



Evaluation of Cowpea Morpho-physiological and Yield Responses to Vegetative and Pre-Anthesis Water-Deficit Stress Tolerance under Greenhouse Conditions

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ABSTRACT

Cowpea production is severely hindered by water scarcity; thus, understanding morpho-physiological response mechanisms of known drought-tolerant cultivars under water-deficit stress is critical to identify and establish representative yield-related traits of climate-hardy cowpeas. To determine cowpea genotypic variability to drought-tolerance, seventeen days-post sowing (DPS) greenhouse plants were subjected to 14-days drought stress without watering, then watered every 10-days at 25%, 50%, and 75% field capacity (FC) until maturity in two-trial experiment. Controls were well-watered at 100% FC every 3-days. Drought stress data were collected on plant height, stem diameter, chlorophyll content and terminal leaflet expansion rate. At maturity, 83 to 119 DPS, pod number, shoot and root biomass, and seed yield per plant were recorded. Data were combined and analyzed using analysis of variance. Drought tolerance was evaluated by percent change in performance and stress tolerance indexes. Drought stress in both trials impacted phenotypic expression. Plant height declined by 74%, stem diameter 18.2%, chlorophyll content, 47.6% terminal leaf length 83.2%, and width 85.2%. Pods per plant were reduced by 73% and seed yield by 98.8%. The estimated correlation between morpho-physiological and other yield-related traits of drought-tolerance indices verified that TVu 11987, LOBIA-I-SEFADE, and TVu 7362 were drought tolerant along with confirmed tolerant commercial cultivars California Blackeye No.5, Big Boy, and Lady. These cultivars exhibited different stress-coping strategies of low water requirements and growth performance to yield reduction. Overall, the genotypic performance recorded as drought-tolerant characteristics may be recommended as potential screening factors for donor cultivar traits in cowpea breeding programs.

Keywords: Cowpea (*Vigna unguiculata*), Water Stress, Field Capacity, Morpho-physiological Traits, Growth and Grain Yield Response.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.], Fabaceae, ($2n = 2x = 22$) is an important legume and inexpensive source of protein, vitamins, minerals and fiber for millions of low-income households where it is consumed as dry grain and leaves as vegetables [1], [2]. According to Food and Agriculture Organization (FAO), 14.5 million ha of land are devoted to cowpea production, mainly in the African countries of Niger, Nigeria, Burkina Faso, Mali, and Sudan, accounting for 75% of global grain production and 78% of cultivated area. However, the average production in most African countries is below $1t\ ha^{-1}$ compared to the potential yield of up to $3t\ ha^{-1}$ [3], [4] due to abiotic factors such as environmental fluctuations and soil and water limiting conditions. These factors are important plant growth and development

determinants, particularly drought, which can severely limit the productivity and quality of cowpeas. In the United States (U.S.), cowpea is an introduced field, and horticultural crop with much of the production predominantly practiced in the southern states where cowpea remains a staple food [4], [5], [6]. Cowpea production in the U.S. is practiced on about 5,220 ha in which 11,750 tons of cowpeas are harvested [3], [7] as dried seed commonly known as black-eyed peas or southern peas, and often cooked, canned or frozen. Furthermore, some cowpeas are harvested while the seeds are high in moisture, and sold fresh [4], [8], [9]. The U.S dry cowpea production has been continuously declining due to changing cropping systems, environmental fluctuations and overall food consumption trends as opposed to increased land devoted for cowpea production in other regions [4], [9]. Other sources suggests that, the collapse of cowpea acreage is associated with increased acreage of soybean due to ease of mechanization and reduced pest problems [10]. However, cowpea is considered more drought-tolerant than soybeans and well adapted to sandy soil types [11]. Due to the importance of cowpea worldwide, versatile end uses and unique grain characteristics, there is a dire need to understand its tolerance performance under soil water deficit and extreme temperatures to help develop hardy crops with higher grain yield stability and better stress adaptation to changes in the global climate.

As global climate change and related problems increase, water shortage is becoming increasingly alarming [12]. These environmental constraints not only impact plant growth, yield, and water relations, but also membrane integrity, pigment content, and photosynthesis [13], [14], especially during pre-anthesis. Cowpea production, especially in Africa, is primarily grown under rainfed conditions thus, its productivity is essentially hindered by erratic rainfall patterns which either come late, at the beginning of the season or stops earlier than usual, leading to severe drought conditions during the growing season [15], [16]. Although cowpea species are naturally well-adapted to growing in drier regions where other legumes do not perform well, many varieties are potentially affected by various environmental factors resulting in a serious reduction of crop yield and quality [17], [18]. Hence, the adaptability of some cultivars to withstand both heat and drought is of significant interest for gaining insight into their resiliency in extreme environmental conditions.

Cowpea exhibits inherent genotypic variations in response to drought stress. Some cultivars are comprised of discrete physiological and morphological traits that enhance their ability to adapt to different environmental conditions [19]. Purushothaman et al., [20] indicated that root traits like thick cortex aid cowpea to effectively absorb re-introduced soil moisture after drought stress which enhances recovery. The application of efficient screening techniques would facilitate the identification of key traits for breeding to improve drought stress adaptation, yield, and quality. Phenotypic evaluation is the first step in the screening of desirable and promising cowpea genotypes with drought adaptive characteristics [21], [22]. Two methods employed include the empirical or performance approach that uses grain yield and its components as the main criteria, since yield is the integrated expression of the entire array of traits related to productivity under stress. The second method is the physiological approach that identifies a specific physiological or morphological trait that significantly contributes to growth and yield in the event of drought [23]. Physiological parameters like water potential, relative turgidity, diffusion pressure deficit, chlorophyll stability index and carbon isotope determination are technically difficult and are associated with a high cost of time and investment, especially when large numbers of breeding populations are involved [24],

[25]. Field screening is difficult due to uncertainties of rainfall differences in photoperiod and temperatures especially in the dry seasons [26]. Pot screening method under greenhouse conditions is an effective and controlled method for evaluating cowpea agronomic performance under water-deficit stress and identification of drought-tolerant characteristics of resistant genotypes [27]. We present in this study a systematic greenhouse evaluation of 15 selected cowpea cultivars under pre- and post-anthesis water deficit to gain an understanding of genotypic variation in stress adaptation to explore the possible associations among studied traits for their potential in developing enhanced phenotypic pools that can be used in breeding. We investigated the impact of no-watering, different types of soil moisture stresses on the physiological, morphological, and yield response of cowpea to identify high-performing germplasm.

MATERIALS AND METHODS

Plant Materials, Soil, and Conditioning

Fifteen cowpea cultivars with known and unknown responses to drought stress were selected for this work (Table 1). They were comprised of six plant introductions (PIs) requested from the United States (U.S.) Department of Agriculture (USDA) National Plant Germplasm System (NPGS) and nine commercial cultivars grown by U.S. farmers. The soil was collected from Tuskegee University's George Washington Carver Agricultural Experiment Station situated in Tuskegee, Alabama (32° 26' 15.59" N 85° 44' 0.870' W) in October 2019. The taxonomic classification of the soil is fine loamy, kaolinitic, thermic Typic Kanhapludults. The soil belongs to the Marvyn soil series, consisting of very deep, well-drained dark greyish brown loamy sand at 0-7 inches, a yellowish-brown sandy loam at 7-15 inches, and a sandy clay at a depth below 15 inches. This geographic location has a dominant slope range of 2-5%, mean annual temperature of 65°F, and annual precipitation of about 54 inches. The soil series are non-saline (0.5-2.0 mohms/cm) with strong to moderate acid reaction.

