

## Effect of water stress on the nutrient uptake of cocoa genotypes in Southwest of Nigeria

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### Abstract

SCA 6, SCA 9, NA 32, ICS 96, N 38, PA 7 and PA 150 were either self or cross pollinated to form six cocoa genotypes namely: SCA 9 \* SCA 12, NA 32 \* NA 32, ICS 95 \* ICS 95, N 38 \* N 38, PA 7 \* PA 150 and SCA 12 \* SCA 9. The selection of the parent cocoa clones were based on their reported varied genotypic and traits to water stress conditions. The seeds of the six cocoa genotypes were pre-germinated for two weeks before their seedlings were transplanted into 4 kg topsoil inside each of the plastic pots in a greenhouse where they were examined for their responses to three different watering regimes at 25, 50 and 100 field capacity (fc) for 30 weeks. Data were collected on the physico-chemical properties of the experimental soil as the leaf contents for N, P, K, Ca, Mg and Fe nutrients. The leaf N, P, Ca and Fe contents showed higher concentrations at lower fc while K and Mg contents increased at higher water regimes. The results showed genotypic differences in the nutrient uptake performances of the cocoa seedlings.

**Keywords:** Cocoa; Genotypes; Water Regimes; Leaf Nutrient Contents.

### 1. Introduction

Cocoa is tropical woody species grows in areas with high annual rainfall of between 1,500 and 2,000 mm/year [1] but as a result of seasonal rainfall patterns that sometimes results in long dry spell, the production of the crop is prone to periodic drought in Nigeria. Most often, the first three years of establishing a new cocoa farm is the most challenging period in terms of the survival and growth of the crop. This is because the young cocoa trees are easily affected by water availability during their early growth and development [2]. Depending on their genes expression, cocoa have some adaptation mechanisms to various physiological levels for their survival and growth during water stress or high temperature conditions, yet the selection of cocoa clones based on how they respond physiologically to drought is meagre [3]. Species or cultivars of plants that have different tolerance abilities to water stress varied in their mechanism for survival and growth under limited water supply [4]. Due to climate change, water deficit poses a serious negative impact on most crops' growth and development, and the ability to maintain adequate water status during drought is now becoming an essential strategy for growth of cocoa [5]. This is achieved through effective stomatal regulation as well as the accumulation of compatible solutes that will become the osmolytes to effect the retention of enough water in their cytoplasm during water stress [6, 7]. However, many research trials that relate the response of most plants with specific water stress conditions are difficult at field level [8] as observations are dependent, to a large extent, on the rate of crop development during an induced/controlled water stress period [9]. Hence, in order to control water

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stress levels and facilitate their reproducibility and statistical verifications, most studies on plant–water relations under drought has to be performed in potted plants and controlled glasshouse conditions while knowledge on roots functions and anatomy of cocoa has been basically carried out on their seedlings responses [10]. Consequently, this paper presents a greenhouse experiment that investigated the effect of water stress condition on the nutrient uptake of six genotypes of cocoa seedlings under a controlled environment in Nigeria.

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## 2. Materials and Methods

### 2.1. Experimental design

The pot experiment was carried out in a greenhouse of Cocoa Research Institute of Nigeria, Ibadan (Latitude 7.26°, Longitude 3.54° and 122m above sea level). The greenhouse temperature and relative humidity were recorded (Fig. 1). There were 6 cocoa genotypes, 3 watering regimes at 100%, 50% and 25 % field capacity, 3 experimental blocks as well as 4 plants per genotype per block to make a total of 216 experimental pots, all of which were sampled. The seedlings were produced from 6 cocoa crosses (SCA 9 \* SCA 12, NA 32 \* NA 32, ICS 95 \* ICS 95, N 38 \* N 38, PA 7 \* PA 150 and SCA 12 \* SCA 9) and examined for their various responses to water stress conditions in the greenhouse. The selected cocoa genotypes were based on their reported varied genotypic water responses and traits to water stress conditions [11, 12].

