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Research Article

Screening for drought tolerance in cowpea at the flowering stage

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Abstract

Drought is one of the major threats to cowpea productivity in tropical countries, and understanding its impacts is germane in ensuring food security in a global context. The present study was established to screen some accessions of cowpea for drought tolerance at the flowering stage in pots under the controlled conditions of a screen house. High significant differences were observed among accessions for wilting and recovery traits, stomatal conductance, relative water content (RWC), terminal leaflet length (TLL) and width (TLW), stem girth, and yield parameters under drought stress. In addition, drought stress caused a significant reduction in morphological traits and RWC between the initial and the final values. Based on cluster and Principal Component Analysis (PCA), accessions were separated into different classes of tolerance. Direct selection for wilting traits, stomatal conductance, morphological traits, and recovery parameters showing high heritability (≥ 60%), GAM (≥ 20%), and PCA (≥ 0.4) will be effective. Hence, four major classes of tolerance were determined: AC03, AC08, and AC10 were highly susceptible. AC01 and AC04 were moderately susceptible. AC06, AC07, and AC09 were moderately tolerant, while AC02 and AC05 were the highly tolerant accessions. The moderately tolerant and the highly tolerant accessions showed a combination of superior resistance to wilting, superior recovery rates, and superior yield attributes. They also showed lower stomatal conductance, higher RWC, and low reduction of RWC, TLW, and stem girth under drought stress compared to the susceptible ones.

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1. Introduction

Drought is one of the greatest threats to crop productivity, including cowpea (*Vigna unguiculata* L. Walp) in the tropical and subtropical countries of the world. Understanding the impacts of

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drought on crop productivity is germane to ensuring food security in a global context (Leng & Hall, 2019). Drought refers to a condition of sustained moisture deficit in soil capable to hinder crop growth and development with a significant reduction in yield (Ajayi et al., 2018). Drought is one of the main implications of climate change (Santos et al., 2020; Choudhary et al., 2021; Cirillo et al., 2021; Onyemaobi et al., 2021; Shanmugam et al., 2021). It is significant to agricultural production, with a disastrous effect projected for many plant species worldwide (Tebeje et al., 2017; Cirillo et al., 2021; Wasae, 2021). Crops under drought stress resist evapotranspiration by stomata closure, but as a result, carbon absorption is cut down during photosynthesis, thereby stunting productivity (Gomes et al., 2020; Adusei et al., 2021). The continuous growth of the global population (Khatun et al., 2021), foreseen to climax at nine billion people by the year 2050 (Santos et al., 2020), with Nigeria contributing significantly to it (Ajayi et al., 2022), is expected to double the global food demand by 2050 (Gomes et al., 2020). In the global context, vis-a-vis attaining food security, cowpea has been recognized as one of the key crops in realizing such a feat. Specifically, both economic, social, and environmental dimensions of the sustainable development goals of the Food Agriculture Organization (FAO) recognize the potential of cowpea in meeting food security (Nunes et al., 2022). Consequently, there is a need for an adequate understanding of the source of genotypic differences for drought tolerance among available cowpea varieties, especially in Nigeria that can be deployed to breeding for drought tolerance to improve its productivity.

In the tropical and subtropical regions of the world, for ages, one of the major sources of protein especially among the poor is cowpea (Ezin et al., 2021). This is because, compared to animal protein, it is cheaper; and has been found to contain around 25% protein in its seeds (Ajayi et al., 2018). Aside from this, it is rich in vitamins, minerals, fiber, and carbohydrates. It is useful in an intercropping system for being able to fix atmospheric nitrogen in the soil for soil replenishment (Santos et al., 2020). It is also one of the major sources of protein for animals as the vegetative parts are fed to cattle (Nunes et al., 2022). Cowpea thrives well in the tropical ecosystem; it has a higher tolerance to a couple of abiotic stresses including heat, drought, and soil acidity compared to cereals, however, a high level of genotypic differences exists among available varieties regarding these abiotic stresses, especially as regards drought. Drought is a major threat to the crop's productivity especially during the flowering and grain filling stage (Nunes et al., 2022), despite its good attributes. Many high-yielding varieties are susceptible to drought making yield loss of devastating magnitude a common occurrence in tropical and subtropical Africa, most especially in Nigeria. It is, therefore, necessary to gather adequate information regarding the genetic differences among available varieties for drought tolerance to deploy such information in the plant breeding program of the crop. Presently, Nigeria accounts for the highest production of cowpea in the world, contributing about 2.61 metric tons per annum accounting for about 36% of the global production of the crop (Ajayi et al., 2022).

Drought at the flowering stage of crops as the consequence of heavy reliance of the agricultural systems in tropical Africa on rain-fed agriculture is devastating to yield. Rain-fed agriculture is characterized by uneven distribution and uncertainty at the reproductive stage, hence causing terminal drought stress with accompanying reduction in yield (Lemma et al., 2021), which may climax between 70 and 80% (Harshani & Fernando, 2021; Wang et al., 2021). The devastating effects of drought stress during the reproductive stage have been confirmed in several crop species including beans (Wasae, 2021), cowpea (Ezin et al., 2021; Santos et al., 2020), maize (Al-Naggar et al., 2011; Badu-Apraku et al., 2021; Khan et al., 2021), mung bean (Singh et al., 2021), rice (Garrity & Toole, 1994; Yang et al., 2019; Hussain et al., 2021), soybean (Moloi & van der Merwe, 2021), tomato (Sivakumar & Srividhya, 2016), wheat (Onyemaobi et al., 2021). Meanwhile, information regarding the mechanisms of their responses to drought during flowering is to a large scarce, making breeding for drought tolerance a difficult task (Yang et al., 2019). Therefore, screening for drought tolerance must consider the reproductive stage and also adopt simple, cheap, and non-destructive screening methods with high efficiency in identifying the level of differences as well as sources of such variations among available germplasm (Ajayi et al., 2018). Simple methods that have proven successful include stomata behavior (Nkouannessi, 2005; Agbicodo, 2009), root traits (Matsui and Singh, 2003; Santos et al., 2020), leaf rolling (Matthew, et al., 1990), leaf wilting scales, and indices (Mai-kodomo et al., 1999; Pungulani et al., 2013; Ajayi et al., 2020), plant wilting scales and percentage of wilted plants and recovery parameters under drought stress (Nkouannessi, 2005; Ajayi et al., 2018). Pot evaluation methods under controlled environments have been proven more reliable in pinpointing the level of genotypic differences for different growth stages including reproductive stage drought tolerance in crop species (Goufo et al., 2017). This is because the inducement of drought can be reliably done in a controlled environment compared to what is obtainable under field conditions (Fatokun et al., 2012; Nkomo et al., 2020; Moloi & van der Merwe, 2021).

The present study was executed in line with the following objectives: 1). to screen accessions of cowpea for drought tolerance at the flowering stage by utilizing their shoot, physiological, and yield traits. 2). to estimate the level of variability, heritability, and association of the drought-responsive traits of cowpea under drought stress at the flowering stage. 3). to confirm the consistency of the flowering stage drought tolerance of the accessions to their previous levels of tolerance at the seedling and vegetative stages.

2. Materials and Methods

2.1. Materials

The ten accessions used in the present study are presented in Table 1. These accessions had previously been screened at the seedling (Ajayi et al., 2018) and the vegetative stages (Ajayi et al., 2020) for their tolerance to drought. Pre-planting soil analysis was done; topsoil was collected, air-dried, and thoroughly mixed, sieved, and physicochemical properties were determined by spectrometry method (Table 2).

