficient stomatal closure without affecting the photosynthetic rate. This is a favourable feature to tolerate drought in cocoa (Balasimha *et al.* 1991 and Balasimha 1999) [4-5]. In the present study, among the various clones evaluated, it was observed that relatively lesser conductance was observed in VTLCH 3 and VTLCH 4 clones under stress compared with 100% field capacity. Balasimha and Rajagopal (1988) found that stomatal conductance was reduced by high photosynthetically active radiation, low relative humidity and moisture stress in cocoa. The genotypes, irrigation regime treatments and their interactions significantly influenced the Pn/$g_s$ ratio (Table 1). Between genotypes, VTLCH 3 and VTLCH 4 showed the highest ratio (more than 72) while CCRP 1 had the lowest Pn/$g_s$ ratio (35.15). When compared to 100% field capacity (48.73), it was found that the ratio was higher under stress regime (53.51). At 100% field capacity, VTLCH 3 and VTLCH4 showed the highest Pn / gs ratio (64.87 and 59.44), and the same trend continued even under 50% of field capacity (89.49 and 85.97), indicating the ability of these clones to withstand drought. The relationship between Pn to $g_s$ is another parameter that will normally increase under stress conditions. In the present investigation, it was found that this ratio was higher under stress condition than 100% field capacity. Although stomatal closer limits the rate of photosynthesis, there might be a small change in the water use efficiency under stress conditions compared to non-stress conditions in these clones. This relationship has been attributed to the adaptation for water deficit stress conditions in cocoa (Balasimha and Rajagopal 1988) [3].

The trend in photosynthesis and stomatal conductance is almost similar to that of transpiration. Transpiration rate (TR), recorded during this study indicated that TR was higher at 100% FC than in plants under 50% FC (Table 2). The lowest transpiration rate is linked to drought tolerance and was observed in VTLCH 4 (3.58) followed by CCRP 2 (3.63) and TNAUCC 1 (3.70). The interaction between genotype × irrigation regime showed that VTLCH 2 recorded a significantly higher rate of transpiration (6.05) followed by CCRP 1 (5.92) and TNAUCC 7 (5.88) at 100% field capacity and the lowest rate was observed in TNAUCC 1 (4.51) followed by CCRP 4 (4.58) and VTLCC 1(4.58), while under 50% FC treatment, VTLCH 4 and VTLCH 3 recorded the

lowest transpiration rate (2.16 and 2.27 respectively) which indicates their drought tolerant nature. Reduced transpiration rate under stress is possible because of the partial closure of the stomata. At the onset of stress, extension growth and leaf expansion are first affected, followed by a decrease in rates of transpiration due to partial stomatal closure and restricts the entry of $CO_2$ ultimately reduction of photosynthesis (Bradford and Hsiao 1982; Chartzoulakis *et al.* 1993) [9, 11]. The adaptive mechanism of plant species to reduce water losses due to transpiration is achieved by a closing of stomata (Tardieu and Davies 1993) [24].

The chlorophyll fluorescence Fv /Fm ratio declined at 50% FC in twenty cocoa genotypes. The results presented in Table 2 showed that the Fv/Fm value was lower in plants subjected to water stress than in well-watered plants. At 50% FC, VTLCH 3, VTLCH 4, CCRP 2 and VTLCH 1 recorded significantly higher chlorophyll fluorescence (Fv/Fm) (0.558, 0.550, 0.510 and 0.489 respectively). The interaction between water regimes and genotypes on Fv /Fm was not significant. Similarly, the percent reduction in chlorophyll fluorescence at 50% FC was minimum in VTLCH 3 and 4 were expressed the minimum chlorophyll fluorescence reduction (4.62 and 4.01 respectively).The application of chlorophyll florescence as a tool to screen cocoa for drought tolerance has been reported in earlier studies in cocoa (Balasimha and Namboothiri, 1996 and Balasimha *et al.* 2013) [2, 6]. Cocoa genotypes showing higher water potential and Fv/ Fm ratio can be considered as drought tolerant (Balasimha *et al.* 2013) [6]. Apshara *et al.* (2013) [17] reported that chlorophyll fluorescence indices decreased due to stress suggesting the photochemical reaction was highly affected Screening of 20 genotypes for drought tolerance revealed that the genotype VTLCH 3 followed by VTLCH 4 had relatively high chlorophyll florescence, indicating their possible genotypic drought tolerance.