The collected topsoil (0-15cm) was sorted to remove large debris (plant residue and pebbles) manually, then passed through a 0.5cm mesh sieve. Tap water was added to the soil to evenly moisten, mixed thoroughly and then steam sterilized (Pro-Grow electric soil sterilizer, Model SS-60R, Brookfield, WI) at 82.2°C for 48 hours. The sterilized soil was air dried for four days followed by thorough mixing with potting soil (SUNGRO:#52 2.8 CUFT 42/PLT; Sun Gro Horticulture, Agawam, MA, USA) in a ratio of 2:1, respectively. The potted mixed soil moisture was determined gravimetrically as described in [28], [29]. Briefly, a 1000 ml graduated cylinder was drilled in the bottom to allow air to escape when water was added. The cylinder was filled with a random subsample of air-dried soil, which was tamped to a similar consistency as used in the pots. The surface of the soil was covered with a paper towel, and 100 ml of water was poured slowly onto the surface to obtain an even distribution through the column. The cylinder was covered by aluminum foil to avoid evaporation and allowed to equilibrate for 24 hours. Soil samples were collected from the cylinder in triplicate about 5 cm above the wetting front and dried at 105°C to a constant weight, after which dry weight was recorded and gravimetric moisture fraction was determined using the fresh to dry weight ratio. This information was used to determine the amount of oven-dry soil per pot and watering to field capacity. Watering treatments were calculated based on soil moisture equivalent to field capacity (FC) in percentages; that is 5.5kg of the air-dried mixed soil was transferred to each pot (22cm height x 22cm diameter) and watered with 935ml tap water to achieve 100% FC. Three soil water-stress treatments were established and classified as 25% FC, 50% FC, 75% FC, and the control

or optimum conditions at 100% FC. These FC treatment levels were applied to the plants after a fourteen-day non-watering period as severe drought.

Table 1. The list of fifteen cowpea cultivars evaluated for drought stress tolerance at Tuskegee University Agricultural Experiment Station, AL, USA (2019/2020)

SN	Plant name	Av. 1 Seed size (mg)	Status	Origin	Drought sensitivity	Other traits
1.	Mississippi Silver	163.33	Comm. Var	United States	Susceptible	Fusarium wilt (R), Root knot nematodes (R)
2.	Top Pick Brown Crowder	186.67	Comm. Var	United States	Tolerant	Resistant to diseases
3.	Top Pick Cream	133.33	Comm. Var	United States	Susceptible	Unknown
4.	Big Boy	340.00	Comm. Var	United States	Tolerant	Unknown
5.	California Blackeye No.5	186.67	Comm. Var	United States	Tolerant	Fusarium wilt (R), Nematodes (R)
6.	Lady	90.00	Comm. Var	United States	Tolerant	Unknown
7.	Pinkeye Purple Hull BVR	176.67	Comm. Var	United States	Tolerant	Blackeye Cowpea Mosaic Virus (R)
8.	Black Crowder	196.67	Comm. Var	United States	Tolerant	Unknown
9.	TVu 7362	120.00	PI	Nigeria	Unknown	Unknown
10.	TVu 11987	143.33	PI	Sudan	Unknown	Unknown
11.	LOBIA-I-SEFADE	243.33	PI	Afghanistan	Unknown	Unknown
12.	UCR 242	160.00	PI	Tanzania	Unknown	Unknown
13.	TVnu 113	30.00	PI	Tanzania	Unknown	Unknown
14.	K929	146.67	PI	Iraq	Unknown	Unknown
15.	White Acre	130.00	Comm. Var	United States	Tolerant	Early maturity

Av, average; Comm var, commercial variety; PI, plant introduction; R, Resistant. Phenotype information were obtained from [30], [31]

Experimental Design and Drought Stress Treatment

Experiments were conducted in 2019 (October 2019 – January 2020) at Tuskegee University, Agricultural Experiment Station (TU-AES) greenhouse. The experimental layout was a Randomized Complete Block Design (RCRD) as a split-plot with a 4 x 15 x 3 factorial treatments arrangement. The drought stress treatment levels (25% FC, 50% FC, 75% FC) and the 100% FC control were the main plots while sub-plots consisted of cowpea cultivars (Table 1) in both experiments. After filling each pot with mixed soil described above, all pots were watered to field capacity (100%) by adding 935 ml of tap water and left to sit for 24 hours. Five seeds of each cultivar were sown in each pot according to experimental design. Following seedlings emergence, thinning was performed to two fairly vigorous growing seedlings per pot, and soil moisture was maintained at field capacity until the first trifoliate leaves were fully expanded at 17 days after sowing. These 17 days post sowing (DPS) plants, except for the controls, were then subjected to a drought stress treatment by suspending watering for 14 days to mimic severe drought conditions. On the 15th day following the drought stress, watering resumed at 25% FC, 50% FC, 75% FC every 10-days per experimental layout. The controls were well-watered at 100% FC every three days without pre-drought treatment. These treatments were maintained throughout the remainder of the experimental period until maturity. Plants were

protected from insects by a weekly spray of pyrethrin concentrate (15cc/gallon of water) from flowering to maturity, and no fertilizers were applied.

Greenhouse Conditions

Weather parameters such as temperature and relative humidity were recorded using a HOBO U12-012 data logger (Onset Computer Corporation, MA, USA) throughout the experimental period. The average maximum and minimum temperature and relative humidity of the greenhouse are shown in Table 2. The average maximum temperature was either below or within the optimal range for cowpea (22 °C and 35 °C) as described by Singh, [9].

Table 2. Mean monthly greenhouse weather data during the experimental period

Year	Month	T _{max} (°C)	T _{min} (°C)	RH _{max} (%)	RH _{min} (%)
2019/2020	October	30.81	18.74	65.00	34.09
	November	36.87	13.23	87.32	42.07
	December	28.79	8.27	87.05	35.11
	January	32.15	13.42	84.43	43.24

T_{max}, average maximum temperature; T_{min}, average minimum temperature; RH_{max}, average maximum relative humidity; RH_{min}, average minimum relative humidity.

Data Collection and Statistical Analysis

Data on growth parameters were assessed at weekly intervals from drought treatments initiation at 18 DPS through the water-stress levels for four weeks except for terminal leaflet expansion rate, which was assessed at 3-day intervals on a tagged leaf from the time the leaf opened until when the leaf attained full expansion. Traits measured included plant height (PH) from 1 cm above soil level to the apex of the plant, stem diameter (St. D) measured 1.5cm above the soil level, chlorophyll content (Ch. C) by using chlorophyll concentration meter, Model MC-100 (Apogee instruments Logan, UT, USA), and expansion rate: which was determined by measuring the length (Tl. L) and width (Tl. W) of terminal leaflet of the second trifoliate leaf. At maturity, plants were harvested to determine yield components and seed yield. These were number of pods per plant (NP/P), seed yield per plant (SY/P) after shelling the dried pods and the seeds were weighed and weight recorded. Shoot (SDW) and root (RDW) dry biomass were determined by separating the shoots and roots that were carefully cleaned and then dried at 70°C until constant weight was attained, and dry weight recorded. All data were recorded as the average of two plants for each pot in Microsoft Office Excel and utilized for statistical analysis. The impact of drought stress on the performance of the cowpea cultivars was assessed based on the percent reduction for each of the studied traits across treatment levels [32], [33]. This assessment enables the estimation of the extent of reduction in performance for a given trait using the following formula:

$$\% \text{ Reduction } (\% \text{ Red}) = \frac{\text{performance without stress} - \text{performance with stress}}{\text{performance without stress}} \times 100\%$$

Performance was also assessed under drought stress and normal conditions using a stress tolerance index (STI) to enable relative comparison of the cultivars under drought stress conditions. STI of the fifteen cowpea cultivars was calculated based on cultivars means for morpho-physiological and yield-related traits in 25%, 50% and 75% FC by using the formula demonstrated by Fernandez, [34];

$$\text{Stress tolerance index; STI} = \frac{(Y_s)(Y_p)}{(Y\bar{p})^2}$$

Where Y_s , Y_p and $\bar{Y}$ are the performance of cultivars under drought stress, the performance of cultivars under optimum conditions and the overall mean of cultivars in optimum condition, respectively.

Data were combined and subjected to statistical analysis of variance using GenStat Release 16.1 (PC/Windows 8) software (www.genstat.kb.vsnl.co.uk). Means differences among the cultivars were observed at $P \leq 0.05$, whereby Fisher's unprotected least significant difference test was performed to separate the differences among the treatment means. Trait-effect by environment biplot (GGE-biplot) analysis was carried out for seed yield trait to determine the yield response of cultivars in each of the applied treatments. Pearson's correlation analysis was performed to examine the magnitude of the relationships among the studied traits. Correlation coefficients (r) were separately calculated for each treatment condition and control by using the same software.

