Effects of Soil Moisture Stress on Seedlings of Three Cocoa (Theobroma Cacao L.) Varieties and Selected Genotypes in Ghana

Derick Oduro[1], Nana Opoku Agyeman[2] and Ebenezer Jeremiah Durosimi Belford[1]*

[1]Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

***Corresponding Author**

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ABSTRACT

Soil moisture stress is a major constraint to cocoa production in West Africa, particularly under changing climatic conditions. This study evaluated the response of the seedlings of three cocoa varieties and selected genotypes to soil moisture stress under greenhouse conditions. The experiment was arranged in a Completely Randomized Design (CRD) involving seven cocoa genetic materials (three varieties and four selected genotypes) subjected to four soil moisture stress treatments, each replicated four times. Pots were randomly arranged on greenhouse benches to minimize environmental variation. Cocoa seedlings were exposed to controlled soil moisture levels representing drought stress conditions. Morphological and physiological parameters, including plant height, leaf area, stem thickness, leaf and soil relative water content, chlorophyll content (NDVI), and root anatomical characteristics, were assessed.

Soil moisture stress significantly affected seedling growth and physiological performance ($p < 0.05$). Seedlings exposed to reduced moisture levels exhibited decreases in plant height, leaf area, and chlorophyll content compared to well-watered plants. However, certain genotypes maintained relatively higher NDVI values and improved root development under moisture stress, suggesting enhanced drought tolerance. Genotypes PA150 and Forastero showed superior drought tolerance, while PA7 and C42 were highly susceptible. Based on percent reduction from control, Forastero (23% reduction from 20.5 to 15.8 leaves) was more tolerant than Criollo (28% reduction from 21.8 to 15.8 leaves), despite similar absolute leaf counts under severe stress.

These findings highlight the importance of identifying drought-tolerant cocoa varieties or genotypes for breeding programs to improve climate resilience in cocoa production systems in Ghana and across West Africa.

Keywords: Cocoa seedlings, drought tolerance, soil moisture stress, Normalized Difference Vegetation Index (NDVI), leaf relative water content, soil relative water content, drought-tolerant genotypes.

INTRODUCTION

Agriculture plays a vital role in the socio-economic development of many countries, serving as a major source of employment and livelihood for the majority of the rural population. In Ghana, agriculture contributes significantly to national development through the cultivation of various crops across diverse climatic zones, ranging from the dry savanna to the moist

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forest regions. Crops such as grains, cocoa, oil palm, and cola nuts constitute an important component of the country's agricultural economy. Among these, cocoa (*Theobroma cacao* L.) remains the most important cash crop and a leading export commodity (Kongor *et al.,* 2016).

Cocoa is cultivated mainly in the humid tropical regions of Ghana and serves as a key raw material for producing chocolate, beverages, confectionery, cosmetics, and pharmaceuticals. Historically, cocoa cultivation in Ghana is widely attributed to Tetteh Quarshie in 1879, although earlier introductions by European missionaries have been documented (COCOBOD, 2016). Currently, three main cocoa varieties are cultivated in Ghana: Forastero, Criollo, and Trinitario, the latter being a hybrid of the first two (Argout *et al.,* 2008)

Cocoa is a tropical perennial species belonging to the family Malvaceae and thrives under warm, humid conditions with well-distributed rainfall (Wicks, 2003; Amoah, 1995). Optimal growth occurs under annual rainfall between 1,500 and 2,000 mm, with a short dry season (Anim-Kwapong and Frimpong, 2005). However, changing climatic conditions have resulted in prolonged dry seasons and irregular rainfall patterns, leading to soil moisture deficits that adversely affect cocoa production and sustainability.

Soil moisture stress, commonly referred to as drought stress, is one of the most important abiotic factors limiting crop productivity worldwide (Farooq *et al.,* 2009). It affects plant growth through its impact on physiological and metabolic processes, including reduced cell expansion, impaired photosynthesis, and decreased nutrient uptake (Mahajan and Tuteja, 2005; Taiz and Zeiger, 2002). In cocoa, drought stress leads to reduced leaf area, wilting, premature leaf abscission, and overall growth retardation (Carr and Lockwood, 2011). The response of cocoa to drought and other environmental stress conditions varies considerably among different varieties and genotypes. At the physiological level, drought stress reduces stomatal conductance, chlorophyll content, and photosynthetic efficiency, while also altering plant water relations (Flexas and Medrano, 2002; Cornic and Massacci, 1996). It may also lead to the accumulation of osmolytes such as proline, which play a role in osmotic adjustment under stress conditions (Hayat *et al.,* 2012; Balasimha, 1988). Furthermore, prolonged soil moisture deficit negatively affects root development, reducing root biomass, root length density, and water uptake efficiency (Salih *et al.,* 1999; Blum *et al.,* 1997).

The response of plants to drought varies significantly depending on species, variety, and genotype. In several crops such as maize, rice, and cowpea, extensive research has led to the identification of drought-tolerant varieties. However, in cocoa, relatively limited research has been conducted to evaluate drought tolerance across different varieties and genotypes, despite its economic importance (Padi *et al.,* 2013; Adu-Ampomah *et al.,* 2001).

In Ghana, the Cocoa Research Institute of Ghana (CRIG) has undertaken efforts to develop improved cocoa genotypes with enhanced tolerance to environmental stresses, including drought. Studies have identified cocoa genotypes with potential drought tolerance based on traits such as leaf water status, root architecture, and carbohydrate accumulation (Adu-Ampomah and Frimpong, 2002; Amponsah, 1973). However, comprehensive comparative evaluations of multiple cocoa varieties and genotypes under controlled soil moisture stress conditions remain limited.

This gap in knowledge poses a significant challenge for cocoa farmers, particularly during the dry season when high seedling mortality is common due to inadequate water availability. Many farmers lack information on which cocoa varieties or genotypes can withstand drought conditions. Identifying drought-tolerant cocoa seedlings is therefore essential for improving productivity, ensuring sustainability, and safeguarding the livelihoods of farmers in cocoa-growing regions.

Therefore, the aim of this study is to evaluate the effects of soil moisture stress on seedlings

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of selected cocoa varieties and genotypes by assessing key morphological, physiological, and anatomical traits under greenhouse conditions. To achieve this, the specific objectives were to:

i. assess the effects of moisture stress on morphological characteristics (leaf number, plant height, stem thickness, leaf area, and appearance of leaves showing drought symptoms).
ii. evaluate the effects of moisture stress on physiological traits, including relative water content and chlorophyll content.
iii. examine the effects of moisture stress on root anatomical features of cocoa seedlings.

MATERIALS AND METHODS

Description of the Study Area

The experiment was conducted in the greenhouse of the Department of Horticulture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The greenhouse environment allowed controlled evaluation of soil moisture stress effects on cocoa seedlings. Greenhouse benches with a height of 80 cm were used to support the experimental pots. The relative humidity of the experimental site throughout the study ranged between 29 and 89%, and the temperature was between 28ºC and 36ºC. A hygrometer was used to measure the relative humidity, and a thermometer was used to measure the ambient air temperature of the study site on a tri-daily basis (at 08:00GMT, 12:00 GMT, and 16:00GMT) throughout the study period.

