

A possible role of potassium in mediating cacao seedling responses to soil water stress

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Abstract

Moisture stress is one of the key constraints to establishment of cocoa, particularly on degraded soils as in the case of replanting after removal of aged cocoa tree stocks. Potassium (K) availability has been found to be critical for survival to water stress in many crop species. In two separate experiments, 13 cacao seedling varieties were evaluated under four levels of applied potassium (0, 1, 2, or 3 g/plant of muriate of potash) to identify any differential physiological mechanisms in the responses of cocoa progenies to applied potassium that may lead to improvement in drought tolerance. In the first experiment, four varieties known in Ghana for their field tolerance to water stress were evaluated whereas in the second experiment, 10 varieties were used. Thirty days after K application, water was withheld from three-month old pot-grown seedlings for a period of 31 days. Measurements were taken on plant growth, dry matter partitioning and various physiological parameters. Water stress significantly impacted on all measured traits from 8 days after stress imposition leading to reduced leaf membrane stability and leaf chlorophyll fluorescence. Leaf polyphenol and proline contents however increased. Though liberal application of K under well-watered conditions tend to negatively impact plant function, K application was beneficial for growth and integrity of physiological functions following water stress. Under water stress, K-treated plants had increased root and shoot biomass, higher leaf water content, higher chlorophyll fluorescence and reduced electrolyte leakage from leaves leading to improved vigour. In the first experiment, there was little evidence for variety x K interaction for specific traits, but the expression of a particular physiological function appeared to have been influenced by the parentage of the varieties. Varieties having Scavina parentage showed reduced membrane stability relative to those with PA 7 parentage, but relative water contents were in the reverse order in these varieties under water stress. Leaf total polyphenol increased much more in the varieties with Scavina parentage than those with PA 7 parentage. The results suggest that different mechanisms may account for their putative drought tolerance. In the second experiment, significant interaction of variety and potassium for the studied traits suggests that not all varieties may benefit from the possible drought mitigation influences of applied potassium.

Introduction

In the major production belt of cocoa (*Theobroma cacao* L.) that lies within 10 °N to 10 °S of the equator, rainfall distribution and amount have a major influence on productivity of the crop (Alvim, 1977; Balasimha, 1988). Having evolved in the Amazon basin with high rainfall and humidity levels, short periods of drought in the production regions impact severely on the crop's physiological functions and yield (dos Santos et al., 2014; Ruf et al., 2015). Key physiological process of the crop that leads to flushes of leaf and shoot growth, rhythms of root initiation and expansion, and periodicity of flowering are all influenced by the patterns of rainfall (Carr and Lockwood, 2011). In addition to the effects of increasing dry spells on cocoa production in West Africa (Schroth et al., 2016) high intensity of land-use have led to loss of carbon, reducing soil water holding capacities and further exacerbating the effects of limited water availability on production (Guo and Gifford, 2002; Anim-Kwapong and Frimpong, 2006). The approaches adopted to reduce the effects of drought on cocoa growth and yield include planting under various shade regimes (Oppong et al., 1999; Rajab et al., 2016), application of various types of mulch (Oppong et al., 1998) and the selection of varieties with better seedling survival following post-rainy seasons in field evaluations (Padi et al., 2013; Ofori et al., 2014). These approaches have had varying levels of success under farmers'

production conditions (Asare and Ræbild, 2016), and an integrated approach may be required to reduce cocoa seedling losses in the field.

Increased concentrations of micronutrients and macronutrients have been implicated in enhancing plants adaptation to moisture stress (Cakmak, 2005). For example, under water stress plants given higher doses of phosphorus showed an increase in photosynthetic efficiency compared with those given lower doses (Rodriguez and Goudriaan, 1999). Potassium is known to be involved in stomatal opening, osmoregulation, cell expansion and increase in dry matter production (Jensen, 1982; Elumalai et al., 2002). Drought stress limits both root growth and the rates of potassium diffusion in the soil towards the roots thereby limiting potassium acquisition (Cakmak, 2005). The reduced potassium availability within the rooting zone further depresses the plant resistance to drought stress, as well as potassium absorption (Wang et al., 2013). Cakmak 2005 observed that potassium application can minimize the production of reactive oxygen species (ROS). Under drought stress, photosynthesis is limited by impairment in stomata regulation, conversion of light energy into chemical energy and phloem export of photosynthates from source leaves into sink organs. As the impairment in photosynthetic carbon dioxide fixation occurs, molecular oxygen is activated leading to oxidative degradation of chlorophyll and membranes (Egilla et al., 2005). Under adequate potassium nutrition, there is inhibition of the transfer of photosynthetic electrons to molecular oxygen, thus reducing ROS production (Cakmak, 2005). In plants supplied with adequate amounts of soil potassium, leaf water relations were influenced such that turgor potential, as a function of relative water content, is increased and such plants are generally found to have better hydrated tissues than potassium stressed plants (Mengel and Kirby, 2001). De La Guardia and Benlloch (1980) observed that accumulation of potassium in the cell favours water uptake thus generating the necessary cell turgor required for growth. Under field conditions Studer and Blanchet (1963) found that high additions of K increased yield and water use efficiency of Italian ryegrass. In olive trees, potassium starvation favored stomatal conductance and transpiration as well as inhibiting shoot growth (Benlloch-Gonzalez et al., 2008).