RESULTS AND DISCUSSION

Significant phenotypic variations ($P < 0.001$) for the studied traits were observed (Table 3) among cultivars under well-watered and drought stress conditions, suggesting the likelihood of obtaining an effect at least as large as the one that was observed under the experimental conditions. That is, the likelihood of the observed effect was caused by some variable other than the one being studied or by chance. The cultivars x drought interaction was significant for chlorophyll content, shoot dry weight, number of pods per plant, and seed yield per plant. No significant effect was found for the interaction of cultivars and drought treatments for plant height, stem diameter, terminal leaflet length and width, and root dry weight.

Effects of drought stress on the morpho-physiological performance of cowpea cultivars

Generally, the performance of cowpea cultivars under well-watered conditions was better than under drought-stressed conditions. The imposition of water stress to plants slowed the rate of growth and influenced morpho-physiological characters like plant height, stem diameter, chlorophyll content, terminal leaf length and terminal leaf width. Visible effects of drought stress, which included slow growth, reduction of plant height and unifoliate and trifoliate leaf wilting, were observed from day 7 of withholding water during the 14 days prior to the initiation of water treatments (Figure 1). On day 14, other symptoms, including scorching and unifoliate leaf abscission, were observed in most of the cultivars. In contrast, cultivar TVnu 113 was initially characterized by slow growth in both conditions, which resulted in delayed depletion of soil moisture in water-stressed pots containing TVnu 113. As a result, this cultivar delayed the manifestation of drought stress symptoms; however, later, during experiment and treatment application, the pace of growth continued relative to other cultivars. Following the initiation of 25%, 50%, 75% field capacity (FC) treatments on the 15th day, recovery of all parameters was noticed but compared to the well-watered condition, performance was slower in the water-stressed pots. As anticipated, plant height, stem diameter, chlorophyll content, and terminal leaflet expansion rate were significantly decreased under water stress compared to well-watered pots. The reduced performance of the cultivars was more obvious in 25% FC at the reproductive stage, including increased leaf senescence, abscission, marginal chlorosis of leaves, flower, and premature pod drop but symptoms decreased with the reduction of soil moisture stress. On the contrary, the plant height for cultivar TVu 11987 in 25% FC was severely reduced. However, TVu 11987 and other cultivars including TVnu 113, White Acre and

LOBIA-I-SEFADE displayed a stay-green phenotype. Mississippi Silver (susceptible check) displayed more chlorotic leaves in 25% FC compared to other cultivars.

Table 3. Mean squares and significance tests for analysis of variance for nine morpho-physiological and yield traits of the 15 cowpea cultivars evaluated for drought stress tolerance under three drought treatment levels and well-watered conditions.

SOV	DF	PH (cm)	St. D (mm)	Ch. C (μmolm^{-2})	Tl. L (cm)
Cultivars (Cult.)	14	2,605.2***	2.522***	7773.6***	16.078***
Drought treatments (DT)	3	7,271.2***	0.951***	5526.4***	19.789***
Cult*DT	42	114.1 ^{ns}	0.0861 ^{ns}	504.9**	0.844 ^{ns}
Residual	118	126.3	0.0881	296.7	1.177
Grand mean		65.52	3.60	237.86	8.78

SOV	DF	Tl. W (cm)	SDW (g)	RDW (g)	NP/P	SY/P (g/p)
Cultivars (Cult.)	14	9.733***	8.367***	0.219***	2.868***	2.585***
Drought treatments (DT)	3	9.493***	20.494***	2.051***	20.586***	23.749***
Cult*DT	42	0.5698 ^{ns}	1.1612**	0.0843 ^{ns}	1.3445*	0.8724*
Residual	118	0.6217	0.6354	0.0677	0.9070	0.5611
Grand mean		5.76	2.26	0.43	2.20	1.98

SOV, source of variations; DF, degrees of freedom; PH, plant height; St. D, stem diameter; Ch. C, chlorophyll content; Tl. L, terminal leaflet length; Tl. W, terminal leaflet width; NP/P, number of pods per plant; SY/P, seed yield per plant; SDW, shoot dry weight; RDW, root dry weight; *P < 0.05; **P < 0.01; ***P < 0.001; ns, non-significant.



Figure 1. Picture of cowpea cultivars after (a) 7 days without watering in drought treated pots, (b) 14th day without watering, (c) Scorching and chlorosis of trifoliolate leaves as a result of drought stress on Big Boy in 50% FC, (d) Increasing drought stress impacted plant height and other traits, (e) Yield components of Mississippi Silver were impacted by increasing drought stress, (f) Cultivar TVu 11987 (left) displayed stay green phenotype whereas Mississippi Silver displayed more chlorotic leaves in 25% FC (right).

Percent reduction in performance for growth traits and chlorophyll content

Percent reduction in performance of plant height

There were variations among cultivars based on the percent reduction (% Red) for plant height due to drought stress from 25% FC to 100% FC. Plant height was reduced from -2.05 to 48.54% (Table 4). In 25% FC TVnu113 showed the lowest % Red (13.13%) for plant height while TVu 11987 had the highest (48.54%). Drought stress has been reported to cause significant reduction of plant height in cowpea [35], [36], which is consistent with the results of the present study. Cultivars with the lowest reduction of plant height under severe drought stress conditions can be considered promising drought-tolerant based on plant height trait.

Table 4. Percent reduction in performance of plant height for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean plant height performance (cm)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	46.03	54.48	55.36	73.45	37.33	25.83	24.63
Top Pick Brown Crowder	29.73	33.30	36.21	51.97	42.79	35.92	30.33
Top Pick Cream	51.62	56.37	61.14	84.12	38.64	32.99	27.32
Big Boy	51.61	68.15	68.29	86.33	40.22	21.06	20.90
California Blackeye No.5	67.5	65.35	66.75	92.37	26.92	29.25	27.74
Lady	44.37	45.96	70.61	69.19	35.87	33.57	-2.05
Pinkeye Purple Hull BVR	57.69	66.43	72.92	98.36	41.35	32.46	25.86
Black Crowder	55.34	51.57	79.76	93.94	41.09	45.10	15.09
TVu 7362	66.65	70.40	74.66	100.88	33.93	30.21	25.99
TVu 11987	48.38	58.38	64.53	94.02	48.54	37.91	31.37
LOBIA-I-SEFADE	60.85	83.62	75.95	94.29	35.47	11.32	19.45
UCR 242	37.05	45.44	48.50	69.14	46.41	34.28	29.85
TVnu 113	85.55	94.34	99.59	98.48	13.13	4.20	-1.13
K929	62.43	70.68	73.10	78.4	20.37	9.85	6.76
White Acre	34.08	36.99	38.65	59.81	43.02	38.15	35.38

Percent reduction in stem diameter performance

The stem diameter of the cultivars was influenced by drought treatment levels (Table 5). Drought stress reduced stem diameter by -5.12% (K929 in 25% FC) to 19.71% (UCR 242 in 25% FC). K929 showed the least %Red in stem diameter, followed by California Blackeye No. 5, which had 1.79%Red in 25% FC, meaning that these cultivars were more tolerant to drought stress based on stem diameter. In another study that involved soybean (*Glycine max*), drought stress-induced water retention in stem tissues which acts as buffering stock for growth. In turn, it reduces the distribution of water from the stem to other parts of the plant [37], [38]. The variation in stem diameter for cowpea cultivars under different drought treatment levels is an indication that, stem diameter may also be an excellent phenotypic parameter for evaluation of drought tolerance in cowpea.

