2017 International Symposium on Cocoa Research (ISCR), Lima, Peru, 13-17 November 2017.

IMPACT OF DROUGHT ON MORPHOLOGICAL, PHYSIOLOGICAL AND NUTRIENT USE EFFICIENCY OF ELITE CACAO GENOTYPES FROM BAHIA-BRAZIL, TARAPOTO-PERU AND PUERTO RICO-USA.

V. C. Baligar[1], A.-A. F. Almeida[2], D. Ahnert[2], J. L. Pires[3], E. Arévalo-Gardini[4], R. Goenaga[5], Z. He[6], M. Elson[1]

[1]USDA-ARS-Beltsville Agricultural Research Center (BARC), Beltsville, MD, USA.

[2]State University of Santa Cruz (UESC), Ilhéus, BA, Brazil.

[3] Cocoa Res. Center, Executive Committee of the Cocoa Plan (CEPEC/CEPLAC), Ilhéus, BA, Brazil

[4] Institute of Tropical Crops (ICT), Tarapoto, Perú,

[5]USDA-ARS Tropical Agricultural Research Station (TARS), Mayaguez, PR, USA.

[6] Univ. of Florida, Indian River Research and Education Center (IRREC), Fort Pierce, FL, USA.

Abstract:

Worldwide, drought is considered one of the most limiting abiotic stress factors for cacao growth, development and production. A series of greenhouse and growth chamber experiments were undertaken to assess drought effects on early cacao morphological and physiological traits and nutrient use efficiency of elite cacao genotypes of Brazil, Peru and Puerto Rico. Cacao genotypes showed varying degrees of intra-specific variations for growth (shoot and root biomass, leaf area, specific leaf area, stem height and diameter, root length, relative growth rate), physiology (photosynthesis, chl a/b, net assimilation rate, water use efficiency) and macro nutrient use efficiency under drought. Understanding of growth, morphology, physiology and nutrient use efficiency plant traits influenced by drought will facilitate identification of cacao genotypes tolerant to drought. Such drought tolerant genotypes could be useful in crop improvement programs to breed superior cultivars for drought stressed ecosystem.

Introduction

Worldwide, drought is considered one of the most limiting factors for cacao growth, development, nutrition and production (Almeida and Valle, 2007; Balasimha, 2011). Cacao is very much affected by drought and in recent years, inconsistent and reduced rainfall patterns in many cacao growing regions have impacted yields raising major concerns for sustainable cacao production (Abo-Hamed et al. 1983; Balasimha et al. 1988; Mohad Razi et al. 1992; Belsky and Siebert 2003; Almeida and Valle, 2007). Furthermore, infertility and low water holding capacity of the soils in cacao growing regions are the other main causes of irregularity in annual production.

Drought stress influences an array of processes in cacao including growth, physiology and nutrient uptake and nutrient use efficiency, and such changes affect yield and sustainability of cacao (Alvim 1977; Orchard and Saltos, 1988; Almeida and Valle, 2007; Balasimha, 2011; Joly, 1988; Santos et al., 2014, 2016; Ofori et al., 2015; Kacou et al., 2016). Morphological changes appear to be good traits for selection of cacao in its early stages for tolerance to drought (Moser et al., 2010; Sale, 1970).

Very little is known about cacao's ability to adapt to short or long duration drought (Almeida and Valle, 2007; Balasimha et al. 1988; Frimpong et al. 1996). Limited studies have been conducted to assess the adaptation strategies of cacao genotypes to short or long duration water deficit (Balasimha and Rajgopal, 1988; Balasimha et al., 1988; Rada et al., 2005; Carr and Lockwood, 2011; Padi et al., 2013). Santos et al. (2014) evaluated the performance of 36 cacao genotypes at two soil water regimes (control and drought) in greenhouse conditions. Mutivariate analysis showed that growth variables, leaf and total dry biomass, relative growth rate as well as Mg content of the leaves were the most important factors in identification of drought tolerance. Santos et al. (2016) evaluated 21 diallel crosses for response to drought and found stem diameter (CD), total leaf area (TLA), leaf dry biomass (LDB), stem dry biomass (SDB), root dry biomass (RDB), total dry biomass (TDB), root length (RL), root volume (RV) and root diameter (RD) were the most important growth parameters separating tolerant and intolerant cacao genotypes to soil water deficiencies. Such plant variables appear to be reliable traits for selection of plants tolerant to drought (Santos et al., 2014 and 2016). Effective regulation of stomatal aperture is attributed to drought tolerance in cacao which helps to maintain higher leaf Ψ (Balashima et al., 1991). There is an urgent need to identify cacao genotypes with drought tolerance and most of the current cacao germplasm has not been screened for either short or prolonged drought tolerance.