The objective of the study reported in this paper was to examine the role of soil-applied potassium on water-stress responses in cocoa seedlings.

Materials and Methods

Plant Materials and growth conditions

Two experiments were conducted in the gauze-house at Cocoa Research Institute of Ghana, New Tafo, Eastern Region - Ghana. Four cocoa varieties were used in the first experiment whereas 10 varieties were used in the second experiment, with one variety T85/799 x PA 7 common to both experiments. Seed pods were generated by manual pollination and seedlings were raised in 7 kg of soil in a 25 x 30 cm polyethene bags for three months before the imposition of potassium and moisture treatments.

Potassium and moisture treatments

The experiment was laid in a Completely Randomized Design (CRD) with four levels of the potassium and two watering regimes. Ten seedlings were used per treatment with three replications. The potassium treatments were applied as dry granules of muriate of potash (MOP, 60% K₂O) at 0g, 1g, 2g, and 3 g/plant to three-month old cocoa seedlings in nursery bags. One month after potassium application, moisture stress treatments were imposed either as watering to field capacity three times a week or water withheld for a period of 31 days.

Data collection

Stem diameter (5cm above the soil) was measured using vernier caliper at the start and end of the experiment. Leaf chlorophyll content and chlorophyll fluorescence measurements were done between 007-010 hours on the fifth and sixth leaves (from the youngest fully expanded leaf) per plant. Leaf chlorophyll content was measured with CL-01 Chlorophyll Content Meter (Hansatech Instruments Ltd., UK). The photosystem II quantum yield (F_v/F_M) was measured using leaf chlorophyll fluorescence meter (FP100; Photon Systems Instruments, Drasov, Czech Republic) on leaves that had been dark-adapted for 30 minutes before measurement. Relative water content (RWC) was determined using ten leaf discs of 1.5 cm diameter

excised using a cork borer from each of three leaves collected from the mid-section of seedlings in each treatment following the method of Yamasaki and Dillenburg (1999). Total polyphenols content in leaves were determined by the Folin-Ciocalteu method, described in Ragazzi and Veronese, 1973. Free proline was determined as described by Bates et al. (1973). Leaf cell membrane stability was assessed through electrolyte leakage of leaf cell content as described by Lutts et al. (1996) using 10 leaf discs of 1.5 cm diameter per treatment. Visual vigour ratings of cocoa seedlings were made after imposition of water stress on an eight point scale at weekly intervals. The number of seedlings that survived the period of stress imposition was assessed at 31 days after stress imposition. The plants were destructively sampled at five months after planting and data was taken on dry weights of leaves, stem and roots.

Soil moisture contents were monitored on weekly basis for the various treatments using the soil moisture meter SM200 (Delta T devices, UK).

Statistical analyses

Experimental data on cocoa seedlings were subjected to analysis of variance (ANOVA) using GenStat Statistical software 9th edition and significant differences among the means at $p < 0.05$ were compared by the least significant difference (LSD). Analyses also focused on the amount of soil water taken up by each variety under varying degrees of water stress.

Results

Two experiments were conducted to understand the influence of potassium on reaction of cocoa varieties to moisture stress during the seedling stage. The first experiment examined four varieties, two with SCA 9 common in their parentage, and the other with PA 7 as a common parent. The ANOVA indicated significant effects of variety and potassium for all plant traits studied, under both well-watered and water-stressed conditions but the interactions between variety and potassium were not significant under either condition.

Variation in soil water content under varieties

In experiment 1, the rates of potassium applied did not significantly influence the soil volumetric water content (SVWC) over the period of the experiment. Figure 1 shows the relative rates of soil water depletion under the four varieties under well-watered conditions. Though plants were watered at two-day intervals, SVWC taken before each irrigation event showed an increase loss of water with plant growth. SVWC was higher under the two varieties with PA 7 in their parentage indicating reduced rates of soil water depletion compared with the two varieties with SCA 9 in their parentage. T63/967 x SCA 9 in particular had very high rates of soil water extraction. The pattern of soil water depletion under water stress (Figure 2) was similar among the varieties as recorded under well-watered conditions, though the SVWC were much lower under stress. Significant differences in SVWC were evident only at 6 and 14 days after moisture stress during which the varieties with PA 7 had much higher values.