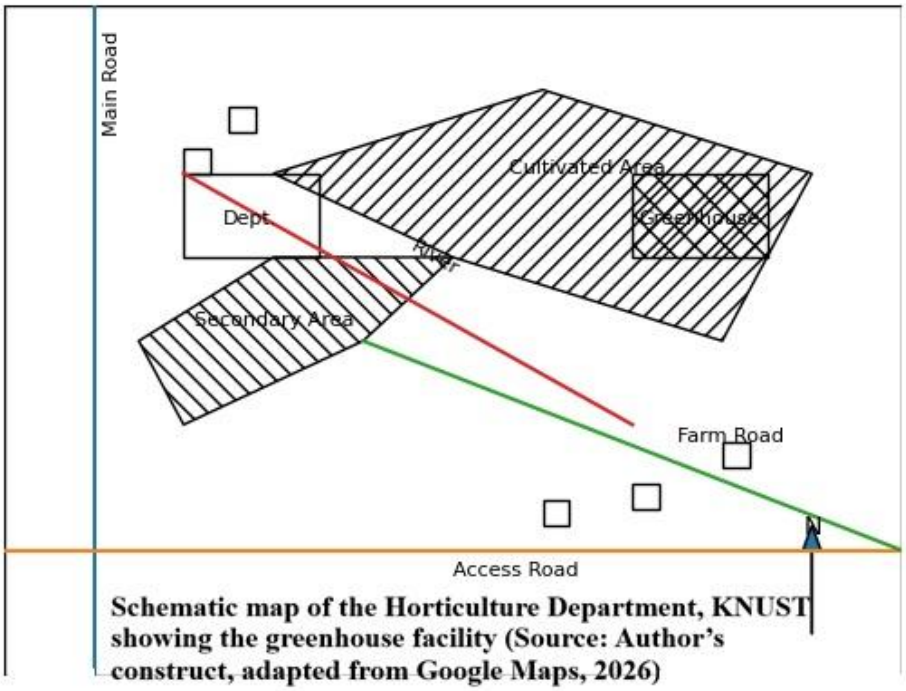

Figure 1. Schematic map of the Horticulture Department, KNUST, showing the greenhouse facility

Experimental materials

Three cocoa varieties (Forastero, Trinitario, and Criollo) and four selected genotypes (PA150, PA7, C42, and C75) were used in the experiment. These cocoa seeds were obtained from selected cocoa farms in Bibiani-Anhwiaso-Bekwai municipal and the certified Seed Production Division (SPD) in Western North, respectively, and germinated under controlled conditions before transplanting into experimental pots.

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Experimental design

The experiment was arranged in a Completely Randomized Design (CRD) involving seven cocoa genetic materials (three varieties and four selected genotypes) subjected to four soil moisture stress treatments, each replicated four times. Seedlings were grown in pots filled with a standard soil mixture suitable for cocoa growth. Uniform seedlings were selected and maintained under greenhouse conditions before the imposition of moisture stress treatments.

Sample Treatment/Plant Materials Preparation

Cocoa pods from each variety and genotype were first weighed collectively using a weighing balance, and the weights were recorded. The pods were then split open to release the seeds. Seeds from each pod were placed into sachets and weighed using an electronic balance. Seeds belonging to the same variety or genotype were collected together in a bucket, cleaned to remove excess pulp, and allowed to germinate. Approximately 550 kg of sandy loam soil was collected from a cocoa farm that had been under cocoa cultivation for over 20 years and transported to the Department of Horticulture, KNUST, but only 480kg was used to fill the 96 pots. Soil samples were collected from a depth of 25 cm using a pickaxe and shovel. The soil was air-dried and passed through a metallic sieve to remove stones, roots, and other debris. A representative soil sample was then analyzed at the Department of Soil Science, College of Agriculture and Natural Resources, KNUST, to determine its physicochemical properties. The prepared soil was transferred into plastic buckets (17 cm in height and 20 cm in diameter), which served as pots for the experiment. Each pot was filled with 5 kg of soil and provided with at least three drainage holes at the bottom to allow excess water to drain. All pots were saturated with water until water drained from the bottom, indicating full saturation. The pots were then allowed to drain overnight to reach field capacity, which corresponded to approximately 1200 ml of water. Germinated cocoa seeds were sown at a depth of 3 cm and spaced approximately 2 cm apart, with three seeds planted per pot to ensure the survival of at least one healthy seedling.

Plate 1. Styrofoam provides shade to prevent sunlight from reaching the seeds

Determination of Percentage Germination

Seven styrofoam sheets (30 cm × 19 cm) were used to provide shade and prevent direct sunlight from reaching the seeds during tabletop germination. Plastic sacks of a similar size were placed beneath the styrofoam sheets to assist with water retention on the greenhouse benches. Three cloth pieces (7 cm × 7 cm), corresponding to the three replications, were placed on the plastic sacks and moistened with water. Seeds of each cocoa variety and

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genotype were counted and divided into three replicates. The seeds were placed on the moist cloth and watered daily for two weeks. Germination counts were recorded throughout this period to determine the percentage germination.

Soil Moisture Stress Treatments

Soil moisture stress treatments were applied by controlling irrigation levels to simulate varying moisture conditions. Soil moisture levels were monitored regularly with a soil moisture meter to ensure consistency across treatments.

Treatment	Description
T1	Well-watered condition (100% field capacity)
T2	Moderate moisture stress (50% field capacity)
T3	High moisture stress (25% field capacity)
T4	Severe moisture stress (0% field capacity), no watering

The Completely Randomized Design resulted in 96 experimental units (pots). After sowing, the seedlings were watered at three-day intervals with 200 ml of water until they were fully established. Seedlings began to emerge within the first and second weeks after sowing. At three weeks after sowing (WAS), when the seedlings had reached the second leaf stage, they were thinned to two seedlings per pot to minimize competition for water and nutrients. Each pot was labeled according to the cocoa variety or genotype and the stress treatment applied.

Application of Soil Moisture Stress

Soil moisture stress treatments were applied nine weeks after sowing (9 WAS), when the seedlings were well established, and controlled by irrigation based on water volume. Field capacity was predetermined by saturating pots and allowing free drainage overnight; the volume of water retained was approximately 1200 ml per pot. Treatments were as follows: Control (100% field capacity = 1200 ml water per week), Moderate Stress (50% = 600 ml per week), High Stress (25% = 300 ml per week), and Severe Stress (0 ml per week). Water was measured using a graduated cylinder and applied evenly to the soil surface once weekly. Pots were not reweighed during the experiment; instead, the fixed-volume irrigation regime was maintained consistently.

Data Collection

Morphological Parameters

Not all parameters were measured at every time point to avoid excessive disturbance to seedlings. Stem thickness was recorded at 10, 15, and 20 days after stress application (DAS). Leaf area was measured at 0, 10, and 20 DAS using the graph method on fully expanded fourth leaves; this 10-day interval minimized leaf removal. Plant height was measured up to 20 DAS because treatment effects were already evident by day 20, and further measurement was unnecessary for demonstrating the trend.

Morphological parameters measured included plant height, stem thickness, leaf area, and number of leaves. Data were recorded at 9, 10, 11, 12, and 13 weeks after sowing. Plant height was measured from the soil surface to the tip of the shoot using a measuring tape. Stem thickness was measured using a vernier caliper, while the number of leaves was determined by direct counting. Leaf area was determined using the graph method. Fully expanded fourth leaves were collected, traced on graph paper, and their length and width measured using a ruler. Leaf area was calculated as:

Leaf Area = Leaf Length × Leaf Width × correction factor (0.75)

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Physiological Parameters

Leaf relative water content was determined using fully expanded fourth leaves from the apex of each seedling. Leaf discs were obtained using a 2 cm cork borer and weighed immediately to obtain the fresh weight (FW). The discs were floated on distilled water at 25°C in darkness for four hours to obtain the turgid weight (TW). The discs were then oven-dried at 50°C for 24 hours to determine the dry weight (DW).

LRWC was calculated as:

$$LRWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Soil samples were collected from each replicate at a depth of 5 cm using a 2 cm diameter sampling tube. The moist soil was weighed (M), oven-dried at 105°C overnight, cooled, and weighed again to obtain the dry weight (D).

$$SRWC\ (\%) = [(M - D) / D] \times 100$$

Chlorophyll content was estimated using a PlantPen NDVI 300 device. NDVI values are unitless and relative, scaled by 100 for presentation. The device was calibrated against a white reference standard before each measurement session. NDVI readings were taken directly from the physiologically expanded third leaf from the apex of each seedling without detaching the leaf.

Root Anatomical Analysis

Roots were carefully removed from the soil and washed thoroughly to remove adhering soil particles. Thin root sections (2–5 cm from the root apex to the root-shoot junction) were collected and immersed briefly in distilled water. Root sections were hand-sectioned using a microtome knife and stained with iodine for 3–5 minutes. The sections were mounted on microscope slides with glycerine and examined under a light microscope at 100× magnification. Images were captured using an MU-series camera connected to the AmScope 3.7 software. Anatomical features measured included the pith, cortex, epidermis, and phloem tissues.

Data Analysis

The collected data were analyzed using analysis of variance (ANOVA) to determine the effects of soil moisture stress on the different cocoa varieties and genotypes. Means were separated using Tukey's Honestly Significant Difference (HSD) test at a 5% probability level. Results were presented in tables and graphical formats. ANOVA was run on replicate means.