Productivity and survivability of cacao are greatly influenced by the levels of essential nutrients in tropical soils (Wood and Lass, 2001; Hartemink, 2005; Pohlan and Perez, 2010; Snoeck et al., 2016). Nutrient deficiencies are common for cacao, especially N, P, Ca, Mg, Fe, B and Zn (Wessel, 1980; Hartemink, 2005; Snoeck et al., 2016). Generally, drought reduces nutrient use efficiency because of its negative effects on both nutrient uptake by the roots and transport from the roots to the shoots. Restricted transpiration rates and impaired active transport and membrane permeability under drought coupled with a decline in soil moisture results in a decrease in the diffusion and mass flow rates of nutrients in the soil to the absorbing root surface are the major factors for reduced nutrient use efficiency (Barber, 1995; Baligar et al., 2001; Hu and Schmidhalter, 2005). Therefore, screening and identification of genotypes that efficiently utilize nutrients under drought stress are prerequisites for a successful cacao improvement program.

Objectives of the current research were to evaluate the effects of adequate and deficit (drought) soil moisture levels on growth and physiological traits and macro-nutrient use efficiency of cacao genotypes from the germplasm banks of Puerto Rico USA, Ilhéus, Bahia, Brazil, and Tarapoto, San Martin, Peru.

MATERIALS AND METHODS

Drought Experiments at Beltsville MD, USA, with genotypes from germplasm banks of Puerto Rico (PR) and Brazil.

Two cacao genotypes from USDA-ARS Tropical Agricultural Research Station (TARS), Mayaguez, Puerto Rico were evaluated: TARS-14 and Amelonado. Three genotypes from the cacao germplasm banks from Brazil (CEPLAC) were evaluated: ICS 9, EET 103 and CC 40.

Growth Conditions**:** Plants were grown in acidic Porter soil (coarse-loamy, mixed mesic, Umbric Dystrochrept Typic Hapludult). Soil was amended with lime to increase soil pH to 5.1 and supplemented with macro and micro nutrients to provide nutrients for adequate growth. Plants were grown in a climatically controlled growth room with day/night temperatures of 30/28°C with 75% RH, 400cm^{-3} m^{-3} CO_2 and photosynthetic photon flux density (PPFD) of 400 ± 50 µmol m^{-2} s^{-1}). For the first 64 days, plants were grown at -33 kPa soil moisture. On the 65th day, plants were divided into two groups and plants in the first group (control) were grown at -33 kPa soil moisture content. Plants in the second group (drought) were subjected to drought treatment by withholding water addition until the leaf stomatal conductance reached less than 10 % of the plants grown at -33 kPa (usually around 4 mmol m^{-2} s^{-1} determined by an SC-1 Leaf Porometer (Decagon Devices, Pullman, WA)).

Growth and Nutrient Use Efficiency Traits: Just before harvest, stem height (SH) and stem diameter (SD) were measured. After harvest the total leaf area (LA, cm^2/plant) was measured by LI-3100 Area Meter (Li-Cor, Inc., Lincoln, NE). The roots were removed from the soil, washed and root lengths (RL, cm/plant) were determined with a Comair Root Length Scanner (Hawker de Haviland, Melbourne, Victoria, Australia). Shoots and roots were washed in de-ionized water, freeze-dried and dry biomass of shoot (SDB, g/plant), root (RDB, g/plant) and Total (TDB, g/plant) were recorded. Leaf area ratio (LAR, cm^2/g) was determined as:

LAR = [LA /TDB]

Dried plant samples were digested in concentrated HNO_3 and elemental concentrations were determined by ICP-OES. Nutrient Use Efficiency (UE) was calculated as:
NUE = [(SDB mg/plant) / (mg of any given element in shoot/ plant)]

Physiological and Water Use Efficiency Traits:
One week before the final harvest, net photosynthesis rate (P_N, µmol CO_2 m^{-2} s^{-1}) was measured on the fully expanded sixth leaf from the top of each plant with a LI-6400 portable photosynthesis system (LI-Cor, Inc., Lincoln, NE). Leaf Chl a and Chl b contents were determined spectrophotometrically by the methods of Lichtenthaler (1987) and Chappelle et al. (1992). The following physiological traits were determined:
Relative growth rate (RGR) = [ln (TDB_2/TDB_1)/ (T_2-T_1)],
where T is time in days and subscripts1 and 2 refer to initial and final harvest.

Net assimilation rate (NAR) = [RGR / LAR]

Total Water Use Efficiency (WUE_{TOTAL}) was determined as follows:
WUE_{TOTAL} = [(shoot biomass,g $plant^{-1}$) /(water transpired during the growth, g $plant^{-1}$)].