Plant physiological responses

The general effects of water stress on plant function (recorded 31 days after stress imposition) were reductions in leaf chlorophyll content and fluorescence, relative water content, leaf membrane stability (increased electrolyte leakage) and increases in leaf proline and phenol contents (Table 1). Under well-watered conditions, application of potassium reduced plant function as manifested in the reduction of all physiological traits measured. Under stress however, application of potassium was beneficial to plant function, with increasing rates of potassium increasing plant physiological function.

Among the varieties, differences in the traits measured was related to their parentage with SCA 9 varieties having slightly enhanced leaf chlorophyll content and better leaf water status under stress whereas the varieties with PA 7 parentage maintained better membrane stability and accumulated lower levels of phenol in their leaves under water stress (Table 1). For proline accumulation, T63/971 x SCA 9 that had the least accumulation under well-watered condition, but had highest accumulation under water stress, with the three other varieties accumulating about one-half the levels in T63/971 x SCA 9 under stress.

Plant growth responses

Visual rating of plant vigour was marginally improved by addition of potassium, significant only at the highest rate of 3 g MOP per plant (Table 2). Similarly, increasing levels of added potassium increased stem diameter with only the highest rate being significantly different from the control under both well-watered and stressed conditions. Both stem and root biomass increased significantly with addition of potassium under both well-watered and stressed conditions but the increases were much higher under stress compared with under well-watered conditions.

Among the four varieties in experiment 1, visual vigour ratings were marginally higher in the varieties with PA 7 parentage compared with those of SCA 9 parentage (Table 2). Based on stem diameter readings, T63/967 x SCA 9 was significantly more vigorous than the three other varieties under both well-watered and stressed conditions. The varieties with PA 7 in their parentage though produced less biomass compared with those of SCA 9 parentage, loss of biomass due to drought was higher in the varieties with SCA 9 parentage. For brevity, the second experiment focused only on plant vigour based on stem diameter measurements. As observed in experiment 1, increasing potassium application increased plant stem diameter under both stressed and well-watered conditions (Table 3). Among varieties, water stress over a period of 31 days significantly reduced plant vigour (Figure 3). Indication of variety x watering regime interaction is indicated by the ranking of varieties. Ranking of variety performance under stress and well-watered conditions remained same, except for T63/967 x EQX 3338 that was the most vigorous variety under well-watered conditions, but under stress was not significantly different from the least vigorous variety. This observation remained same under all levels of applied potassium under each water regime.

Discussion

The seedling phase of cocoa is particularly susceptible to drought and tolerance of the crop during the early establishment phase remains one of the most important requirements for successful cocoa cultivation (Padi et al., 2013). The observation of the favourable role of applied potassium in enhancing growth and biomass accumulation of cocoa seedlings under water stress is consistent with observations in many other crops (Jensen, 1982; Anderson et al., 1992). These effects on growth may be the reflection of the role of potassium

in mediating key physiological processes. Notably, relative water content and leaf membrane stability in terms of electrolyte leakage were considerably improved in the present study. Leaf relative water content and electrolyte leakage are two key parameters used to assess leaf membrane integrity under moisture stress (Alizade 2002; Pei et al., 1998). Egilla (2005) noted that one of the beneficial effects of sufficient soil potassium in mitigating drought stress was due to its role in facilitating osmotic adjustment, which maintains higher turgor pressure and relative water content.

The two secondary metabolites studied, phenol and proline, both increased several-fold under drought. Delauney and Verma, 1993 noted that the accumulation of proline in leaf tissues under drought acts as osmoprotectants thereby protecting cells from damage. In the current study, increasing levels of applied potassium reduced the level of proline accumulation relative to the control under moisture stress. In many plants, intracellular proline levels have been found to increase more than a hundred-fold during stress (Handa et al., 1983). Yang et al, 2011 observed that under stress, drought tolerant wheat cultivars accumulate lower levels of proline compared with drought sensitive ones due to faster degradation of proline in the tolerant cultivar. Judging from the visual vigour ratings under stress, T63/971 x SCA 9 is the most sensitive to stress, and it accumulated the highest levels of proline under stress, corroborating these earlier observations. Generally, stomatal closure under water stress leads to reduced carbon dioxide uptake and consequently the consumption of reduction equivalents (NADPH and H⁺) declines considerably leading to oxidative stress (de Abreu and Mazzafera, 1995). As a result, metabolic processes are shifted towards synthesis of reduced compounds including phenols (Selmar and Kleinwächter, 2013). The increases in phenol concentration observed under applied potassium during stress might play a role in promoting plant function (Lei et al., 2006).