RESULTS

Soil chemical properties were significantly altered by moisture stress intensity (Table 1). Available phosphorus (P), organic carbon, and organic matter decreased as stress severity increased, particularly under high and severe stress. Conversely, total cadmium (Cd) and zinc (Zn) increased under severe stress, likely due to reduced leaching and concentration effects. Analysis of variance (ANOVA) revealed significant treatment effects for available P ($F = 12.34$, $p = 0.002$), organic carbon ($F = 8.76$, $p = 0.009$), and total Cd ($F = 10.21$, $p = 0.004$). This indicates reduced mineralization and nutrient availability, which can impair seedling development.

Germination rates ranged from 78% to 94% across genotypes, with no significant differences among varieties (data not shown).

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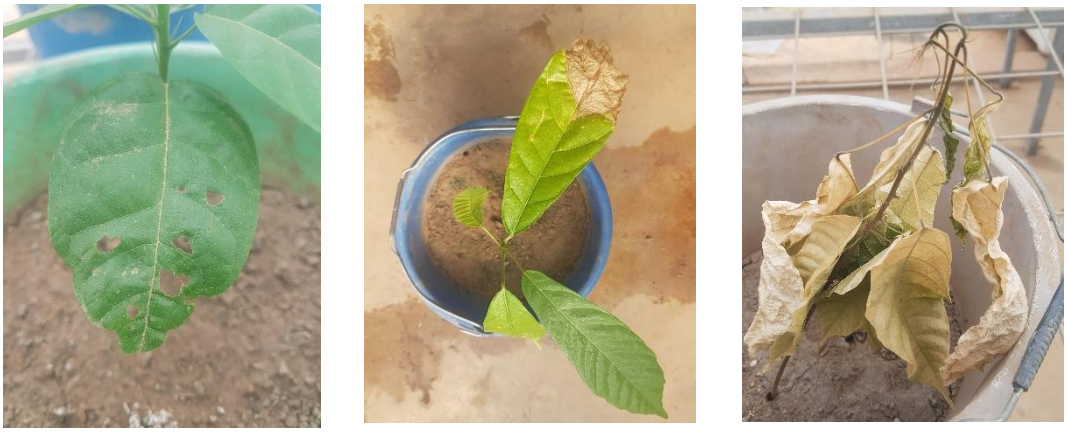
Table 1. Chemical Properties of soil before and after moisture stress application

Soil Property	Initial Content	Final Contents after Moisture Stress			
		Control (T1)	M. Stressed (T2)	H. Stressed (T3)	S. Stressed (T4)
pH	6.293±0.172	6.323±0.091	6.323±0.074	6.503±0.021	6.633±0.015
Available P (mg/kg)	21.937±1.146	21.170±0.175	12.283±0.146	11.803±0.031	13.210±0.105
Total N (%)	0.114±0.001	0.118±0.002	0.113±0.001	0.107±0.002	0.115±0.001
Exchangeable K (cmol/kg)	0.415±0.020	0.265±0.055	0.082±0.003	0.405±0.003	0.535±0.004
Exchangeable Ca (cmol/kg)	4.520±0.087	8.607±0.031	6.100±0.100	8.120±0.072	7.433±0.012
Exchangeable Mg (cmol/kg)	2.547±0.170	5.000±0.020	3.707±0.101	4.500±0.092	3.587±0.031
Exchangeable Na (cmol/kg)	0.059±0.004	0.077±0.012	0.064±0.020	0.088±0.001	0.061±0.005
Organic Carbon (%)	1.484±0.007	1.481±0.003	1.362±0.003	1.284±0.002	1.400±0.002
Organic Matter (%)	2.558±0.012	2.554±0.005	2.349±0.005	2.214±0.004	2.414±0.004
Total Zn (mg/kg)	22.967±2.919	62.473±1.550	67.147±5.906	60.680±2.841	79.893±3.091
Total Ni (mg/kg)	0.733±0.037	0.790±0.037	0.634±0.034	0.700±0.036	0.699±0.015
Total Mn (mg/kg)	7.270±0.419	148.600±4.629	102.033±1.504	163.167±4.389	94.247±0.442
Total As (mg/kg)	0.881±0.029	0.864±0.036	0.738±0.022	0.676±0.023	0.638±0.056
Total Fe (mg/kg)	273.167±45.608	263.667±43.108	240.667±16.442	591.333±58.620	542.000±159.094
Total Cu (mg/kg)	5.877±0.305	64.260±1.502	67.647±6.340	102.763±7.146	57.607±4.939
Total Pb (mg/kg)	0.652±0.028	0.624±0.011	0.551±0.012	0.523±0.009	0.472±0.007
Total Cd (mg/kg)	0.564±0.044	1.847±0.121	1.580±0.327	1.167±0.095	2.480±0.310
Total Hg (mg/kg)	0.393±0.044	0.521±0.006	0.434±0.025	0.557±0.038	0.370±0.023

Morphological Responses to Moisture Stress

Visual Drought Symptoms

Visual symptoms of drought stress appeared progressively with increasing stress intensity. These included: rapid leaf yellowing beginning at the leaf margin and/or lamina, necrosis of leaf lamina, drooping and death of young tender leaves, wilting, paling starting from the leaf margin, and in severe cases, leaf perforation and browning (Plate 2). Symptoms were more pronounced in genotypes PA7 and C42 compared to PA150 and Forastero.



(A) **(B)** **(C)**

(D) **(E)**

Plate 2. Various visual symptoms of drought stress observed in seedlings of the different genotypes and varieties of cocoa, indicative of drought symptoms (A) Perforation of mature green leaves, (B) Necrosis on leaf apex, (C) Browning of leaves, (D) Paling starting from leaf margin and lamina, (E) Shrinking of physiologically immature leaves

Effects of soil moisture stress on the number of leaves

Moisture stress significantly reduced leaf number across all varieties and genotypes ($p < 0.05$, Table 2). At Day 0, all genotypes within a treatment did not show any significant difference. Genotype PA150 consistently maintained the highest leaf number across all treatments, peaking at 25.5 leaves under control conditions at 25 days after stress application (DAS). In

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contrast, PA7 recorded the lowest leaf counts under severe stress (8.5 leaves at 25 DAS). ANOVA revealed a significant genotype-by-treatment interaction ($F = 4.32$, $p = 0.008$). At day 5 after stress application, no significant differences in leaf number were observed among treatments (all $p > 0.05$), indicating that stress effects required more than 5 days to manifest. Although Criollo and Forastero both had 15.75 leaves under severe stress at day 25, their relative reductions differed. Forastero showed a 23% reduction from its control (20.50 to 15.75), whereas Criollo showed a 28% reduction (21.75 to 15.75). This indicates that Forastero is more drought-tolerant based on the percent reduction from optimal conditions.

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Table 2. Effects of soil moisture stress on the number of leaves of cocoa seedlings at 25 days intervals after stress application (Mean ± SE; n = 4. Different superscript letters indicate significant differences at $p < 0.05$, Tukey's HSD.)

Variety/Genotype	Treatments	Day after the application of moisture stress					
		0	5	10	15	20	25
Criollo	**Control**	12.750±4.19	16.000±4.69^{a}	18.500±4.43^{abc}	19.500±4.20^{bcd}	21.250±3.59^{cde}	21.750±3.86^{cd}
	MS	9.500±0.58	12.500±0.58^{a}	14.750±1.71^{abc}	17.250±3.50^{abcd}	18.000±3.16^{abcde}	18.750±3.10^{bcd}
	HS	10.250±1.71	13.250±1.50^{a}	14.500±1.73^{abc}	15.250±1.71^{abcd}	15.750±2.22^{abcde}	16.250±2.22^{abcd}
	SS	12.250±2.22	14.250±2.06^{a}	15.000±2.00^{abc}	15.500±1.73^{abcd}	15.750±1.59^{abcde}	15.750±1.89^{abcd}
Forastero	**Control**	11.750±2.22	14.250±3.59^{a}	17.000±4.97^{abc}	18.500±3.87^{abcd}	20.000±3.16^{cde}	20.500±2.38^{cd}
	MS	12.500±2.65	16.000±2.94^{a}	17.500±3.11^{abc}	19.000±2.31^{bcd}	19.750±2.63^{bcde}	20.250±2.06^{cd}
	HS	13.500±1.73	16.750±2.06^{a}	18.250±0.96^{abc}	19.000±1.83^{bcd}	19.750±1.71^{bcde}	20.000±1.41^{cd}
	SS	11.750±0.96	14.250±2.06^{a}	14.750±2.22^{abc}	15.000±1.83^{abcd}	15.250±1.71^{abcde}	15.750±1.50^{abcd}
Trinitario	**Control**	11.750±1.71	16.250±4.27^{a}	18.250±5.12^{abc}	23.250±4.43^{d}	24.500±5.32^{e}	25.000±5.35^{d}
	MS	10.500±1.29	14.500±1.73^{a}	16.250±2.63^{abc}	19.000±0.82^{bcd}	19.750±0.96^{bcde}	20.000±1.41^{cd}
	HS	11.000±2.16	14.750±3.59^{a}	17.500±3.87^{abc}	19.500±4.12^{bcd}	19.750±4.11^{bcde}	20.000±4.08^{cd}
	SS	9.750±1.71	15.500±3.11^{a}	16.500±2.65^{abc}	17.250±1.89^{abcd}	17.250±1.89 abcde	17.250±1.89^{abcd}
PA150	**Control**	13.750±3.20	17.750±2.99^{a}	20.250±3.40^{c}	22.000±3.16^{cd}	24.250±2.87^{de}	25.500±3.11^{d}
	MS	13.250±2.87	16.500±1.00^{a}	18.250±0.96^{abc}	19.500±3.08^{bcd}	21.000±1.63^{cde}	21.750±1.50^{cd}