Drought Experiment with Seminal Cacao Genotypes at UESC, Bahia Brazil:

Thirty six seminal cacao genotypes belonging to genetic groups Forastero, Criollo and Trinitario were selected from open pollinated seeds collected from clonal accessions at the Cacao Germplasm Bank of the Cacao Research Center (CEPEC), Ilhéus, Bahia, Brazil. Cacao genotypes were grown for six months in a greenhouse at Ilhéus. The maximum values of PPFD inside the greenhouse ranged from 800 to 1200 µmol photons m^{-2} s^{-1}, the air temperature averaged 27±2°C and relative humidity averaged 80±3% during the experimental period. At the end of six months growth, plants were divided into two groups, the first group was subjected to drought by gradually reducing the soil water content by withholding water addition until the dawn leaf water potential (Ψ_{WL}) reached -2.0 to -2.5 MPa, these leaf water potentials were reached approximately 40–60 days after the beginning of the drought cycle. The second group of plants (control) were irrigated daily to maintain soil moisture near field capacity and Ψ_{WL} averaged between -0.1 to -0.5 MPa. Measurements of Ψ_{WL} were done at the second or third mature leaf from the apex of the orthotropic axis between 2:00 and 4:00 am, using a pressure chamber (Model 1000, PMS Instrument Company, Albany, OR, USA). Dried leaf plant samples of each genotype were digested in nitro-percloric acid (3:1). After digestion, macronutrient concentrations were determined; Ca and Mg by atomic absorption spectrophotometery, P by colorimetry and K by flame emission photometry (EMBRAPA, 1997). Nitrogen was determined by the Kjeldahl method after sulphosalicylic digestion (Jones et al., 1991). Leaf mineral content was expressed as g/plant for each genotype and treatment. Determination and calculations of plant traits for growth, physiology and nutrient use efficiency were similar those described for BARC research above. Root volume was estimated after immersion of roots in a known water volume and observing the displacement.

Drought Experiment with Wild and National and International Cacao Clones at ICT, Peru

Two months old rooted cuttings of 57 cacao clones were grown in greenhouse for 6 months. Plants were grown for first 3 months at field capacity soil moisture then divided into two groups and for the first group soil moisture was maintained at field capacity and plants in the second group were subjected to drought treatment by withholding water addition. Experiment was terminated when plants reached wilting state in drought treatment and at harvest drought tolerant index (DTI) was determined:
DTI = [(Shoot +root DB at field capacity)/[(Shoot +root DB at drought)] x 100.
Cacao clones were grouped as follows:
DTI < 70% Intolerant to Drought;
DTI 70 % to ≤ 90% Moderately Tolerant to Drought
DTI > 90% Tolerant to drought

RESULTS AND DISCUSSION

Morphology and Growth Traits of Cacao Genotypes:

Inadequate soil moisture (drought) promotes significant alterations in the morphological, growth and development of cacao (Alvim, 1977; Almeida and Valle, 2007; Balasimha and Rajagopal, 1988; Balasimha, 2011; Ofori et al., 2015; Santos et al, 2014 and 2016). With few exceptions overall, the Puerto Rico and Brazil cacao genotypes showed significant intra-specific differences for morphology

(LA, SH, SD) and growth (TDB, RDB, RL, LAR, RGR, R/S ratio) traits under varying soil moisture levels (Table 1 and 2).

Drought reduced total dry biomass (TDB), leaf area (LA) and stem diameter (SD) in all genotypes; however the reduction of TDB in drought conditions was minimal in the two Brazilian genotypes (EET 103 and CC 40). Under water stress considerable reduction in leaf area expansion has been reported in cacao (Joly and Hahn, 1989; Deng et al., 1989). In Puerto Rico genotypes TDB, R/S, SH, SD, RL, LA, RGR traits were significantly influenced by drought. Whereas, in Brazilian genotypes significant effects of drought were observed on R/S and TDB. In young cacao seedlings, withholding water addition reduced RDB and TDB by 50% (Mohd Razi et al., 1992). In all genotypes, drought increased root length (RL). In cacao and in other plants, drought is known to increase biomass allocation to the roots, that induces longer root length which facilitates exploration of a larger soil volume and consequently could lead to increased water and nutrients absorption (Almeida et al., 2002; Fageria, 2012; Santos et al., 2016).

Table 1. Morphological and growth traits of Puerto Rico (PR) genotypes subjected to adequate and deficit (drought) soil moisture*.

Genotype	Soil Moist	TDB	RDB	R/S	SH	SD	RL	LA	LAR	RGR
TARS-14	Control	11.71	2.47	0.27	35.6	7.19	4138	1540	133.0	2.01
TARS-14	Drought	8.37	2.14	0.34	26.0	4.73	5199	1040	124.5	0.92
Amelonado	Control	7.60	1.53	0.26	33.3	5.54	3213	1107	144.3	0.75
Amelonado	Drought	5.88	1.32	0.29	30.0	4.43	3448	755	128.4	0.07
Significance										
Genotype		**	**	NS	NS	**	**	**	NS	NS
Water		**	NS	**	**	**	**	**	NS	**

*TDB = Total Dry Biomass (g plant^{-1}); RDB = Root Dry Biomass (g plant^{-1}); R/S = Root / Shoot Ratio; SH = Stem Height (cm plant^{-1}); SD = Stem Diameter (mm); RL = Total Root Length (cm plant^{-1}); LA = Leaf Area (cm^2 plant^{-1}); LAR = Leaf Area Ratio (cm^2 g^{-1}); RGR = Relative Growth Rate (g g^{-1} d^{-1}) (x10^{-2})

Table 2. Morphological and growth traits of Brazilian genotypes subjected to adequate and deficit (drought) soil moisture*.