## Conclusion

Present results have shown that photosynthesis, gas exchange and chlorophyll fluorescence parameters measured through the initial growth phase under induced water stress, could be used as a valuable measure to determine the severity of stress. The results of the experiment showed that VTLCH 3 and VTLCH 4 performed better than other genotypes under water deficit condition. Therefore, these genotypes can be used in further breeding program to develop varieties and hybrids suitable for Tamil Nadu condition.

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**Table 1:** Effect of irrigation regime on photosynthetic rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$), stomatal conductance (mol $H_2O$ $m^{-2}$ $s^{-1}$) and photosynthetic rate: stomatal conductance (Pn / gs) ratio of cocoa genotypes

| Genotypes (G) | Photosynthetic rate(Pn) (µmol $CO_2$ $m^{-2}$ $s^{-1}$) | | | Stomatal conductance (gs) (mol $H_2O$ $m^{-2}$ $s^{-1}$) | | | Photosynthetic rate : stomatal conductance ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | Irrigation Regime (I) | | | Irrigation Regime (I) | | | Irrigation Regime (I) | | |
| | 100% FC | 50% FC | Percentage over control | 100% FC | 50% FC | Percentage over control | 100% FC | 50% FC | Mean |
| TNAUCC 1 | 7.24 | 4.92 | 32.04 | 0.125 | 0.081 | 35.20 | 57.92 | 60.74 | 59.33 |
| TNAUCC 2 | 4.85 | 3.23 | 33.40 | 0.129 | 0.072 | 44.19 | 37.60 | 44.86 | 41.23 |
| TNAUCC 3 | 5.39 | 3.20 | 40.63 | 0.135 | 0.088 | 34.81 | 39.93 | 36.36 | 38.14 |
| TNAUCC 4 | 6.71 | 3.58 | 46.65 | 0.132 | 0.074 | 43.94 | 50.83 | 48.38 | 49.61 |
| TNAUCC 5 | 6.25 | 3.89 | 37.76 | 0.167 | 0.098 | 41.32 | 37.43 | 39.69 | 38.56 |
| TNAUCC 6 | 6.89 | 4.61 | 33.09 | 0.131 | 0.074 | 43.51 | 52.60 | 62.30 | 57.45 |
| TNAUCC 7 | 6.94 | 4.52 | 34.87 | 0.125 | 0.087 | 30.40 | 55.52 | 51.95 | 53.74 |
| TNAUCC 8 | 5.96 | 3.85 | 35.40 | 0.135 | 0.071 | 47.41 | 44.15 | 54.23 | 49.19 |
| TNAUCC 9 | 5.50 | 3.13 | 43.09 | 0.158 | 0.084 | 46.84 | 34.81 | 37.26 | 36.04 |
| TNAUCC 10 | 6.63 | 3.02 | 54.45 | 0.142 | 0.075 | 47.18 | 46.69 | 40.27 | 43.48 |
| CCRP 1 | 5.49 | 2.63 | 52.09 | 0.137 | 0.087 | 36.50 | 40.07 | 30.23 | 35.15 |
| CCRP 2 | 7.41 | 5.07 | 31.58 | 0.146 | 0.084 | 42.47 | 50.75 | 60.36 | 55.56 |
| CCRP 3 | 5.21 | 3.47 | 33.40 | 0.118 | 0.075 | 36.44 | 44.15 | 46.27 | 45.21 |
| CCRP 4 | 7.35 | 4.85 | 34.01 | 0.138 | 0.078 | 43.48 | 53.26 | 62.18 | 57.72 |
| CCRP 5 | 6.20 | 4.01 | 35.32 | 0.124 | 0.089 | 28.23 | 50.00 | 45.06 | 47.53 |
| VTLCC 1 | 7.42 | 5.11 | 31.13 | 0.129 | 0.069 | 46.51 | 57.52 | 74.06 | 65.79 |
| VTLCH 1 | 7.12 | 4.65 | 34.69 | 0.140 | 0.077 | 45.00 | 50.86 | 60.39 | 55.62 |
| VTLCH 2 | 6.25 | 3.38 | 45.92 | 0.135 | 0.084 | 37.78 | 46.30 | 40.24 | 43.27 |
| VTLCH 3 | 7.46 | 5.28 | 29.22 | 0.115 | 0.059 | 48.70 | 64.87 | 89.49 | 77.18 |
| VTLCH 4 | 7.37 | 5.33 | 27.68 | 0.124 | 0.062 | 50.00 | 59.44 | 85.97 | 72.70 |
| Mean | 6.48 | 4.09 | - | 0.134 | 0.078 | - | 48.73 | 53.51 | 51.12 |
| | G | I | G X I | G | I | G X I | G | I | G X I |
| SE(d) | 0.117 | 0.037 | 0.166 | 0.0026 | 0.0008 | 0.0037 | 1.03 | 0.32 | 1.46 |
| CD (P=0.05) | 0.232** | 0.073** | 0.328** | 0.0052** | 0.0016** | 0.0073** | 2.05** | 0.64** | 2.90** |