Based on overall vigour rating, the varieties with PA 7 in their parentage appeared more tolerant to water stress compared with those of SCA 9 parentage. Both clones, however, have been found to have good combining abilities to seedling survival under water stress in Ghana (Padi et al., 2013; 2016). The high rates of soil water extraction observed in the SCA 9 varieties may pose difficulties under conditions of severe moisture stress and may be responsible for their relatively lower tolerance observed in this study. The relatively smaller increases in leaf phenol content under drought and better membrane stability of the PA 7 derived varieties suggest that these two traits may be key in identifying more tolerant varieties in a wider number of varieties. Moreover, reduction in biomass accumulation under drought was lower in the PA 7 derived varieties than the SCA 9 derived types. Given the higher rates of soil water depletion under varieties with SCA 9 parentage, we found their higher plant water status relative to those of PA 7 parentage rather surprising. Ericson et al., (1999) noted that the internal water status of plants growing in dry soils depends primarily on soil water availability and only secondarily on transpiration. However, Ritchie et al., (1990) observed that early decline in stomatal conductance in response to decline soil water content is responsible for maintenance of high leaf RWC under drought. The higher RWC of varieties with SCA 9 parentage may indicate different pathways to soil water stress tolerance in the different genetic groups.

Regardless of the ranking of the varieties in the first experiment for tolerance to soil water stress, the physiological functions under water stress of all four varieties benefited from application of potassium. With wider genetic base, it is expected that not all varieties may respond positively to potassium application under water stress. The growth responses of T63/967 x EQX 3338 is indicative of the need to further define for which genetic groups potassium application under drought stress will be beneficial for survival. Moreover, the optimal level of potassium to be applied to cocoa seedlings for mitigating water stress could not be established from this study as increasing levels of potassium linearly improved plant physiological functions, survival and growth.

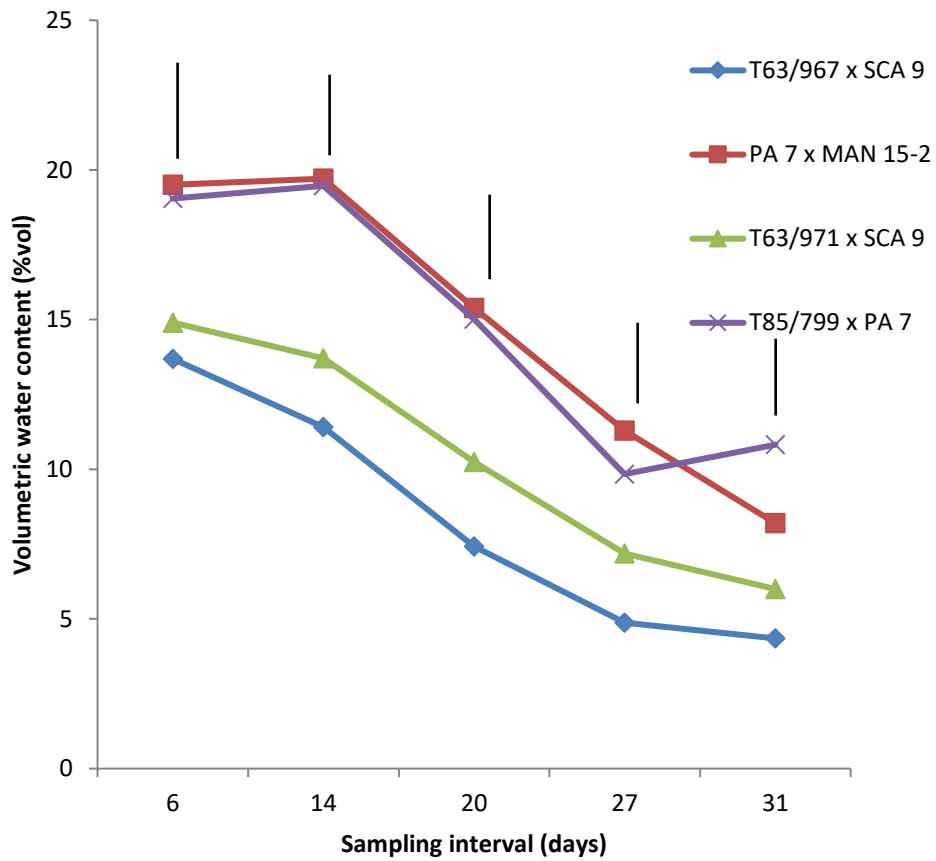


Figure 1: Changes in volumetric soil water contents under four cocoa varieties evaluated in a gauze-house facility

Sampling interval refers to days after four months from sowing. Plants were watered at two days intervals. Vertical LSD bars represent differences between varieties on a given day of assessment at $p = 0.05$.