Table 5. Percent reduction in performance of stem diameter for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean stem diameter performance (mm)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	3.53	3.46	3.71	3.92	9.95	11.73	0.29
Top Pick Brown Crowder	3.89	4.09	3.75	4.18	6.94	2.15	0.83
Top Pick Cream	3.49	3.40	3.61	3.72	6.18	8.60	0.13
Big Boy	4.01	4.40	4.31	4.42	9.28	0.45	0.13
California Blackeye No.5	3.85	3.75	3.67	3.92	1.79	4.34	0.27
Lady	3.65	3.57	3.61	4.04	9.65	11.63	0.62
Pinkeye Purple Hull BVR	3.44	3.66	3.68	3.93	12.47	6.87	0.25
Black Crowder	3.61	4.04	3.84	3.86	6.48	-4.66	0.02
TVu 7362	3.40	3.55	3.64	3.97	14.36	10.58	0.33
TVu 11987	3.96	3.98	4.02	4.25	6.82	6.35	0.24
LOBIA-I-SEFADE	3.55	3.56	2.85	3.72	4.57	4.30	0.92
UCR 242	3.38	3.59	3.65	4.21	19.71	14.73	0.81
TVnu 113	2.42	2.35	2.50	2.85	15.09	17.54	0.36
K929	2.67	2.66	2.95	2.54	-5.12	-4.72	-0.52
White Acre	3.40	3.33	3.46	3.69	7.86	9.76	0.38

Percent reduction in chlorophyll content

Among cowpea cultivars, reduction in chlorophyll content reduction was lower in TVu 11987, White Acre, LOBIA-I-SEFADE and TVnu 113. These cultivars displayed stay green phenotypes under 25% FC drought conditions which enhanced delayed leaf senescence for these cultivars. Leaf chlorophyll content percent reduction ranged from -16.23% for Top Pick Cream in 75% FC to 65.16% for LOBIA-I-SEFADE in 75% FC. On the other hand, the average chlorophyll content for the cultivars ranged from 172.3 μmolm^{-2} for TVnu 113 at 50% FC to 293.15 μmolm^{-2} for TVu 11987 in 100% FC (Table 6.). Top Pick Cream recorded the lowest %Red (1.92%) for chlorophyll content in a more drought condition, whereas California Blackeye No.5 had the highest percent reduction (21.67%) in the same treatment condition. Under well-watered conditions, the minimum average chlorophyll content was 195.38 μmolm^{-2} for K929, while the maximum chlorophyll content was 293.15 μmolm^{-2} for TVu 11987. These results are consistent with the findings of Sheikh et al., [39]. In other studies, chlorophyll content has been correlated with drought tolerance in crop plants, therefore, this parameter can be used as selection criteria, especially in the assessment of the degree of leaf damage under drought stress conditions [40]. The stay-green trait could have implication on the regulation of plant size, and hence water demand and utilization by the crop, and the environment that regulates water supply by the soil [41]. Therefore, the stay-green trait could be considered as an important selection criterion for cowpea cultivars with the ability to cope with drought stress conditions as also noted in this study.

Table 6. Percent reduction in performance of chlorophyll content for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean chlorophyll content (μmolm^{-2})				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	221.62	255.6	241.12	259.42	14.57	1.48	24.91
Top Pick Brown Crowder	243.76	234.6	258.83	254.21	4.11	7.70	-8.89
Top Pick Cream	239.2	270.5	257.53	243.88	1.92	-10.91	-16.23
Big Boy	224.3	223.7	246.85	263.32	14.80	15.04	19.08
California Blackeye No.5	225.67	250.2	244.96	288.09	21.67	13.15	46.69
Lady	229.33	242.1	232.98	258.50	11.28	6.35	36.88
Pinkeye Purple Hull BVR	242.2	245.0	246.68	270.18	10.36	9.33	23.89
Black Crowder	240.83	257.3	248.53	251.36	4.19	-2.36	3.01
TVu 7362	221.18	208.8	230.21	235.74	6.18	11.42	5.48
TVu 11987	274.35	274.3	291.69	293.15	6.41	6.44	1.55
LOBIA-I-SEFADE	238.48	222.9	184.76	246.20	3.14	9.47	65.16
UCR 242	204.4	204.6	202.49	244.44	16.38	16.29	60.67
TVnu 113	182.58	172.3	189.43	216.75	15.76	20.51	27.74
K929	188.13	178.8	202.29	195.38	3.71	8.48	-8.81
White Acre	257.57	256.1	252.96	289.07	10.90	11.41	60.37

Percent reduction in terminal leaflet expansion rate

The percent terminal leaf length and width reduction, due to water stress, varied from -9.09% to 24.61% and -13.12% to 28.90%, respectively (Table 7 and 8). Cultivars that had the lowest %Red for terminal leaf length and width at 25% FC were LOBIA-I-SEFADE (-0.60%), White Acre (1.75%), and Lady (6.15%) for terminal leaf length and LOBIA-I-SEFADE (-13.12%), Lady (3.43%) and White Acre (4.04%) for terminal leaf width. Under no stress conditions, Mississippi silver had the highest average leaf length (11.50 cm), and the lowest average value for leaf length was found on TVnu 113 (6.56 cm). These results indicate that drought stress impacted cell division and expansion, causing reduction of terminal leaf length and terminal leaf width due to smaller and more compact cells and greater specific leaf weight [42]. Also, Verelst et al., [43] found that, the reduction of leaf length was not due to reduction in cell numbers of the drought-stressed leaves of *Brachypodium distachyon*, but instead, the smaller length was almost entirely accounted for by a reduced mature cell size. Similar mechanisms could be accounted for reduction of leaf width observed under the present study.

Table 7. Percent reduction in performance of terminal leaf length for cowpea cultivars under drought stress treatment levels

Cultivars	Mean terminal leaf length (cm)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	8.67	9.32	10.15	11.50	24.61	18.96	11.74
Top Pick Brown Crowder	8.59	9.35	8.44	10.73	19.94	12.86	21.34
Top Pick Cream	8.80	8.31	8.50	10.15	13.30	18.13	16.26
Big Boy	9.12	9.72	9.61	10.97	16.86	11.39	12.40
California Blackeye No.5	9.44	9.51	8.20	10.81	12.67	12.03	24.14
Lady	8.70	8.82	9.09	9.27	6.15	4.85	1.94
Pinkeye Purple Hull BVR	8.32	8.79	9.41	10.56	21.21	16.76	10.89
Black Crowder	9.21	9.37	9.31	10.59	13.03	11.52	12.09
TVu 7362	8.27	8.87	9.05	10.41	20.56	14.79	13.06
TVu 11987	9.04	8.73	8.13	10.16	11.02	14.07	19.98
LOBIA-I-SEFADE	8.36	7.45	6.51	8.31	-0.60	10.35	21.66
UCR 242	8.29	8.78	8.15	9.85	15.84	10.86	17.26
TVnu 113	5.18	5.59	5.96	6.56	21.04	14.79	9.15
K929	6.32	5.92	7.68	7.04	10.23	15.91	-9.09
White Acre	9.53	8.65	9.00	9.70	1.75	10.82	7.22

Table 8. Percent reduction in performance of terminal leaf width for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean terminal leaf width (cm)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	5.85	6.16	6.71	7.72	24.22	20.21	13.08
Top Pick Brown Crowder	5.59	6.20	5.68	7.29	23.32	14.95	22.09
Top Pick Cream	4.97	4.97	5.13	6.46	23.07	23.07	20.59
Big Boy	6.15	6.73	6.56	7.42	17.12	9.30	11.59
California Blackeye No.5	6.28	6.37	5.87	7.23	13.14	11.89	18.81
Lady	6.20	6.20	5.83	6.42	3.43	3.43	9.19
Pinkeye Purple Hull BVR	4.86	5.38	5.51	6.17	21.23	12.80	10.70
Black Crowder	5.55	6.19	6.01	6.80	18.38	8.97	11.62
TVu 7362	5.77	6.13	6.40	7.65	24.58	19.87	16.34
TVu 11987	6.21	5.75	5.29	6.93	10.39	17.03	23.67
LOBIA-I-SEFADE	6.38	5.13	4.01	5.64	-13.12	9.04	28.90
UCR 242	5.36	6.11	5.66	6.66	19.52	8.26	15.02
TVnu 113	3.15	3.37	3.36	3.63	13.22	7.16	7.44
K929	4.24	3.81	4.84	4.48	5.36	14.96	-8.04
White Acre	5.94	5.48	5.63	6.19	4.04	11.47	9.05

Percent reduction in performance for yield-related traits

Yield-related traits are critical components for increasing cowpea yield. Seed yield increase can be achieved through the manipulation of yield-related traits such as the pod length, pod number, and number of seeds per pod[44]. Significant differences in yield components and seed yield among cultivars under well-watered and in drought stress conditions are shown in Tables 9 and 10. Cultivar LOBIA-I-SEFADE had the highest seed yield across all treatment conditions

