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Genotypic responses of cacao (*Theobroma cacao* L.) Rootstock-scion combinations to soil moisture deficit using Morpho-physiological indicators

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Abstract

Cacao (*Theobroma cacao* L.) production is increasingly threatened by soil moisture deficit (SMD) driven by climate variability and recurring El Niño events, making the identification of planting materials with robust early drought-adaptive responses essential for sustainable production. This study evaluated the early genotypic responses of 21 cacao rootstock-scion combinations (RSCs) to SMD using morpho-physiological indicators, using a split-plot RCBD with 3 blocks. Morphological and physiological traits were evaluated. Under SMD, UF 18/BR-25 (84.19%), PBC123/BR-25 (82.81%), and UF 18/W10 (82.17%) maintained the highest relative water content (RWC) values. The lowest visual severity scores (VSS) were recorded for UF 18/UF1 8 (1.17), PBC123/W10 (1.22), and PBC123/PBC123 (1.24), indicating fewer drought symptoms. In contrast, PBC123/ICS95 (1.80), UF 18/PBC123 (1.71), and BR-25/ICS95 (1.67) exhibited greater sensitivity to SMD. Based on percent change and stress tolerance index ranking using RWC, VSS, PBC123/W10, BR-25/UF 18, BR-25/PBC123, UF 18/UF 18, BR-25/DOM30, PBC123/PBC123 and UF 18/BR-25 showed the highest early drought-response rank and were identified as promising candidates for further long-term drought-tolerance evaluation. These findings show variation in drought responses among cacao RSCs and highlight promising materials for drought-tolerance breeding and rootstock improvement in the Philippines.

Introduction

Cacao (*Theobroma cacao* L.) is an economically important perennial crop and the primary raw material for chocolate production worldwide. According to the Department of Agriculture (2022), cacao is a priority crop for rural development, employment generation, and export promotion. Global cocoa production reached approximately 5 million metric tons during the 2022/2023 season, with Côte d'Ivoire and Ghana accounting for nearly two-thirds of the total (ICCO, 2023). However, cacao production remains constrained by climate change, declining soil fertility, aging plantations, and diseases that reduce productivity and threaten farmers' livelihoods (Läderach et al., 2016). In the Philippines, the cacao industry has expanded through government and private-sector initiatives, with Mindanao serving as the major production area due to its favorable climate (PSA, 2023).

Climate change and prolonged drought associated with El Niño events have become major challenges to cacao production in the Philippines. Dry spells have become more frequent and severe in recent decades (Lasco et al., 2016), while the recent 2023-2024 El Niño episode caused significant agricultural losses nationwide (FAO, 2024). Drought stress negatively affects cacao physiology by reducing leaf water availability, stomatal conductance, and photosynthetic activity, resulting in reduced vegetative growth, poor pod development, and lower productivity (Baby et al., 2025; Osorio-Zambrano et al., 2021). Previous studies have also reported substantial genetic variation among cacao genotypes in their ability to maintain leaf water potential and photosynthetic efficiency under water stress, highlighting the importance of developing drought-tolerant planting materials (Osorio-Zambrano et al., 2021; Santos, 2014).

Grafting using suitable rootstocks is considered a promising strategy to improve drought tolerance in perennial crops because rootstock-scion interactions influence water uptake, nutrient acquisition, and stress adaptation (Nawaz, 2016; Warschefsky et al., 2016). However, the role of rootstock-scion combinations (RSCs) in improving drought tolerance in cacao remains insufficiently studied in the Philippines, where localized information on genotypic responses under soil moisture deficit (SMD) is limited (Lasco et al., 2016). Specifically, while government distribution programs promote grafted seedlings across Mindanao, the interactive role of specific RSCs in modulating early physiological water-retention mechanisms and root development remains undocumented under local Philippine agro-climatic conditions. This localized screening is critical because mismatched scion and rootstock cultivars can suffer from graft-incompatibility under moisture stress. Therefore, this study aimed to evaluate the drought responses of different cacao RSCs using morpho-physiological indicators to identify promising combinations for drought-tolerance screening and future climate-resilient cacao breeding programs in the Philippines.

Several studies have highlighted the importance of physiological and agro-morphological traits in drought-tolerance screening. Suchithra et al. (2022) reported that drought stress limits cacao photosynthesis through stomatal closure and metabolic disruption, while Marenco et al. (2014) observed reduced photosynthetic efficiency under water stress. Relative water content (RWC) has also been identified as a reliable indicator of drought tolerance, with tolerant genotypes maintaining higher water status under stress conditions (Dzandu et al., 2021; Baby et al., 2025; Araque et al., 2012). Similarly, chlorophyll retention and root development have been associated with improved drought adaptation in cacao (De Almeida et al., 2016; Baligar et al., 2017; Osorio-Zambrano et al., 2021). Studies by Santos et al. (2014) and Niether et al. (2020) further demonstrated substantial genetic variation among cacao genotypes in their response to drought stress, supporting the need for integrated screening approaches.

Rootstock-scion interactions also play an important role in drought adaptation in grafted cacao seedlings. Warschefsky et al. (2016) emphasized that rootstocks influence water uptake, nutrient acquisition, and the scion's stress physiology. Jaimez et al. (2023) reported significant variation among cacao RSCs in terms of stomatal conductance, RWC, and growth under water-deficit conditions. Moreover, Fernandez (2022) identified UF 18 and BR-25 as promising rootstocks because of their graft compatibility and nursery performance, while Juwahir et al. (2025) reported that PBC123 exhibited superior root growth and biomass production under stress conditions. Rootstock-scion interactions may substantially influence the response of cacao to water deficit. Jaimez et al. (2021) demonstrated differences in physiological and root responses among cacao rootstock-scion combinations subjected to water deficit, with some combinations maintaining greater water potential and physiological performance than others. Similarly, recent evidence of

genotype-specific graft incompatibility in cacao demonstrates that rootstock-scion interactions can influence graft success, vigor, nutrient status, and other physiological traits (DuVal et al., 2025). These findings support the importance of evaluating RSCs under SMD to identify drought-resilient planting materials that can contribute to climate-resilient cacao breeding and sustainable cacao production in the Philippines.