Table 1. Selected accessions of cowpea and their previous drought-tolerant statuses at the seedling and vegetative stages

S/N	Accession ID	Biological status	Seedling stage	Vegetative stage	Code
1	**TVu-199**	Breeding material	Drought tolerant	Moderately tolerant	AC01
2	**TVu-207**	Breeding material	Drought tolerant	Moderately tolerant	AC02
3	**TVu-218**	Breeding material	Highly susceptible	Highly susceptible	AC03
4	**TVu-235**	Breeding material	Drought tolerant	Moderately tolerant	AC04
5	**TVu-236**	Breeding material	Moderately tolerant	Moderately tolerant	AC05
6	**TVu-241**	Breeding material	Drought tolerant	Drought tolerant	AC06
7	**IT98K-205-8**	Unknown	Moderately tolerant	Drought tolerant	AC07
8	**IT98K-555-1**	Unknown	Highly susceptible	Highly susceptible	AC08
9	**TVu-4886**	Landrace	Moderately tolerant	Moderately tolerant	AC09
10	**TVu-9256**	Landrace	Highly susceptible	Highly susceptible	AC10

Table 2. Pre-planting properties of the topsoil used to screen the accessions of cowpea for drought tolerance at the flowering stage

S/N	Parameters	
1	pH	6.50
2	Total organic matter (%)	2.35
3	Available P (c mol/kg)	2.50
4	Total N (%)	0.36
5	H^+ (mmol)	1.05
6	Al^{3+} (mmol)	2.20
7	Na (ppm)	25.30
8	Cu (ppm)	0.90
9	Mg (ppm)	51.00
10	Pb (ppm)	0.60
11	Mn (ppm)	79.00
11	Sand (%)	78.80
12	Silt (%)	10.56
13	Clay (%)	10.64
14	Texture class	Sandy Loam

2.2. Procedures

The evaluation of the accessions was performed in pots (with three perforations each for draining excess moisture) in the screen house during the flowering stage between June and September 2016 as described by Nkouannessi (2005) with minor modifications. The seeds were planted in plastic pots filled with 7 kg of sieved topsoil with no added fertilizer. At the emergence of seedlings, plants were thinned to three (3) fairly identical plants in each pot, with five (5) pots per accession in three replicates using a Completely Randomized Design (CRD); a total of 450 plants were contained in the screen house. Each pot was watered with 500 ml of water per day till more than 80 percent of the plants in the screen house have flowered; thereafter watering was terminated for 21 days to impose drought stress. The 500 ml was predetermined according to Ogbaga et al. (2014) as the amount required to bring each pot to 100% field capacity.

2.3. Shoot wilting parameters

On days 14 and 21 of drought stress, susceptibility was scored for plants based on the 1983 Descriptors for cowpea of the International Board for Plant Genetic Resources (IBPGR). One plant per pot was tagged for relative water content (RWC), while the two remaining plants per pot were untagged. The drought susceptibility score (DSS) on a 1 – 7 scale was used on the untagged plants, where 1 to 3 indicated low susceptibility (plant growing well with green leaves); 4 to 5 indicated medium susceptibility (a plant having most of the leaves turned yellow / or wilting), and 6 to 7 indicated high susceptibility (dead and dry plant). The mean score of plants per replicate and across the three replicates was respectively calculated. Accordingly, the percentage of wilted plants (PPW) was documented for each accession. However, the leaf wilting index (LWI) was determined for days 7, 14, and 21 under drought stress, as the ratio of leaves per plant showing wilting signs or wilted to the total number of leaves according to Pungulani et al. (2013). Accessions were ranked from 1 to 10 based on their superiority for each of the wilting traits where 1 signified the best and 10, the poorest.

2.4. Measurement of morphological parameters

Morphological traits like the terminal leaflet length (TLL) and width (TLW) of fully expanded middle terminal leafleafletsplants were determined on the initial day of imposing stress and day 7 utilizing a meter rule on two-terminal leaflets per pot per replicate. The girth of the stem of each accession per replicate was measured at 2 cm above the surface of the soil on two plants per pot using a digital vernier caliper, to the nearest millimeter at the initial day of imposed stress; 7 days, 14 days, and 21 days of the imposition of drought stress. The ranking of accessions for these traits was based on the percentage reduction in mean performance between initial and final values, where 1 signified the most superior (least reduction in mean value) and 10, the poorest (highest reduction in mean value).

2.5. Yield parameters

To the determination of the percentage pod set, twenty (20) flowers per accession per replicate were tagged on the last day of watering before the imposition of drought stress on only the matured flowers. The percentage pod set was calculated as the ratio of the number of pods formed and survived to maturity to the number of flowers tagged and expressed as a percent according to Kumar et al. (2008). The total number of pods per plant was done by counting the number of pods per plant per replicate at maturity. A mean number of pods of two plants per pot was recorded, then the mean across replicates was determined. Pod weight was determined by selecting the ten (10) best pods per replicate after the removal of seeds. The average pod weight was determined per replicate for each accession, after which the mean pod weight for three replicates was determined. The number of seeds per pod was determined by averaging the number of seeds per pod of two plants per pot. The mean number of seeds per pod for each accession was recorded and the mean for the three replicates was determined. Seed yield per plant was determined by averaging the yield of two plants in each pot. The mean seed yield for each accession was recorded and the mean for three replicates was determined. Accessions were ranked for each trait between 1 and 10, where 1 designated the most superior (highest mean performance), and 10 designated the most inferior (lowest mean performance).

2.6. Physiological parameters

The relative water content (RWC) was done by the methods of Kumar et al. (2008). This was done for the initial, 7 days, and 14 days after the termination of watering from two young fully expanded leaflets from the top of each plant per pot per replicate. They were detached and weighed for the fresh weight (FW), afterwards, they were placed in small bags containing distilled water and kept in the refrigerator for 12 hours. Turgid weights (TW) were determined by first blotting the leaves to dryness after removing from water and weighing, as well as dry weight (DW) after drying the leaves in the oven for 48 hours at 60°C. RWC (%) was determined on each leaflet by using the formula: RWC = (FW – DW)/ (TW – DW) × 100. The average value for each replicate was determined per accession, after which the mean value of each accession was determined on the three replicates. Stomatal conductance was done only on the 14th day using a steady-state Leaf Porometer. This was done between 11.30 am and 5.00 pm utilizing surviving leaflets from among those used for TLL and TLW on only three selected plants of each accession per replicate. The average value of each parameter was determined for each accession per replicate and the mean for the three replicates was determined. The ranking of accessions for RWC was based on the percentage reduction in mean performance between initial and final values, where 1 signified the most superior (least reduction in mean value) and 10, the poorest (highest reduction in mean value). However, accession with the highest stomatal conductance was ranked 1 (best) while the one with the lowest mean performance was ranked 10 (poorest).

2.7. Determination of recovery parameters

Watering was recommenced after twenty-one (21) days of drought stress. Fourteen (14) days later, the percentage of plants that recovered in each accession was documented. Centered on this percentage, plants were ranked between 1 (highest percentage recovery) and 10 (lowest recovery percentage). Stem greenness was scored on day 14 using a scale of 1–5, where 1 indicated the recovered plant is yellow and 5 was a completely green plant. The stem re-growth was scored in three categories: 1 signified plant recovered, but with no re-growth; 3 signified plant with re-growths from auxiliary buds; and 5 signified re-growth from the shoot apexes (Pungulani et al., 2013). Accessions were ranked between 1 and 10 based on their overall mean performance for stem greenness and stem re-growth.

2.8. Statistical analysis

Analysis of variance (ANOVA) was performed in SPSS version 20. Means were divided at a $P \leq 0.05$ level of significance using the Duncan Multiple Range Test (DMRT). The mean rank (MR) of all parameters, their standard deviation (SDR), as well as the grand mean rank (GMR) were determined. Estimates of genetic parameters were done according to the procedures cited in Ajayi et al., (2017a). The data on the ranking of accessions were subjected to cluster analysis using the Paleontological statistics software package for data analysis (PAST) version 4.0 (Hammer et al., 2001). All final data for wilting, morphological, physiological, recovery and yield data were used for Principal Component Analysis (PCA) employing PAST.