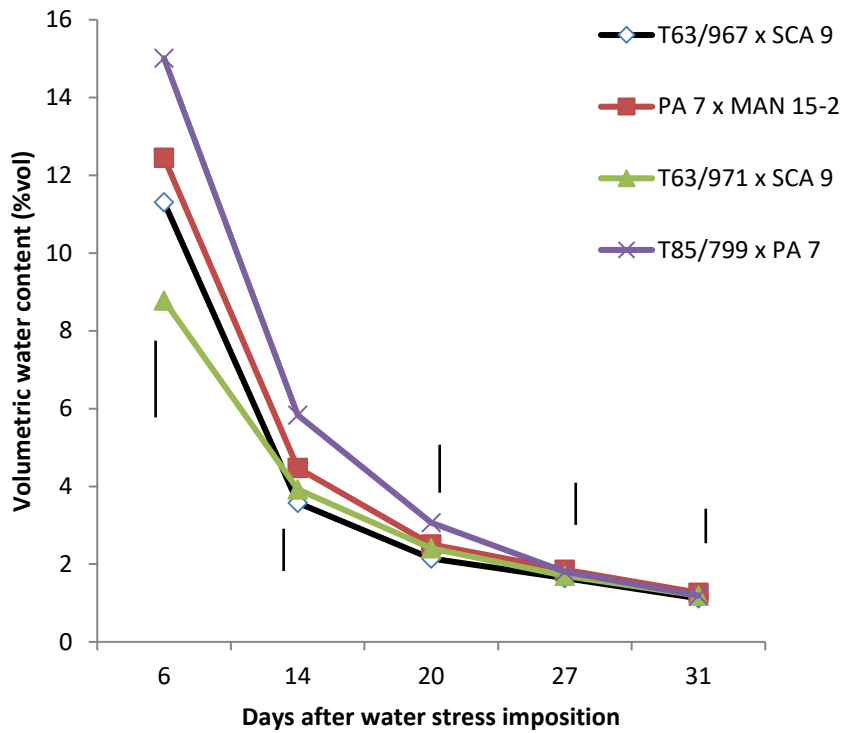


Figure 2: Changes in volumetric soil water contents under four cocoa varieties nursed in a gauze-house facility under moisture stress.

Stress imposition started four months after sowing. Vertical LSD bars represent differences between varieties on a given day of assessment at $p = 0.05$.

Table 1. Physiological response of four cocoa varieties to varying potassium levels under contrasting soil moisture regimes

Potassium (g MOP/plant)	Leaf chlorophyll content (mmol/cm ²)		Leaf chlorophyll fluorescence (mg/l)		Relative water content (%)		Electrolyte leakage (%)		Leaf phenol content (mg/g)		Leaf Proline content (µg/g)	
	Without* stress	Stress	Without stress	Stress	Without stress	Stress	Without stress	Stress	Without stress	Stress	Without stress	Stress
0	11.1	8.3	0.68	0.56	70.3	39.5	8.4	30.3	10.5	13.1	10.7	705.7
1	11.1	8.2	0.66	0.61	72.4	40.7	9.4	22.3	11.4	13.8	12.7	711.3
2	9.8	7.4	0.64	0.63	74.7	43.3	9.4	23.1	12.0	15.2	13.2	612.8
3	9.6	7.0	0.63	0.61	72.1	43.6	8.9	13.2	12.5	16.4	14.0	667.9
LSD	1.12	1.03	0.021	0.039	ns	2.16	ns	2.37	0.83	0.57	1.10	10.00
Varieties												
T63/967 × SCA 9	12.5	9.0	0.63	0.58	73.5	44.2	9.9	25.2	12.1	16.0	13.2	509.1
T63/971 × SCA 9	10.3	7.6	0.68	0.60	73.3	42.5	8.2	30.3	12.0	18.1	7.1	1067.9
PA 7 × MAN 15-2	9.3	7.2	0.64	0.59	69.5	41.0	9.4	20.2	11.4	11.2	10.6	550.0
T85/799 × PA 7	9.4	6.9	0.67	0.64	73.3	39.4	8.7	13.3	10.8	13.2	19.7	570.7
LSD	1.12	1.03	0.02	ns	ns	2.16	0.81	2.37	0.83	0.57	1.10	10.00

*Data under stress were taken at 31 days after water stress imposition.