This study aimed to assess the genotypic responses of cacao RSCs to SMD using morpho-physiological indicators. Specifically, the study evaluated the agro-morphological performance and physiological responses of 21 cacao RSCs under WW and SMD conditions to identify promising combinations that can withstand SMD. The study also aimed to combine morphological and physiological traits as selection criteria for drought tolerance in cacao seedlings.

Materials and Methods

Study site

The study was conducted from September 2025 to May 2026 at the screenhouse of the University of Southern Mindanao Agricultural Research and Development Center (USMARDC), Kabacan, Cotabato.

Experimental Design and Treatments

The experiment was conducted using a split-plot RCBD with three blocks and 21 treatment combinations arranged in a 2 × 21 factorial structure. Each treatment consisted of 10 seedling samples, a total of 1260 seedlings. The main plot had 2 levels of soil moisture: well-watered (WW) and soil moisture deficit (SMD). The subplots comprised 21 RSCs from the rootstocks UF 18, BR-25, and PBC123, and the scions UF 18, BR-25, PBC123, W10, ICS 95, SEBP 571, and DOM30.

Experimental Procedure

Rootstock seedlings were from cacao seeds of UF 18, BR-25, and PBC123 obtained from the cacao genebank of USMARDC. These rootstocks were selected based on previous studies showing their good graft compatibility, root vigor, and nursery performance (Fernandez, 2022; Juwahir et al., 2025). Seeds were sown using polyethylene bags (6x10") filled with sterilized growing media composed of topsoil, vermicompost, and rice hull (2:1:1) under the screenhouse installed with UV plastic and 70% garden nets. Seedlings were maintained for 3-4 months until they were suitable for grafting (Cena, 2016).

Cleft grafting (Isele et al., 2020) using the cacao clones UF 18, BR-25, PBC123, W10, ICS95, SEBP571, and DOM30 as scions. The clones UF 18, BR-25, and PBC123 were selected for their reported high-yield performance according to the Philippine Cacao Roadmap 2021-2025, while W10, as a widely adopted clone in Philippine cacao production (Juvinal et al., 2024), and three additional internationally recognized cacao clones-ICS 95, SEBP 571, and DOM 30- were selected to determine their performance under controlled soil moisture conditions. Grafted seedlings were labeled and carefully maintained.

Measurements were taken 60 days after grafting. Twenty-one RSCs were subjected to soil moisture deficit (SMD) by maintaining the soil at approximately 40% field capacity, while the other 21 combinations received regular watering. Measurements were taken 60 days after grafting. Twenty-one RSCs were subjected to soil moisture deficit (SMD) by maintaining the soil at approximately 40% field capacity, while the other 21 combinations received regular watering. Soil moisture content was monitored daily for 2 weeks using a

Yago 6-in-1 soil detector meter, and drip irrigation was applied as needed to maintain the target 40% field capacity with a calibrated nipple drip (2 L/hour) throughout the stress period. To ensure technical accuracy, the volumetric moisture readings of the Yago detector were pre-calibrated against the moisture-retention curve of the specific 2:1:1 topsoil-vermicompost-rice-hull growing medium to identify the exact sensor threshold corresponding to 40% field capacity.

The experiment was conducted during months with no La Niña forecast, according to advisories from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA). This precaution was taken to ensure that moisture stress resulted solely from imposed treatment.

Data Gathered

The number of leaves (NL), shoot height (SH), stem diameter (SD), and leaf area (LA) were measured on seven randomly selected following Schmildt et al. (2017), physiologically uniform seedlings per treatment at 2 weeks after the imposition of soil moisture stress. While each treatment was established with 10 seedlings to safeguard against nursery losses, measurements were strictly confined to these seven fully successful grafts per treatment to exclude any confounding effects from minor physical handling damage or graft-union callousing.

Visual severity score (VSS): A visual scoring scale was created in this study to assess the severity of SMD symptoms in cacao seedlings (Figure 1). Seven seedlings per treatment were assessed at the 2nd week of imposed SMD based on observable symptoms. This was calculated using the formula: VSS = total score/total no. of observed plants.

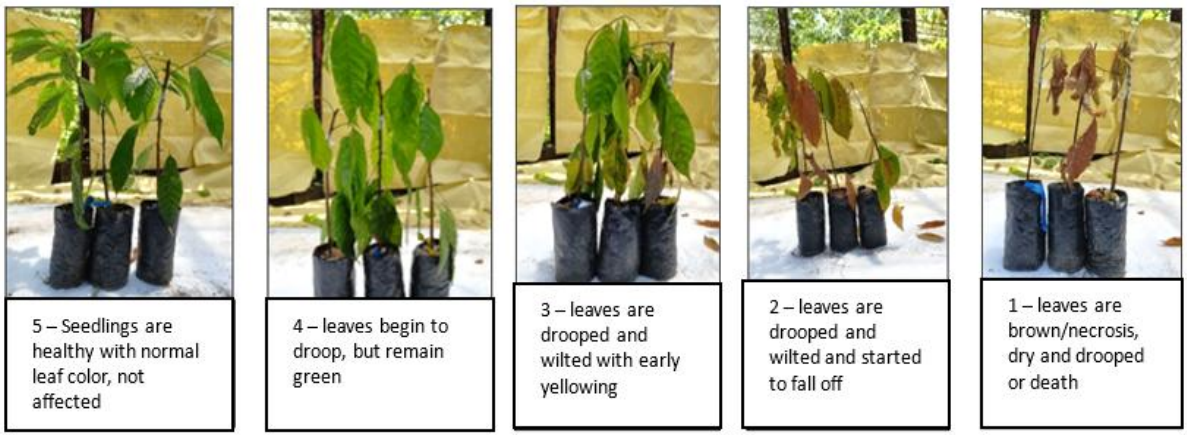

Figure 1. Score chart for visual severity on plants under soil moisture conditions.