3. Results

3.1. Variability among accessions of cowpea under drought stress at the flowering stage

Results from ANOVA revealed highly significant differences among accessions for all traits. The coefficient of variation among traits ranged between 4.71 in leaf wilting index (LWI) on day 21 and 42.17% in the percentage of wilted plants (PPW) on day 14 (Table 3). Wilting parameters of the accessions are presented in Table 4. The drought susceptibility score (DSS) and PPW, respectively ranged between 2.61 and 26.33% in AC06 to 6.84 and 97.33% in AC03 as of day 21 of drought stress. However, LWI ranged between 0.81 in AC06 and 1.00 in AC01, AC03, and AC09.

Table 3. Mean square values for all parameters of accessions of cowpea screened under drought stress at the flowering stage

Parameters	Accession (DF = 9)	Error (DF = 20)	CV (%)
DSS14	4.37**	0.60	27.66
DSS21	7.59**	0.99	20.23
PPW14 (%)	1186.82**	76.70	42.17
PPW21 (%)	2080.62**	262.25	24.30
LWI7	0.05**	0.01	40.00
LWI14	0.04**	0.03	21.92
LWI21	0.01**	0.002	4.71
IRWC (%)	29.32**	23.42	10.19
RWC7 (%)	163.33**	73.96	12.06
RWC14 (%)	235.38**	86.45	17.47
SCND (mmol $m^{-2}s^{-1}$)	685.34**	106.06	11.55
PREC (%)	1812.87**	29.50	23.54
STG	17.28**	0.24	17.69
STR	4.61**	0.26	36.68
ITLL (cm)	2.29**	1.34	9.86
TLL7 (cm)	2.21**	1.37	10.16
ITLW (cm)	5.09**	0.76	11.89
TLW7 (cm)	5.27**	0.74	12.01
ISG (mm)	0.26**	0.06	11.25
SG7 (mm)	0.28**	0.24	10.45
SG14 (mm)	0.94**	0.28	13.96
PPSET (%)	534.81**	56.67	19.47
TPDP	7.11**	1.00	25.38
SPP	13.48**	1.35	10.08
SDPL	1034.66**	188.89	30.86
PDL (cm)	8.68**	0.82	5.88
SYDPL (g)	19.82**	2.33	23.52
PODW (g)	0.05**	0.008	24.17

**: Significant at $P \leq 0.05$; DF: Degree of freedom

DSS14, 21: Drought susceptibility score at day 14 and 21; PPW14, 21: Percentage of plants wilted at day 14 and 21; LWI7, 14, 21: Leaf wilting index at day 7, 14, 21; IRWC, RWC7, RWC14: Initial relative water content, relative water content at day 7, relative water content at day 14; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; ITLL: Initial terminal leaflet length; TLL7: Leaflet length at day 7; ITLW: Initial terminal leaflet width; TLW7: Leaflet width at day 7; ISG: Initial stem girth; SG7, 14, 21: Stem girth at day 7, 14 and 21; PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SYDPL: Seed yield per plant; PODW: Pod weight

Table 4. Wilting parameters (means ± standard error) of accessions of cowpea screened under drought stress at the flowering stage

ACCESSION	DSS 14	DSS 21	PPW 14	PPW 21	LWI 7	LWI 14	LWI 21
AC01	2.68±0.39[ab]	6.16±0.47[c]	13.33±3.33[ab]	97.00±1.73[e]	0.24±0.03[abc]	0.93±0.06[a]	1.00±0.00[c]
AC02	2.54±0.69[ab]	5.02±0.77[bc]	20.00±5.77[bc]	64.00±15.50[cd]	0.37±0.07[cd]	0.83±0.04[a]	0.92±0.04[bc]
AC03	3.57±0.77[bc]	6.84±0.11[c]	50.67±10.53[ef]	97.33±2.67[e]	0.52±0.12[d]	0.87±0.12[a]	1.00±0.00[c]
AC04	1.75±0.13[a]	2.85±0.23[a]	8.67±0.67[ab]	28.33±7.26[ab]	0.14±0.04[ab]	0.67±0.04[a]	0.85±0.02[ab]
AC05	2.07±0.29[a]	3.60±0.32[ab]	0.00±0.00[a]	53.33±3.33[abc]	0.21±0.04[abc]	0.82±0.07[a]	0.98±0.02[c]
AC06	1.91±0.35[a]	2.61±0.35[a]	0.00±0.00[a]	26.33±4.91[a]	0.22±0.07[abc]	0.64±0.12[a]	0.81±0.05[a]
AC07	1.97±0.29[a]	5.05±0.60[bc]	28.00±4.36[cd]	65.33±6.89[cd]	0.28±0.05[bc]	0.67±0.09[a]	0.94±0.00[bc]
AC08	5.37±0.55[d]	6.77±0.12[c]	60.00±5.77[f]	90.27±5.00[bc]	0.25±0.06[abc]	0.94±0.03[a]	0.97±0.02[c]
AC09	1.96±0.17[a]	4.00±1.16[ab]	0.00±0.00[a]	56.67±17.64[bc]	0.04±0.01[a]	0.69±0.17[a]	1.00±0.00[c]
AC10	4.21±0.41[cd]	6.29±0.65[c]	37.00±3.79[de]	87.66±12.33[de]	0.23±0.06[abc]	0.92±0.07[a]	0.99±0.01[c]

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using DMR.
DSS14, 21: Drought susceptibility score at day 14 and day 21; PPW14, 21: Percentage of wilted plants at day 14 and 21; LWI7, 14, 21: Leaf wilting index at day 7, 14, and 21.

The morphological parameters of the accessions, such as terminal leaf length (TLL), terminal leaf width (TLW), and stem girth are presented in Table 5. The final TLL measured on day 7 of stress was highest (13.07 cm) in AC08 and lowest (10.03 cm) in AC07. However, the percentage reduction between initial and final ranged between the lowest (-0.36%) in AC05 and the highest (8.24%) in AC02. Also, TLW ranged between 5.30 cm in AC07 and 9.30 cm in AC04, with the lowest percentage reduction (-0.35%) in width in AC05 and the highest reduction (8.77%) in AC01. Further, the highest reduction (33.17%) of stem girth as of day 21 of stress occurred in AC01 with the least stem girth of 2.80 mm, meanwhile, both the lowest percentage reduction of stem girth (6.51%) and biggest stem girth (4.74 mm) respectively were obtained in AC09.

The yield traits of the accessions under drought stress are presented in Table 6. The highest performance for seed yield pod length (18.30 cm), (9.95 g), and pod weight (0.56 g) was obtained in AC01 while the lowest performance for seed yield (2.75 g), pod length (12.27 cm), and pod weight, respectively was obtained in AC10 and AC09. Performance for the number of seeds per pod (1.27) and the number of seeds per plant (82.37) was highest in AC03 while the lowest performance in the two traits respectively was obtained in AC07 (8.93) and AC04 (19.66). However, the percentage of pod set per plant ranged from the lowest (16.67%) in AC06 to the highest (56.66%) in AC05 while the total number of pods per plant ranged from the lowest (1.73) in AC04 to the highest (6.48) in AC07.

The results for physiological parameters such as relative water content (RWC) and stomatal conductance (SCND) are presented in Table 7. The final RWC observed on day 14 of drought stress was highest (63.79%) in AC07 and lowest (41.01%) in AC10, consequently, the highest reduction in the trait (47.84%) between the initial and final value was obtained in AC03 while the lowest reduction (14.05%) was obtained in AC09. SCND on the hand was observed to range from the lowest (72.58 mmol $m^{-2}s^{-1}$) in AC09 and (119.38mmol $m^{-2}s^{-1}$) in AC10.

