Research Article

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Phenotyping cowpea accessions at the seedling stage for drought tolerance in controlled environments

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Abstract: One of the most important screening techniques used in cowpea selection for drought tolerance is screening at the seedling stage. The objective of this study was to phenotype 60 cowpea genotypes for seedling drought tolerance in screen houses (glasshouse and greenhouse). Principal component analysis revealed that of the 14 variables, the first 4 expressed more than 1 eigenvalue. Data showed that PC1, PC2, and PC3 contributed 39.3, 15.2, and 10%, respectively, with 64.68% total variation. A PCA plot and biplot showed that the number of pods (NP), seeds per pod (SP), survival count (SC), pod weight (PWT), and stem wilting in week 1 (WWK1) had the most significant contributions to genetic variability to drought tolerance and to yield after stress imposition based on the PCA, biplot, and cluster plot, the accessions IT 07-292-10, IT 07-274-2-9, IT90K-59, 835-911, RV 343, and IT 95K-2017-15 had the maximum variability in terms of NP, SP, SC, PWT, and WWK1 after drought imposition. Cowpea accessions 835-911, IT 07-292-10, RV 344, *Kangorongondo*, and IT 90K-59 were the major individuals that contributed mainly to domain information model (DIM) 1 and 2. The accessions that contributed the least were IT 89KD288, *Chibundi mavara*, and TVU12746. Thirty-six cowpea accessions from both screen houses were tolerant to drought, 15 were moderately tolerant, while 23 were susceptible. The findings of the study provided a useful tool for screening and determining drought-tolerant and susceptible accessions at the seedling stage.

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1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], Fabaceae, ($2n = 2x = 22$) is an important leguminous crop in developing countries, especially in sub-Saharan Africa, Asia, and Latin America, with a genome size of about 620 million base pairs [1]. The improvement in cowpea is mainly dependent on breeding and selection from existing landraces according to the existence of phenotypic variability, which is largely influenced by environmental conditions.

According to the Food and Agriculture Organisation of the United Nations (FAO), cowpea was grown on 1 million ha in Africa in 2014, with the bulk of production occurring in West Africa, particularly in Niger, Nigeria, Burkina Faso, Mali, and Senegal [2]. The global cowpea production was 5.59 million and the average yield was 443.20 kg/ha [3]. Africa leads in both area and production, accounting for about 95% of each. Niger and Nigeria are the leading producers of cowpea, together accounting for about 70% of the area and 67% of production worldwide. Most cowpea cultivars have relatively short growth and maturation cycles of 60–80 days, which makes them suitable for drought-prone regions [4].

Drought is one of the most serious environmental stresses, and it has a significant negative impact on crop yield. Authors of ref. [5] recommended the use of water-efficient varieties in combination with good crop husbandry practices. Cowpea plants exposed to temperatures of 30–38°C from 8 days after emergence to maturity had very limited vegetative growth and reproductive potential. Authors of refs. [6,7] observed that there is a great need to screen and breed for drought-tolerant and water-efficient varieties in Africa, as cowpea is grown mostly under rain-fed conditions, with frequent exposure to intermittent droughts. Authors of refs. [5,8] recommended the use of well-adapted, early maturing cultivars in the smallholder farming sector to escape losses from late season droughts. In an experiment by the author of ref. [9], to ascertain the growth of cowpea cultivars under osmotic stress, seeds of three cowpea cultivars (BRS Tumucumaque, BRS Aracê, and BRS Guariba) were germinated at five osmotic potentials after three pre-treatments: pre-soaking in deionised water, pre-soaking in salicylic acid, and without pre-soaking. It was observed that salicylic acid promoted a reduction in abiotic stress, and BRS Guariba was more tolerant to water deficits and adjusted its cellular electrolyte leakage to increase its proline content under induced water stress.