Genotype	Soil Moist.	TDB	RDB	R/S	SH	SD	RL	LA	LAR	RGR
ICS 9	Control	1.84	0.38	0.26	14.6	3.68	217	240	134.6	4.15
ICS 9	Drought	1.59	0.37	0.34	13.4	2.88	252	201	124.0	3.45
EET 103	Control	2.47	0.68	0.39	17.9	4.39	628	335	131.8	1.50
EET 103	Drought	2.45	0.76	0.48	16.1	3.71	730	245	102.6	1.71
CC 40	Control	2.92	0.79	0.38	21.0	4.63	560	335	119.0	0.99
CC 40	Drought	2.92	0.93	0.48	16.6	4.36	827	277	95.0	0.97
Significance										
Genotype		*	**	**	*	**	**	NS	NS	**
Water		NS	NS	**	NS	*	NS	NS	NS	NS

*See table 1

In the cacao seminal genotypes in Brazil, overall, average morphological and growth traits (TDB, RDB, R/S, SH, SD, SD, RV, LA, LAR, RGR, and NAR) were reduced under drought stress (Table 3) and the detailed responses of these 36 cacao genotypes for two soil moisture (adequate and drought) were reported earlier by Santos et al. (2014). Reduction in SH, LA and LAR under drought appears to be an

early indication of drought effect. Drought reduced RGR and NAR considerably. In these genotypes drought reduced root dry biomass (RDB) similarly to earlier studies conducted at BARC. Drought increases the biomass allocation to roots and thereby increases root length which facilitates larger exploration of soil to improve the water and nutrient absorption (Kozlowski and Pallardy, 2002; Santos et al., 2014, Fageria 2012). Therefore these root traits are reliable plant traits in the selection of cacao genotypes tolerant to drought.
Santos et al. (2014) further stated that growth variables, such as leaf and total dry biomass, relative growth rate as well as Mg content of the leaves were the most important factors in identification of drought tolerant cacao genotypes. Therefore, these variables are reliable plant traits in the selection of plants tolerant to drought. Cacao genotypes that maintain good growth and productivity under drought could be exploited to identify drought tolerant genotypes useful in cacao improvement programs for drought prone areas.

.

Table 3. Average morphological and growth traits of 36 Brazilian seminal cacao genotypes subjected to adequate and deficit (drought) soil moisture stress for 40-60 days where predawn leaf water potential of the drought treatments reached –2.0 to –2.5 MPa.

Genotype	Soil Moist.	TDB	RDB	R/S	SH	SD	RV	LA	LAR	RGR	NAR
Brazilian	Control	119.8	24.7	0.26	132.4	19.9	105.0	9544	79.8	4.95	6.27
	Drought	92.0	19.1	0.26	123.0	17.6	65.9	7275	79.2	4.42	5.64

RV = Root Volume (cm^3) NAR = Net Assimilation Rate

Drought experiment carried in greenhouse at ICT, Peru showed intra-specific differences for drought tolerance among national and international cacao clones (Fig 1). Based on drought tolerance index (DTI) 33 accessions were intolerant to drought, 21 accessions were moderately tolerant to drought and only 3 accessions (H10, ICT 1087, CA 14) were tolerant to drought.

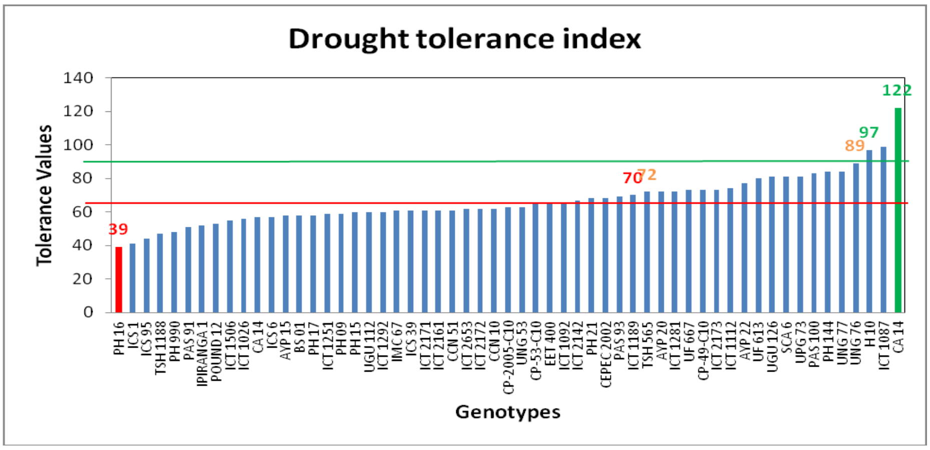

Fig 1: Range of DTI observed in wild and national and international cacao clones at ICT, Peru

Physiological Traits of Cacao Genotypes:

The genotypes from Puerto Rico and Brazil showed intra-specific differences for physiological traits (P_N, Chl a/b, WUE, NAR) under varying soil moisture levels from adequate to deficit (drought) (Fig. 2). Invariably, in all genotypes, significant reduction in P_N, Chl a/b and NAR was recorded under drought stress. Drought reduced the total leaf area (LA) in all the genotypes and this contributed to reduced P_N. The decrease in P_N in cacao under drought can be attributed to lower leaf water potentials (Balasimha et al., 1991; Deng et al., 1990; Mohd Razi et al., 1992). Reduction in soil water (drought) leads to immediate closure of stomata thereby preventing excess water loss from leaves and improves water use efficiency but this could limit significantly CO_2 uptake (Chaves et al., 2002). Drought had significant effects on the Chl a/b ratio and overall in all genotypes Chl a/b was lower under drought. The ratio of

Chl a/b is considered an appropriate indicator of stomatal limitation of photosynthesis (Farquhar and Sharkey, 1982).