Table 5. Effect of drought stress on terminal leaflet length and width (cm), and stem girth (mm) (means ± standard error) of accessions of cowpea at the flowering stage

ACCESSION	ITLL	TLL7	RD (%)	ITLW	TLW7	RD (%)	ISG	SG7	SG14	RD (%)
AC01	11.50±0.75^{ab}	11.47±0.58^{ab}	0.26	6.50±0.46^{abc}	5.93±0.49^{ab}	8.77	4.19±0.25^{a}	4.15±0.21^{a}	2.80±0.43^{a}	33.17
AC02	12.13±0.75^{ab}	11.13±1.17^{ab}	8.24	6.60±0.70^{abc}	6.53±0.69^{ab}	1.06	4.31±0.05^{ab}	4.41±0.37^{ab}	3.31±0.41^{ab}	23.2
AC03	11.70±0.72^{ab}	11.30±0.55^{ab}	3.42	7.90±0.55^{cde}	7.57±0.43^{bc}	4.18	4.87±0.24^{cd}	4.62±0.23^{ab}	3.71±0.14^{abc}	23.81
AC04	12.63±0.86^{b}	12.57±0.83^{b}	0.48	9.37±0.47^{e}	9.30±0.46^{d}	7.47	4.71±0.19^{bcd}	4.69±0.28^{ab}	3.89±0.55^{bcd}	17.41
AC05	11.23±0.62^{ab}	11.27±0.64^{ab}	-0.36	8.60±0.70^{de}	8.63±0.77^{cd}	-0.35	4.59±0.04^{abc}	4.66±0.15^{ab}	3.70±0.14^{abc}	19.39
AC06	11.93±0.63^{ab}	11.80±0.66^{ab}	1.08	8.70±0.46^{de}	8.50±0.47^{cd}	2.29	5.09±0.08^{d}	5.22±0.19^{b}	4.60±0.09^{cd}	9.63
AC07	10.03±0.23^{a}	10.03±0.23^{a}	0.00	5.30±0.32^{a}	5.30±0.32^{a}	0.00	4.67±0.04^{bcd}	4.67±0.18^{ab}	3.83±0.03^{bcd}	17.99
AC08	13.27±1.01^{b}	13.07±0.86^{b}	1.51	6.10±0.46^{ab}	5.97±0.49^{ab}	2.13	4.81±0.14^{cd}	4.60±0.26^{ab}	3.59±0.34^{abc}	25.36
AC09	11.83±0.35^{ab}	11.80±0.36^{ab}	0.25	6.70±0.15^{abc}	6.60±0.06^{ab}	1.49	5.04±0.04^{cd}	5.09±0.21^{ab}	4.74±0.19^{d}	6.51
AC10	11.17±0.46^{ab}	10.80±0.32^{ab}	3.31	7.53±0.52^{bcd}	7.23±0.40^{bc}	3.98	4.94±0.08^{cd}	4.83±0.52^{ab}	3.77±0.29^{abcd}	23.68

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ DMRT. RD: Percentage reduction. ITLL: Initial terminal leaflet length; TLL 7: Terminal leaflet length at day 7; ITLW: Initial terminal leaflet width; TLW 7: Terminal leaflet width at day 7; ISG: Initial stem girth; SG7, 14: Stem girth at day 7, and 14.

Table 6. Yield traits (means ± standard error) of accessions of cowpea screened under drought stress at the flowering stage

ACCESSION	PPSET (%)	TPDP	SPP	SDPL	PDL (cm)	SYDPL (g)	PODW (g)
AC01	53.33 ± 3.33^{ef}	4.73 ± 0.24^{cde}	11.13 ± 0.07^{bcd}	52.72 ± 2.90^{bc}	18.30 ± 0.88^{e}	9.95 ± 0.36^{d}	0.56 ± 0.02^{e}
AC02	43.33 ± 6.67^{def}	4.87 ± 0.47^{cde}	11.47 ± 0.35^{bcd}	55.87 ± 6.08^{c}	15.36 ± 0.24^{bcd}	9.49 ± 1.03^{cd}	0.42 ± 0.05^{cde}
AC03	50.00 ± 5.77^{ef}	5.35 ± 1.28^{de}	15.27 ± 0.47^{f}	82.37 ± 21.63^{d}	16.63 ± 0.16^{d}	$6.82\pm1.90b^{bc}$	0.27 ± 0.01^{abc}
AC04	26.66 ± 3.33^{abc}	1.73 ± 0.27^{a}	11.78 ± 1.64^{cd}	19.66 ± 2.07^{a}	14.44 ± 0.15^{bc}	2.78 ± 0.22^{a}	0.53 ± 0.12^{de}
AC05	56.66 ± 3.33^{f}	3.27 ± 0.51^{abc}	13.10 ± 0.45^{de}	42.55 ± 6.19^{abc}	16.87 ± 0.41^{de}	5.98 ± 0.95^{c}	0.40 ± 0.04^{bcde}
AC06	16.67 ± 3.33^{a}	1.89 ± 0.11^{a}	14.29 ± 0.46^{ef}	27.04 ± 2.03^{ab}	15.78 ± 0.11^{cd}	4.55 ± 0.32^{ab}	0.38 ± 0.07^{bcd}
AC07	43.33 ± 3.33^{def}	6.48 ± 0.52^{e}	8.93 ± 0.24^{a}	57.79 ± 4.11^{c}	13.76 ± 0.29^{ab}	8.99 ± 0.83^{cd}	0.29 ± 0.03^{abc}
AC08	23.33 ± 3.33^{ab}	4.43 ± 0.54^{bcd}	9.93 ± 0.44^{abc}	43.71 ± 4.07^{abc}	15.68 ± 0.65^{cd}	6.65 ± 0.58^{bc}	0.44 ± 0.06^{cde}
AC09	40.00 ± 5.77^{cde}	4.00 ± 0.58^{bcd}	9.33 ± 0.88^{ab}	36.67 ± 4.17^{abc}	12.27 ± 0.91^{a}	6.98 ± 0.80^{bc}	0.17 ± 0.03^{a}
AC10	33.33 ± 3.33^{bcd}	2.67 ± 0.44^{ab}	10.10 ± 0.10^{abc}	26.91 ± 4.37^{ab}	14.85 ± 0.57^{bc}	2.75 ± 0.48^{a}	0.25 ± 0.02^{ab}

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using DMRT. PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SYDPL: Seed yield per plant; PODW: Pod weight.

Table 7. Effect of drought stress on the physiological parameters (means ± standard error) of accessions of cowpea at the flowering stage

ACCESSION	IRWC (%)	RWC 7 (%)	RWC 14 (%)	RD (%)	SCND (mmol $m^{-2}s^{-1}$)
AC01	86.47±0.48^{ab}	80.51±6.71^{bc}	47.59±9.58^{abc}	44.96	90.43±3.45^{a}
AC02	89.50±0.78^{b}	77.04±7.19^{bc}	59.98±8.82^{bcd}	32.98	85.52±1.81^{a}
AC03	84.66±0.70^{ab}	59.09±8.94^{a}	44.16±0.59^{ab}	47.84	86.79±7.07^{a}
AC04	80.46±1.82^{ab}	75.56±5.17^{abc}	59.72±7.07^{bcd}	25.78	77.08±4.69^{a}
AC05	83.88±1.48^{ab}	69.96±0.48^{abc}	51.56±2.55^{abcd}	38.53	86.21±2.09^{a}
AC06	85.24±2.09^{ab}	70.88±4.41^{abc}	50.56±3.95^{abcd}	40.69	82.83±3.42^{a}
AC07	83.16±2.17^{ab}	66.66±1.89^{abc}	63.79±2.81^{cd}	23.29	78.72±8.60^{a}
AC08	84.52±2.44^{ab}	64.72±3.12^{ab}	46.72±2.19^{abc}	44.72	112.48±12.49^{b}
AC09	78.02±0.73^{a}	82.06±1.44^{c}	67.06±0.74^{d}	14.05	72.58±2.09^{a}
AC10	84.55±7.45^{ab}	66.61±2.84^{abc}	41.01±5.72^{a}	51.49	119.38±3.99^{b}

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using DMRT. RD: Percentage reduction. IRWC: Initial relative water content; RWC 7, 14: Relative water content at days 7 and 14; SCND: Stomata conductance.