### 2.2. Experimental soil

The experimental soil was topsoil of Sandy loam Alfisol (USDA Soil Classification) in a virgin land which is moderately rich in organic matter content and collected between 0-15 cm depths of the soil.

### 2.3. Production of cocoa seedlings

Hand pollination of the cocoa flowers took place at the International Cocoa Germplasm Plot of the Cocoa Research Institute of Nigeria, Ibadan and the pods were harvested at 6 months after pollination when they had shown signs of maturity and ripening. The cocoa seeds were extracted from their pods, pre-germinated in sawdust for 2 weeks and later transplanted into pots containing about 4 kg top soil collected between 0-15 cm of the soil surface, and passed through a 2 cm sieve to remove all stubbles, stones and other debris before being used to fill the seedlings pots. The seedlings that had their two cotyledons opened were transferred into the experimental pots in the greenhouse from 2 weeks after sown and later raised in the greenhouse until they were 8 months old. All seedlings were initially watered at the same rate (2-day interval) before the imposition of the different water treatments at exactly 3 weeks after transplanting (WAT) into the greenhouse pots which equivalents to 5 weeks after sowing. The soil field capacities were determined using the method described by Ayegboyin [11].

### 2.4. Data collection

The leaf nutrient uptake were measured destructively. All stands of seedlings per treatment per genotype were harvested (uprooted) for sampling at the end of the trial at 32<sup>nd</sup> weeks after transplanting (WAT). After uprooting cocoa seedlings, their shoots were separated from their roots with secateurs and all leaves detached into different paper envelopes and dried in the oven at 65°C until a constant weight was reached. The dried leaves were then ground to prepare them for their nitrogen, phosphorus, potassium, calcium, magnesium and iron nutrient content analyses in a standard laboratory.

### 2.5. Statistical analysis

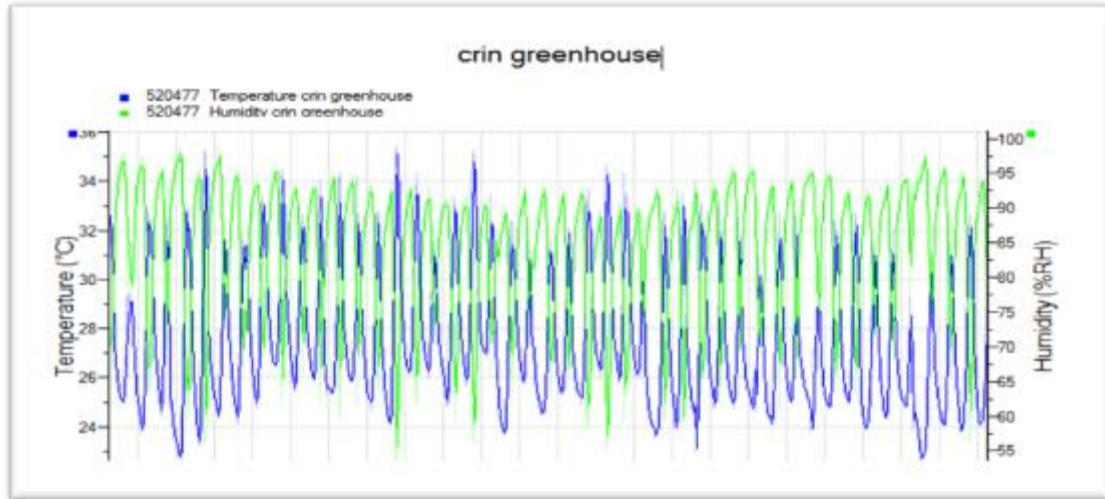
Data collected were subjected to Analysis of Variance before significant means were determined by least significant difference (LSD) at P = 0.05 value.

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## 3. Results and Discussion

### 3.1. The temperature and relative humidity of the greenhouse

The temperature ranged from 22 to 36°C while the relative humidity ranged from 53 to 98% during the period of data collection (Fig. 1).



**Figure 1** Environmental conditions of the greenhouse as recorded by a data logger (Tinytag, Gemini Data Loggers, Chichester, UK).