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	HS	12.000±2.16	15.500±5.07^{a}	17.000±5.94^{abc}	19.500±5.74^{bcd}	20.500±5.26^{cde}	21.250±5.56^{cd}
	SS	12.250±3.64	16.750±2.22^{a}	17.000±2.71^{abc}	17.000±2.71^{abcd}	17.000±1.71^{abcde}	17.000±2.71^{abcd}
PA7	**Control**	10.000±1.41	12.750±0.96^{a}	14.250±0.96^{abc}	15.750±1.89^{abcd}	16.500±1.91^{abcde}	17.000±2.16^{abcd}
	MS	8.000±0.58	11.250±2.65^{a}	9.500±5.51^{ab}	12.500±3.51^{abc}	13.250±3.51^{abc}	13.500±4.00^{abc}
	HS	10.250±1.50	11.750±1.89^{a}	12.250±1.50^{abc}	13.500±1.29^{abcd}	13.500±1.29^{abc}	14.000±0.82^{abc}
	SS	5.500±2.89	7.500±4.58^{a}	8.250±4.58^{a}	8.500±4.16^{a}	8.500±4.16^{a}	8.500±4.16^{a}
C42	**Control**	8.000±1.83	11.500±4.20^{a}	13.500±4.20^{abc}	15.500±4.20^{abcd}	16.500±3.70 abcde	17.250±3.77^{abcd}
	MS	8.000±1.83	13.500±3.70^{a}	15.500±4.20^{abc}	16.000±4.97^{abcd}	16.500±4.80^{abcde}	16.750±4.99^{abcd}
	HS	9.000±1.83	11.750±0.96^{a}	13.000±1.15^{abc}	14.000±1.63^{abcd}	14.000±1.63^{abcd}	14.000±1.63^{abc}
	SS	8.500±1.29	12.500±3.32^{a}	14.250±2.63^{abc}	14.500±2.38^{abcd}	14.500±2.38^{abcde}	14.500±2.38^{abc}
C75	**Control**	12.250±2.87	15.000±1.41^{a}	18.500±2.89^{abc}	20.250±2.87^{cd}	21.000±2.83^{cde}	21.500±3.11^{cd}
	MS	11.750±1.89	16.000±4.40^{a}	17.250±4.65^{abc}	18.750±4.92^{bcd}	19.500±4.20^{bcde}	20.000±3.92^{cd}
	HS	10.000±1.41	14.250±2.36^{a}	16.750±2.06^{abc}	17.250±2.22^{abcd}	17.750±2.63^{abcde}	17.750±2.63^{abcd}
	SS	8.250±2.65	9.750±3.46^{a}	9.750±3.46^{ab}	9.750±3.46^{ab}	9.750±3.46^{ab}	9.750±3.46^{ab}

Different superscript letters indicate significant differences at $p < 0.05$. Day 0 values represent the pre-stress baseline; treatment effects are assessed by change over time.

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Effects of soil moisture stress on stem thickness of cocoa seedlings

Stem thickness did not differ significantly across treatments for most genotypes, although Trinitario (4.35 mm) and Forastero (4.13 mm) showed robust stem development under control and moderate stress. PA7 again performed poorly (1.70 mm under severe stress). This suggests that while stem diameter is a relatively stable trait, it can still indicate stress susceptibility in sensitive genotypes. ANOVA revealed significant genotype by treatment interaction for stem thickness ($F = 3.21$, $p = 0.04$), but pairwise differences were not consistent across all stress levels.

Table 3. Effects of soil moisture stress on stem thickness of cocoa seedlings

Variety	Treatments	Day after the application of moisture stress		
		10	**15**	**20**
Criollo	**Control**	2.450±0.66^{a}	3.025±0.43^{abc}	3.500±0.42^{cdef}
	MS	2.950±0.72^{a}	3.275±0.69^{abc}	3.400±0.59^{bcdef}
	HS	2.350±0.58^{a}	2.625±0.54^{abc}	2.725±0.54^{abcde}
	SS	2.650±0.31^{a}	2.525±0.24^{abc}	2.450±0.17^{abc}
Forastero	**Control**	3.175±0.31^{a}	3.900±0.32^{c}	4.350±0.42^{f}
	MS	3.525±0.59^{a}	3.850±0.65^{c}	4.125±0.67^{def}
	HS	3.400±0.33^{a}	3.700±0.34^{c}	3.875±0.38^{cdef}
	SS	3.000±0.51^{a}	2.900±0.55^{abc}	2.850±0.51^{abcdef}
Trinitario	**Control**	3.475±0.34^{a}	3.825±0.38^{c}	4.350±0.33^{f}
	MS	3.525±0.34^{a}	3.700±0.33^{c}	4.125±0.29^{def}
	HS	3.275±0.22^{a}	3.500±0.12^{bc}	3.875±0.10^{cdef}
	SS	2.875±0.30^{a}	2.675±0.24^{abc}	2.850±0.17^{abcdef}
PA150	**Control**	3.425±0.30^{a}	3.875±0.34^{c}	4.225±0.39^{ef}
	MS	3.700±0.27^{a}	3.900±0.22^{c}	4.125±0.24^{def}
	HS	2.875±0.41^{a}	3.125±0.46^{abc}	3.325±0.41^{bcdef}
	SS	2.875±0.34^{a}	2.800±0.29^{abc}	2.675±0.32^{abcd}
PA7	**Control**	2.725±0.31^{a}	3.025±0.31^{abc}	3.225±0.31^{bcdef}
	MS	2.300±0.21^{a}	2.450±0.31^{abc}	2.550±0.26^{abc}
	HS	2.725±0.39^{a}	2.875±0.39^{abc}	2.975±0.36^{abcdef}
	SS	2.125±0.72^{a}	1.850±0.49^{a}	1.700±0.42^{a}
C42	**Control**	2.125±0.22^{a}	2.475±0.22^{abc}	2.875±0.19^{abcdef}
	MS	2.375±0.38^{a}	2.450±0.34^{abc}	2.575±0.35^{abc}
	HS	2.350±0.19^{a}	2.375±0.17^{abc}	2.400±0.18^{abc}
	SS	2.325±0.33^{a}	2.100±0.24^{ab}	1.950±0.21^{ab}
C75	**Control**	2.800±0.33^{a}	3.075±0.25^{abc}	3.525±0.29^{cdef}
	MS	2.875±0.50^{a}	3.125±0.46^{abc}	3.400±0.37^{bcdef}
	HS	2.975±0.10^{a}	3.150±0.13^{abc}	3.250±0.10^{bcdef}
	SS	2.275±0.15^{a}	2.100±0.17^{ab}	1.950±0.26^{ab}

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Effect on Leaf Area and Leaf Development

Leaf area was significantly reduced under moisture stress, with genotype C42 showing the smallest leaf area (31.05 cm²) under severe stress, while PA150 maintained the largest area (157.71 cm²) under control conditions (Table 4). The reduction was more pronounced under severe stress across all genotypes.

Table 4. Effects of soil moisture stress on leaf area (cm²) of cocoa seedlings at 20-day intervals after stress application (Mean ± SE; n = 4. Different superscript letters indicate significant differences at p < 0.05, Tukey's HSD.)