The recovery parameters such as percentage of recovered plants (PREC), stem re-growth (STR), and greenness (STG) are presented in Table 8. Accessions AC01, AC03, AC08, and AC10 did not recover. AC06, on the other hand, had the highest PREC (65.00%), the highest STR (3.11), and the highest STG (4.89).

Accession ranks based on wilting parameters, reduction in morphological traits, RWC, stomatal conductance, and yield traits are presented in Table 9. The mean of ranks (MR) and standard deviation of ranks (SDR) respectively, were highest in AC10 (7.39) and AC09 (3.18), while the lowest values were obtained in AC05 (4.00) and AC02 (1.85). Based on the MR and SDR, accessions with a mean rank lower than the grand mean (GM) of ranks (5.32) and its SDR (2.59) were classified as the highly tolerant accessions; these included AC02 and AC05. Moderately tolerant accessions included those with lower MR values but higher SDR compared to GM, such as AC06, AC07, and AC09. Moderately susceptible accessions were those with MR a bit higher than the GM and with higher SDR, including AC01 and AC04. Highly susceptible accessions were AC03, AC08, and AC10 with higher MR (>5.99) compared to GM.

Table 8. Recovery parameters (means ± standard error) of accessions of cowpea screened under drought stress at the flowering stage after two weeks of re-watering

ACCESSION	PREC (%)	STG	STR
AC01	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
AC02	20.00±3.00^{b}	4.72±0.15^{bc}	1.83±0.44^{b}
AC03	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
AC04	43.33±5.77^{c}	4.68±0.09^{bc}	2.07±0.52^{b}
AC05	21.66±7.64^{b}	4.67±0.17^{bc}	2.33±0.33^{bc}
AC06	65.00±8.66^{d}	4.89±0.11^{c}	3.11±0.11^{c}
AC07	24.00±6.08^{b}	3.92±0.85^{b}	2.22±0.39^{bc}
AC08	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
AC09	56.66±9.07^{d}	4.83±0.17^{bc}	2.33±0.33^{bc}
AC10	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using DMRT. PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth.

Table 9. Ranking of accessions of cowpea screened under drought stress at flowering stage

ACC	DSS21	PPW21 (%)	LWI21	RRWC (%)	PREC (%)	STG	STR	SCND (mmol $m^{-2}s^{-1}$)	RTLL (%)	RTLW (%)	RSG (%)	PPSET (%)	TPDP	SPP	SDPL	PDL	SYDPL (g)	PODW (g)	MR	SDR
AC01	7	9	8	8	7	7	6	3	4	10	10	2	4	6	4	1	1	1	5.44	3.05
AC02	5	5	3	4	6	3	5	6	10	3	6	4	3	5	3	6	2	4	4.61	1.85
AC03	10	10	8	9	7	7	6	4	9	8	8	3	2	1	1	3	5	8	6.06	3.04
AC04	2	2	2	3	3	4	4	9	5	9	3	7	10	4	10	8	9	2	5.33	3.07
AC05	3	3	6	5	5	5	2	5	1	1	5	1	7	3	6	2	7	5	4.00	2.03
AC06	1	1	1	6	1	1	1	7	6	6	2	9	9	2	8	4	8	6	4.39	3.13
AC07	6	6	4	2	4	6	3	8	2	2	4	4	1	10	2	9	3	7	4.61	2.64
AC08	9	8	5	7	7	7	6	2	7	5	9	8	5	8	5	5	6	3	6.22	1.93
AC09	4	4	8	1	2	2	2	10	3	4	1	5	6	9	7	10	4	10	5.11	3.18
AC10	8	7	7	10	7	7	6	1	8	7	9	6	8	7	9	7	10	9	7.39	2.00
GM																			**5.32**	**2.592**

ACC: Accessions; MR: Mean of rank; SDR: Standard deviation of rank; GM: Grand mean of rank.

DSS21: Drought susceptibility score at day 21; PPW21: Percentage of plants wilted at day 21; LWI21: Leaf wilting index at day 21; RRWC: Percent reduction in relative water content by day 14; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; RTLL: Percent reduction in leaflet length by day 7; RTLW7: Percent reduction in leaflet width by day 7; Percent reduction in stem girth by day 14; PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SYDPL: Seed yield per plant; PODW: Pod weight.

3.2. Cluster analysis based on the ranking of accessions of cowpea screened under drought stress at the flowering stage

The ranking of accessions under drought stress produced a Dendrogram that grouped the accessions into three major clusters (Figure 1). Cluster I consisted of AC10, AC08, AC01, and AC03, both highly susceptible and moderately susceptible accessions. Cluster II had three sub-clusters; sub-cluster II-a consisted of AC07 and AC09, moderately tolerant accessions. Sub-cluster II-b and c respectively, consisted of AC02 and AC05, highly tolerant accessions. However, cluster III consisted of AC06 (moderately tolerant accession) and AC04 (a moderately susceptible accession).

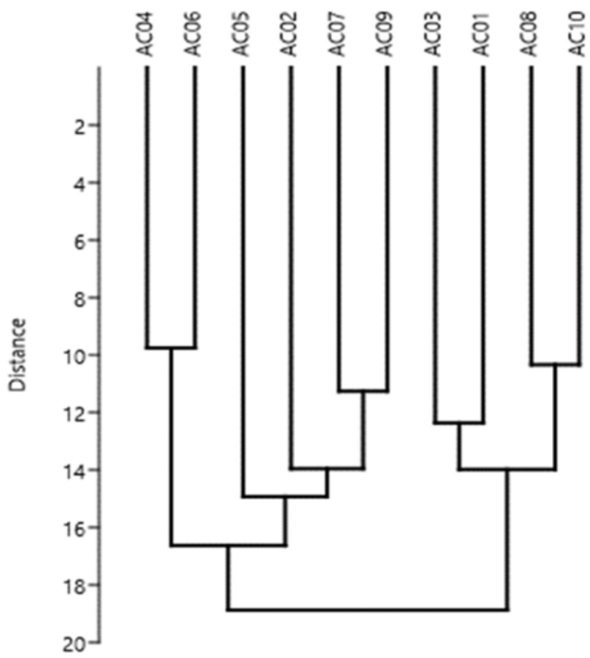

Figure 1. Dendrogram (UPGMA) based on the ranking of accessions of cowpea screened under drought stress at the flowering stage

3.3. Estimates of genetic parameters of accessions of cowpea screened under drought stress at the flowering stage

The results for estimates of genetic parameters are presented in Table 10. Genotypic variance (GV) ranged from 0.003 in LWI14 and 21 to 606.12 in PPW21, while the phenotypic variance (PV) fell between 0.005 in LWI21 and 868.37 in PPW21. Genotypic coefficient of variation (GCV = 105.69%) and phenotypic coefficient of variation (PCV = 108.28%) respectively were highest in PREC. However, the lowest GCV (1.67%) and PCV (5.99%) were obtained in the IRWC. Heritability in the broad sense (H^2b) ranged from 7.76% in IRWC to the highest (95.95) in STG. Furthermore, genetic advance as a percent of the mean (GAM) ranged from the lowest (0.01%) in IRWC to the highest (173.64%) in PPW14. Heritability was high in all wilting traits except in LWI7 and 14. It was high for all recovery parameters and yield traits except for the number of seeds per plant and low for all morphological and physiological parameters except for TLW and stomatal conductance.