(Table 10). Drought stress reduced yield component traits and seed yield; however, cultivars responded differently to stress treatments. The number of pods per plant was reduced by up to 72%. At 25% FC the number of pods per plant for cultivar TVnu 113, Black Crowder, Top Pick Brown Crowder were reduced by -275.00, 33.33 and 40.00, respectively. These were the top three cultivars that had a lower reduction in number of pods per plant and were considered the better drought adapted cultivars. The reduction in the number of pods per plant under drought stress conditions for most of the cultivars could be associated with abscission of flowers and young pods as a result of drought stress during the early stages of reproductive growth. In contrast cultivar TVnu 113 attained the lowest mean number of pods per plant and seed yield in control conditions (0.8 pods/plant) as compared to when in drought conditions (3.0, 3.5 and 4.5 pods/plant ni 25% FC, 50% FC, and 75% FC respectively). It is probable that, TVnu 113 performs better in average soil moisture conditions and the cultivar might have better water use efficiency (WUE) under stress conditions when compared to well-watered conditions. Cultivar LOBIA-I-SEFADE had the highest mean seed yield in high-stress conditions (2.37g/plant) and showed similar trends across all treatments such as 2.42 g/plant, 2.05 g/plant and 3.23 g/plant in 50 %FC, 75% FC and in 100 % FC, respectively. The better seed yield stability of LOBIA-I-SEFADE across treatments is of significant concern in regard to drought stress tolerance. Other cultivars, including susceptible check (Mississippi Silver), displayed a higher percent reduction for the number of pods and seed yield with increasing soil moisture stress. Observations of this kind were reported in other studies [30], [45]–[47].

Table 9. Percent reduction in performance of number of pods per plant for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean number of pods per plant				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	0.50	1.20	1.80	1.80	72.22	33.33	0.00
Top Pick Brown Crowder	1.80	2.50	2.30	3.00	40.00	16.67	23.33
Top Pick Cream	1.50	2.00	2.80	2.80	46.43	28.57	0.00
Big Boy	1.00	1.80	1.70	2.30	56.52	21.74	26.09
California Blackeye No.5	1.70	2.20	2.30	3.30	48.48	33.33	30.30
Lady	1.20	2.80	3.50	4.30	72.09	34.88	18.60
Pinkeye Purple Hull BVR	1.00	1.30	1.50	2.80	64.29	53.57	46.43
Black Crowder	1.00	1.30	1.70	1.50	33.33	13.33	-13.33
TVu 7362	1.00	1.70	3.20	3.70	72.97	54.05	13.51
TVu 11987	1.20	1.80	2.50	2.50	52.00	28.00	0.00
LOBIA-I-SEFADE	1.30	1.50	2.30	4.00	67.50	62.50	42.50
UCR 242	1.00	1.30	3.70	2.70	62.96	51.85	-37.04
TVnu 113	3.00	3.50	4.50	0.80	-275.00	-337.50	-462.50
K929	1.50	2.20	3.00	2.80	46.43	21.43	-7.14
White Acre	1.30	2.00	2.80	3.50	62.86	42.86	20.00

Table 10. Percent reduction in performance of seed yield per plant for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean seed yield (g/plant)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	0.56	2.34	2.64	2.76	79.71	15.22	4.30
Top Pick Brown Crowder	1.09	2.09	2.26	3.08	64.61	32.14	26.60
Top Pick Cream	0.96	1.82	2.57	1.94	50.52	6.19	-32.50
Big Boy	0.59	1.65	2.21	3.69	84.01	55.28	40.10
California Blackeye No.5	1.43	2.02	2.90	3.82	62.57	47.12	24.10
Lady	0.70	1.52	2.33	3.31	78.85	54.08	29.60
Pinkeye Purple Hull BVR	0.94	2.14	2.24	3.72	74.73	42.47	39.80
Black Crowder	0.82	1.25	2.25	1.29	36.43	3.10	-74.40
TVu 7362	0.50	1.16	2.89	2.53	80.24	54.15	-14.20
TVu 11987	1.34	2.17	2.62	3.15	57.46	31.11	16.80
LOBIA-I-SEFADE	2.37	2.42	2.05	3.23	26.63	25.08	36.50
UCR 242	0.87	1.70	2.59	2.65	67.17	35.85	2.30
TVnu 113	0.83	0.82	1.03	0.03	-2666.67	-2633.33	-3333.3
K929	1.18	2.13	2.98	1.75	32.57	-21.71	-70.30
White Acre	1.33	2.01	2.82	2.95	54.92	31.86	4.40

Based on the seed yield attribute, the ranking of the average performance of cultivars in each treatment by GGE biplot analysis (Figure 2) enabled the identification of cultivars with greater yield stability under drought stress conditions. The blue vectors in Figure 1 denote the treatments and well-watered conditions applied in the experiments (25% FC, 50% FC, 75% FC, and 100% FC). Cultivars with like performance tend to group together for each of the conditions, while the ones close to vectors origin are classified to have average performance. Hence, in that regard, cultivars LOBIA-I-SEFADE, TVu 11987, White Acre and Top Pick Brown Crowder were clustered in 25% FC condition, meaning that these cultivars had high performance and stability for seed yield trait under the environment of high soil moisture stress (25% FC).

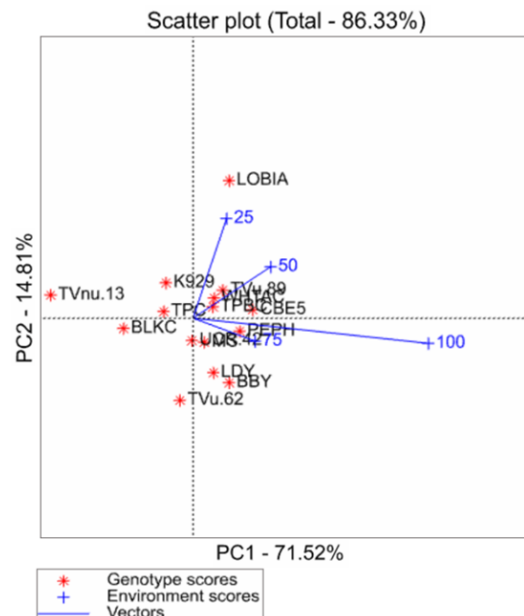


Figure 2: GGE biplot showing cultivar main effect and cultivar by drought stress treatments interaction for seed yield. The cowpea cultivars are MS, Mississippi silver; TPBC, Top Pick Brown Crowder; TPC, Top Pick Cream; BBY, Big Boy; CBE5, California Blackeye #5; LDY, Lady; PEPH, Pinkeye Purple Hull; BLKC, Black Crowder; TVu.62, TVu7362; TVu.87, TVu11987; LOBIA, Lobia-I-Sefade; UCR.42, UCR242; TVnu.13, TVnu113; K929 and WHTA, White Acre.

Ahmed & Suliman, [44] indicated that the final cowpea yield is dependent upon the number of pods per plant, the number of grains per pod and the extent to which grains are filled. Tolerant cultivars tend to accumulate high total biomass and pod yield as compared to susceptible cultivars [48]. Our results indicate that cultivar TVnu 113 had an exceptional low reduction of the shoot and root biomass (-47.95%) under drought conditions. Other cultivars displaying reduced percent reduction for biomass in high soil moisture stress were Mississippi Silver (40.35%), and Lady (47.52% and 8.51% respectively) (Tables 11 and 12). The highest reduction was found in K929 with 71.77%. Generally, the percent reduction for biomass traits increased with increasing moisture stress. However, some cultivars exhibited low reduction for the shoot and root biomass at high-stress conditions. Other studies suggest that increased root biomass under drought conditions may be an indication of increased plant ability to maintain its water content in water stress conditions [20], [21]. In that regard, cultivars with a low percent reduction for these traits indicates their ability to accumulate high plant biomass under drought conditions, and they may be considered drought-tolerant cultivars.