Variety	Treatments	Day after the application of moisture stress		
		0	10	20
Criollo	**Control**	91.8867±6.43 ^{ab}	114.6200±16.00^{cde}	132.5433±18.79^{ghi}
	MS	94.2067±4.97 ^{ab}	98.4667±3.38^{bcde}	101.4867±5.23^{cdefgh}
	HS	79.9900±13.72 ^{ab}	77.8500±13.21^{abc}	78.4467±13.34^{bcde}
	SS	82.7033±8.70 ^{ab}	78.3533±7.29^{abc}	67.5567±10.27^{abcd}
Forastero	**Control**	107.9833±30.04 ^{ab}	128.5167±36.16^{de}	143.2267±38.48^{hi}
	MS	101.3200±17.70 ^{ab}	107.0967±16.73^{cde}	110.4633±18.44^{defgh}
	HS	97.6967±12.74 ^{ab}	97.7733±11.67^{bcde}	98.3433±11.22^{cdefg}
	SS	93.2167±5.86 ^{ab}	86.3333±5.96^{bcd}	72.8767±11.52^{bcde}
Trinitario	**Control**	85.6567±19.20 ^{ab}	102.9233±24.66^{bcde}	113.0567±27.36^{defgh}
	MS	89.3567±3.76 ^{ab}	91.4900±4.51^{bcd}	92.7900±4.43^{bcdefg}
	HS	79.6133±5.33 ^{ab}	79.8567±5.69^{abc}	79.9233±4.74^{bcde}
	SS	85.5067±5.46 ^{ab}	78.6167±7.41^{abc}	68.2933±7.48^{abcd}
PA150	**Control**	112.4567±10.93^{b}	135.7267±13.09^{e}	157.7100±23.10^{i}
	MS	108.2267±10.61 ^{ab}	112.7733±9.82^{cde}	116.0433±10.41^{efgh}
	HS	95.3300±9.71 ^{ab}	96.8400±10.27^{bcde}	97.4367±10.00^{cdefg}
	SS	89.6667±4.08 ^{ab}	83.4233±3.34^{abc}	76.9400±4.89^{bcde}
PA7	**Control**	84.2633±13.35 ^{ab}	89.6067±11.60^{bcd}	101.1433±15.27^{cdefgh}
	MS	90.4700±5.96 ^{ab}	92.0033±6.58^{bcd}	93.4800±6.87^{bcdefg}
	HS	78.8800±12.64 ^{ab}	78.2867±12.76^{abc}	78.0900±12.89^{bcde}
	SS	72.6433±14.80^{a}	60.0200±23.90^{ab}	50.5233±21.04^{ab}
C42	**Control**	88.9400±4.37 ^{ab}	103.8133±7.22^{cde}	115.0967±6.83^{efgh}
	MS	77.6067±18.81 ^{ab}	81.6200±18.30^{abc}	85.4400±18.22^{bcdef}
	HS	82.0600±6.28 ^{ab}	81.1100±5.38^{abc}	80.5400±4.66^{bcde}

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	SS	72.0767±21.29[a]	43.2100±27.54[a]	31.0500±13.46[a]
C75	**Control**	89.2533±7.02 [ab]	105.7500±8.87[cde]	128.1333±10.04[fghi]
	MS	84.4000±7.54 [ab]	88.3867±8.43[bcd]	97.1300±10.79[cdefg]
	HS	88.2933±3.52 [ab]	89.4867±3.73[bcd]	89.5400±3.86[bcdefg]
	SS	85.6933±3.63 [ab]	75.4433±4.53[abc]	63.2633±9.54[abc]

Effect of Soil Moisture Stress on Plant Height

At day 0 (before stress application), initial plant height varied among genotypes and treatments due to natural biological variation. However, the critical comparison is the growth increment from day 0 to day 20, which clearly shows suppression under moisture stress (Table 5). Plant height was significantly suppressed under moisture stress ($p < 0.05$). PA150 achieved the greatest height under control conditions (39.0 cm at 20 DAS), while PA7 showed severe stagnation under severe stress (11.25 cm). Criollo exhibited moderate height retention under severe stress (31.0 cm), indicating better tolerance than PA7 (Table 5). ANOVA revealed a significant genotype-by-treatment interaction ($F = 9.12$, $p = 0.003$).

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Table 5. Effects of soil moisture stress on plant height (cm) of cocoa seedlings at 20-day intervals after stress application (Mean ± SE; n = 4. Different superscript letters indicate significant differences at p < 0.05, Tukey's HSD.)

Variety	Treatments	Day after the application of moisture stress				
		0	5	10	15	20
Criollo	**Control**	23.5000±7.14 ^{abcd}	26.0000±7.12 ^{abcd}	28.0000±8.29 ^{abc}	30.0000±8.91 ^{abc}	31.5000±8.10 ^{bcd}
	MS	19.0000±2.45 ^{abcd}	22.0000±4.24 ^{abcd}	24.0000±6.00 ^{abc}	26.5000±6.56 ^{abc}	27.5000±6.61 ^{abcd}
	HS	19.5000±2.65 ^{abcd}	21.7500±2.63 ^{abcd}	23.25000±3.30 ^{abc}	25.0000±3.27 ^{abc}	26.5000±3.11 ^{abcd}
	SS	27.2500±3.59^{cd}	28.2500±2.36^{bcd}	29.2500±1.71 ^{bc}	30.5000±1.91 ^{abc}	31.000±2.45 ^{bcd}
Forastero	**Control**	24.2500±2.99^{bcd}	27.5000±5.45 ^{bcd}	29.7500±8.06 ^{bc}	33.0000±8.29^{bc}	35.2500±8.22 ^{bcd}
	MS	22.7500±2.99 ^{abcd}	25.500±3.51 ^{abcd}	27.7500±3.50 ^{abc}	29.7500±4.27 ^{abc}	31.0000±4.32 ^{bcd}
	HS	29.2500±2.99^{d}	32.0000±4.16^{d}	34.2500±4.11^{c}	36.2500±5.32^{c}	37.2500±6.18 ^{cd}
	SS	23.5000±3.79 ^{abcd}	24.2500±3.86 ^{abcd}	25.5000±4.43 ^{abc}	26.5000±4.51 ^{abc}	26.7500±4.99 ^{abcd}
Trinitario	**Control**	28.0000±4.32 ^{cd}	29.7500±4.57 ^{bcd}	30.7500±4.79 ^{bc}	33.5000±4.80 ^{bc}	35.0000±5.72 ^{bcd}
	MS	24.2500±1.71 ^{bcd}	25.5000±2.52 ^{abcd}	28.0000±2.83 ^{abc}	29.7500±3.30 ^{abc}	30.7500±3.86 ^{abcd}
	HS	20.0000±2.58 ^{abcd}	21.5000±3.00 ^{abcd}	23.0000±3.56 ^{abc}	24.500±4.36 ^{abc}	25.2500±4.27 ^{abcd}
	SS	19.2500±7.37 ^{abcd}	20.0000±8.12 ^{abcd}	20.7500±9.60 ^{abc}	21.5000±10.50 ^{abc}	21.7500±10.31 ^{abcd}
PA150	**Control**	26.2500±6.95 ^{bcd}	30.5000±7.14^{cd}	32.0000±7.87 ^{bc}	36.0000±8.41^{c}	39.0000±8.83 ^{d}
	MS	27.0000±7.16 ^{cd}	28.2500±7.63 ^{bcd}	29.7500±6.70 ^{bc}	32.0000±6.38^{bc}	34.2500±6.34 ^{bcd}