3.4. PCA and biplot based on all final parameters of accessions of cowpea screened under drought stress at the flowering stage

The PCA and biplot respectively are presented in Table 11 and Figure 2. Nine PC axes were derived, with the first four having eigenvalues above 1.00 and accounting for 88.26% of the total variation. PCs 1 and 2 respectively, accounted for 44.70% and 21.36% of the variability, totaling 66.06%. All parameters had high contributions in PC1 except TLL7, seeds per pod, and pod weight. However, all the wilting parameters, SG14 and PREC the ones with low contributions in PC2. Biplot based on PCs 1 and 2 divided accessions into four major groups. Group, I consisted of two, AC07 (moderately tolerant) and AC02 (highly tolerant). Group II consisted of four, AC01 (moderately susceptible), AC03, AC08, and AC10 (highly susceptible). Group III consisted of three, AC06 (moderately tolerant), AC04 (moderately susceptible), and AC05 (highly tolerant). Group IV consisted of one, AC09 (moderately tolerant). All the yield parameters except pod length and weight were highly positively correlated with LWI21 in group I and weakly correlated with DSS21 and PPW21 in group II. However, pod length and stomatal conductance were highly positively correlated with each other and also weakly positively correlated with DSS21 and PPW21 in group II. In group III, TLW7 and TLL7 were highly positively correlated and also weakly correlated with pod length and pod weight, and highly negatively correlated with all other yield parameters. All recovery parameters in group IV were highly positively correlated and correlated positively with SG14 and RWC14, and highly negatively correlated with all the yield and wilting parameters. Vertex accession for yield traits in group I was AC07, while the vertex accessions in group II for the wilting parameters and SCND were AC10, AC08, and AC01. The vertex accessions in group III were AC06 and AC04 for TLW7 and TLL7, while the vertex accession for recovery and RWC14 was AC09. The most stable accessions under drought stress were AC05 and AC02 because of their very close positions to the origin of the biplot.

Table 10. Estimates of genetic parameters of accessions of cowpea screened under drought stress at flowering stage

TRAIT	MEAN	GV	PV	GCV (%)	PCV (%)	H^2B (%)	GAM (%)
DSS14	2.80	1.26	1.86	40.09	48.71	67.74	67.97
DSS21	4.92	2.20	3.19	30.15	36.30	68.97	51.58
PPW14 (%)	20.77	370.04	446.74	92.61	101.76	82.83	173.64
PPW21 (%)	66.63	606.12	868.37	36.95	44.23	69.79	63.58
LWI7	0.25	0.01	0.02	40.00	56.57	50.00	58.27
LWI14	0.79	0.003	0.03	6.93	21.92	31.61	14.28
LWI21	0.95	0.003	0.005	5.77	7.44	60.00	9.19
IRWC (%)	84.05	1.97	25.39	1.67	5.99	7.76	0.01
RWC7 (%)	71.31	29.79	103.75	7.65	14.28	28.71	8.45
RWC14 (%)	53.22	49.64	136.09	13.26	21.97	36.48	16.47
SCND	89.20	193.00	299.06	15.57	19.39	64.54	25.77
PREC (%)	23.07	594.46	623.96	105.69	108.28	95.27	212.61
STG	2.77	5.68	5.92	86.04	87.84	95.95	173.61
STR	1.39	1.45	1.71	86.63	94.08	84.79	164.32
ITLL (cm)	11.74	0.32	1.66	4.82	10.97	19.28	4.36
TLL7 (cm)	11.52	0.28	1.65	4.59	11.15	16.97	3.89
ITLW (cm)	7.33	1.44	2.20	16.37	20.24	65.45	27.28
TLW7 (cm)	7.16	1.51	2.25	17.16	20.95	67.11	28.96
ISG (mm)	4.74	0.07	0.13	11.83	16.57	53.85	8.44
SG7 (mm)	4.69	0.01	0.25	2.13	10.66	4.00	0.87
SG14 (mm)	3.79	0.22	0.50	12.38	18.66	44.00	16.91
PPSET (%)	38.67	159.38	216.05	32.65	38.01	73.77	57.76
TPDP	3.94	2.04	3.04	36.25	44.25	67.11	61.17
SPP	11.53	4.04	5.39	17.43	20.14	74.95	31.09
SDPL	44.53	281.92	470.81	37.71	48.73	59.88	60.11
PDL (cm)	15.39	2.62	3.44	10.52	12.05	76.16	18.91
SDYPL (g)	6.49	5.83	8.16	37.2	44.01	71.45	64.78
PODW (g)	0.37	0.014	0.022	31.98	40.09	63.63	52.55

GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H^2B: Heritability; GAM: Genetic advance as percent of the mean. DSS14, 21: Drought susceptibility score at days 14 and 21; PPW14, 21: Percentage of plants wilted at day 14 and 21; LWI7, 14, 21: Leaf wilting index at day 7, 14, and 21; IRWC: Initial relative water content; RWC 7, 14: Relative water content at day 7 and 14; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; ITLL: Initial terminal leaflet length; TLL7: Terminal leaflet length at day 7; TLW7: ITLW: Initial terminal leaflet width; Terminal leaflet width at day 7; ISG: Initial stem girth; SG, 7, 14: Stem girth at day 7, and 14; PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SDYPL: Seed yield per plant; PODW: Pod weight.

Table 11. PCA of accessions of cowpea screened under drought stress at flowering stage

Parameters	PC1	PC2	PC3	PC4
DSS21	**0.95**	-0.05	-0.24	0.02
PPW21 (%)	**0.98**	-0.01	-0.16	0.06
LWI21	**0.77**	0.24	-0.22	0.22
RWC14 (%)	**-0.54**	**0.75**	-0.03	-0.29
SCND (mmol $m^{-2}s^{-1}$)	**0.55**	**-0.60**	**-0.45**	-0.05
TLL7 (cm)	-0.22	**-0.55**	-0.07	**-0.40**
TLW7 (cm)	**-0.62**	**-0.55**	0.32	0.36
SG14	**-0.74**	0.12	**-0.43**	0.39
PREC (%)	**-0.94**	0.21	-0.01	0.03
STG	**-0.85**	**0.41**	0.21	-0.05
STR	**-0.88**	0.37	0.19	0.02
PPSET (%)	**0.52**	**0.46**	**0.46**	0.23
TPDP	**0.65**	**0.70**	0.06	-0.05
SPP	-0.12	**-0.40**	**0.66**	**0.55**
SDPL	**0.67**	**0.43**	0.36	0.28
PDL (cm)	**0.50**	**-0.46**	**0.68**	-0.01
SYDPL (g)	**0.49**	**0.68**	0.35	-0.30
PODW (g)	0.03	**-0.42**	**0.59**	**-0.68**
Eigen-values	8.05	3.85	2.42	1.58
Variance (%)	44.70	21.36	13.42	8.78
Cum. Variance (%)	44.70	66.06	79.48	88.26

High contribution (≥4.0).

DSS21: Drought susceptibility score at day 21; PPW21: Percentage of plants wilted at 21; LWI21: Leaf wilting index at day 21; RWC14: Relative water content at day 14; SCND: Stomatal conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; TLL7: Terminal leaflet length at day 7; TLW7: Terminal leaflet width at day 7; SG14: Stem girth at day 14; PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SYDPL: Seed yield per plant; PODW: Pod weight.