Volumetric soil water content (% vol)

Well-watered condition: 26.55

Stress: 1.75

Table 2. Growth responses of four cocoa varieties to varying potassium levels under contrasting soil moisture regimes

Potassium (g MOP/plant)	Visual vigour rating		Stem diameter (mm)		Dry weight of stem (g)		Dry weight of roots (g)	
	Stress at 15 days	Stress at 31 days	Without stress	Stress	Without stress	Stress	Without stress	Stress
0	6.2	1.8	8.2	8.7	7.3	5.2	5.7	2.9
1	6.8	2.1	8.1	8.9	7.4	5.7	5.3	3.1
2	6.9	1.8	8.3	9.1	8.1	5.3	6.1	3.7
3	7.2	2.7	8.8	9.7	8.6	6.4	6.3	4.4
LSD	0.40	0.3	0.42	0.38	1.36	0.76	1.25	0.44
Varieties								
T63/967 × SCA 9	6.5	2.0	9.6	9.2	10.1	6.4	6.9	3.6
T63/971 × SCA 9	6.8	1.7	8.7	8.0	7.3	5.3	4.7	2.8
PA 7 × MAN 15-2	7.0	2.2	9.1	8.3	7.2	5.7	5.8	3.9
T85/799 × PA 7	6.9	2.5	9.0	7.9	6.7	5.1	5.9	3.8
LSD	ns	0.3	0.42	0.38	1.36	0.76	1.25	0.44

*Volumetric soil water content (% vol):

Well-watered condition: 26.55

15 days after water stress: 4.45

31 days after water stress: 1.75

Table 3. Main effect of potassium on the stem diameter of 10 cocoa varieties evaluated under well-watered or water stress conditions in a gauze-house facility

Potassium	Watered	Stressed*
no K	12.4	6.7
1g K	13.1	6.7
2g K	12.7	6.9
3g K	13.1	7.1
LSD	0.43	0.29