	HS	22.5000±8.35 abcd	24.2500±8.77 abcd	27.2500±9.95 abc	28.500±10.15 abc	29.7500±10.56 abcd
	SS	26.2500±2.99 bcd	27.7500±3.59 bcd	28.7500±4.35 bc	29.5000±3.70 abc	29.5000±3.70 abcd
PA7	**Control**	19.5000±5.07 abcd	21.5000±4.51 abcd	22.7500±5.12 abc	24.7500±5.12 abc	26.0000±4.97 abcd
	MS	12.2500±4.62 ab	14.7500±5.13 abc	17.0000±7.02 abc	18.7500±7.55 abc	19.7500±7.51 abcd
	HS	19.5000±4.20 abcd	20.7500±3.77 abcd	21.7500±4.57 abc	23.0000±5.48 abc	23.2500±5.85 abcd
	SS	9.7500±6.56 a	10.2500±6.66 a	10.5000±7.21 a	11.2500±6.24 a	11.2500±6.24 a
C42	**Control**	14.2500±5.38 abc	16.5000±7.05 abcd	17.5000±7.85 abc	19.0000±8.68 abc	20.2500±8.62 abcd
	MS	17.5000±3.11 abcd	21.0000±5.29 abcd	23.5000±6.45 abc	25.7500±6.55 abc	26.5000±5.74 abcd
	HS	14.7500±4.19 abc	16.5000±4.65 abcd	18.0000±4.97 abc	18.5000±4.80 abc	19.0000±5.29 abc
	SS	16.7500±2.63 abcd	18.0000±2.71 abcd	18.5000±3.00 abc	19.2500±3.59 abc	19.2500±3.59 abcd
C75	**Control**	24.7500±6.65 bcd	27.7500±8.34 bcd	29.7500±10.34 bc	31.7500±11.53 bc	33.2500±11.67 bcd
	MS	23.7500±4.03 abcd	25.7500±4.43 abcd	27.2500±5.68 abc	28.7500±5.56 abc	29.5000±5.97 abcd
	HS	17.7500±1.89 abcd	19.7500±1.71 abcd	21.2500±2.50 abc	21.7500±2.22 abc	22.5000±3.70 abcd
	SS	13.500±1.00 abc	14.2500±0.00 ab	14.5000±0.58 ab	15.0000±1.00 ab	15.5000±1.53 ab

Different superscript letters indicate significant differences at $p < 0.05$. Day 0 values represent the pre-stress baseline; treatment effects are assessed by change over time.

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Physiological Responses

Effect on Leaf Relative Water Content (LRWC)

LRWC declined progressively with increasing moisture stress ($p < 0.05$). Criollo maintained the highest LRWC (83.71%) under control conditions, while PA7 showed the lowest (42.76%) under severe stress. LRWC was strongly correlated with leaf area retention ($r = 0.82$, $p = 0.01$). LRWC is a direct measure of plant water status and a reliable screening tool for drought tolerance. High LRWC under stress suggests efficient stomatal control or osmotic adjustment.

Effect on Soil Relative Water Content (SRWC)

SRWC varied widely among genotypes under the same irrigation regime, indicating differential water extraction capacity. Forastero exhibited low SRWC (1.23% under severe stress) despite relatively high LRWC, suggesting efficient soil water extraction, maintaining leaf turgor even as soil dries.

Effect on Chlorophyll Content (NDVI)

NDVI values (scaled ×100 for presentation) decreased significantly under stress ($p < 0.05$). Trinitario maintained the highest NDVI under high stress (41.23), while PA7 showed the sharpest decline (7.41 under severe stress). Chlorophyll retention was positively correlated with LRWC ($r = 0.79$, $p = 0.02$). Chlorophyll retention under stress is critical for continued carbon assimilation. Genotypes that maintain green leaf area and chlorophyll content are better equipped to withstand and recover from drought (Table 6). Notably, Forastero showed a transient increase in NDVI at day 5 under severe stress (41.03) compared to its control (34.42), possibly due to early stress responses such as reduced leaf water content, concentrating chlorophyll, or stomatal closure, altering light reflectance. This initial rise was followed by a sharp decline by day 25 (20.93)

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Table 6: Chlorophyll content (NDVI ×100) of cocoa seedlings at 25-day intervals after stress application (Mean ± SE; n = 4. Different superscript letters indicate significant differences at p < 0.05, Tukey's HSD.)

Variety	Treatments	Day after the application of moisture stress				
		5	10	15	20	25
Criollo	**Control**	33.3025±3.23 ^{a}	41.9900±1.99 ^{de}	51.8975±6.33 ^{fgh}	59.1975±2.52 ^{j}	63.2275±2.40 ^{e}
	MS	34.3350±2.94 ^{a}	37.2775±5.12 ^{bcde}	44.8625±6.16 ^{defgh}	50.1700±4.63 ^{efghij}	54.1500±3.51 ^{cdef}
	HS	34.5850±1.78 ^{a}	35.6425±2.58 ^{abcde}	37.1400±2.95 ^{cdefg}	38.4925±2.53 ^{bcdef}	39.4900±2.84 ^{ab}
	SS	36.3050±6.83 ^{a}	30.3275±3.31 ^{abcde}	28.3525±3.54 ^{abcd}	25.6550±2.56 ^{abc}	18.3425±2.10 ^{a}
Forastero	**Control**	34.4225±3.44 ^{a}	40.9400±1.24 ^{de}	54.1225±4.28 ^{gh}	60.1150±6.17 ^{j}	64.2700±6.30 ^{e}
	MS	31.6175±0.63 ^{a}	38.9475±2.88 ^{cde}	48.7725±5.42 ^{efgh}	52.6475±3.62 ^{fghij}	57.0175±1.57 ^{ef}
	HS	31.2850±2.93 ^{a}	32.6500±2.94 ^{abcde}	33.6925±2.64 ^{bcde}	35.9400±2.64 ^{bcde}	37.7050±3.13 ^{b}
	SS	41.0250±9.76 ^{a}	35.6200±8.78 ^{abcde}	30.1250±4.83 ^{abcd}	27.2175±5.06 ^{abcd}	20.9275±4.25 ^{a}
Trinitario	**Control**	28.4950±5.81 ^{a}	39.6675±2.36 ^{cde}	48.7700±1.86 ^{efgh}	59.1625±1.53 ^{j}	65.0900±3.75 ^{e}
	MS	30.1150±3.82 ^{a}	40.3175±3.07 ^{cde}	50.4125±3.58 ^{efgh}	53.4775±3.42 ^{fghij}	55.5675±3.30 ^{def}
	HS	37.2725±2.88 ^{a}	37.7750±2.89 ^{bcde}	38.8875±2.89 ^{defgh}	39.7350±2.62 ^{bcdefg}	41.2250±3.03 ^{bcd}
	SS	37.0125±8.18 ^{a}	27.9925±5.25 ^{abcde}	21.7425±4.16 ^{abc}	20.3600±4.89 ^{a}	15.5350±3.95 ^{a}
PA150	**Control**	33.7125±2.88 ^{a}	38.5300±2.19 ^{cde}	54.1725±1.58 ^{gh}	58.0350±2.79 ^{ij}	62.4875±2.53 ^{e}

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	MS	34.4450±2.82 [a]	43.6900±2.46 [de]	53.3800±5.55 [fgh]	55.2525±3.70 [ghij]	56.9000±3.66[ef]
	HS	34.3075±1.47 [a]	35.0225±1.48 [abcde]	36.5150±2.21 [cdefg]	37.7600±2.33 [bcdef]	39.7725±2.84 [ab]
	SS	25.6950±8.37 [a]	22.6375±6.41 [abc]	19.1325±6.92 [ab]	16.2250±5.77 [a]	12.5725±4.78 [a]
PA7	**Control**	36.5925±6.65 [a]	45.2800±4.99 [e]	50.0550±3.99 [efgh]	54.8525±4.85 [ghij]	58.0575±4.57 [e]
	MS	25.4850±3.69 [a]	30.7100±3.11 [abcde]	36.1300±1.33 [cdef]	38.5800±1.69 [bcdef]	39.3650±1.03 [ab]
	HS	34.5875±3.59 [a]	35.4875±3.24 [abcde]	36.5675±3.84 [cdefg]	37.8750±3.55 [bcdef]	38.7800±3.65 [b]
	SS	24.1225±8.92 [a]	20.5650±7.93 [ab]	14.6775±7.53 [a]	11.8750±5.87[a]	7.4100±3.12 [a]
C42	**Control**	35.4575±4.07 [a]	43.7550±3.61 [de]	54.9875±5.15 [h]	58.9575±5.65 [j]	62.3525±5.10 [e]
	MS	33.6275±2.90 [a]	41.0500±5.98 [de]	48.1800±5.36 [efgh]	56.3275±5.02 [hij]	57.4100±4.77 [ef]
	HS	38.3400±4.90 [a]	39.2075±5.08 [cde]	40.1475±5.04 [defgh]	40.6800±4.49 [cdefgh]	41.8875±3.82 [bcd]
	SS	33.1000±6.60 [a]	26.4300±2.29 [abcd]	16.6050±3.44 [a]	13.6900±2.47 [a]	9.1350±0.70 [a]
C75	**Control**	28.5600±7.43 [a]	38.5900±5.05 [cde]	55.0375±3.99 [h]	58.8325±2.32 [j]	62.3275±2.97 [e]
	MS	30.9075±3.60 [a]	41.2900±7.85 [de]	49.8125±8.00 [efgh]	53.2425±6.68 [fghij]	54.5225±6.03[def]
	HS	39.1200±2.46 [a]	39.7650±2.09 [cde]	40.6975±2.33 [defgh]	41.9925±2.56 [defghi]	42.5825±2.72 [bcde]
	SS	24.2775±10.50 [a]	19.4250±7.46 [a]	14.6475±3.06 [a]	12.5275±2.85 [a]	8.2250±1.59 [a]