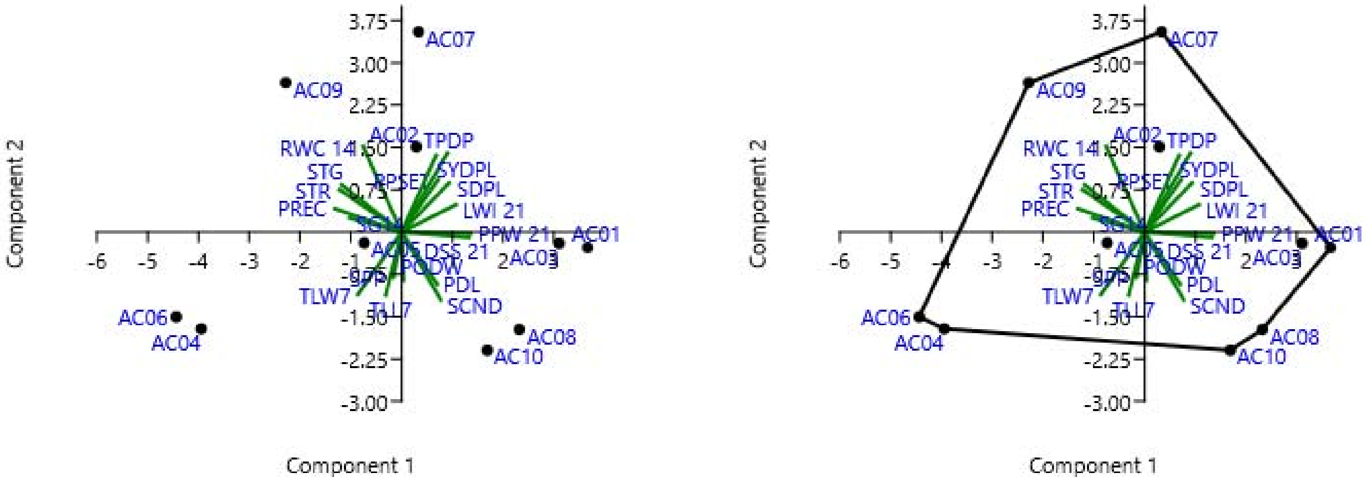

Figure 2. Biplot of PCA (a) and polygon view of biplot (b) of accessions of cowpea screened under drought stress at flowering stage. ACC01 – ACC10 are codes for accessions. DSS21: Drought susceptibility score at day 21; PPW21: Percentage of plants wilted at 21; LWI21: Leaf wilting index at day 21; RWC14: Relative water content at day 14; SCND: Stomatal conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; TLL7: Terminal leaflet length at day 7; TLW7: Terminal leaflet width at day 7; SG14: Stem girth at day 14; PPSET: Percentage pod set; TPDP: Total pods per plant; SPP: Seeds per pod; SDPL: Seeds per plant; PDL: Pod length; SYDPL: Seed yield per plant; PODW: Pod weight.

4. Discussion

4.1. Accession differences for measured parameters under drought stress at the flowering stage

The high significant variability revealed by the ANOVA among accessions for all traits studied at the flowering stage indicated the existence of sufficient genetic variations among the accessions. This agrees with Nkouannesssi (2005). Visual observation of wilting resulting from drought stress is possible in cowpea, and this allows the classification of genotypes based on the intensity of wilting. It was observed from the results that responses were accession-specific and time-dependent. Wilting parameters showed that as early as day 7, accessions AC02 and AC03 were already showing signs of wilting as conveyed in Nkouannessi (2005), Muchero et al., (2008), Agbicodo (2009), Nkomo et al. (2020), suggesting that susceptible accessions can easily be identified within the first week of withholding moisture (Pungulani et al., 2013). However, the most susceptible on day 21 were AC03, AC08, AC01, and AC10 with a combination of the highest PPW and LWI. This is similar to the responses of the accessions at the seedling and vegetative stages (Ajayi et al., 2018; 2020).

Re-watering of accessions for two weeks after the stress resulted in the recovery of some plants among six accessions excluding AC01, AC03, AC08, and AC10 in which all plants have died and dried. Among accessions, those in which wilting was slow at the beginning with consistently lower DSS and lower PPW at the end such as AC04, AC06, and AC09 had very high recovery rates. Therefore, these accessions must have had an in-built mechanism to retard their tissue moisture loss by restricting the rate of transpiration that the highly susceptible accessions did not possess. This backed their reduced predisposition to drought stress and their possession of higher greenness at recovery. The importance of stem greenness to drought tolerance in cowpea has been previously emphasized (Guofo et al., 2017). Similar results were recorded during the seedling and the vegetative stages (Ajayi et al., 2018; 2020) and also in line with other reports on soybean, common bean, and cowpea (Ries et al., 2012; Fatokun et al., 2012; Mukeshimana et al., 2014; Ibitoye, 2015; Nkomo et al., 2020).

Pungulani et al. (2013) stated that the physiological and morphological traits promoting water loss from leaf tissues might be a key factor responsible for early wilting among the accessions showing droopiness within seven days of being stressed. In sorghum, drought stress was reported to negatively affect most morphological and physiological traits (Bibi et al., 2010; 2012). Stomata behavior at the initiation of drought stress might be a crucial factor since late wilting accessions were able to regulate stomatal opening to prevent water loss (Agbicodo, 2009).

Generally, the accessions had high stomatal conductance. Plants of the accessions with the slowest rate of wilting (AC04, AC05, AC06, AC07, and AC09) were among those with the lowest stomatal conductance. These results show that these accessions can maintain photosynthesis under restricted moisture through minimal stomatal openings as reported by Mukeshimana (2013). Other mechanisms of restricting moisture loss during drought stress also include an osmotic adjustment in leaves (White et al., 1992) which encourages the maintenance of turgor and survival in plants under drought stress and ensures plant recovery after re-introduction of watering (Mukeshimana, 2013).

In the present study, RWC was reduced among accessions at the flowering stage up to day 14 of drought stress. Furthermore, an RWC of above 40% among cowpea genotypes is considered one of the best indicators of drought tolerance (Alidu *et al*., 2019). In the present study, the tolerant accessions possessed an RWC of between 50 and 67% in line with Alidu et al. (2019) for inbred lines of cowpea. Accessions with slow wilting had the highest RWC on day 14 of drought stress. Reduction of RWC between the initial and day 14 was generally the lowest among accessions with low wilting parameters. Maintenance of higher water status among tolerant accessions could be linked to lower stomatal conductance exhibited by them in line with the findings of Shanmugam et al. (2021) in rice. This result contradicts the findings of Agbicodo (2009) who observed no correlation between the leaf water status of cowpea plants and stomatal conductance under water deficit. Inconsistent reports have emanated from many workers as regards relationships between RWC and stomatal conductance in cowpea plants under drought stress (Anyia and Herzog, 2004; Souza *et al*., 2004; Hamidou *et al*., 2007). RWC is a vital agronomic parameter that governs better growth and physiological functions in plants. Plants growing under drought conditions can experience increased osmotic potential leading to a decrease of 60 – 80% in RWC, which can be most pronounced among the highly sensitive accessions (Panda et al., 2022) in agreement with the findings of this study. For instance, the most sensitive accessions; AC10, AC03, and AC08, were among those in which RWC reduced the most as a result of drought.

Observable differences were noted among the accessions for morphological traits such as TLL and TLW under drought stress until day 7. The significant reduction in the TLL and TLW among the most susceptible accessions at day 7 indicates that drought stress led to reduced leaf area of existing leaves which in turn led to leaf shedding on the plants because many of the measured terminal leaflets did not survive beyond day 7. The highly sensitive accessions were amongst those with the highest reduction in TLL and TLW (AC03, AC08, and AC10). These are similar to the findings of Samson and Helmut (2007) and also consistent with their responses at the seedling and vegetative stages (Ajayi et al., 2018; 2020). Also, the most susceptible at the flowering stage were among accessions with the highest reduction of stem girth resulting from drought stress. These findings are in agreement with the findings of Abdou Razakou (2013) who conveyed that drought imposed on cowpea plants reduced their stem girth between 2.43% and 50%. As stated by Mitchell et al., (1998) and Abdou Razakou (2013), reduction of plant size and leaf area has been found as mechanisms controlling water use and reducing injury under drought stress. Genotypes of maize also differed significantly for stem diameter at different drought stages (Salami et al., 2007).