*Stress imposition was for a period of 31 days after plants were 5 months old

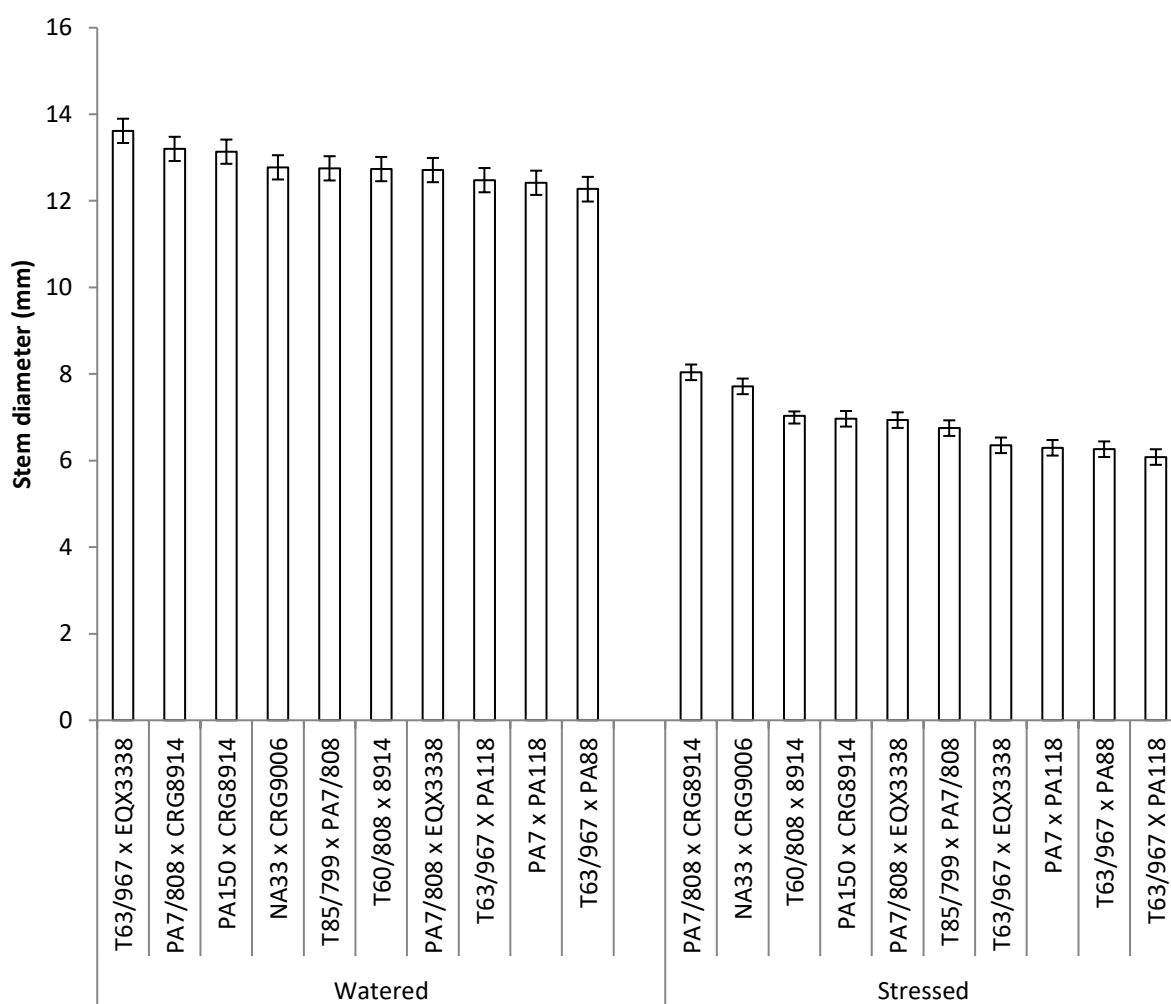


Figure 3. Stem diameter of 10 cocoa varieties evaluated under well-watered or water-stressed conditions in a gauze-house facility

REFERENCES

- Alizade, A. (2002). *Soil, Water and Plant Relationship*. Mashhad, Iran: Emam Reza University Press.
- Alvim, P.T. (1977) Cacao. In: Alvim PT, Kozlowski TT (Ed), *Ecophysiology of tropical crops* (279–313). New York: Academic Press.
- Anderson, M. N., Jensen, C. R., & Losch, R., (1992). The interaction effects of potassium and drought in field-grown barley. I. Yield, water-use efficiency and growth. *Acta Agriculturae Scandinavica, Section B- Soil and Plant Science*, 42, 34–44.
- Anim-Kwapong, G. J., & Frimpong, E. B. (2006) Vulnerability of agriculture to climate change- impact of climate change on cocoa production. In: Report on vulnerability and adaptation assessment under the Netherlands climate change studies assistance programme phase 2 (NCCSAP2). Cocoa Research Institute of Ghana. 44 pp.
- 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, pp.215-217.
- Bates, L. S., Waldren, R. P. & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.
- Benlloch-Gonzalez, M., Arquero, O., Fournier, J.M., Barranco, D., & Benlloch, M. (2008). K⁺ starvation inhibits water-stress-induced stomatal closure. *J. Plant Physiol.*, 165, 623–630.
- Cakmak, I. (2005): The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J. Plant Nutr. Soil Sci.*, 168, 521–530.
- Carr, M., & Lockwood, G. (2011). The water relations and irrigation requirements of cocoa (*Theobroma cacao* L.): A review. *Experimental Agriculture*, 47(4), 653-676. doi:10.1017/S0014479711000421
- de Abreu I. N., & Mazzafera, P. (2005). Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiology*, 43, 241 – 248
- De La Guardia, M. D., & Benlloch, M. (1980). Effects of potassium and gibberellic acid on stem growth of whole sunflower plants. *Physiol Plant*, 49, 443–8.
- Delauney, A. J., & Verma, D. P. S. (1993). Proline biosynthesis and osmoregulation in plants. *Plant J.*, 4, 215–223.
- dos Santos, I. C, Furtado de Almeida, A., Anher, D., da Conceição, A. S., Pirovani, C. P., Pires, J. L., Valle, R. René, & Baligar, V. C. (2014). Molecular, Physiological and Biochemical Responses of *Theobroma cacao* L. Genotypes to Soil Water Deficit. *Plos One*, 9(12), 31. doi: [10.1371/journal.pone.0115746](https://doi.org/10.1371/journal.pone.0115746)
- Egilla, J. N., Davies, F. T., & Boutton, T. W. (2005). Drought stress influences leaf water content, photosynthesis, and water-use efficiency of hibiscus rosa-sinensis at three potassium concentrations. *Photosynthetica*, 43, 135–140.
- Elumalai, R. P., Nagpal, P., & Reed, J. W. (2002). A mutation in Arabidopsis KT2/KUP2 potassium transporter gene affects shoot cell expansion. *Plant Cell*, 14, 119–131.
- Erickson, P.I., Ketring, D.L., & Stone, J.F., (1991). Response of internal tissue water balance of peanut to soil water. *Agronomy Journal*, v.83, p.248-253.
- Guo, L. B., & Gifford, R. M. (2002). Soil Carbon Stocks and Land Use Change—A Meta Analysis. *Global Change Biology*, 8, 345-360. <http://dx.doi.org/10.1046/j.1354-1013.2002.00486.x>
- Handa, S., Bressan, R. A., Handa, A. K., Carpita, N. C., & Hasegawa, P. M. (1983). Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiology*, 73, 834 - 843.
- Jensen, P. (1982). Effects of interrupted K⁺ supply on growth and uptake of K⁺, Ca²⁺, Mg²⁺ and Na⁺ in spring wheat. *Physiol. Plant.*, 56, 259–265.
- Lei, Y., Yin, C., & Li, C. (2006). Differences in some morphological physiological and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. *Physiologia Plantarum*, 127, 182– 191.
- Lutts, S., Kinet, J.M. & Bouharmont, J. (1996). NaCl-induced Senescence in Leaves of Rice (*Oryza sativa* L.) Cultivars Differing in Salinity Resistance. *Annals of Botany* 78, 389-398
- Mengel, K., & Kirkby, E. A. (2001). *Principles of Plant Nutrition*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Ofori, A., Padi, F. K., Acheampong, K., & Lowor, S. (2015). Genetic variation and relationship of traits related to drought tolerance in cocoa (*Theobroma cacao* L.) under shade and no-shade conditions in Ghana. *Euphytica*, 201(3) Page 411
- Oppong, F. K., Opoku-Ameyaw, K., Osei-Bonsu, K., Amoah, F. M., Brew, K. M., & Acheampong, K. (1999). The effect of time of planting at stake on cocoa seedling survival. *Ghana Journal of Agricultural Science*, 32, 79-86.