In a wooden box experiment, to screen cowpea recombinant inbred lines (RILs) for seedling drought tolerance [10], 200 inbred lines were used. It was observed that 12 RILS performed well for recovery, 13 RILS were susceptible to drought stress, and 11 RILS had higher relative water and chlorophyll contents. Authors of ref. [11] analysed 10 cowpea accessions under screen house conditions and observed significant differences among accessions for percentage plant recovery, stem regrowth, and stem greenness. For the evaluation of four Mozambican cowpea landraces for drought tolerance, authors of ref. [12] determined that variability exists among the landraces in terms of growth under drought conditions, with *Timbawene moteado* having considerably higher leaf dry biomass, leaf and nodule protein content, and symbiotic nitrogen fixation compared to those of other landraces, as well as the lowest increase in proteolytic activity.

In a screen house experiment, to select drought-tolerant cowpea seedlings, authors of ref. [8] evaluated 23 cowpea accessions at the seedling stage in the 2013 and 2014 growing seasons. They observed that the plant height, number of leaves, and stem greenness were all affected by drought stress. It was found that five varieties, Kanannado, Danila, IT07K-297-13, IT03K-378-4, and Aloka local, were highly tolerant to drought. In addition, six varieties IT07K-322-40, IT07K-313-41, IT07K-291-92, IT06K-270, IT07K-244-1-1, and IT06K-275 were classified as highly susceptible to drought and the remaining 12 varieties were found to be neither tolerant nor susceptible to drought. Most cowpea plants exposed to moisture variation during the vegetative or reproductive stages perform poorly; hence, seedling-stage screening is ideal in this scenario. The objective of this study was to phenotype 60 cowpea genotypes for seedling drought tolerance in screen houses.

2 Materials and methods

2.1 Plant material

Sixty cowpea accessions collected from three geographic origins were used in this study (Table 1). Out of these, 33 accessions were from the International Institute of Tropical Agriculture (IITA) in Nigeria, 19 accessions were from the Agricultural Research Council – Grain Crops in South Africa, and 8 accessions were from smallholder farmers in Buhera District in Zimbabwe.

2.2 Planting and data collection

Seeds of cowpea accessions were planted in 20 cm diameter pots in topsoil mixed with compost (3:1) in a greenhouse (environment 1) at the Agriculture Research Council – Grain Crops in Potchefstroom, South Africa, in January 2019. The experiment was repeated in a glasshouse (environment 2) in February 2019. An alpha lattice design with four blocks was used for both the experiments. Sixty accessions were carefully selected for drought tolerance at the seedling stage and were used in the experiments. A triplicated 10×6 alpha lattice design was used for the experiments. After planting, the pots were watered to field capacity for their establishment, thereafter which watering was completely withheld for 3 weeks after planting (WAP), when plants were at the three-leaf stage [10]. Thereafter, wilted plants of each variety were counted daily until all the plants of the susceptible lines had wilted. Stress was measured by observing all dead plants in the susceptible group. Watering resumed at 3 weeks after stressing in both the greenhouse (environments 1 and 2) experiments until harvest. After the resumption of watering, the number of recovered seedlings were rated for recovery. Based on the days to wilting and percentage recovery, the accessions were rated as either drought-tolerant or -susceptible. The longer an accession took to wilt as well as its ability to recover after being subjected to drought stress were very important determinants in the experiment. Drought-related traits were recorded at the seedling stage on days to emergence (DTE), recovery rate (RR), survival count (SC), stem greenness from week 1–3 (SGWK1, SGWK3, and SGWK3) and wilting in week 1–3 (WWK1, WWK2, and WWK3). Yield-related traits were recorded as average seeds per pod (AVSD), pod length (PL), pod width (PWDTH), and pod weight (PWT).