**3.2. Laboratory analysis of experimental soil.**

**Table 1** Result of laboratory analysis of experimental soil.

	Parameter	Unit	Value
	pH		6.9
Particle size	Sand	%	85.40
	Silt	%	12.00
	Clay	%	2.60
Exchangeable Bases	Ca	cmol/kg	5.36
	Mg	cmol/kg	1.08
	K	cmol/kg	0.364
	Na	cmol/kg	0.494
	Al+H	cmol/kg	0.07
	ECEC	cmol/kg	7.37
Base	Salinity	%	99.05
Total	N	%	0.149
Total	Organic C	%	2.49
Available	P	mg/kg	10.79
Micro nutrients	Mn	mg/kg	66.30
	Fe	mg/kg	1.35
	Cu	mg/kg	6.47
	Zn	mg/kg	12.75

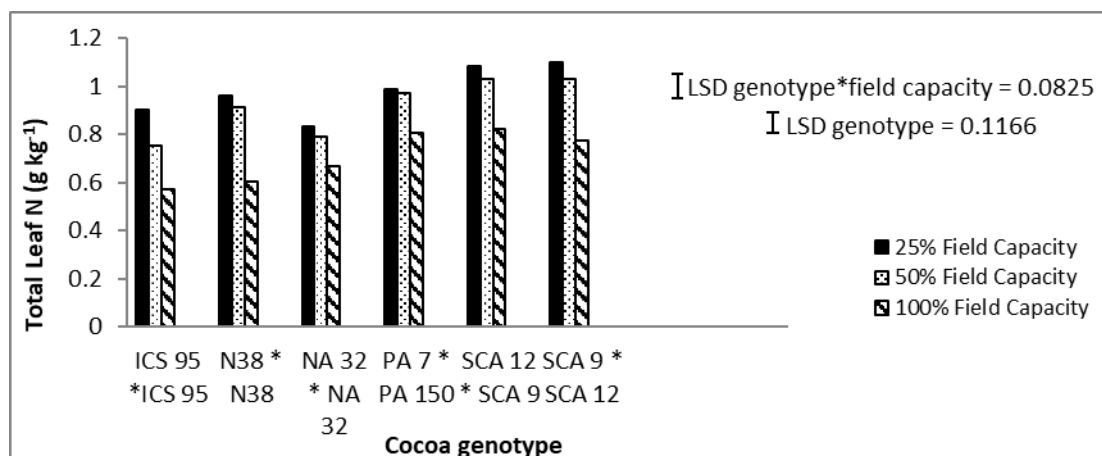
The physical and chemical properties of the soil are presented in Table 1. The experimental soil has a pH of 6.9 which is ideal for cocoa growth. Cocoa grows well in soils with pH that ranges from 5.0 to 7.5. Although, if the nutrient content is high enough, cocoa can still cope with some levels of high acidic and alkaline soils, except excessive acidity and alkalinity at pH 4.0 or less and pH of 8.0 or higher, respectively. Based on the established critical levels for soils in

Southwest Nigeria, the experimental soil was good in organic content (OC) since the critical OC level for cocoa is 2.49 % [13]. The total N of 0.15 % was considered optimal for most crops according to Sobulo and Osiname [14]. The available P was 10.79 mgkg<sup>-1</sup> and higher than 10 mgkg<sup>-1</sup> P regarded as adequate for crop production [13]. The exchangeable K, Ca, Mg and Na were higher than 0.2 cmol/kg regarded as the critical levels for cocoa seedlings [15] and thus, indicating rich soil fertility. The results of the exchangeable bases, Ca, Mg, K and Na in the sampled soil showed that the soil had enough of Calcium, Magnesium and Potassium nutrients for good cocoa production. The term “exchangeable bases” or “total exchangeable bases” refers to the sum of the bases (calcium, magnesium, potassium, and sodium) in exchangeable form expressed as milligram equivalents per 100 g of soil. The 0.07 exchangeable Al+H showed a very low exchangeable Aluminum hydride. However, hydrated Aluminum species (combined with hydroxyl [OH<sup>-</sup>]) usually are not toxic to plants because their charge is too weak to displace basic cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>) from soil exchange sites. As soil pH becomes lower, decreasing soil pH provides increasing H<sup>+</sup> ion activity, which reacts with OH<sup>-</sup> ions combined with the Al<sup>3+</sup> ion, stripping the OH<sup>-</sup> away from the Al<sup>3+</sup>, thereby increasing the charge on the Al-species to a +2 or +3 charge. The distribution of particle sizes of Sand (85.04 %), Silt (12.00 %) and Clay (2.60 %) showed that the sampled soil was Sandy loam. This results revealed that the soil used for the experiment was ideal for cocoa production. Cocoa requires deep and well drained soils. Poorly drained soil affects growth of plants. Majority of area under cocoa cultivation is on clay loam and sandy loam soil.