Drought increased WUE_{TOTAL} in genotypes TARS-14 and Amelonado from PR and CC40 from Brazil but the effect of drought was only significant in Puerto Rico genotypes. It appears that cacao is efficient in using water under drought conditions. Stomatal closure under drought reduces the transpiration water loss and leads to increased WUE (Rada et al., 2005). With the exception of Brazilian genotypes EET-103 and CC 40, NAR in the other genotypes was reduced under drought.

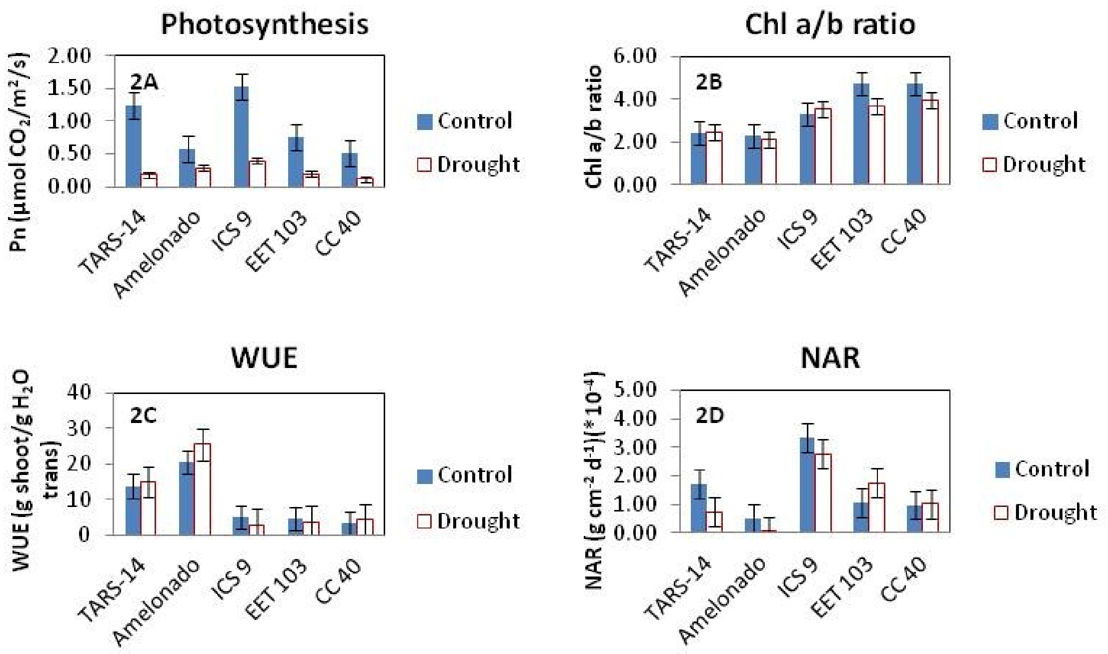

Fig.2: Adequate and deficit (drought) soil moisture effects on photosynthesis ($P_{N,}$), Chlorophyll a/b ratio (Chl a/b), total water use efficiency (WUE_{TOTAL}) and net assimilation rate (NAR) of PR (TARS-14, Amelonado) and Brazil (ICS 9, EET 103, CC 40) cacao genotypes.

Macro-Nutrient Use Efficiency (UE) traits of cacao genotypes:

Deficiencies of essential nutrients such as N, P, Ca, Mg, Fe, B and Zn greatly influence growth and development of cacao (Wessel, 1980; Wood and Lass, 2001, Hartemink, 2005; Snoeck et al., 2016). In drought conditions, availability of soil nutrients is drastically reduced which might induce higher nutrient deficiency in cacao. The UE values are useful in assessing the plants ability to use absorbed nutrients efficiently or non-efficiently especially under drought conditions. The effect of drought on nutrient use efficiency in cacao is very much unknown. Therefore, the screening and identification of genotypes that are efficient utilizers of absorbed nutrients under drought stress is a prerequisite for successful cacao improvement programs for drought prone areas. Cacao genotypes that are efficient in use of absorbed nutrients under moderate drought stress might grow well and maintain their productivity and such genotypes have advantage over the inefficient nutrient utilizers in overcoming drought stress. The genotypes grown from Puerto Rico and Brazil showed intra-specific differences for Macro-nutrient use efficiency (EU), under varying soil moisture levels from adequate to deficit (drought), however the effects were only significant with Brazilian genotypes (Fig 3). Overall, in Puerto Rico genotypes, UE for N, P, K, Ca, and Mg increased with drought. However, in Brazilian genotypes except for CC 40, only the UE for N increased with drought, while the UE for P, K, Ca and Mg decreased with drought.
Nutrient use efficiency (NUE) for N, P, K, Ca, and Mg in the average of 36 cacao genotypes from Ilhéus, Bahia, Brazil was influenced by adequate and deficit (drought) soil moisture content (Fig 4). Overall, NUE for N, P, K decreased and NUE for Ca and Mg increased under drought. Under drought stress,