Total Chlorophyll Content (TCC): Fresh leaf samples were collected and processed at the 2nd week of imposed SMD. Each sample was sliced into small pieces (<0.25 mm2, 1g) and chlorophyll was extracted with 96% (v/v) ethanol. The solutions were covered with aluminum foil and stored in the dark for 24 hours to prevent pigment degradation and ensure complete extraction. TCC was measured by absorbance at 665 nm and 645 nm with a spectrophotometer (Shimadzu 1800 model) as described by Raza et al. (2022). The concentration (mg/g) was calculated using the formula:

$$\text{Chlorophyll a+b} = \frac{(5.1 \times A665 + 20.24 \times A649 \times V)}{m}$$

Where A665 and A649 represent the absorbance value at the corresponding wavelength, V refers to the volume (liter) of chlorophyll solution, and m is the weight (gram) of the leaf sample.

Relative water content (RWC): 20 leaf discs (1 cm in diameter) were excised after the 2nd week of imposed SMD from the youngest fully expanded leaves, and their fresh weight (FW) was recorded immediately. The discs were then placed in mini cups containing distilled water and kept at room temperature for 4 hours to ensure full hydration. After gently blotting excess surface moisture, the turgid weight (TW) was measured. The samples were subsequently oven-dried at 80 °C for 24 hours, and DW was recorded (Barrs, 1968) as applied by Baby et al. (2025). RWC was calculated using the formula: $RWC = [(FW - DW) / (TW - DW)] \times 100$.

Shoot and root weight (SW and RW): Shoots and roots were separated, and the fresh weight of each was recorded. Samples were subsequently oven-dried at 70 °C for 48 h to a constant weight, yielding dry biomass.

Root volume (RV): measured by the water displacement method with a volumetric flask.

Statistical Analysis

Data were analyzed using the Statistical Tool for Agricultural Research (STAR V. 2.0.1). Analysis of variance (ANOVA), descriptive statistics, correlation analysis, principal component analysis (PCA; JASP V 0.96.0), and cluster analysis were performed to evaluate the responses of cacao RSCs under SMD. To simplify the factor loadings and improve biological interpretability, a promax oblique rotation was applied; consequently, these factors are reported as Rotated Components (RC1, RC2, RC3) rather than standard unrotated PCs, following established multivariate protocols. Data standardization prior to clustering was conducted in RStudio (2026 04.00 Build 526) using the *scale()* function.

The 21 RSCs were ranked based on their drought response (Table 6) using Percent Change (PC) and Stress Tolerance Index (STI) of the trait VSS and RWC, following the approaches described by Frimpong et al. (2021) and Kumar et al. (2023). PC was calculated as $[(SMD - WW) / WW] \times 100$, while STI was calculated as $(SMD \times WW) / (Mean\ WW)^2$.

Results and Discussion

The analysis of variance revealed that water condition did not significantly affect the number of leaves (NL) and leaf area (LA) after the second week of evaluation (Table 1). Similarly, fresh weight (FW), shoot fresh weight (SFW), and root fresh weight (RFW) remained statistically comparable between well-watered (WW) and soil moisture deficit (SMD) treatments after two weeks. These results suggest that the imposed SMD was not severe enough to induce immediate changes in leaf production, leaf expansion, or fresh biomass accumulation. The maintenance of these traits may indicate that cacao seedlings initially relied on existing water reserves and physiological adjustments to sustain growth despite reduced soil moisture availability. The absence of significant differences in FW, SFW, and RFW further suggests that short-term water limitation did not substantially affect tissue hydration or biomass accumulation during the evaluation period.

Table 1. Analysis of variance (ANOVA) for morphological and physiological traits of different cacao rootstock-scion combinations under two soil moisture conditions after 2 weeks.

SV	Week	NL	SH	SD	LA	VSS	RWC	TCC	FW	SFW	RFW	DW	SDW	RDW	RV
Block	W2	0.081^{ns}	0.119^{ns}	0.177^{ns}	0.381^{ns}	0.500	0.929^{ns}	0.860^{ns}	0.229^{ns}	0.361^{ns}	0.216^{ns}	0.2596^{ns}	0.259^{ns}	0.254^{ns}	0.850^{ns}
Water condition	W2	0.117^{ns}	0.015*	0.023*	0.063^{ns}	0.000**	0.046*	0.009**	0.898^{ns}	0.759^{ns}	0.759^{ns}	0.0038**	0.004**	0.004**	0.790^{ns}
RSCs	W2	0.770^{ns}	0.955^{ns}	0.483^{ns}	0.225^{ns}	0.000**	0.001**	0.146^{ns}	0.719^{ns}	0.273^{ns}	0.559^{ns}	0.6767^{ns}	0.676^{ns}	0.676^{ns}	0.000**
Water condition: RSC	W2	0.383^{ns}	0.484^{ns}	0.58^{ns}	0.444^{ns}	0.000**	0.000**	0.407^{ns}	0.671^{ns}	0.508^{ns}	0.941^{ns}	0.151^{ns}	0.1516^{ns}	0.151^{ns}	0.1054^{ns}
CV(a)%		17.01	12.38	10.04	29.79	5.48	15.21	6.21	42.93	47.83	81.91	12.84	12.81	12.71	51.28
CV(b)%		24.02	27.44	15.42	34.03	5.87	3.33	4.51	32.40	32.34	52.04	38.30	38.30	38.28	35.36

**: Highly significant at 1%; *: Significant at 5%; ns = not significant; RSC = rootstock-scion combinations; SV = sources of variation; NL = number of leaves; SH = shoot height; SD = stem diameter; LA = leaf area; VSS= visual severity score; RWC = relative water content; MC = moisture content; TCC = total chlorophyl content; DW = dry weight; SDW = shoot dry weight; RDW = root dry weight; RV = root volume; CV(a)% =coefficient of variation for error A; CV(b)% = coefficient of variation for error B

Shoot Height (SH) and Stem Diameter (SD)

Water condition significantly affected shoot height after two weeks of treatment (Table 1). The overall mean shoot height was significantly higher under WW (16.09 cm) than under SMD (12.59 cm) conditions (Table 2). This reduction indicates that limited water availability restricted shoot elongation. SMD reduces cell expansion and turgor pressure, which are essential for stem growth, resulting in shorter plants under drought conditions.

Table 2. Mean comparison for morphological and physiological traits of different cacao rootstock-scion combinations under two soil moisture conditions after 2 weeks.