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Root Anatomical Adaptations

Root xylem architecture varied significantly under stress. PA150 exhibited larger, concentrated xylem vessels under stress, suggesting enhanced water transport capacity. PA7 and C75 showed xylem absence or reduction under severe stress, indicating structural vulnerability. Forastero and Trinitario maintained abundant, angular xylem tissues, supporting their relatively better performance. Xylem structure directly influences hydraulic conductivity. Genotypes with robust xylem networks are better able to sustain water flow under limited soil moisture, a key trait for drought tolerance.

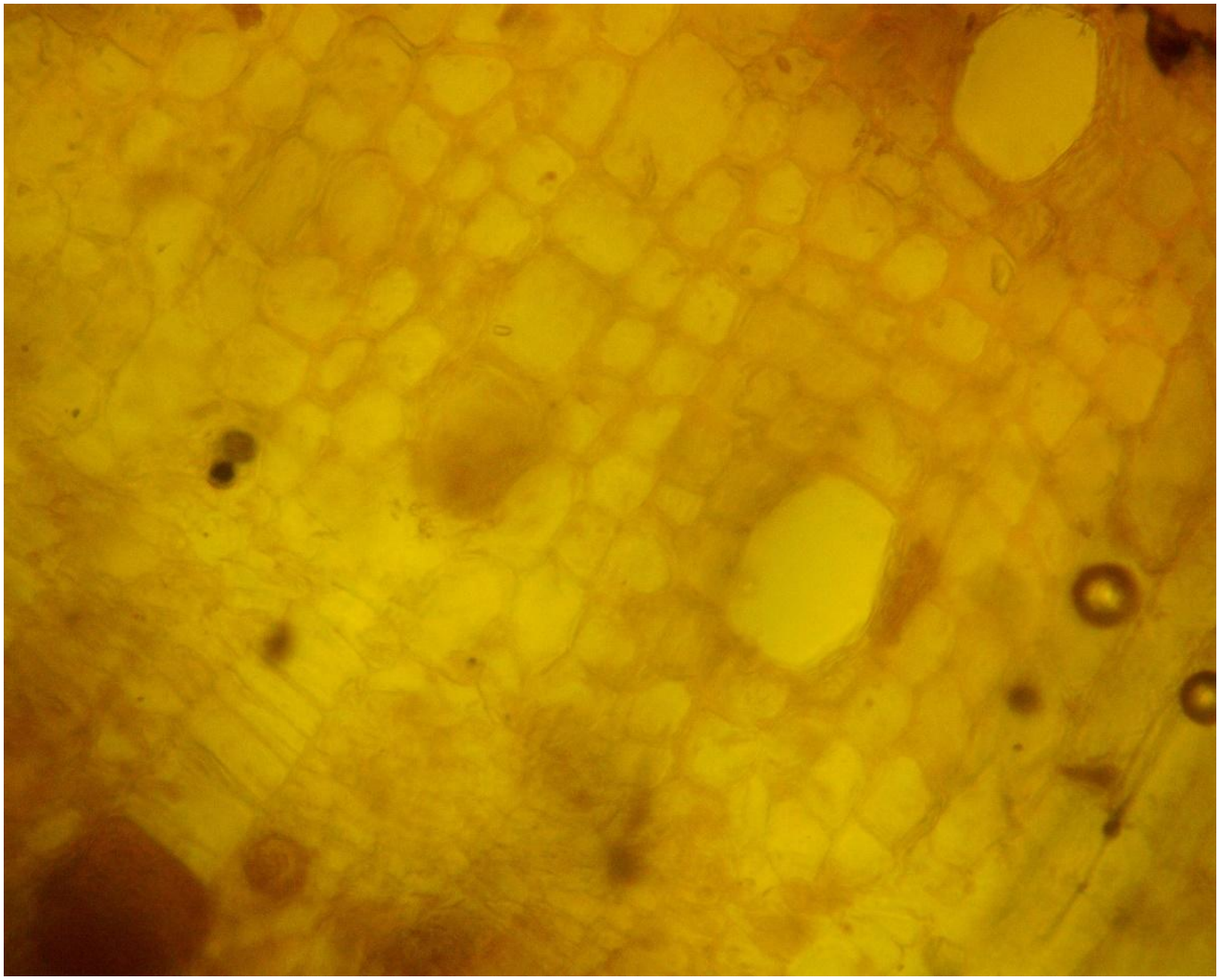

Plate 3. Cross-section of cocoa root (PA150, severe stress) at 100× magnification showing xylem vessels (X), cortex (C), and epidermis (E). Scale bar = 100 µm.

Table 7. Root anatomical characteristics of selected genotypes under severe stress (Mean ± SE; n = 4. Different superscript letters indicate significant differences at p < 0.05, Tukey's HSD.)

Genotype/Varieties	Xylem vessel diameter (µm)	Cortex thickness (µm)	Epidermis thickness (µm)
PA150	45.3 ± 3.2^{a}	210.4 ± 12.3^{a}	28.7 ± 2.1^{a}
Forastero	42.1 ± 2.8^{a}	195.6 ± 11.2^{ab}	27.4 ± 2.0^{a}
Trinitario	40.8 ± 3.0^{ab}	188.3 ± 10.5^{b}	26.9 ± 1.9^{ab}
Criollo	38.4 ± 2.7^{ab}	179.2 ± 9.8^{bc}	25.3 ± 2.2^{ab}
C42	30.2 ± 2.5^{ab}	145.7 ± 8.9^{cd}	21.4 ± 1.8^{bc}
C75	28.7 ± 2.3^{c}	138.5 ± 8.2^{d}	20.1 ± 1.7^{c}
PA7	25.1 ± 2.1^{c}	121.3 ± 7.6^{e}	18.6 ± 1.5^{c}

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DISCUSSION

Effects of soil moisture stress on morphological characteristics

The results demonstrated considerable genotypic variability in response to soil moisture stress, indicating that some cocoa varieties and genotypes possess inherent adaptive mechanisms that enhance their tolerance to drought conditions. This finding aligns with previous reports in cocoa (Padi et al., 2013; Adu-Ampomah et al., 2001) and other crops such as sorghum and maize (Saddam *et al.,* 2014; Obeng-Bio, 2010). The consistent superior performance of PA150 across morphological, physiological, and anatomical parameters (Tables 2–6) suggests that this genotype combines multiple drought tolerance mechanisms, including efficient water use, osmotic adjustment, and robust xylem architecture. Drought stress symptoms observed in this study, leaf drooping, yellowing of basal leaves, necrosis, and leaf perforation (Plate 2), agree with earlier reports by Bae et al. (2009) and Carr and Lockwood (2011). The delayed expression of symptoms in PA150 and Forastero, compared to rapid symptom development in PA7 and C42, indicates that thicker, waxy leaf cuticles may mask early stress, as noted by Osei-Bonsu (2011).

Leaf number reduction under drought (Table 2) was significant ($p < 0.05$), with PA150 maintaining the highest leaf count (25.5 under control, 17.0 under severe stress) and PA7 the lowest (17.0 under control, 8.5 under severe stress). This differential response is consistent with reports in field beans, barley, and sunflower (Karamanos, 1983; Marc and Palmer, 1976). The absence of significant treatment effects in some genotypes (e.g., Forastero under moderate and high stress) aligns with observations in flax and sugar beet (Morton and Watson, 1948; Milthorpe, 1945), suggesting inherent tolerance mechanisms in specific genetic materials. Percent reduction from control provides a more meaningful comparison of drought tolerance than absolute stressed values, as it accounts for genotypic differences in growth potential under optimal conditions. Water stress may reduce leaf production by affecting leaf initiation, stomatal regulation, and carbohydrate availability for shoot development (Anjum *et al.,* 2011; Marc and Palmer, 1976; Brix, 1962). Similarly, stem thickness was generally unaffected, although genotype-specific differences were observed. Reduced stem growth under drought is associated with reduced carbon assimilation and decreased turgor-driven cell expansion (Anjum *et al.,* 2011; Bibi *et al.,* 2010).