Table 1: List of cowpea accessions used in the study obtained from three geographic regions

Entry	Name	Source	Origin
1	Dr Saunders	ARC-GC	South Africa
2	IT96D-610	IITA	Nigeria
3	RV 574	ARC-GC	South Africa
4	RV 342	ARC-GC	South Africa
5	Pan 311	ARC-GC	South Africa
6	Bechuana white	ARC-GC	South Africa
7	Barapara jena	Buhera	Zimbabwe
8	TVU 9443	IITA	Nigeria
9	95K-589-2	IITA	Nigeria
10	RV 344	ARC-GC	South Africa
11	Agrinawa	ARC-GC	South Africa
12	IT 95K-207-15	IITA	Nigeria
13	Orelo	IITA	Nigeria
14	TVU 9671	IITA	Nigeria
15	Mutonono	Buhera	Zimbabwe
16	UAM-14-143-4-1	IITA	Nigeria
17	98K-503-1	IITA	Nigeria
18	RV 503	ARC-GC	South Africa
19	86 D 1010	IITA	Nigeria
20	TVU 9620	IITA	Nigeria
21	RV 202	ARC-GC	South Africa
22	RV 351	ARC-GC	South Africa
23	Encore	ARC-GC	South Africa
24	TVU 14190	IITA	Nigeria
25	IT 89KD-288	IITA	Nigeria
26	RV 551	ARC-GC	South Africa
27	IT 82E-18	IITA	Nigeria
28	Barapara purple	Buhera	Zimbabwe
29	Kangorongondo	Buhera	Zimbabwe
30	835-911	IITA	Nigeria
31	ITOOK 76	IITA	Nigeria
32	98K-476-8	IITA	Nigeria
33	Ziso dema	Buhera	Zimbabwe
34	Chibundi mavara	Buhera	Zimbabwe
35	90K-284-2	IITA	Nigeria
36	RV 221	ARC-GC	South Africa
37	RV 343	ARC-GC	South Africa
38	IT 98K-506-1	IITA	Nigeria
39	Oleyin	IITA	Nigeria
40	IT 07-292-10	IITA	Nigeria
41	IT 08K-150-27	IITA	Nigeria
42	RV500	ARC-GC	South Africa
43	IT 90K-277-2	IITA	Nigeria
44	98D-1399	IITA	Nigeria
45	ITOOK 1263	IITA	Nigeria
46	RV 563	ARC-GC	South Africa
47	IT 18	Buhera	Zimbabwe
48	RV 194	ARC-GC	South Africa
49	335-95	IITA	Nigeria
50	TVU 12746	IITA	Nigeria
51	IT 07-274-2-9	IITA	Nigeria
52	97K-499-35	IITA	Nigeria
53	IT 07-318-33	IITA	Nigeria
54	IT89-KD-288	IITA	Nigeria
55	RV558	ARC-GC	South Africa
56	IT 99K-573-2-1	IITA	Nigeria
57	Mupengo dema	Buhera	Zimbabwe
58	CH47	ARC-GC	South Africa
59	TVU 13004	IITA	Nigeria
60	IT 90K-59	IITA	Nigeria

Table 1: *Continued*

IITA – International Institute of Tropical Agriculture; ARC-GC – Agriculture Research Council Grain Crops.

2.3 Data collection

2.3.1 Temperature conditions of the screen houses

The daily minimum and maximum temperatures of the screen houses were captured using temperature loggers. The loggers were placed in the screen houses and set to record the temperature at hourly intervals for the whole period of the experiment. The highest and lowest day temperatures recorded in the greenhouse (environment 1) were 35.75°C and 27.67°C, respectively. The highest and lowest night temperatures recorded in the greenhouse (environment 1) were 26.87°C and 19.99°C, respectively. The highest and lowest daytime temperatures recorded in the glasshouse (environment 2) were 36.4°C and 19°C, respectively. The highest and lowest night temperatures recorded in the glasshouse (environment 2) was 23.64°C and 18.5°C, respectively.

2.3.2 Agronomic traits

Drought tolerance was estimated using the wilting score (WS) as the degree of wilting severity, based on the 0–4 score scale as described in ref. [5]. Data were collected on number of days to seedling emergence, stem greenness, and wilting at 14, 21, and 30 days after planting (DAP), and rated on a scale of 0–4 [13].

Stem greenness (SGWK)

- 0 – leaves and stem completely yellow;
- 1 – 75% of the leaves yellow, brown either from the base or tip of the stem;
- 2 – 50% yellow or pale green, stem not turgid;
- 3 – 25% yellow, 75% green, stem less turgid;
- 4 – completely green, stem turgid.