RSCs	SH (cm)		SD (mm)		VSS		RWC (%)		TCC		DW (g)		SDW		RDW		RV Mean
	WW	SMD	WW	SMD	WW	SMD	WW	SMD	WW	SMD	WW	SMD	WW	SMD	WW	SMD	
UF 18/UF 18	13.31	14.00	3.14	3.33	5.00^{a}	4.14^{j}	88.77^{a}	77.54^{abc}	0.368	0.318	16.01	4.96	12.01	3.72	4.00	1.24	6.67^{ab}
BR-25/BR-25	15.40	12.07	3.32	3.19	5.00^{a}	2.82^{abcd}	85.52^{a}	79.57^{abc}	0.370	0.327	12.49	11.97	9.36	8.98	3.12	2.99	4.25^{ab}
PBC123/PBC123	14.45	12.45	3.44	3.50	5.00^{a}	3.95^{hij}	91.32^{a}	77.84^{abc}	0.368	0.301	9.51	11.03	7.14	8.27	2.38	2.76	6.33^{ab}
UF 18/BR-25	16.66	11.55	3.11	2.86	5.00^{a}	2.92^{abcde}	90.43^{a}	84.19^{a}	0.370	0.341	12.13	4.91	9.10	3.68	3.03	1.23	3.33^{b}
UF 18/PBC123	13.39	12.31	2.93	3.03	5.00^{a}	2.56^{ab}	85.56^{a}	81.54^{abc}	0.370	0.334	12.46	12.07	9.35	9.05	3.12	3.02	5.17^{ab}
UF 18/W10	16.30	13.63	3.49	3.55	5.00^{a}	2.91^{abcd}	91.92^{a}	82.17^{ab}	0.370	0.312	9.81	7.63	7.36	5.73	2.45	1.91	3.50^{b}
UF 18/ICS 95	20.32	11.59	3.42	2.97	5.00^{a}	3.30^{cdefg}	91.11^{a}	74.51^{bc}	0.370	0.338	11.59	7.20	8.69	5.40	2.90	1.80	4.00^{b}
UF 18/SEBP 571	17.28	12.41	3.76	2.84	5.00^{a}	2.82^{abcd}	86.34^{a}	77.86^{abc}	0.369	0.335	12.05	6.02	9.04	4.51	3.01	1.50	4.25^{ab}
UF 18/DOM 30	19.69	13.05	4.02	2.96	5.00^{a}	3.59^{efghij}	91.56^{a}	79.51^{abc}	0.349	0.308	13.40	7.85	10.05	5.88	3.35	1.96	7.83^{a}
BR-25/UF 18	13.76	15.26	3.93	3.04	5.00^{a}	3.77^{ghij}	85.98^{a}	80.58^{abc}	0.368	0.330	12.00	3.36	9.00	2.52	3.00	0.84	4.50^{ab}
BR-25/PBC123	16.53	15.30	4.01	2.72	5.00^{a}	3.86^{ghij}	87.97^{a}	79.62^{abc}	0.370	0.323	9.20	5.89	6.90	4.42	2.30	1.47	4.00^{b}
BR-25/W10	12.76	12.14	3.35	3.12	5.00^{a}	3.26^{bcdefg}	87.42^{a}	78.01^{abc}	0.369	0.332	11.93	5.90	8.95	4.42	2.98	1.47	4.17^{b}
BR-25/ICS 95	12.75	14.50	3.52	3.23	5.00^{a}	2.72^{abc}	87.08^{a}	74.59^{bc}	0.369	0.342	8.75	12.61	6.56	9.46	2.19	3.15	6.17^{ab}
BR-25/SEBP 571	14.48	12.89	3.39	3.06	5.00^{a}	3.39^{cdefgh}	89.91^{a}	79.23^{abc}	0.359	0.344	14.86	7.19	11.14	5.40	3.71	1.80	4.75^{ab}
BR-25/DOM 30	15.44	12.75	3.26	2.73	5.00^{a}	3.5^{defghi}	90.7^{a}	81.72^{ab}	0.368	0.319	10.50	8.96	7.88	6.72	2.63	2.24	6.83^{ab}
PBC123/UF 18	17.80	11.33	3.43	3.10	5.00^{a}	3.01^{abcdef}	92.89^{a}	75.5^{abc}	0.368	0.319	9.89	6.32	7.42	4.74	2.47	1.58	3.83^{b}
PBC123/BR-25	19.49	13.23	3.65	3.25	5.00^{a}	2.86^{abcd}	92.07^{a}	82.81^{ab}	0.369	0.325	12.93	10.83	9.70	8.12	3.23	2.71	4.83^{ab}
PBC123/W10	15.54	11.75	3.53	3.05	5.00^{a}	4.00^{ij}	90.09^{a}	80.67^{abc}	0.369	0.321	14.55	7.37	10.91	5.53	3.64	1.84	4.17^{b}
PBC123/ICS 95	18.99	9.81	3.97	3.23	5.00^{a}	2.27^{a}	91.95^{a}	73.27^{c}	0.370	0.321	11.45	10.84	8.59	8.13	2.86	2.71	4.50^{ab}
PBC123/SEBP 571	18.19	11.80	3.77	3.49	5.00^{a}	3.63^{fghij}	87.30^{a}	77.23^{abc}	0.369	0.353	10.03	9.41	7.52	7.06	2.51	2.35	5.17^{ab}
PBC123/DOM 30	15.28	10.63	4.18	3.34	5.00^{a}	3.35^{cdefgh}	86.38^{a}	78.54^{abc}	0.369	0.347	11.45	7.42	8.59	5.56	2.86	1.86	3.67^{b}
Mean	**16.09^{a}**	**12.59^{b}**	**3.55^{a}**	**3.12^{b}**	**5.00^{a}**	**3.27^{b}**	**89.16**	**78.89**	0.368a	0.329b	**11.76^{a}**	**8.08^{b}**	**8.82^{a}**	**6.06^{b}**	**2.94^{a}**	**2.02^{b}**	**4.85**

Means with the same letter are not significantly different; RSCs = rootstock-scion combinations; WW = well-watered; SMD = soil moisture deficit; VSS =visual severity score; RWC = relative water content; TCC = total chlorophyl content; DW= dry weight; RV = root volume. *Note: Arithmetic means are presented. The pooled standard errors (SE) for the key traits are: SH = ±0.84 cm, SD = ±0.15 mm, VSS = ±0.05, RWC = ±1.12%, TCC = ±0.008 mg/g, DW = ±0.45 g, and RV = ±0.32 cm^{3}."*

SD was also significantly affected by water conditions. Seedlings grown under WW conditions produced significantly thicker stems (3.55 mm) than those exposed to SMD (3.12 mm). Reduced stem thickening under drought stress may be associated with decreased cambial activity and limited cell enlargement caused by insufficient water availability. Since stem diameter contributes to plant vigor and structural support, the

reduction observed under SMD indicates that drought negatively influences overall seedling development. The findings agree with Santos et al. (2014), Agele et al. (2019), and Oduro et al. (2026), who reported that drought in cacao mainly restricts vegetative growth and biomass accumulation.