### 3.3. Leaf nutrients contents of cocoa seedlings

Data on the leaf nutrient contents of the cocoa seedlings under different field capacities are shown in the values of the Total N (Fig. 2), Available K (Fig. 3), Phosphorus (Fig. 4), Calcium (Fig. 5), Magnesium (Fig. 6) and Iron (Fig. 7).

#### 3.4. Cocoa total leaf nitrogen content

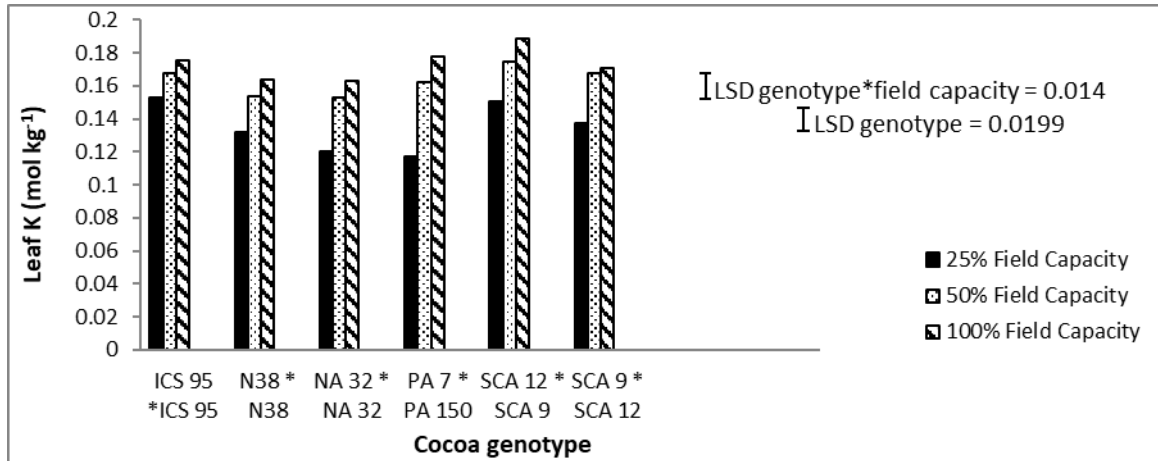


**Figure 2** Total leaf nitrogen content of cocoa seedlings under different field capacities. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

The genotype \* field capacity interactions on the nitrogen (N) content of the cocoa was on the borderline of significance at P = 0.05. Leaf N contents increased with decrease in the quantities of water applied. While the values of leaf N contents decreased with higher field capacities, the response slightly varied between genotypes (Fig.2). Leaf N content of the unstressed cocoa leaves at 100% fc were significantly lower than those of the severely stressed plants at 25% fc, but there were no significant differences among the treatment means at 25% fc and 50% fc. The overall highest N concentration of 1.098 g kg<sup>-1</sup> was produced by SCA 9 \* SCA 12 at 25% fc but was significantly higher than the 0.835 g kg<sup>-1</sup> produced by NA 32 \* NA 32 under the same field capacity. At 100% fc, ICS 95 \* ICS 95 produced the overall lowest value of N content (0.574 g kg<sup>-1</sup>) which was significantly lower than the value of 0.807 g kg<sup>-1</sup> produced by PA 7 \* PA 150 genotype under the same field capacity at the same period of time.