Wilting (WWK)

- 0 – no sign of wilting;
- 1 – 25% wilting;
- 2 – moderate wilting, 50%;

3 – yellow and brown leaves with 75% wilting;

4 – completely wilted.

After re-watering, data were collected on the SC: the number of surviving plants per genotype.

Recovery type

0 – no recovery;

0.5 – recovery from the basal meristem;

1 – recovery from the apical meristem;

The Recovery rate (RR) was computed as follows: (No. of dead plants/No. of emerged plants) × 100

2.4 Data analysis

A two-way analysis of variance (ANOVA) was used to determine significant differences in DTE, wilting scores, SC, and yield-related traits. GenStat (version 19) software (www.genstat.kb.vsni.co.uk) was used for the statistical analysis of data. Statistical analysis was performed using [14] (www.ibm.com/support/pages/spss-statistics-20-available-download) statistical computer package for Bartlett's test of sphericity, principal component analysis, scree plot, Kaiser–Meyer–Olkin measure of sampling of adequacy and rotated component plot.

3 Results

There were significant differences among most drought-related traits at the seedling stage on DTE, RR, SC, SGWK1, SGWK3, and SGWK3 and WWK1, WWK2, and WWK3). There were also significant differences at $p < 0.01$ between Envt × Genotype and RR, SGWK2, SGWK3,WWK1, WWK2, and WWK3 (Table 2).

There were significant differences among most yield-related traits on average seeds per pod (SP), PL, PWDTH, and PWT. There were also significant differences at $p < 0.01$ between Envt × Genotype and SP, NP, number of seeds (NSDS), PL, PWDTH, and PWT (Table 3).

3.1 Principal component analysis

Principal component analysis (PCA) revealed that of the 14 component variables (PCs) only 4 PCs with eigenvalue

Table 2: Mean square of traits from the analysis of variance from two screen houses for 60 cowpea accessions under drought stress conditions

		Trait								
Source	**DF**	**DTE**	**RR**	**SC**	**SGWK1**	**SGWK2**	**SGWK3**	**WWK1**	**WWK2**	**WWK3**
Envt	1	36.74**	9343.2**	137.52**	52.90**	62.5**	46.94**	154.71**	18.68**	122.50**
Envt × Genotype	118	0.46 ns	1012.2**	0.30 ns	0.44 ns	0.78**	0.72**	0.52**	1.29**	0.57**
Rep	2	0.81 ns	3432.5**	84.94**	9.29**	4.84**	0.58**	3.10**	3.94**	34.70**
Residual	238	0.34	317.4	0.81	0.31	0.38	0.39	0.2292	0.39	0.31

**– significant at $p < 0.01$; ns – not significant; DTE – date to emergence; RR – recovery rate; SC – survival count; SGWK1 – stem greenness in week 1 after imposition of water stress; SGWK2 – stem greenness in week 2 after imposition of water stress; SGWK3 – stem greenness in week 3 after imposition of water stress; WWK1 – level of wilting in week 1 after imposition of water stress; WWK2 – level of wilting in week 2 after imposition of water stress; WWK3 – level of wilting in week 3 after imposition of water stress.

Table 3: Mean square of yield-related traits from the analysis of variance from two screen houses for 60 cowpea accessions after drought stress

		Trait					
Source	**DF**	**SP**	**NP**	**NSDS**	**PL**	**PWDTH**	**PWT**
Envt	1	59.211**	30.044 ns	20.07 ns	226.768**	1.22267**	512.298**
Envt × Gen	118	28.831**	17.574**	1006.99**	51.074**	0.33401**	215.105**
Rep	2	20.4	16.3**	284.63**	31.63**	0.2433**	132.764**
Residual	238	1.936	3.462	62.35	2.453	0.01265	7.068

**– significant at $p < 0.01$; ns – not significant; AVSD – average seeds per pod; NP – number of pods; NSDS – number of seeds; PL – pod length; PWDTH – pod width; PWT – pod weight.

greater than 1 were maintained (Table 4). The 4 PCs explained 72.1% of the total phenotypic variation. The first principal component (PC) was positively influenced by PWT, with a value of 0.358, as well as by PL (0.286), SP (0.263), SWT (0.255), and NP (0.181). PC2 was influenced by SGWK3, with a value measuring 0.332, and SC, with a value of 0.232. In PC3, SGWK1 had the highest value (0.384), followed by SGWK2 (0.295), and PWT (0.109). In PC4, the DTE had a positive influence (0.926), as did SGWK2 (0.194).