Visual Severity Score (VSS)

The visual severity score was highly significantly affected by water condition, RSCs, and their interaction. Under WW conditions, all combinations exhibited the same VSS value (5.00), indicating the absence of visible stress symptoms. However, under SMD, substantial variation was observed among combinations. The highest VSS values were recorded in UF 18/UF 18 (4.14), PBC123/W10 (4.00), and PBC123/PBC123 (3.95), indicating fewer visible drought symptoms and better tolerance to SMD. In contrast, PBC123/ICS 95 (2.27), UF 18/PBC123 (2.56), and BR-25/ICS95 (2.72) exhibited higher VSS values, suggesting greater susceptibility to drought stress.

The significant interaction between water condition and RSCs indicates that the extent of drought symptoms depended on the specific RSCs This is consistent with the findings of Oduro et al. (2026), who noted similar symptoms in cacao seedlings under stress among genotypes, with tolerant lines delaying symptom development due to adaptive traits such as thicker cuticles and efficient water use.

Relative Water Content (RWC)

Relative water content was significantly influenced by water condition, RSCs, and their interaction. The overall mean RWC declined from 89.16% under WW conditions to 78.89% under SMD, demonstrating the effect of drought on plant water status. However, considerable variation existed among combinations under stress.

Among the evaluated combinations, UF 18/BR-25 maintained the highest RWC under SMD (84.19%), followed by PBC123/BR-25 (82.81%) and UF 18/W10 (82.17%). These combinations were more effective in maintaining tissue hydration despite reduced soil moisture. Conversely, PBC123/ICS 95 exhibited the lowest RWC (73.27%), followed by UF 18/ICS95 (74.51%) and BR-25/ICS95 (74.59%), indicating a reduced ability to conserve water under drought conditions.

The significant interaction between water conditions and RSCs suggests that the ability to maintain leaf water content varied with genetic background. According to Suchithra et al. (2022), cacao genotypes that maintain RWC above 80% under water stress are considered drought-tolerant due to improved osmotic adjustment and cellular protection.

Total Chlorophyll Content (TCC)

Total chlorophyll content was significantly affected by water condition but not by RSCs. The overall mean chlorophyll content decreased from 0.37 368 under WW conditions to 0.32933 under SMD. This reduction indicates that drought stress negatively affected chlorophyll accumulation or accelerated chlorophyll degradation. Although numerical differences among combinations were observed, the absence of significant RSC effects suggests that all combinations responded similarly to water deficit with respect to chlorophyll content.

Dry Weight (DW) and Root Volume (RV)

The analysis of variance (ANOVA) showed that soil moisture condition had a highly significant effect on DW, SDW, and RDW, while the effect of RSCs and the interaction

between RSCs and soil moisture condition were not significant (Table 1). This indicates that biomass accumulation was mainly influenced by water availability rather than by genetic differences among the evaluated combinations.

Mean comparison revealed that seedlings grown under WW conditions had significantly greater total dry matter accumulation than those exposed to SMD. The mean DW decreased from 11.76 g under WW to 8.08 g under SMD, representing approximately 31.3% reduction in total dry biomass under drought conditions. Similarly, SDW significantly declined from 8.82 g (WW) to 6.06 g (SMD). For RDW, seedlings under WW produced significantly (2.94 g) than those under SMD (2.02 g). Reduced shoot dry matter accumulation under SMD is commonly associated with lower photosynthetic activity, reduced cell expansion, and restricted assimilate production due to stomatal closure and reduced carbon fixation.

For RV, ANOVA showed that neither soil moisture condition nor the interaction between RSCs and moisture condition significantly affected RV (Table 1). However, the effect of RSCs was highly significant, indicating that differences in RV were primarily controlled by genetic factors rather than water treatment.

Mean comparison (Table 2) showed that RV ranged from 3.33 to 7.83, with an overall mean of 4.85. The top three highest RV values were observed in UF 18/DOM 30 (7.83), BR-25/SEBP 571 (6.83), and UF 18/UF 18 (6.67). In contrast, the lowest three RV values were recorded in UF 18/BR-25 (3.33), UF 18/PBC123 (3.50), and PBC123/DOM 30 (3.67).

The superior RV observed in UF 18/DOM 30 suggests enhanced root system expansion compared with the other combinations. This combination also maintained relatively strong biomass performance under both moisture conditions, indicating a more vigorous belowground growth pattern. This can help sustain plant growth and physiological activity during periods of limited moisture availability.

Correlation analysis

The correlation analysis (Table 3) revealed several significant relationships among agro-morphological, physiological, and biomass traits under SMD. NL was positively correlated with SH, SD, and LA, indicating that plants with more leaves generally had greater vegetative growth. Likewise, SH was positively associated with SD and LA, and SD was positively associated with LA, suggesting that vigorous plants maintained better growth under moisture stress.