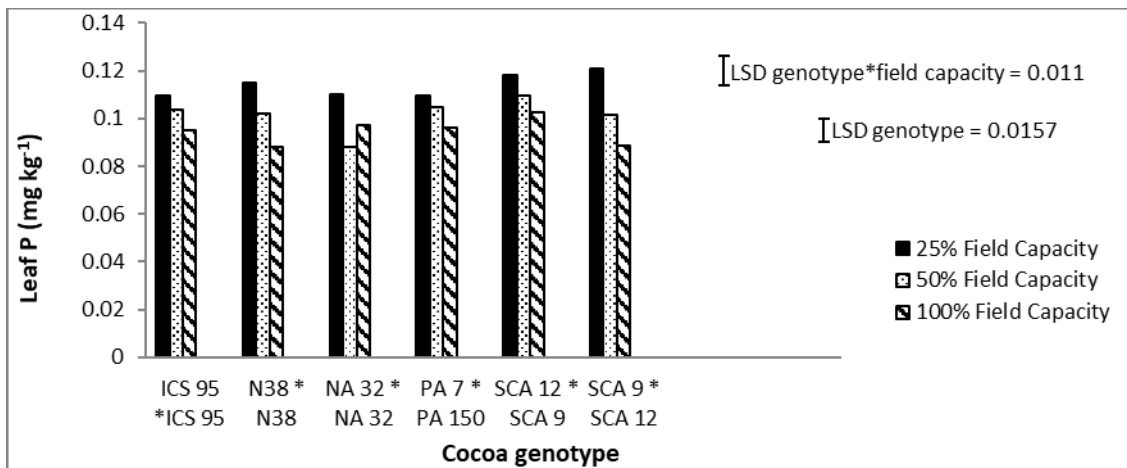
#### 3.4. Cocoa leaf potassium content

A significant genotype \* field capacity interactions was found in the values of potassium (K) content of the seedlings (P = 0.05). The leaf K contents increased with the quantities of water applied and there was significant differences between treatments means at 25% fc and 100% fc (Fig. 3). However, in contrast to the other genotypes, there were no significant differences among the severely stressed at 25% fc and moderately stressed 50% fc leaves of ICS 95 \* ICS 95 and N 38 \* N 38.



**Figure 3** Potassium content of cocoa seedlings leaf under different field capacities. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

### 3.5. Cocoa leaf phosphorus content

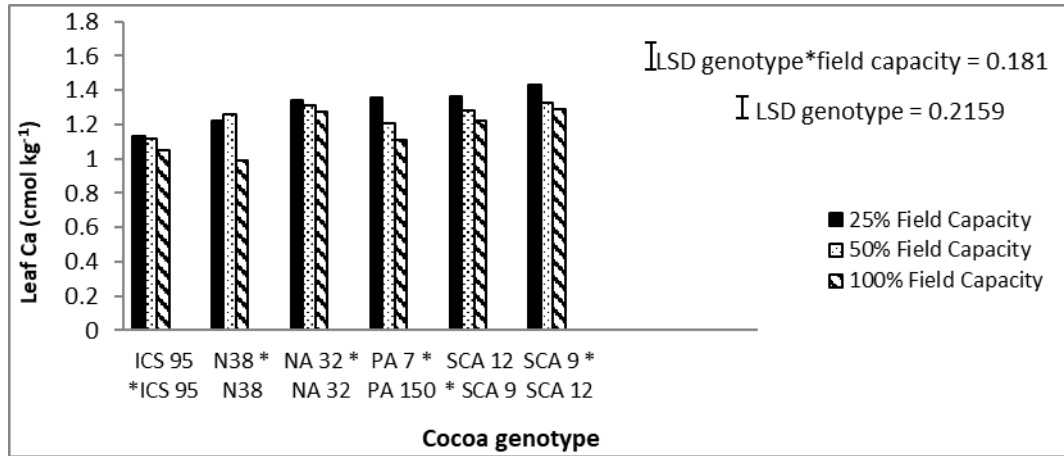


**Figure 4** Phosphorus content of cocoa seedlings leaf under different field capacities. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