Leaf area reduction (Table 4) was most severe in C42 (31.05 cm^2 under severe stress against 115.10 cm^2 under control, a 73% reduction) and least in PA150 (76.94 cm^2 under severe stress against 157.71 cm^2 under control, a 51% reduction). This reduction is consistent with drought-induced inhibition of cell expansion and stomatal closure, which limits photosynthesis and growth (Kesiime *et al.,* 2016; Capell *et al.,* 2004). Genotypes like PA150 that maintain larger leaf area under stress may sustain higher photosynthetic activity, offering a growth advantage when water becomes available.

Plant height suppression under stress (Table 5) was most pronounced in PA7 (11.25 cm under severe stress against 26.00 cm under control, a 57% reduction) and least in Criollo (31.00 cm under severe stress against 31.50 cm under control, only 1.6% reduction). Water deficit restricts xylem transport and cell elongation (Farooq *et al.,* 2009; Taiz and Zeiger, 2002), and reduced assimilate allocation to shoots further limits height (De Souza and Da Silva, 1987). The remarkable height retention in Criollo under severe stress suggests efficient assimilate partitioning.

Effects of soil moisture stress on physiological traits

Leaf relative water content (LRWC) differed significantly among genotypes ($p < 0.05$), with Criollo maintaining the highest LRWC under control (83.71%) and PA7 the lowest under severe stress (42.76%). The use of LRWC as a drought tolerance indicator is well established in cocoa and other crops (Zakariyya *et al.,* 2017; Boughalleb *et al.,* 2016). The strong positive correlation between LRWC and leaf area retention ($r = 0.82$, $p = 0.01$) confirms LRWC as a reliable proxy for overall plant water status under stress. Notably, C42 maintained relatively high LRWC under severe stress (63.30%) despite poor morphological performance (leaf area 31.05 cm^2, plant height 19.25 cm). This discrepancy suggests that LRWC alone is insufficient for selecting drought-tolerant genotypes without complementary indicators such as chlorophyll content or root anatomy (Osei-Bonsu, 2011).

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Soil relative water content (SRWC) varied widely among genotypes under the same irrigation regime, indicating differential water extraction capacity. Forastero exhibited low SRWC (1.23% under severe stress) despite relatively high LRWC (42.89%), suggesting efficient soil water extraction and retention. In contrast, C75 showed higher SRWC (1.56% under severe stress) but lower LRWC (39.89%), indicating poor water uptake efficiency.

This finding supports Grossiord *et al.* (2020), who demonstrated that plant water status is not solely dependent on soil moisture but is also influenced by physiological factors such as stomatal regulation and root hydraulic conductivity. Therefore, SRWC should not be used as a standalone selection criterion.

NDVI decreased significantly under stress ($p < 0.05$), with Trinitario maintaining the highest values under high stress (41.23) and PA7 showing the sharpest decline (7.41 under severe stress). The positive correlation between NDVI and LRWC ($r = 0.79$, $p = 0.02$) indicates that chlorophyll retention is linked to overall plant water status. The reduction in NDVI under drought is associated with chloroplast damage, inhibition of chlorophyll biosynthesis, and oxidative stress caused by reactive oxygen species (Foyer *et al.,* 1994; Zhang and Kirkham, 1996). Genotypes that maintain green leaf area and chlorophyll content under stress (e.g., Trinitario, PA150) are better equipped to withstand and recover from drought. Genotype C42 exhibited poor retention of both leaf area (27% of control) and chlorophyll content (23% of control) under severe stress, consistent with its susceptibility ranking. The transient NDVI increase observed in Forastero at early stress (day 5) may represent an adaptive response, as seen in other species where drought initially concentrates photosynthetic pigments before degradation begins.

Effects of soil moisture stress on root anatomical features

Soil moisture stress significantly altered root anatomical characteristics ($p < 0.05$, Table 7). PA150 exhibited a larger xylem vessel diameter (45.3 μm) and greater cortex thickness (210.4 μm) under severe stress, suggesting enhanced water transport capacity and mechanical strength. In contrast, PA7 showed severely reduced xylem development (25.1 μm diameter) and the thinnest cortex (121.3 μm), indicating structural vulnerability. The absence of xylem vessels in PA7 and C75 under severe stress reflects reduced cell division and expansion due to decreased turgor pressure (Anjum *et al.,* 2011; Bibi *et al.,* 2010). Forastero and Trinitario maintained abundant, angular xylem tissues, supporting their relatively better morphological performance. Xylem structure directly influences hydraulic conductivity; genotypes with robust xylem networks are better able to sustain water flow under limited soil moisture (Manivannan *et al.,* 2007).

Effects of soil moisture stress on soil chemical properties

Soil moisture stress significantly reduced available phosphorus, organic carbon, and organic matter ($p < 0.05$, Table 1), indicating reduced mineralization and nutrient availability. These changes can impair seedling development, as phosphorus is critical for root growth and energy transfer (Ashraf *et al.,* 1998).

The increase in total cadmium (Cd) under severe stress (from 0.564 to 2.480 mg/kg) is concerning, as Cd accumulation may exacerbate stress through toxicity. Reduced leaching under drought conditions likely concentrates heavy metals in the root zone (Sardans *et al.,* 2008). This finding highlights the bidirectional impact of drought: not only does moisture stress directly affect plant physiology, but it also degrades the soil environment, creating feedback loops that exacerbate stress.

PA150 and Forastero consistently outperformed other entries across morphological, physiological, and anatomical parameters. Their ability to maintain leaf production, plant height, leaf area, and chlorophyll content under stress suggests a combination of drought avoidance (e.g., efficient water use, stomatal regulation) and tolerance mechanisms (e.g., osmotic adjustment, xylem adaptation). In contrast, PA7 and C42 exhibited pronounced susceptibility, with sharp declines in all measured parameters. Their poor performance under even moderate stress raises concerns about their suitability for rain-fed systems in drought-prone areas.

CONCLUSION

This study demonstrates that cocoa genotypes exhibit pronounced variability in their response to soil moisture stress. PA150 and Forastero emerged as the most promising candidates for drought-prone environments, combining robust growth, physiological resilience, and adaptive root anatomy. In contrast, PA7 and C42 are highly susceptible and should be avoided in regions with limited water availability. Importantly, mean soil moisture content alone was found to be insufficient as a selection criterion for identifying drought-tolerant cocoa genotypes. It should instead be integrated with other physiological and anatomical indicators such as free proline accumulation, stomatal characteristics, trichome presence, cuticle thickness, mesophyll structure, and epidermal features. The findings have direct implications for Ghana's cocoa sector, offering evidence-based guidance for breeding, seed selection, and farm management in the face of climate change. By integrating drought-tolerant genotypes into national programs and promoting soil health practices, Ghana can safeguard its cocoa industry and the livelihoods that depend on it amid climate change.

RECOMMENDATION

Based on the findings of this study, the following recommendations are made:

i. Further research should focus on the effects of soil moisture stress on the reproductive development of cocoa, as this aspect was not covered in the present study but is critical for yield performance.
ii. Additional studies should investigate physiological mechanisms such as transpiration rate, stomatal behaviour, and water-use efficiency among cocoa genotypes to better explain differences in drought tolerance.
iii. Future research should also consider detailed leaf anatomical traits, including cuticle thickness, stomatal density, and mesophyll structure, to improve screening and selection of drought-tolerant cocoa varieties.
iv. Breeding programmes should prioritize genotypes such as PA150 and Forastero for further evaluation and potential use in developing drought-resilient cocoa planting materials.

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SUPPLEMENTARY MATERIALS

Table S1. Summary of ANOVA results (F-values and p-values) for key parameters

Parameter	Factor	F-value	p-value
Leaf number	Genotype × Treatment	4.32	0.008
Stem thickness	Genotype × Treatment	3.21	0.04
Plant height	Genotype × Treatment	9.12	0.003
Available P (soil)	Treatment	12.34	0.002
Organic C (soil)	Treatment	8.76	0.009
Total Cd (soil)	Treatment	10.21	0.004