Table 3. Correlation analysis of agro-morphological, physiological and biomass traits under soil moisture condition

	NL	SH	SD	LA	VSS	RWC	TCC	FW	SFW	RFW	DW	SDW	RDW	RV
NL	-													
SH	0.40**	-												
SD	0.37**	0.78**	-											
LA	0.28**	0.69**	0.62**	-										
VSS	-0.17	-0.44**	-0.42**	-0.30**	-									
RWC	0.16	0.36**	0.34**	0.28**	-0.76**	-								
TCC	0.14	0.29**	0.28**	0.20*	-0.71**	0.55**	-							
FW	0.19	0.11	0.01	0.03	-0.06	0.08	-0.06	-						
SFW	0.13	0.18*	0.18*	0.04	-0.06	0.08	-0.02	0.94**	-					
RFW	0.02	0.09	-0.09	-0.00	0.04	0.40	-0.11	0.68**	0.38**	-				
DW	0.11	-0.19*	-0.10	-0.17	0.39**	-0.33**	-0.38**	0.51**	0.45**	0.42**	-			
SDW	0.11	-0.19*	-0.10	-0.17	0.39**	-0.33**	-0.38**	0.51**	0.45**	0.42**	1.00**	-		
RDW	0.11	0.19*	-0.10	-0.17	0.34**	-0.33**	-0.38**	0.51**	0.45**	0.42**	1.00**	1.00**	-	
RV	-0.01	0.01	0.04	0.023	-0.10	0.07	-0.05	0.59**	0.42**	0.68**	0.42**	0.42*	0.42**	-

ns = not significant; * significant; ** highly significant at p< 0.05; NL = number. of leaves; SH = shoot height; SD = stem diameter; LA = leaf area; VSS = visual severity score; RCW = relative water content; TCC = total chlorophyl content; FW = Fresh weight; SFW = shoot fresh weight; RWF = root fresh weight; DW = dry weight; SDW = shoot dry weight; RDW = root dry weight; and RV = root volume

VSS showed significant negative correlations with SH, SD, and LA, indicating that more stressed plants had poorer growth. VSS was also negatively correlated with RWC and total TCC, showing that severe stress reduced water status and chlorophyll retention.

RWC was positively correlated with SH (0.36), SD (0.34), LA (0.28), and TCC (0.55), indicating that plants with better hydration-maintained growth and chlorophyll under stress. Similarly, TCC was positively associated with SH (0.29), SD (0.28), and RWC (0.55), highlighting the importance of water status in maintaining photosynthetic activity.

Fresh weight (FW) had strong positive correlations with shoot fresh weight (SFW; 0.94), root fresh weight (RFW; 0.68), dry weight (DW; 0.51), shoot dry weight (SDW; 0.51), and root dry weight (RDW; 0.51). Root volume (RV) was positively associated with DW, SDW, and RDW (0.42), while RFW showed a strong correlation with RV (0.68), indicating that larger root systems supported greater biomass production under SMD.

Therefore, better water status was associated with improved growth and biomass accumulation, whereas higher stress reduced plant performance. The strong relationships among RWC, TCC, and RV suggest that these traits are useful indicators of drought tolerance in cacao.

Principal component analysis (PCA) under soil moisture conditions

Similarly, Principal component analysis (PCA) of soil moisture conditions (Table 4) identified the main relationships among morphological, physiological, and biomass traits. Three rotated components (RC1, RC2, and RC3) explained most of the variation among the evaluated samples.

Table 4. PCA Component loadings of agro-morphological and physiological traits under soil moisture conditions.

Parameters	Component Loadings			
	RC1	RC2	RC3	Uniqueness
NL			0.712	0.551
SH			0.854	0.207
SD			0.857	0.247
LA			0.793	0.377
VSS	0.663			0.335
RWC	-0.632			0.428
TCC	-0.596			0.537
MC	-0.828			0.325
FW		0.970		0.086
SFW		0.904		0.191
DW	0.803	0.486		0.055
SDW	0.803	0.486		0.055
RDW	0.803	0.486		0.055
RV		0.730		0.482

Applied rotation method is promax, NL = no. of leaves; SH = shoot height; SD = stem diameter; LA = leaf area; VSS = visual severity score; RCW = relative water content; TCC = total chlorophyl content; MC = moisture content; FW = Fresh weight; SFW = shoot fresh weight; RWF = root fresh weight; DW = dry weight; SDW = shoot dry weight; RDW = root dry weight; RV = root volume

RC1 was mainly associated with physiological stress responses. Visual stress score (VSS) showed a positive loading (0.663), while relative water content (RWC; -0.632), total chlorophyll content (TCC; -0.596), and moisture content (MC; -0.828) had strong negative loadings. The large negative loading of MC indicates that RC1 primarily represents the moisture-stress response, in which increased stress was associated with lower water status, chlorophyll content, and moisture retention.

RC2 was dominated by biomass traits, with fresh weight (FW) showing the highest loading (0.970), followed by shoot fresh weight (SFW; 0.904) and root volume (RV; 0.730). Dry weight traits (DW, SDW, and RDW) also showed moderate positive loadings (0.486), indicating that biomass accumulation was closely linked to shoot growth and root development.

RC3 represented vegetative growth traits, including shoot height (SH; 0.854), stem diameter (SD; 0.857), leaf area (LA; 0.793), and number of leaves (NL; 0.712). The high loadings of SH and SD suggest that structural growth traits were strongly interconnected under SMD.

The component characteristics (Table 5) showed that the first three principal components explained 96.3% of the total variation under SMD, indicating that these components effectively summarized the drought-response traits of the cacao RSCs.

Table 5. Component characteristics of the PCA

	Unrotated solution			Rotated solution		
Components	**Eigen value**	**Proportion var.**	**Cumulative**	**SumSq. Loadings**	**Proportion var.**	**Cumulative**
Component 1	3052	0.764	0.764	1150	0.288	0.288
Component 2	571.8	0.143	0.907	951.2	0.238	0.526
Component 3	224.0	0.056	0.963	1617	0.405	0.931
Component 4	97.84	0.024	0.987	227.6	0.057	0.987

RC1 represented drought stress adaptation, explaining 28.8% of the variance and comprising visual severity score, relative water content, moisture content, and total chlorophyll content. The clustering of RWC, MC, and TCC suggests that seedlings with better water retention were able to maintain chlorophyll content and physiological function under drought stress.

RC3 explained the largest proportion of variance (40.5%) and was associated with vegetative growth traits, including shoot height, stem diameter, leaf area, and number of leaves. This indicates that growth responses were distinct from physiological stress responses.

The PCA highlights the importance of water status, chlorophyll retention, biomass accumulation, and vegetative growth as key indicators for drought tolerance screening and selection of superior cacao RSCs.