The leaf phosphorus (P) content of cocoa genotypes as well as genotypes \* field capacity interactions were significant ( $P = 0.05$ ). The leaf P content increased with reduced water supply for all cocoa plants except NA 32 \* NA 32 that have their seedlings at 100% higher than those seedlings at the same period of time. All genotypes have higher leaf P concentrations at 25% fc than at 100% fc (Fig. 4).

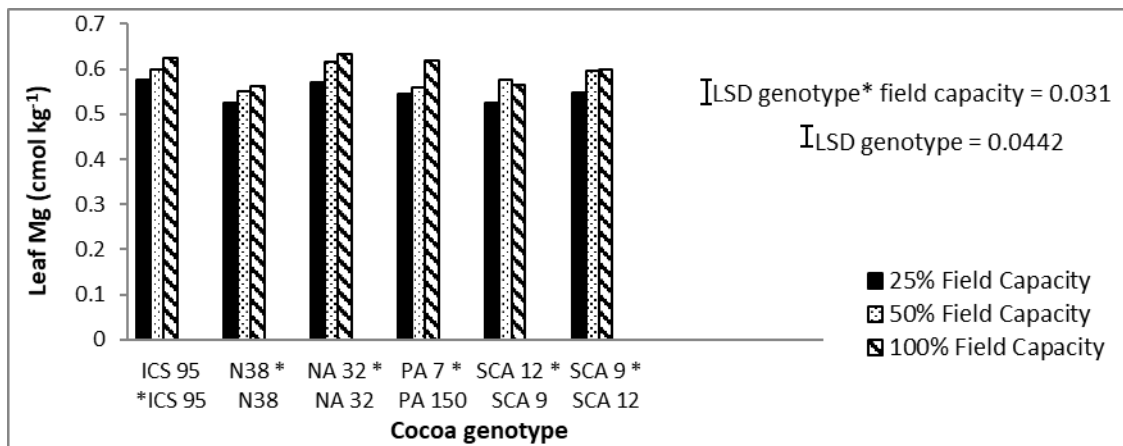
### 3.6. Cocoa leaf calcium content

Similar to the N, P and K values, the interaction between genotype\*quantity of water on Ca content was on the borderline of significance ( $P = 0.05$ ). The genotypic behaviour of Ca accumulation varied and did not follow any specific pattern (Fig. 5). The highest Ca content was recorded with PA 7 \* PA 150 ( $1.759 \text{ cmol kg}^{-1}$ ) at 25% fc which was significantly higher than those produced by other genotypes under the same field capacity. The overall effect of genotypes was not significant.



**Figure 5** Calcium content of cocoa seedlings leaf under different field capacities. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