Severe drought has been found to cause a significant reduction in seed yield which is linked to a reduction in plant water status in many legumes (Garg et al., 2005). Significant genotypic differences were observed among the accessions for the number of pods, percentage of pod set, the total number of pods, seeds per pod, seeds per plant, pod length, seed yield, and pod weight at the flowering stage. The percentage pod set and the number of pods ranged from low to moderate among highly susceptible accessions. Accession with the highest reduction of RWC (AC10) produced the lowest seed yield under imposed drought, while accessions exhibiting a lesser decrease in RWC set higher pod percentage, formed a higher number of seeds, and higher seed yield. Comparable outcomes were conveyed by Kumar et al., (2008). Agbicodo (2009) reported a significant reduction in grain, fodder, and total yields of cowpea, and stated that imposed drought at the early stages of flowering and pod formation enforced significant damage to plant functions and consequently total biomass yield. Similarly, a decrease in yield under drought stress was reported in cowpea (Aniya and Herzog, 2004a and b; Hamidou et al., 2007). In this study, AC04 and AC06 which displayed the least levels of drought susceptibility at the flowering stage had a low percent pod set, the lowest number of pods, and were among accessions with the lowest seed yield. This indicated that these accessions maintained more tissue moisture by redirecting moisture to tissue rather than exhausting it on pod production. This attribute can be linked to the drought avoidance mechanism of cowpea (Shavrukov et al., 2017), however, one of the major difficulties in classifying cowpeas into different classes based on drought avoidance and escape is the overlap that characterizes the two groups.

4.2. Ranking and cluster analysis based on key traits of accessions of cowpea screened for drought tolerance at the flowering stage

A high level of variations for all traits amongst accessions was responsible for the strength of the accession ranking in clearly separating accessions into different classes of tolerance; this is also indicated in the dendrogram. Determination of the most desirable drought-tolerant genotypes employing mean rank, the standard deviation of ranks, and rank-sum of parameters has been previously reported in crop species (Al-Rawi, 2016; Wasae, 2021). Accordingly, it is common for different traits to rank different accessions as tolerant. Hence, the determination of the most tolerant accessions needs to consider the mean of ranks in conjunction with their standard deviation for all parameters measured. Accessions exhibiting the lowest mean of ranks coupled with the lowest standard deviation of ranks are selected as the most drought–tolerant. The most susceptible accessions of cowpea were separated by the dendrogram from the moderate and the highly tolerant accessions. A similar trend was also observed by Ajayi et al., (2018) for the seedling stage. Cluster analysis is a powerful tool that has been employed in the selection of several drought-tolerant crop species (Zdravkovic et al., 2013; Al-Rawi, 2016).

4.3. PCA and biplot of traits of cowpea screened under drought stress at the flowering stage

According to Hammer et al., (2001), an effective PCA is the one in which most of its variance is accounted for by the first one or first two-component axes. The possession of more than 66 percent of the variance by the first two PCs agrees with Panda et al., (2021) contrary to the findings of (Nkomo et al., 2020). This indicates high variability for drought responses of accessions. All traits except TLL7, seeds per pod, and pod weight were high contributors to PC1. However, only the wilting traits, SG14, PREC, and STR contributed highly to PC2. These traits should be considered in breeding programs for the selection of drought-tolerant cowpea. Li et al., (2015) reported that PCA was effective in selecting the best root parameters governing seedling drought tolerance in maize. The high level of positive correlation shown by the biplot among all the yield parameters, accompanied by the moderate positive correlation with all the wilting parameters indicates that most accessions with high seed yield were more susceptible to drought stress at the flowering stage. Therefore, tolerant accessions (AC07 and AC02) in group I which are highly correlated with the yield traits and LWI21 became sensitive to drought because they produced higher seed yield under drought stress.

High positive correlations among traits such as stomatal conductance and pod length, with their moderate positive correlation with wilting parameters such as DSS21 and PWP21, implies that accessions with high stomatal conductance maintained higher pod length and were more affected by the drought stress. Furthermore, the high negative correlations exhibited between the major yield traits (seed yield, total number of pods per plant, percentage pod set, and seeds per plant) and stomatal conductance suggest that such accessions connected with stomatal conductance in group II (AC08, AC03, AC10, and AC01) were very poor in yield under drought stress. Such accessions can be enhanced through hybridization with the accessions in group I. However, high positive correlations among RWC, recovery parameters, and SG14, imply that ability to maintain high tissue moisture status under drought stress encouraged higher recovery, greenness, regrowth, and higher stem girth of accessions. Therefore, studies on drought tolerance in cowpea should consider wilting parameters with traits such as relative water content, stomatal conductance, yield parameters, and recovery parameters. PCA and biplot align with the findings in both the seedling and vegetative stages (Ajayi et al., 2017b; 2020). Successful selection of drought-tolerant traits and genotypes of sorghum through PCA and biplot have been reported (Bibi et al., 2012).

4.4. Estimates of genetic variation, heritability, and genetic advance of accessions of cowpea screened under drought stress at the flowering stage

It is always helpful to divide phenotypic differences into genetic and non-heritable constituents which include the coefficient of variations on genotypic (GCV), and phenotypic (PCV) levels, heritability, and genetic advance as a percentage of the mean (GAM). These parameters are important for trait improvement and as well as for the effective selection of genotypes for different environmental constraints (Ajayi et al., 2017a). In the present study, GCV was revealed to be lower than PCV for all traits, also, both PCV and GCV were lowered under drought stress compared to the initial values among the wilting parameters except for RWC, TLL, and TLW, similar to the seedling stage (Ajayi et al., 2017a). The reduction of PCV and GCV by drought has been linked to the effect of the environment, suggesting that the performance of genotypes is better under normal growing conditions compared to drought stress (Sabiel et al., 2014). However, GCV and PCV increased under drought stress in the present study in RWC and all morphological traits as reported by Hefny (2013). The marginal differences between GCV and PCV among traits suggest that the traits are mostly governed by the genes. Virtually all traits demonstrated moderate to high heritability under the imposed drought. Heritability was higher under drought stress in traits such as LWI21, RWC14, and TLW7. Wilting parameters (except LWI), stomatal conductance, TLW7, and all the yield parameters (except seeds per plant) showing both high heritability and GAM under stress suggest that these traits can be used directly to select drought-tolerant cowpea. This is applicable in traits with moderate heritability and high GAM such as seeds per plant, LWI7, LWI14, and RCW14. These findings are similar to the reported data among the same accessions screened under drought stress at the seedling stage. While moderate heritability in RWC agrees with Ajayi (2020), the high heritability for stomatal conductance in the present study is in line with Agbicodo et al., (2009). Heritability was also high for morphological attributes such as the total weight of wheat plants and root weight under drought stress (Naeem et al., 2015). Therefore, centered on heritability and genetic advance, selections for attributes such as wilting traits (DSS, PPW, and LWI), recovery parameters (PREC, STG, and STR), stomatal conductance, and morphological attributes such as stem girth and terminal leaflet width under drought stress would be more effective.

5. Conclusion

Accessions of cowpea varied significantly in their responses to drought stress at the flowering stage. Based on cluster analysis, broad-sense heritability, GAM, PCA, and biplot, selection for traits such as the wilting, RWC, stomatal conductance, terminal leaflet width, and stem girth, and yield parameters such as percentage pod set, the total number of pods, seeds per plant, pod length and seed yield per plant and the recovery parameters under drought stress would be effective. Upon these parameters, four major classes of tolerance were determined: accessions such as AC03, AC08, and AC10 were categorized as highly susceptible. AC01 and AC04 were classified as moderately susceptible. Accessions AC06, AC07, and AC09 were moderately tolerant, while AC02 and AC05 were the highly tolerant accessions. The moderately tolerant and the highly tolerant accessions showed a combination of superior resistance to wilting, superior recovery rates, and superior yield attributes. They also showed lower stomatal conductance, higher RWC, and low reduction of RWC and stem girth under the imposed drought compared to the susceptible ones.

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