Hierarchical cluster Analysis under soil moisture conditions

The dendrogram in Figure 2 illustrates the similarity among the 21 cacao RSCs based on their agro-morphological and physiological responses to SMD.

Combinations grouped at shorter Euclidean distances exhibited more similar drought-response characteristics, whereas those separated by larger distances showed greater variation in performance.

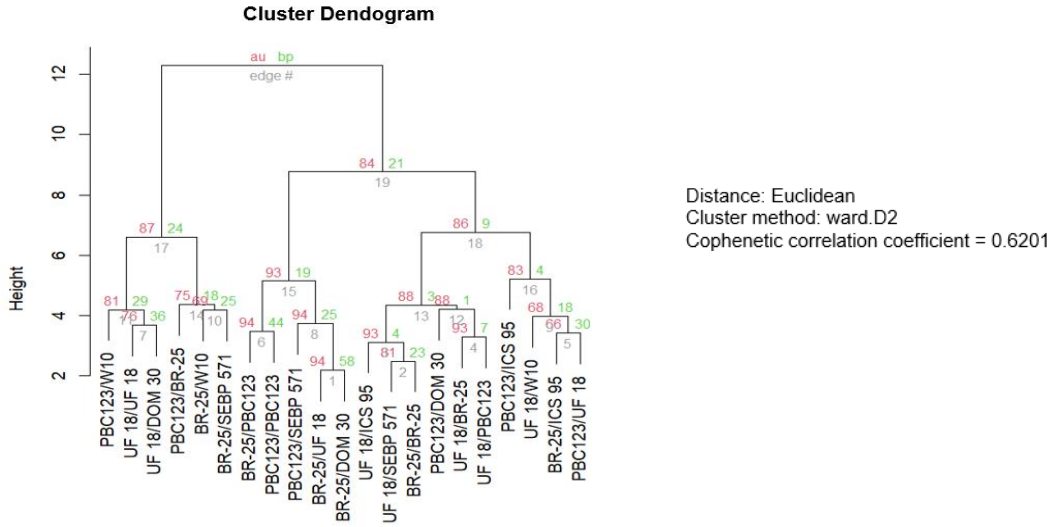

Figure 2. Hierarchical cluster dendrogram of 21 RSCs under SMD showing the cluster relationships among samples. Bootstrap and cophenetic correlation values show the reliability of the cluster formation.

The cluster analysis generally separated the RSCs into groups corresponding to their drought-tolerance classifications derived from the consolidated scoring system (Table 6). The first major cluster consisted mainly of combinations classified as better early drought response, including the first major cluster consisted mainly of combinations, including UF 18/UF 18, UF 18/DOM 30, PBC123/W10, PBC123/BR-25, BR-25/W10, and BR-25/SEBP571. The presence of tolerant better drought response combinations within this cluster suggests that these genotypes shared favorable characteristics such as higher relative water content and higher visual stress symptoms.

Table 6. Percent change and stress tolerance index of visual severity score (VSS) and relative water content (RWC) of cacao rootstock-scion combinations under soil moisture deficit after two weeks.

RSCs	VSS				RWC				Mean	Final Rank
	PC	Rank	STI	Rank	PC	Rank	STI	Rank		
UF 18/UF 18	-17.28	1	0.83	1	-12.65	15	0.87	9	6.5	4
BR-25/BR-25	-43.69	18	0.56	16	-6.96	4	0.86	10	12	16
PBC123/PBC123	-21.00	3	0.79	3	-14.76	18	0.89	7	7.75	6
UF 18/BR-25	-41.59	14	0.58	14	-6.90	3	0.96	1	8	7
UF 18/PBC123	-48.74	20	0.51	18	-4.70	1	0.88	8	11.75	15
UF 18/W10	-41.88	15	0.58	14	-10.61	11	0.95	2	10.5	13
UF 18/ICS 95	-34.02	11	0.66	11	-18.22	19	0.85	11	13	18
UF 18/SEBP 571	-43.61	17	0.56	16	-9.82	7	0.85	11	12.75	17
UF 18/DOM 30	-28.12	7	0.72	7	-13.16	16	0.92	4	8.5	8
BR-25/UF 18	-24.55	5	0.75	5	-6.28	2	0.87	9	5.25	2
BR-25/PBC123	-22.80	4	0.77	4	-9.49	6	0.88	8	5.5	3
BR-25/W10	-34.76	12	0.65	12	-10.76	12	0.86	10	11.5	14
BR-25/ICS 95	-45.63	19	0.54	17	-14.34	17	0.82	12	16.25	20
BR-25/SEBP 571	-32.17	9	0.68	9	-11.88	14	0.90	6	9.5	11
BR-25/DOM 30	-30.03	8	0.70	8	-9.90	8	0.93	3	6.75	5
PBC123/UF 18	-39.84	13	0.60	13	-18.72	20	0.88	8	13.5	19
PBC123/BR-25	-42.90	16	0.57	15	-10.06	9	0.96	1	10.25	12
PBC123/W10	-19.99	2	0.80	2	-10.46	10	0.91	5	4.75	1
PBC123/ICS 95	-54.61	21	0.45	19	-20.32	21	0.85	11	18	21
PBC123/SEBP 571	-27.32	6	0.73	6	-11.53	13	0.85	11	9	9.5
PBC123/DOM 30	-32.90	10	0.67	10	-9.08	5	0.85	11	9	9.5

VSS = visual severity score; RWC = relative water content; PC = percent change; STI = stress tolerance index; Rank 1-7 better early drought response; 8-14 moderate/intermediate early drought response; and 15-21 poorer drought response.

The second major cluster contained a wider range of moderately early drought response and poorer drought response combinations. These combinations were located farther from the better drought response groups, indicating distinct physiological and morphological responses to drought stress. Their separation in the dendrogram is consistent with their lower drought-tolerance scores, reflecting reduced water retention capacity and poorer adaptation to SMD.

The clustering pattern supports the results of the drought-tolerance classification, demonstrating that combinations with similar levels of tolerance tended to group. Better early drought response combinations such as PBC123/W10, BR-25/UF 18, BR-25/PBC123, UF 18/UF 18, BR-25/DOM30, PBC123/PBC123 and UF 18/BR-25 were generally associated with clusters characterized by favorable drought-response traits, whereas poorer combinations formed more distinct groups. The bootstrap values further indicate the reliability of several clusters, particularly those with high approximately unbiased (AU) values, suggesting that these groupings were consistently supported across repeated resampling analyses.