### 3.7. Cocoa leaf magnesium content

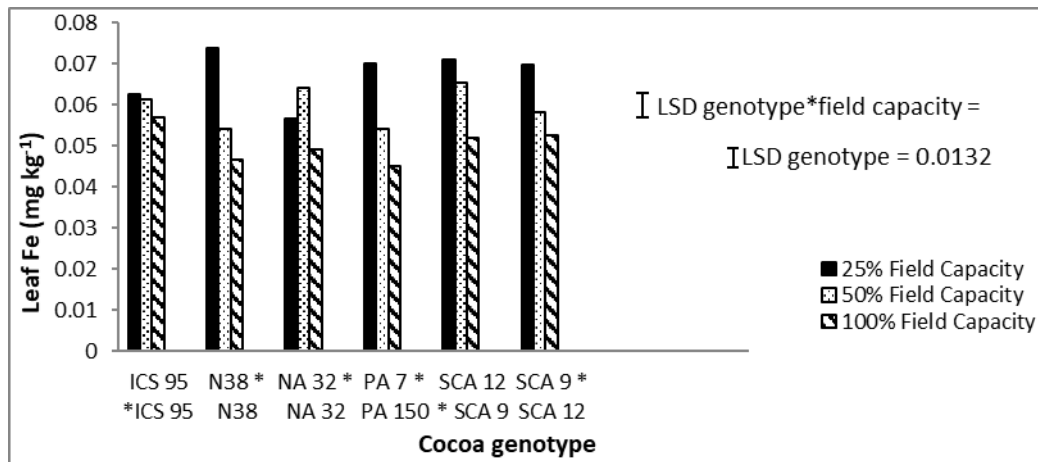


**Figure 6** Magnesium content of cocoa seedlings under different water regimes. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

A significant effect of water regimes on the cocoa genotypes ( $P = 0.05$ ) was recorded for leaf magnesium (Mg) content. Apart from SCA12 \* SCA 9, Mg accumulation progressed with quantities of water supplied (Fig. 6). Mg content of N 38 \* N 38 were the lowest among all genotypes across all water regimes at 25%, 50% and 100% field capacities. There were significant differences in Mg accumulation of all genotypes raised under 100% fc and their counterparts under 25% fc.

### 3.8. Cocoa leaf iron content

There was a significant genotype \* field capacity interaction on leaf iron (Fe) content ( $P = 0.05$ ). Apart from NA 32 \* NA 32, the quantity of leaf Fe decreased with the increase in water application (Fig. 7). N 38 \* N 38 produced  $0.074 \text{ mg kg}^{-1}$  and  $0.0466 \text{ mg kg}^{-1}$  which were the overall highest Fe content value at 25% fc and overall lowest Fe value at 100% fc respectively, and the values were significantly different at  $P = 0.05$ . Generally, the leaf Fe values obtained under 100% fc were significantly lower ( $P = 0.05$ ) than their contents at 25% fc for all tested cocoa genotypes.



**Figure 7** Iron content of cocoa seedlings under different field capacities. Values represent means of 12 seedlings at 32 weeks after transplanting. Bars represent Least Significant Differences.

## 4. Discussion

### 4.1. Soil physico-chemical analyses

The physico-chemical analyses of the experimental soil (Table 1) shows that the soil was slightly acidic with pH (H<sub>2</sub>O) = 6.61 and was suitable for optimum cocoa production [16]. The values of N (5.5 g kg<sup>-1</sup>), P (0.7 mg kg<sup>-1</sup>) and K value (0.26 cmol kg<sup>-1</sup>) were above the critical levels of 1.8 g kg<sup>-1</sup>, 0.13 mg kg<sup>-1</sup> and (0.03cmol/kg) for N, P and K required respectively for cocoa cultivation in Ibadan [17,18]. The soil can be described as sandy loam with its Ca and Mg values (0.36 cmol kg<sup>-1</sup> and 0.28 cmol kg<sup>-1</sup>) above their critical levels of 0.3 cmol kg<sup>-1</sup> and 0.2 cmol kg<sup>-1</sup>, respectively [19].