**Table 2:** Effect of irrigation regime on transpiration rate (mmol $H_2O$ $m^{-2}$ $s^{-1}$) and chlorophyll fluorescence (Fv / Fm) of cocoa genotypes

| Genotypes (G) | Transpiration rate (TR) (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | | | Chlorophyll fluorescence (Fv / Fm) | | |
|---|---|---|---|---|---|---|
| | Irrigation Regime (I) | | | Irrigation Regime (I) | | |
| | 100% FC | 50% FC | Mean | 100% FC | 50% FC | Mean |
| TNAUCC 1 | 4.51 | 2.88 | 3.70 | 0.493 | 0.436 | 0.465 |
| TNAUCC 2 | 5.00 | 2.99 | 4.00 | 0.400 | 0.332 | 0.366 |
| TNAUCC 3 | 5.17 | 3.07 | 4.12 | 0.354 | 0.296 | 0.325 |
| TNAUCC 4 | 5.78 | 3.40 | 4.59 | 0.462 | 0.401 | 0.432 |
| TNAUCC 5 | 5.37 | 3.28 | 4.33 | 0.433 | 0.368 | 0.401 |
| TNAUCC 6 | 5.78 | 3.26 | 4.52 | 0.448 | 0.375 | 0.412 |
| TNAUCC 7 | 5.88 | 3.31 | 4.60 | 0.475 | 0.406 | 0.441 |
| TNAUCC 8 | 5.31 | 3.23 | 4.27 | 0.472 | 0.398 | 0.435 |
| TNAUCC 9 | 5.74 | 3.30 | 4.52 | 0.494 | 0.408 | 0.451 |
| TNAUCC 10 | 4.64 | 2.87 | 3.76 | 0.383 | 0.324 | 0.354 |
| CCRP 1 | 5.92 | 3.31 | 4.62 | 0.460 | 0.396 | 0.428 |
| CCRP 2 | 4.38 | 2.88 | 3.63 | 0.568 | 0.510 | 0.539 |
| CCRP 3 | 5.12 | 3.17 | 4.15 | 0.547 | 0.458 | 0.503 |
| CCRP 4 | 4.58 | 2.88 | 3.73 | 0.510 | 0.448 | 0.479 |
| CCRP 5 | 5.54 | 3.08 | 4.31 | 0.482 | 0.413 | 0.448 |
| VTLCC 1 | 4.58 | 3.00 | 3.79 | 0.456 | 0.389 | 0.423 |
| VTLCH 1 | 5.31 | 3.21 | 4.26 | 0.546 | 0.489 | 0.518 |
| VTLCH 2 | 6.05 | 3.29 | 4.67 | 0.505 | 0.442 | 0.474 |
| VTLCH 3 | 5.38 | 2.27 | 3.83 | 0.585 | 0.558 | 0.572 |
| VTLCH 4 | 5.00 | 2.16 | 3.58 | 0.573 | 0.550 | 0.562 |
| Mean | 5.25 | 3.04 | 4.14 | 0.482 | 0.420 | 0.451 |
| | G | I | G X I | G | I | G X I |
| SE(d) | 0.075 | 0.023 | 0.106 | 0.010 | 0.003 | 0.014 |
| CD (P=0.05) | 0.149** | 0.047** | 0.212** | 0.020** | 0.006** | NS |

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