closing of stomates reduces transpiration rates thereby reducing nutrient influx and transport of ions that are transported through mass-flow mechanism (Barber, 1995; Baligar et al., 2001). The difference in nutrient use efficiency observed between cacao genotypes used in these studies is probably related to age of the plants used for study and nature of seedlings under consideration. Nutrient use efficiency probably is not a good trait to use in selection of cacao genotypes tolerant to drought but it is a good trait in selection of plants that are efficient in utilization of any particular nutrient absorbed under low availability in soil. Such NUE efficient genotypes could be incorporated into cacao improvement program to improve particular nutrient use efficiency to improve the growth and development of cacao in soil that is deficient of any particular nutrient.

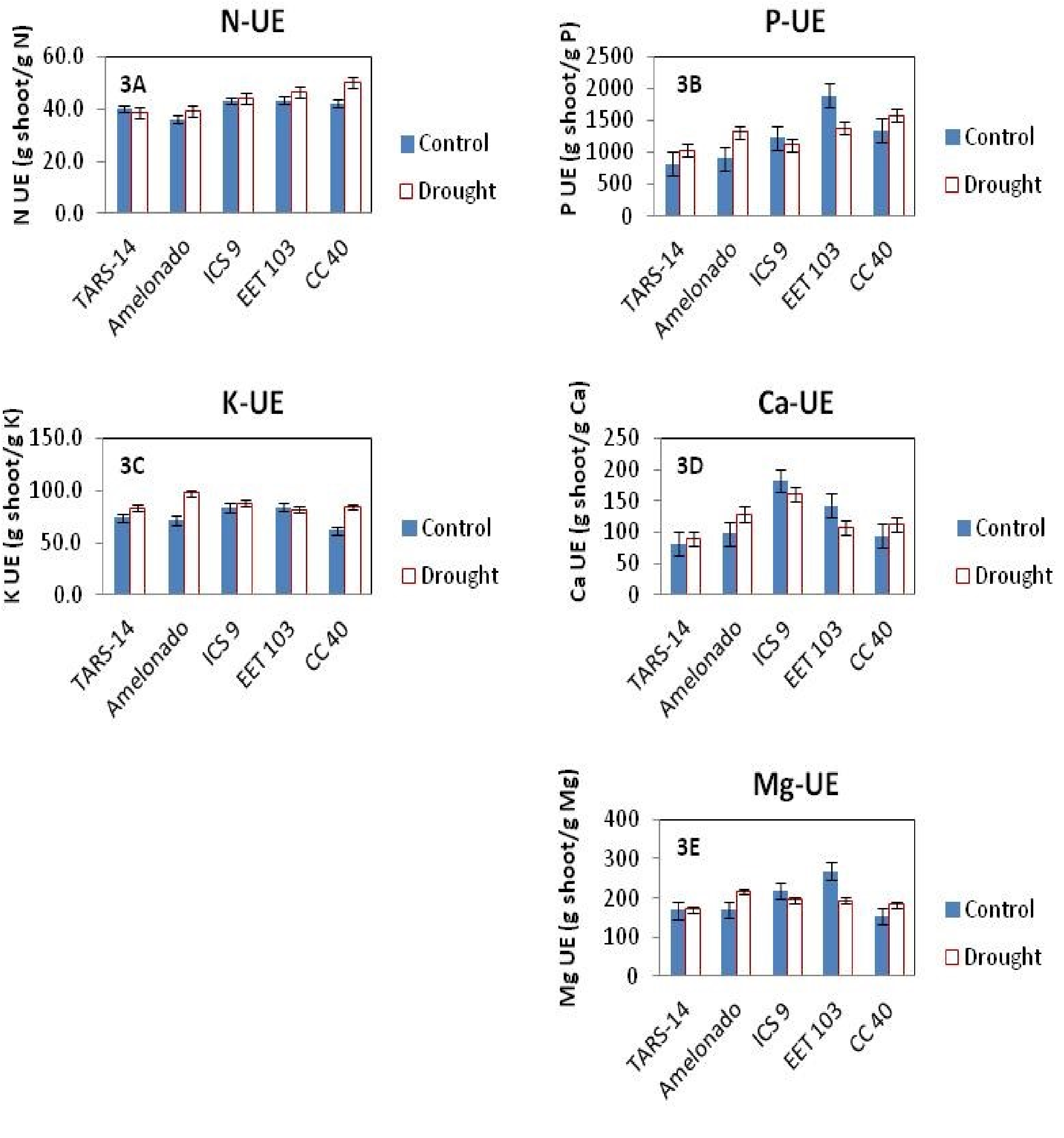

Fig.3. Adequate and deficit (drought) soil moisture effects on nutrient use efficiency (UE) for N, P, K, Ca and Mg traits of PR (TARS-14, Amelonado) and Brazilian (ICS 9, EET 103,CC 40) cacao genotypes.