The hierarchical cluster analysis validated the scoring-based classification and highlighted the existence of distinct early drought response, intermediate, and poorer drought response groups among the evaluated cacao RSCs.

Drought responses of 21 rootstock-scion combinations through percent change and stress tolerance index under SMD.

The percent change (PC) and stress tolerance index (STI) of visual severity score (VSS) and relative water content (RWC) were used to characterize the relative early response of the cacao rootstock-scion combinations (RSCs) to soil moisture deficit (SMD) after two weeks of stress (Table 6). The indices allowed the RSCs to be compared based on the magnitude of change from the well-watered condition and their relative performance under moisture deficit. Individual ranks were assigned for each index, with ranks 1–7 representing a better early drought response, ranks 8–14 representing an intermediate response, and ranks 15–21 representing a poorer early drought response. The mean of the four individual ranks was used to obtain the final ranking of the RSCs.

For VSS, PC values ranged from −17.28% in UF 18/UF 18 to −54.61% in PBC123/ICS 95. Based on the ranking criterion used in the study, UF 18/UF 18 had the most favorable VSS-PC ranking (Rank 1), followed by PBC123/W10 (Rank 2) and PBC123/PBC123 (Rank 3). In contrast, PBC123/ICS 95 (Rank 21), UF 18/PBC123 (Rank 20), and BR-25/ICS 95 (Rank 19) exhibited the poorest rankings for VSS-PC. A similar pattern was observed for STI-VSS, where UF 18/UF 18 recorded the highest value (0.83; Rank 1), followed by PBC123/W10 (0.80; Rank 2) and PBC123/PBC123 (0.79; Rank 3). PBC123/ICS 95 recorded the lowest STI-VSS (0.45; Rank 19), indicating a relatively poor early response based on this index. The agreement between VSS-PC and STI-VSS for several RSCs suggests that these indices captured differences in the response of the combinations to moisture deficit.

Relative water content also revealed considerable variation among RSCs. The PC of RWC ranged from −4.70% in UF 18/PBC123 to −20.32% in PBC123/ICS 95. UF 18/PBC123 recorded the most favorable RWC-PC ranking (Rank 1), followed by BR-25/UF 18 (Rank 2) and UF 18/BR-25 (Rank 3). These combinations experienced relatively smaller reductions in RWC under SMD, indicating greater maintenance of tissue water status during the stress period. In contrast, PBC123/ICS 95 (Rank 21), PBC123/UF 18 (Rank 20), and UF 18/ICS 95 (Rank 19) showed the poorest RWC-PC rankings, reflecting comparatively greater reductions in RWC.

The STI-RWC results further demonstrated differences in the capacity of the RSCs to maintain RWC relative to the WW condition. The highest STI-RWC values were observed in UF 18/BR-25 and PBC123/BR-25 (both 0.96; Rank 1), followed by UF 18/W10 (0.95; Rank 2) and BR-25/DOM 30 (0.93; Rank 3). These combinations therefore showed relatively favorable performance in maintaining RWC under SMD. Conversely, BR-25/ICS 95 recorded the lowest STI-RWC (0.82; Rank 12). Although this value fell within the intermediate ranking category, it was accompanied by relatively poor rankings for the other indices, contributing to its low overall position.

When the four indices were considered simultaneously, substantial variation in early drought response was observed among the RSCs. PBC123/W10 obtained the highest overall ranking, with a mean rank of 4.75, followed by BR-25/UF 18 (5.25), BR-25/PBC123 (5.50), UF 18/UF 18 (6.50), and BR-25/DOM 30 (6.75). These combinations were within the better early drought-response group based on their mean ranks. PBC123/W10 was particularly notable because it ranked among the top combinations for both VSS-PC (Rank 2) and STI-VSS (Rank 2), while also maintaining relatively favorable RWC-related rankings (Ranks 10 and 5). BR-25/UF 18 similarly showed a strong combined response, particularly for RWC-PC (Rank 2), although its VSS-related rankings were intermediate.

Several RSCs exhibited intermediate overall responses. PBC123/SEBP 571 and PBC123/DOM 30 both had a mean rank of 9.50, while BR-25/SEBP 571 had a mean rank of 9.50 and PBC123/BR-25 had a mean rank of 10.25. These combinations generally showed a mixture of favorable and intermediate rankings across the four indices, suggesting that their responses to SMD were not uniformly strong across both visual and physiological indicators.

The poorest overall responses were observed in PBC123/ICS 95 (mean rank = 21.00), BR-25/ICS 95 (16.25), PBC123/UF 18 (13.50), and UF 18/ICS 95 and UF 18/SEBP 571 (13.00 and 12.75, respectively). PBC123/ICS 95 was particularly consistent in its poor performance, ranking 21st for both VSS-PC and RWC-PC and recording a relatively low STI-VSS (0.45). Its mean rank of 21.00 consequently placed it clearly within the poorer early drought-response group. The consistently low ranking of combinations involving ICS 95 as the scion, particularly when grafted onto PBC123 and BR-25, suggests that scion identity may contribute to variation in the early response of cacao graft combinations to soil moisture deficit. However, this observation should be considered an indication of response within the conditions of the present experiment rather than evidence of a general scion effect.

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Ethical Considerations

This study did not involve human participants or animals. All experimental procedures involving cacao plant materials were conducted following established research practices and institutional guidelines.

CRediT Author Statement

Eljean B. Villasista: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft, Writing – Review & Editing. **Gwen Iris Descalsota-Empleo:** Conceptualization, Supervision, Methodology, Validation, Writing – Review and Editing and Validation.

Declaration on Conflict of Interest

The authors declare that they have no conflict of interest regarding the research, authorship, and publication of this manuscript.

Declaration on Generative AI use

The authors declare that generative AI tools were used only to assist in improving language, grammar, and clarity during manuscript preparation. The authors reviewed and verified the content and take full responsibility for the accuracy, originality, and integrity of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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