Table 11. Percent reduction in performance of shoot dry weight for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean shoot dry weight (g)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	1.70	1.40	2.17	2.85	40.35	50.88	23.86
Top Pick Brown Crowder	1.07	1.35	1.77	2.23	52.02	39.46	20.63
Top Pick Cream	0.88	1.03	1.63	2.15	59.07	52.09	24.19
Big Boy	1.27	2.27	2.65	3.48	63.51	34.77	23.85
California Blackeye No.5	0.78	1.75	1.47	2.30	66.09	23.91	36.09
Lady	1.80	2.02	2.78	3.43	47.52	41.11	18.95
Pinkeye Purple Hull BVR	1.33	1.52	1.92	2.85	53.33	46.67	32.63
Black Crowder	1.63	3.20	3.32	3.58	54.47	10.61	7.26
TVu 7362	1.23	1.95	1.63	4.15	70.36	53.01	60.72
TVu 11987	1.72	2.73	3.22	4.42	61.09	38.24	27.15
LOBIA-I-SEFADE	1.18	1.77	1.73	3.27	63.91	45.87	47.09
UCR 242	1.03	1.45	3.48	3.48	70.40	58.33	0.00
TVnu 113	4.32	5.97	5.62	2.92	-47.95	-104.45	-92.47
K929	0.70	1.50	1.68	2.48	71.77	39.52	32.26
White Acre	1.18	1.33	1.72	2.40	50.83	44.58	28.33

Table 12. Percent reduction in performance of root dry weight for cowpea cultivars under three drought stress treatment levels

Cultivars	Mean root dry weight (g)				Percent reduction (%)		
	25%FC	50%FC	75%FC	100%FC	25%FC	50%FC	75%FC
Mississippi Silver	0.25	0.25	0.52	0.78	67.95	67.95	33.33
Top Pick Brown Crowder	0.25	0.25	0.28	0.47	46.81	46.81	40.43
Top Pick Cream	0.17	0.33	0.37	0.77	77.92	57.14	51.95
Big Boy	0.12	0.32	0.38	0.75	84.00	57.33	49.33
California Blackeye No.5	0.17	0.23	0.23	0.32	46.88	28.13	28.13
Lady	0.27	0.23	0.50	0.50	46.00	54.00	0.00
Pinkeye Purple Hull BVR	0.13	0.35	0.23	0.53	75.47	33.96	56.60
Black Crowder	0.13	0.47	0.77	1.20	89.17	60.83	35.83
TVu 7362	0.15	0.28	0.28	1.02	85.29	72.55	72.55
TVu 11987	0.27	0.53	0.92	1.05	74.29	49.52	12.38
LOBIA-I-SEFADE	0.47	0.30	0.40	1.23	61.79	75.61	67.48
UCR 242	0.20	0.28	0.28	0.87	77.01	67.82	67.82
TVnu 113	0.43	0.68	0.70	0.47	8.51	-44.68	-48.94
K929	0.22	0.20	0.32	0.40	45.00	50.00	20.00
White Acre	0.27	0.32	0.38	0.57	52.63	43.86	33.33

Stress tolerance indices

Stress tolerance index (STI) is used to identify genotypes that can produce high yield both under stress and optimum conditions [34]. The STI procedure has successfully been applied in the identification and selection of cowpea genotypes with a high level of tolerance to drought [36]. In this study, the performance for each trait and cultivar was assessed at 25% FC, 50% FC, 75% FC and under normal conditions and averages were calculated, followed by the

determination of grand mean. Cultivars with average STI above the grand mean of STI were considered drought tolerant. Stress tolerance indices of the fifteen cowpea cultivars in 25% FC are presented in Table 13. Based on STI in 25% FC, cultivars TVu 11987, LOBIA-I-SEFADE, and TVu 7362 were identified as drought tolerant along with confirmed tolerant cultivars California Blackeye No.5, Big Boy, and Lady. These cultivars attained mean STI values greater than the grand mean value of STI (0.66) (Table 13). In contrast, other cultivars were categorized as moderately or sensitive to drought. K929 had the least mean STI value (0.43), indicating high sensitivity to drought stress. However, the remaining cultivars were either moderately susceptible or susceptible to drought stress. These results correspond to the findings of Ajayi et al., [36]. Generally, drought-tolerant cultivars exhibited different stress-coping strategies of low water requirements growth performance to yield reduction. Overall, the phenotypic performance recorded as drought-tolerant characteristics may be recommended as potential screening factors for donor cultivar traits in cowpea breeding programs.

Table 13. Stress tolerance indices (STI) based on growth and yield characteristics in 25%

Cultivars	Stress tolerance indices										Status
	SDW	RDW	NP/P	SY/P	PH	St. D	Ch. C	TL. L	TL. W	Av	
Mississippi Silver	0.52	0.37	0.11	0.22	0.49	0.95	0.89	1.04	1.09	0.63	S
Top Pick Brown	0.25	0.22	0.68	0.47	0.22	1.12	0.96	0.97	0.98	0.65	MS
Top Pick Cream	0.20	0.25	0.53	0.26	0.63	0.89	0.90	0.94	0.77	0.60	S
Big Boy	0.47	0.17	0.29	0.31	0.65	1.22	0.92	1.05	1.10	0.69	T
California Blackeye No.5	0.19	0.10	0.71	0.77	0.91	1.04	1.01	1.07	1.09	0.77	T
Lady	0.66	0.25	0.65	0.33	0.45	1.02	0.92	0.84	0.96	0.68	T
Pinkeye Purple Hull BVR	0.40	0.13	0.36	0.49	0.82	0.93	1.01	0.92	0.72	0.64	S
Black Crowder	0.62	0.29	0.19	0.15	0.75	0.96	0.94	1.02	0.91	0.65	MS
TVu 7362	0.54	0.29	0.47	0.18	0.98	0.93	0.81	0.90	1.06	0.68	T
TVu 11987	0.81	0.54	0.38	0.60	0.66	1.16	1.25	0.96	1.04	0.82	T
LOBIA-I-SEFADE	0.41	1.09	0.66	1.08	0.83	0.91	0.91	0.73	0.87	0.83	T
UCR 242	0.38	0.33	0.34	0.33	0.37	0.98	0.77	0.86	0.86	0.58	S
TVnu 113	1.34	0.38	0.30	0.00	1.22	0.48	0.61	0.36	0.28	0.55	S
K929	0.18	0.17	0.53	0.29	0.71	0.47	0.57	0.47	0.46	0.43	S
White Acre	0.30	0.29	0.58	0.55	0.30	0.86	1.15	0.97	0.88	0.65	MS
Grand mean STI	0.66										

SDW., dry weight of the shoot; RDW, root dry weight; NP/P, number of pods per plant; SY/P, seed yield per plant; PH, Plant height; St. D, stem diameter; Ch. C, chlorophyll content; TL. L, terminal leaf length; TL. W, terminal leaf width; Av, Average; MS, moderately susceptible; S, susceptible to drought; T, tolerant to drought.

Correlation analysis

A positive and significant correlation was observed between NP/P and SY/P under both stress and well-watered conditions with coefficients of 0.484*** and 0.705***, respectively (Table 14). Hence, this is an indication that number of pods can serve as an important criterion for the selection of cowpea cultivars with high yield potential in both drought and well-watered conditions. These results are consistent with prior reports [44], which mean that selecting cowpea cultivars with high pod productivity under drought stress conditions will potentially contribute to increased seed yield. Furthermore, highly significant ($P < 0.001$), and positive

associations were found among other traits, including Ch. C and St. D, Tl. L and Tl. W in 25% FC. In 100% FC conditions, significant associations were also revealed between Ch. C. and St. D, Tl. L and Tl. W and SY/P. The positive direct effects of Tl. L and Tl. W. might be associated with increasing leaf sizes and Ch. C. of the leaves. Wider leaves mean more leaf surface area to capture light for photosynthesis, which can contribute to more pods produced and final seed yield. However, reduced leaf sizes may serve as an avoidance mechanism utilized by the plants to protect themselves from the effect of water stress [49], [50]. PH and SDW showed positive and significant association in both 25% FC and 100% FC. In 25% FC RDW was positive and significantly associated with SY/P, but it was not significant under 100% FC, while a positive and significant relationship existed between RDW and SDW in both 25% FC and in 100% FC. St. D., Tl. L and Tl. W were positive and significantly associated with SY/P in 100% FC, but they were not significant in 25% FC. This is an indication that these parameters cannot be relied on for the selection of cowpea cultivar with better yield potential in drought conditions.