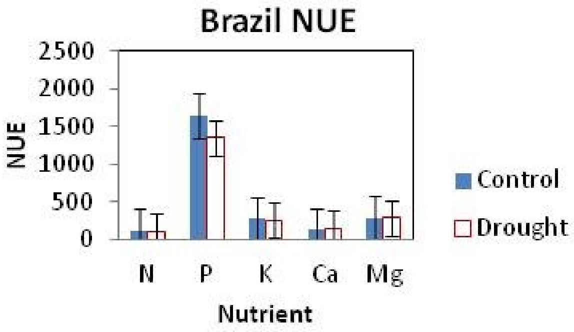

Fig. 4. Adequate and deficit (drought) soil moisture effects on average nutrient use efficiency (NUE, mg of shoot biomass /mg of any given element in shoot) response traits for N (N-UE), P (P-UE), K (K-UE), Ca (Ca-UE) and Mg (Mg-UE) in 36 cacao genotypes from Ilhéus, Bahia, Brazil.

CONCLUSIONS

Cacao genotypes showed varying degrees of intra-specific variations for growth/morphology (leaf, stem, and root parameters), physiology (photosynthesis, Chl a/b, water use efficiency, NAR) and nutrient use efficiency for N, P, K, Ca, and Mg under drought. Understanding of plant growth, morphology, physiology and nutrient use efficiency traits influenced by drought will facilitate identification of cacao genotypes tolerant to drought. Plant traits such as LDB (leaf dry biomass), TDB (total dry biomass), LA (total leaf area), RGR (relative growth rate), RV (root volume), P_n (rate of photosynthesis) WUE (water use efficiency), NUE (nutrient use efficiency) and DTI (drought tolerance index) are effective traits for selection of drought tolerant cacao genotypes. Tolerant genotypes are vital plant types for cacao improvement programs to breed superior cultivars for drought stressed ecosystems of cacao growing regions of the world.

References

Abo-Hamed S, Collin HA, Hardwick K (1983) Biochemical and physiological aspects of leaf development in cocoa (*Theobroma cacao* L.). VII. Growth, orientation, surface structure and water loss from developing flush leaves. New Phytologist 95: 9-17.

Almeida A-AF, Brito RCT, Aguilar MAG, Valle RR (2002) Water relations aspects of *Theobroma cacao* L. clones. Agrotrópica, 14: 35–44.

Almeida A-AF, Valle RR (2007) Ecophysiology of the cacao tree. Brazilian J. Plant Physiol. 19: 425-448.

Alvim PT (1977) Cacao. In: Alvim PT, Kozlowski TT (ed) Ecophysiology of tropical crops. Academic Press, New York, p.279–313.

Balasimha D (1988) Water relations, growth and other indicators of plant water stress in cocoa under drought. In: Proc. 10th Int. Cocoa Res. Conf. Santo Domingo, Dominican Republic 215–217.

Balasimha D (2011) Towards understanding the physiology of cocoa (*Theobroma cacao* L.) J. Plantation Crops 39: 1-10.

Balasimha, D, Daniel EV, Bhat, PG (1991) Influence of environmental factors on photosynthesis in cacao tree. Agricultural and Forest Meteorology 55: 15–21.

Balasimha D, Rajagopal V (1988) Stomatal responses of cocoa (*Theobroma caca*o L) to climatic factors. Indian Journal of Agricultural Sciences 58: 213–216.

Balasimha D, Rajagopal V, Daniel EV, Nair, RV, Bhagavan S (1988) Comparitive drought tolerance of cacao accessions. Trop. Agric. 65: 271-274.

Baligar VC, Fageria NK, He ZL (2001). Nutrient use efficiency in plants. Comm. Soil Sci. and Plant Ana. 32: 921-950.

Barber SA (1995) Soil Nutrient Bioavailability: A Mechanistic Approach. John Wiley &Sons, New York.

Belsky JM, Siebert SF (2003) Cultivating cacao: Implications of sun-grown cacao on local food security and environmental sustainability. Agric. Human Values 20: 277-285.

Carr MKV, Lockwood G (2011) The water relations and irrigation requirements of cocoa (*Theobroma cocoa* L.): A Review. Experimental Agriculture 47: 653–676.

Chappelle EW, Kim MS, McMurtrey JE III. (1992): Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentration of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. Remote Sens Environ 39: 239-247.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field? Photosynthesis and growth. Annals of Botany 89: 907-916.

Deng X, Joly RJ, Hahn DT (1989) Effects of plant water deficit on the daily carbon balance of leaves of cacao seedlings. Physiol Plant 77: 407-412.