### 4.2. Cocoa nutrition during water stress

The leaf N concentration was much lower in the cocoa seedlings that received full watering than those at lower field capacities (Fig. 2) probably due to either a greater leaching and/or higher uptake of N by cocoa under reduced water availability. N availability and solubility are reduced by leaching under high irrigation but accumulations of free amino acids that are not converted to protein due to plant water deficit might also be responsible for higher N content in the cocoa water-stressed leaves. Although, higher N is expected to be associated with higher photosynthesis, as the water stress intensified in the experimental cocoa, their stomatal conductance may have become so low that it would have an overriding effect on N ability to transform into higher photosynthesis and growth of the crop. Meanwhile, the report of the influence of soil moisture availability on cocoa K leaf content in this present study (Fig. 3) is consistent with the observation of Thong and Ng [20] that soil water aids K accumulation in plants although Booshart and Uexkull [21] reported that cocoa trees well supplied with K are more tolerant to adverse effects of water stress under field conditions. While the mineral-nutrient status of plants plays a critical role in increasing plant resistance to environmental stress factors, Cakmak and Engels [22] explained that plants that are suffering from environmental stresses like drought usually require a larger internal requirement for K to maintain the photosynthetic CO<sub>2</sub> fixation, although sometimes during drought, the immobilization of K and/or unavailability of all the extractable K to plants uptake might limit the K content levels in water stressed leaves. Therefore, despite the fact that K level of the experimental soil was above its critical value for cocoa production in Nigeria (Table 1) as explained by Ipinmoroti *et al* [17], soil water deficit during the experiment may have resulted in the immobilisation of K and/or the inability of cocoa genotypes to absorb the extractable K during water stress at 25% fc. Water stress may have had a serious influence on K uptake in cocoa and resulted in declining K concentrations in its water stressed leaves in the present study. Similar results where K content in the cocoa leaves was at variance with the K soil value has been reported [18].

Just like N, it was observed that cocoa leaf Mg content decreased with higher quantity of water supplied (Fig. 6). Mg uptake by fine roots showed an increase in the accumulation of this nutrient as water availability got reducing, indicating promotion of higher Mg uptake during water stress in cocoa. Similar result has been reported on *Cunninghamia lanceolata* by Li *et al* [23]. Mg increases the length and surface area of roots which invariably helps to increase the uptake of water and nutrients by root, improves translocation of photosynthates and reduces the photo-oxidative damage to chloroplast under drought conditions. However, Mg activities might not have been fully activated on the cocoa plants in the present greenhouse study since its major role are in the chlorophyll and cocoa seedlings in the present work were raised under limited sunshine in the greenhouse.

Leaf P (Fig. 4), Ca (Fig. 5) and Fe (Fig. 7) increased with decreased water supply to cocoa. P is a constituent of nucleic acids, phospholipids, phosphor-proteins, dinucleotide and adenosine triphosphate (ATP). While ATP is used in the plants adaptation to water stress conditions [24], P is required for storage and transfer of energy, photosynthesis as well as regulation of some enzymes and the transport of carbohydrates in plants [25, 26]. P deficiency reduces leaf photosynthetic rate and growth of plant. This could be the consequent of reduction in stomatal conductance and ribulose 1, 5 biphosphate (RuBP) carboxylase regeneration capacity as well as the inducement of better root growth while trying to maintain cell turgidity during water stress [27]. Calcium also plays a major role as calmodulin which controls the plant metabolic activities and enhances the plant growth under water stress condition [28]. In the present work, there were higher Ca leaf contents in cocoa with higher quantity of water supply and the observation of higher leaf Fe concentrations in the stressed cocoa leaves might have been the results of slower leaf expansion and less leaching. Also, water stress condition at 25% fc may had led to a complete utilization of available plant water and substantially decreased the physiological processes of cocoa seedlings, it did not result into a complete shutdown of cocoa system during the period. Similar observation had been previously reported on Barnea olives by BenGal *et al* [29]. The present work also showed that the primary sensors of water deficit in cocoa are interconnected with changes in this crop nutrition and carbon dioxide balance which invariably trigger both physiological and biochemical perturbations in the roots and stems. Such phenomenon explains the stunted growth of the cocoa seedlings during water stress condition at 25% fc in the present work. However, we deduced that the supply of K and Mg nutrient elements in adequate and appropriate forms at early stage of cocoa growth may help the sustainability and development of the crop during some water stress conditions.

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## 5. Conclusion

The results of this study further revealed the existence of genotypic differences in the survival and growth of cocoa during water stress conditions at seedlings, level and the ability of application of adequate supply of K and Mg to ameliorate the water stress effect in cocoa. However, more studies are recommended for the evaluation of the nutritional genotypic responses of different mature cocoa plants to water availability on the field.

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## Compliance with ethical standards

### Acknowledgments

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### Disclosure of conflict of interest

The authors declare no conflict of interest on this publication.

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