Table 14. Pearson Correlation coefficients (r) showing the relationship among traits in 100% FC (above diagonal) and 25% FC (below diagonal)

	Ch. C	NP/P	PH	RDW	SY/P	SDW	St. D	Tl. L	Tl. W
Ch. C	-	0.269	0.034	0.071	0.535***	0.143	0.656***	0.627***	0.563***
NP/P	-0.065	-	0.089	0.066	0.705***	0.160	0.339	0.238	0.296
PH	-0.321	0.401	-	0.289	0.055	0.478***	0.127	0.201	0.128
RDW	0.028	0.429	0.357	-	0.080	0.589***	0.257	0.171	0.214
SY/P	0.318	0.484***	0.186	0.516***	-	0.211	0.637***	0.548***	0.566***
SDW	-0.167	0.462	0.529***	0.539***	-0.042	-	0.470	0.303	0.379
St. D	0.656***	-0.081	-0.276	-0.005	0.230	-0.243	-	0.807***	0.824***
Tl. L	0.618***	-0.100	-0.266	0.011	0.319	-0.294	0.826***	-	0.942
Tl. W	0.527***	-0.100	-0.167	0.201	0.423	-0.252	0.800***	0.903***	-

Ch. C, chlorophyll content; NP/P, number of pods per plant; PH, Plant height; RDW, root dry weight; SY/P, seed yield per plant; SDW, dry weight of the shoot; St. D, stem diameter; Tl. L, terminal leaf length; Tl. W, terminal leaf width

CONCLUSION

Fifteen cowpea cultivars were evaluated under well-watered and drought-stress conditions in greenhouse pot experiments with the aim of understanding morpho-physiological response mechanisms of known drought-tolerant cultivars under water-deficit stress. This is critical to identifying and establishing representative growth and yield-related traits needed for breeding climate-hardy cowpeas. The analysis of variance revealed significant variations for the studied morpho-physiological traits that included PH, St. D, Ch. C, Tl. L, and Tl. W. Others were yield-related traits, including the NP/P, SY/P, SDW and RDW. Increasing drought stress resulted in a significant percent reduction for most of the studied traits compared to plants that were subjected to well-watered conditions. Cowpea cultivars that exhibited the lowest percent reduction for the measured trait under high soil moisture stress conditions were considered promising drought-tolerant cultivars. According to our findings, cultivars TVu 11987, LOBIA-I-SEFADE, and TVu 7362 were identified as drought tolerant along with confirmed tolerant cultivars California Blackeye No.5, Big Boy, and Lady. Of all the tested parameters, seed production is most likely warranted by cowpea producers as cultivar LOBIA-I-SEFADE, showed the highest weight of seeds produced at 25% FC. Drought tolerant cultivars exhibited different stress-coping strategies of low water requirements, growth performance to yield reduction.

Overall, the phenotypic performance recorded as drought-tolerant characteristics may be recommended as potential screening factors for donor cultivar traits in cowpea breeding programs.

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References

- [1]O. M. Aliyu, O. O. Lawal, A. Wahab, and U. Y. Ibrahim, "Evaluation of Advanced Breeding Lines of Cowpea (*Vigna unguiculata* L . Walp) for High Seed Yield under Farmers Field Conditions Evaluation of Advanced Breeding Lines of Cowpea (*Vigna unguiculata* L . Walp) for High Seed Yield under Farmers ' Field Cond," no. March, 2019, doi: 10.9787/PBB.2019.7.1.12.
- [2]T. M. Gondwe, E. O. Alamu, P. Mdziniso, and B. Maziya-Dixon, "Cowpea (*Vigna unguiculata* (L.) Walp) for food security: an evaluation of end-user traits of improved varieties in Swaziland," *Sci. Rep.*, vol. 9, no. 1, pp. 1–6, Dec. 2019, doi: 10.1038/s41598-019-52360-w.
- [3]FAOSTAT, "FAOSTAT statistical database," 2019. [Online]. Available: <http://www.fao.org/faostat/en/#data/QC>.
- [4]O. A. Osipitan, J. S. Fields, S. Lo, and I. Cuvaca, "Production Systems and Prospects of Cowpea (*Vigna unguiculata* (L.) Walp.) in the United States," *Agronomy*, vol. 11, no. 2312, pp. 1–10, Nov. 2021, doi: 10.3390/AGRONOMY11112312.
- [5]R. L. Fery, "The cowpea: Production, utilization, and utilization in the United States," *Hortic. Rev. (Am. Soc. Hortic. Sci)*, vol. 12, pp. 197–222, 1990.
- [6]W. F. Wight, *The History of the Cowpea and its Introduction Into America*. Washington, DC, USA: US Government Printing Office, 1907.
- [7]USDA National Agricultural Statistics Service, "United States Summary and State Data," 2014.
- [8]J. Quinn and R. Mayers, "COWPEA A Versatile Legume for Hot, Dry Condi-tions," 2002.
- [9]B. B. Singh, *Cowpea: The Food Legume of the 21st Century*. Madison WI, USA: Crop Science Society of America, 2014.
- [10]D. Riley, "Cowpea profiles," *Newsletter by the Coastal Plain Experiment Station. Tifton, GA 31793*, no. 1, pp. 1–4, 2016.
- [11]Iowa State University extension and Outreach, "Cowpea," *Alternative Crop Production*, 2013. [Online]. Available: <https://www.extension.iastate.edu/alternativeag/cropproduction/cowpea.html>.
- [12]Q. Cui *et al.*, "Evaluation of Drought Tolerance in Arkansas Cowpea Lines at Seedling Stage," *HortScience*, vol. 55, no. 7, pp. 1132–1143, 2020, doi: 10.21273/hortsci15036-20.
- [13]G. Kaur and B. Asthir, "Molecular responses to drought stress in plants," *Biologia Plantarum*, vol. 61, no. 2. Springer Netherlands, pp. 201–209, 01-Jun-2017, doi: 10.1007/s10535-016-0700-9.
- [14]P. R. Shukla *et al.*, "Technical Summary: Climate Change and Land," Prajal Pradhan, 2019.
- [15]C. A. Fatokun, O. Boukar, and S. Muranaka, "Evaluation of cowpea (*Vigna unguiculata* (L.) Walp.) germplasm lines for tolerance to drought," *Plant Genet. Resour. Characterisation Util.*, vol. 10, no. 3, pp. 171–176, 2012, doi: 10.1017/S1479262112000214.
- [16]L. Horn, H. Shimelis, and M. Laing, "Participatory appraisal of production constraints, preferred traits and farming system of cowpea in the northern Namibia: implications for breeding," *Legum. Res.*, vol. 38, no. 5, pp. 691–700, 2015, doi: 10.18805/lr.v38i5.5952.