Deng X, Joly RJ, Hahn DT (1990) The influence of plant water deficit on photosynthesis and translocation of 14C-labeled assimilates in cacao seedlings. Physiol Plant 78: 623-627.

EMBRAPA (1997) Manual de métodos de análise de solos (Manual of soil analysis methods). 2nd ed. Rio de Janeiro, Centro Nacional de Pesquisa de Solos. 212p.

Fageria NK (2012). The Role of Plant Roots in Crop Production, CRC Press, Boca Raton FL.

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Ann. Rev. Plant Phys. 33: 317-345.

Frimpong EB, Adu-Ampomah Y, Karimu A (1996) Efforts to breed for drought resistant cocoa in Ghana. In: 12th Int. Cocoa Res Conference, Salvador, Bahia Brazil. pp. 375-381.

Hartemink AE (2005) Nutrient stocks, nutrient cycling, and soil changes in cocoa ecosystems-a review. Adv. Agron. 86: 227-253.

Hu Y, Schmidhalter U (2005). Drought and salinity: A comparison of their effects on mineral nutrition of plants. J. Plant Nutr. Soil Sci. 168: 541–549.

Joly RJ (1988) Physiological adaptations for maintaining photosynthesis under water stress in cacao. In: Proceedings from the 10th International Cocoa Research Conference, Nigeria, pp. 199-203.

Joly RJ, Hahn DT (1989) An empirical model for leaf expansion in cacao in relation to plant water deficit. Annals of Botany 64:1-8.

Jones JBJ, Wolf B, Mills HA (1991) Plant Analysis Handbook. A practical sampling, preparation, analysis and interpretation guide. Micro-Macro Publishing, Athens, GA, USA.

Kacou Antoine Alba M, Apshara SE, Hebbar KB, Mathias TG, Severin A (2016). Morpho-physiological criteria for assessment of two month old cocoa (*Theobroma cacao* L) genotypes for drought tolerance. Ind. J. Plant Physiol. 21: 23-30.

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Botanical Review 68: 270–334.

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes Method. Enzymol. 148: 350-382.

Mohd Razi I, Abd Halim H, Kamariah D, Mohd Noh J (1992) Growth, plant water relation and photosynthesis rate of young *Theobroma cacao* as influenced by water stress. Pertanika 15: 93-98.

Moser G, Leuschner Ch, Hertel D, Hölscher D, Köhler M, Leitner D, et al. (2010) The response of cacao trees (*Theobroma cacao* L) to a 13-month desiccation period in Sulawesi, Indonesia. Agroforestry Systems79: 171–187.

Ofori A, Padi PK, Acheampong K (2015). Genetic variation and relationship of traits related to drought tolerance in cocoa (*Theobroma cacao* L.) under shade and non-shade conditions in Ghana. Euphytica 201: 411-421.

Orchard JE, Saltos MR (1988) The growth and water status of cocoa during its first year of establishment under different methods of soil water management. In: Proceedings of 10th Int Cocoa Res Conference, Santo Domingo, Dominican Republic, 193–198.

Padi FK, Adu-Gyamfi P, Akpertey A, Alfred A, Ofori A (2013) Differential response of cocoa (*Theobroma cacao* L.) families to field establishment stress. Plant Breeding 132: 229–236.

Pohlan HAJ, Perez VD (2010) Growth and production of cacao. In: Verheye WH (ed.) Soils, Plant Growth and Crop Production, Vol III. Encyclopedia of Life Support Systems, Eolss Publ, Paris, France,

Rada F, Jaimez RE, Garcia-Nuñez C, Azocar A, Ramírez M (2005) Water relations and gas exchange in *Theobroma cacao* var. Guasare under periods of water deficits. Revista de la Facultad de Agronomia de la Universidad del Zulia 22: 112–120.

Sale PJM (1970) Growth, flowering and fruiting of cacao under controlled soil moisture conditions. Journal of Horticultural Science 45: 99–118.

Santos IC dos, Anhert D, Das Conceição AS, Pirovani CP, Pires JL, Valle RR., Almeida A-AF, Baligar VC (2014) Molecular, physiological and biochemical responses of *Theobroma cacao* L. genotypes to soil water deficit. PLOS ONE 9: 1-31. E115746. Doi:10.1371/journal.pone.0115746.

Santos EA, Almeida A-AF, Ahnert D, White MCS, Valle RR, Baligar VC (2016) Diallel analysis and growth parameters as selection tools for drought tolerance in young *Theobroma cacao* plants. PLOS ONE 11(8): 1/22- 22/22. e0160647. doi:10.1371/ journal.pone.0160647.

Snoeck D, Koko L, Joffre J, Bastide P, Jagoret P (2016) Cacao nutrition and fertilization. Sustainable Agric Rev 19: 155-202.

Wessel M (1980) Developments in cocoa nutrition in the nineteen seventies, a review of literature. Cocoa Growers' Bull. 30: 11-24.

Wood GAR, Lass RA (2001). Cocoa, 4th edition. Blackwell Science, Oxford, UK.619 p.