- Oppong, F. K., Osei-Bonsu, K., Amoah, F. M., & Acheampong, K. (1998). Potential use of *Flemingia macrophylla* as mulch for managing weeds in young cocoa in Ghana. *Ghana Journal of Agricultural Science*, 31, 67-72.
- Padi, F. K., Adu-Gyamfi, P., Akpertey, A., Arthur, A., & Ofori A. (2013). Differential response of cocoa families to field establishment stress. *Plant Breeding*, 132, 229 – 236
- Padi, F. K., Ofori, A., & Arthur, A. (2016). Genetic variation and combining abilities for vigour and yield in a recurrent selection program for cacao. *Journal of Agricultural Science (Cambridge)*, 155, 444-464
- Pei Z. M., Ghassemian M., Kwak C. M., McCourt P., & Schroeder J. I. (1998). Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. *Science* 282 287–290. 10.1126/science.282.5387.287
- Ragazzi, E. & Veronese, G. (1973). Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *Journal of Chromatography*, 77, 369-375.
- Rajab, A. Y., Leuschner, C., Barus, H., Tjoa, A., & Hertel, D. (2016) Cacao Cultivation under Diverse Shade Tree Cover Allows High Carbon Storage and Sequestration without Yield Losses. *PLoS ONE*, 11(2): e0149949. <https://doi.org/10.1371/journal.pone.0149949>
- Richard, A., & Ræbild, A. (2016). Tree diversity and canopy cover in cocoa systems in Ghana. *New Forests*, 47, 287-302. <https://doi.org/10.1007/s11056-015-9515-3>
- Ritchie, S.W., Nguyen, H.T., & Holaday, A.S., (1990). Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop Science*, v.30, p.105-111.
- Rodríguez, D., Andrade, F. H., & Goudriaan, J. (1999). Effects of phosphorus nutrition on tiller emergence in wheat. *Plant and Soil*. 209: 283. doi: 10.1023/A:1004690404870
- Ruf, F., Schroth, G., & Doffangui, K. (2015). Climate change, cocoa migrations and deforestation in West Africa — what does the past tell us about the future? *Sustain. Sci.* 10, 101–111. <http://dx.doi.org/10.1007/s11625-014-0282-4>.
- Schroth, G., Läderach, P., Martinez-Valle, A. I, Bunn, C., & Jassogne, L. (2016). Vulnerability to climate change of cocoa in West Africa: Patterns, opportunities and limits to adaptation. *Science of the Total Environment*, 556, 231–241. <http://dx.doi.org/10.1016/j.scitotenv.2016.03.024>
- Selmar, D. & Kleinwächter, M. (2013). Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54, 817 - 826.
- Studer, R., & Blanchet, R. (1963). Irrigations en region temperee a influence oceanique et interactions entre l'alimentation pot-assique et l'alimentation hydrique des plantes. *C. R. Ac. Agric. F.* 49, 339-348.
- Wang, M., Zheng, Q., Shen, Q., & Guo, S. (2013). The Critical Role of Potassium in Plant Stress Response. *International Journal of Molecular Sciences*, 14(4), 7370–7390. <http://doi.org/10.3390/ijms14047370>
- Yamasaki, S., & Dillenburg, L. R., (1999). Measurements of leaf relative water Content in *Araucaria angustifolia*. *Revista Brasileira de Fisiologia Vegetal*, 11(2), 69-75.
- Yang, Y., Zhang Y., Wei, X., You, J., Wang, W., Lu, J., & Shi, R. (2011). Comparative oxidative responses and proline metabolism in two wheat cultivars under short term lead stress. *Ecotoxicological and Environmental Safety*, 74, 733 - 740.