- [17]M. A. M. Barbosa, A. K. da S. Lobato, M. H. L. da Silva, G. M. M. and Marques, and D. José, "Cowpea Breeding for Drought Tolerance — From Brazil to World," in *Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives*, IntechOpen, 2016, pp. 565–584.
- [18]O. Boukar *et al.*, "Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding," *Plant Breeding*, vol. 138. Blackwell Publishing Ltd, pp. 415–424, 2018, doi: 10.1111/pbr.12589.
- [19]W. Muchero, J. D. Ehlers, and P. A. Roberts, "Seedling stage drought-induced phenotypes and drought-responsive genes in diverse cowpea genotypes," *Crop Sci.*, vol. 48, no. 2, pp. 541–552, 2008, doi: 10.2135/cropsci2007.07.0397.
- [20]P. Ramamoorthy, K. Lakshmanan, H. D. Upadhyaya, V. Vadez, and R. K. Varshney, "Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.)," *F. Crop. Res.*, vol. 201, pp. 146–161, Feb. 2017, doi: 10.1016/J.FCR.2016.11.004.
- [21]R. Santos, M. Carvalho, E. Rosa, V. Carnide, and I. Castro, "Root and Agro-Morphological Traits Performance in Cowpea under Drought Stress," *Agronomy*, vol. 10, no. 1601, pp. 1–20, 2020, doi: 10.3390/agronomy10101604.
- [22]L. Mwadzingeni, H. Shimelis, S. Tesfay, and T. J. Tsilo, "Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses," *Front. Plant Sci.*, vol. 7, no. 1276, pp. 1–12, 2016, doi: 10.3389/fpls.2016.01276.
- [23]E. M. Agbicodo, C. A. Fatokun, S. Muranaka, R. G. F. Visser, and C. G. Linden Van Der, "Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects," *Euphytica*, vol. 167, pp. 353–370, Jun. 2009, doi: 10.1007/s10681-009-9893-8.
- [24]S. N. Nigam *et al.*, "Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut," *Ann. Appl. Biol.*, vol. 146, no. 4, pp. 433–439, 2005, doi: 10.1111/j.1744-7348.2005.040076.x.
- [25]T. Matsui and B. B. Singh, "Root Characteristics in Cowpea Related to Drought Tolerance at the Seedling Stage," *Exp. Agric.*, vol. 39, no. 1, pp. 29–38, Jan. 2003, doi: 10.1017/S0014479703001108.
- [26]B. B. Singh, T. Terao, and K. Station, "A simple screening method for drought tolerance in cowpes," *Indian J. Genet.*, vol. 59, no. 2, pp. 211–220, 1999.
- [27]I. Watanabe, S. Hakoyama, T. Terao, and B. B. Singh, "Advances in cowpea research," in *Cowpea, Evaluation methods for drought tolerance of cowpea*, B. B. Singh, D. R. Mohan, K. E. Dashiell, and L. E. Jackai, Eds. IITA, Ibadan, Nigeria: Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), 1997.
- [28]W. H. Gardner, "Water content," in *Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods*, (2nd Editi., Madison, WI 53711: American Society of Agronomy-Soil Science Society of America, 1986, pp. 493–544.
- [29]A. Shukla *et al.*, "Soil Moisture Estimation using Gravimetric Technique and FDR Probe Technique : A Comparative Analysis," *Am. Int. J. Res. Formal, Appl. Nat. Sci. AIJRFANS*, pp. 89–92, 2014.
- [30]R. B. Dadson, F. M. Hashem, I. Javaid, J. Joshi, A. L. Allen, and T. E. Devine, "Crop/ Stress Physiology," *J. Agron. Crop Sci.*, vol. 191, pp. 210–217, 2005.
- [31]O. J. Olorunwa, A. Shi, and T. C. Barickman, "Varying drought stress induces morpho-physiological changes in cowpea (*Vigna unguiculata* (L.) genotypes inoculated with *Bradyrhizobium japonicum*," *Plant Stress*, vol. 2, pp. 1–13, 2021, doi: 10.1016/j.stress.2021.100033.
- [32]J. C. Molina, V. Moda-Cirino, N. S. Fonseca Júnior, R. T. Faria, and D. Destro, "Response of Common Bean Cultivars and Lines to Water Stress," *Crop. Breed. Appl. Biotechnol.*, vol. 1, no. 4, pp. 363–372, 2001, doi: 10.13082/1984-7033.v01n04a05.
- [33]M. A. Ndimbo, S. Nchimbi-Msolla, and E. Semu, "Effect of Nitrogen Fixation on Yield and some Yield Component of Common Bean (*Phaseolus vulgaris* L.) Genotypes under Moisture Stress," *Nat. Sci. Res.*, vol. 5, no. 18, pp. 95–103, 2015.

- [34]G. C. . Fernandez, "Effective Selection Criteria for Assessing Plant Stress Tolerance," in *Asian vegetable research and development centre*, 1992, pp. 257–270.
- [35]R. L. Karuwal, Suharsono, A. Tjahjoleksono, and N. Hanil, "Identification of Drought-tolerant Local Cowpea Varieties of Southwest Maluku (Indonesia)," *Makara J. Sci.*, vol. 22, no. 4, pp. 179–186, 2018, doi: 10.7454/mss.v22i4.10257.
- [36]A. T. Ajayi, A. E. Gbadamosi, V. O. Olumekun, and I. O. Omotuyi, "Variable Expression of the Candidate Gene NCED1 Among Cowpea Accessions under Different Drought Stress Conditions," *J Genet Resour*, vol. 7, no. 1, pp. 133–143, 2021, doi: 10.22080/jgr.2021.20645.1233.
- [37]Y. Ohashi, N. Nakayama, H. Saneoka, P. K. Mohapatra, and K. Fujita, "Differences in the responses of stem diameter and pod thickness to drought stress during the grain filling stage in soybean plants," *Acta Physiol. Plant.*, vol. 31, no. 2, pp. 271–277, 2009, doi: 10.1007/s11738-008-0229-4.
- [38]D. A. Verbree, B. B. Singh, and W. A. Payne, "Genetics and Heritability of Shoot Drought Tolerance in Cowpea Seedlings," *Crop Sci.*, vol. 55, no. 1, pp. 146–153, 2015, doi: 10.2135/cropsci2014.02.0137.
- [39]A. Qayoom Sheikh, A. Kumar Pandit, and B. Ahmad Ganai, "Seasonal Variation in Chlorophyll Content of Some Selected Plant Species of Yousmarg Grassland Ecosystem," *Asian J. Plant Sci. Res.*, vol. 7, no. 2, pp. 33–36, 2017.
- [40]X. Zu *et al.*, "A new method for evaluating the drought tolerance of upland rice cultivars," *Crop J.*, vol. 5, no. 6, pp. 488–498, 2017, doi: 10.1016/j.cj.2017.05.002.
- [41]N. M. Kamal, Y. S. A. Gorafi, M. Abdelrahman, E. Abdellatef, and H. Tsujimoto, "Stay-Green Trait: A Prospective Approach for Yield Potential, and Drought and Heat Stress Adaptation in Globally Important Cereals," *Int. J. Mol. Sci.*, vol. 20, no. 5837, pp. 1–26, Dec. 2019, doi: 10.3390/IJMS20235837.
- [42]P. Arunachalam and P. Kannan, "Screening for drought tolerant groundnut (*Arachis hypogaea* L.) lines suitable for rainfed alfisol," *Asian J. Agric. Res.*, vol. 7, no. 1, pp. 35–42, 2013, doi: 10.3923/ajar.2013.35.42.
- [43]W. Verelst *et al.*, "Molecular and Physiological Analysis of Growth-Limiting Drought Stress in *Brachypodium distachyon* Leaves," *Mol. Plant*, vol. 6, no. 2, pp. 311–322, 2013, doi: 10.1093/mp/sss098.
- [44]F. E. Ahmed and A. S. H. Suliman, "Effect of water stress applied at different stages of growth on seed yield and water-use efficiency of Cowpea," *Agric. Biol. J. North Am.*, vol. 1, no. 4, pp. 534–540, 2010.
- [45]E. A. Bastos, S. P. do Nascimento, E. M. da Silva, F. R. Freire Filho, and R. L. Gomide, "Identification of cowpea genotypes for drought tolerance," *Rev. Ciência Agronômica*, vol. 42, pp. 100–107, 2011, doi: 10.1590/s1806-66902011000100013.
- [46]Y. A. Abayomi and T. O. Abidoye, "Evaluation of cowpea genotypes for soil moisture stress tolerance under screen house conditions," *African J. Plant Sci.*, vol. 3, no. 10, pp. 229–237, 2009.
- [47]D. Yahaya, N. Denwar, M. W. Blair, D. Yahaya, N. Denwar, and M. W. Blair, "Effects of Moisture Deficit on the Yield of Cowpea Genotypes in the Guinea Savannah of Northern Ghana," *Agric. Sci.*, vol. 10, pp. 577–595, Apr. 2019, doi: 10.4236/AS.2019.104046.
- [48]P. Anantharaju and A. R. Muthiah, "Screening for drought tolerance in cowpea *Vigna unguiculata* (L.) walp.," *Legum. Res.*, vol. 31, no. 4, pp. 283–285, 2008.
- [49]A. O. Anyia and H. Herzog, "Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought," *Eur. J. Agron.*, vol. 20, pp. 327–339, 2004, doi: 10.1016/S1161-0301(03)00038-8.
- [50]P. Goufo *et al.*, "Cowpea (*Vigna unguiculata* L. Walp.) metabolomics: Osmoprotection as a physiological strategy for drought stress resistance and improved yield," *Front. Plant Sci.*, vol. 8, pp. 1–22, Apr. 2017, doi: 10.3389/FPLS.2017.00586/BIBTEX.