A scree plot to show the relationship between eigenvalues and principal components was constructed to summarise the contribution of PCs (Figure 1). The plot showed that maximum variation was present in variable 1 with the highest eigenvalue of 5.8 followed by variable 2 (2.1), variable 3 (1.4), and variable 4 (1). Variable 14 had the lowest eigenvalue (0).

A further PCA with VARIMAX rotation was conducted to assess how the variables were clustered (Figure 2). The component plot in rotated space (Figure 2) highlights the important variables in order when all three components are compared. WWK1, WWK2, and SC are the most important variables of the three components, respectively. Bartlett's test of sphericity was significant at $p < 0.05$, while the Kaiser–Meyer–Olkin measure of sampling of adequacy was 77, indicating sufficient items for each factor.

Table 4: Eigen-values, proportions of variability, and morphological traits that contributed to the first four PCs of cowpeas

	PC1	PC2	PC3	PC4
Eigen values	5.51	2.13	1.41	1.04
Proportion of variance (%)	39.4	15.2	10.1	7.4
Cumulative variance (%)	39.38	54.6	64.7	72.1
DTE	0.034	0	−0.008	0.926
SGWK1	−0.05	−0.101	0.384	−0.169
SGWK2	−0.045	0.032	0.295	0.194
SGWK3	−0.123	0.332	−0.004	0.088
WWK1	−0.07	0.13	−0.339	−0.039
WWK2	0.069	−0.117	−0.216	0.063
WWK3	0.111	−0.277	−0.052	0.004
SC	0.004	0.232	−0.178	0.025
Recovery Rate	0.095	−0.322	0.055	0.095
NP	0.181	0.063	−0.036	−0.047
SP	0.263	−0.012	−0.074	0.033
PL	0.286	−0.048	−0.074	0.035
PWT	0.358	−0.283	0.109	0.022
SWT	0.255	−0.034	0.027	0.012

SGK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; SWT – seed weight.

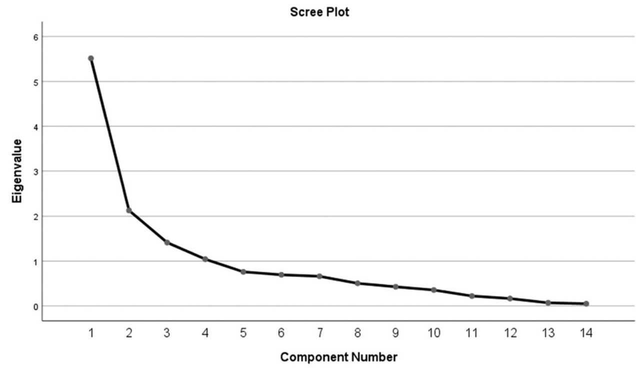

Figure 1: Scree plot showing contributions of PCs to variability.

The day and night temperature ranges in both the screen houses varied during the three-week period when the plants were stressed. The highest and lowest daytime temperatures recorded in the greenhouse (environment 1) were 35.75 and 27.67°C, respectively, with a mean daytime temperature of 32.24°C (Figure 3). The highest and lowest night-time temperatures recorded in the greenhouse (environment 1) were 26.87 and 19.99°C, with a mean night-time temperature of 23.98°C, respectively. The highest and lowest daytime temperatures recorded in the glasshouse (environment 2) was 36.4 and 19°C, with a mean daytime temperature of 26.06°C respectively. The highest and lowest temperatures recorded in the glasshouse (environment 2) was 23.64 and 18.5°C, with a mean night-time temperature of 21.42°C, respectively.

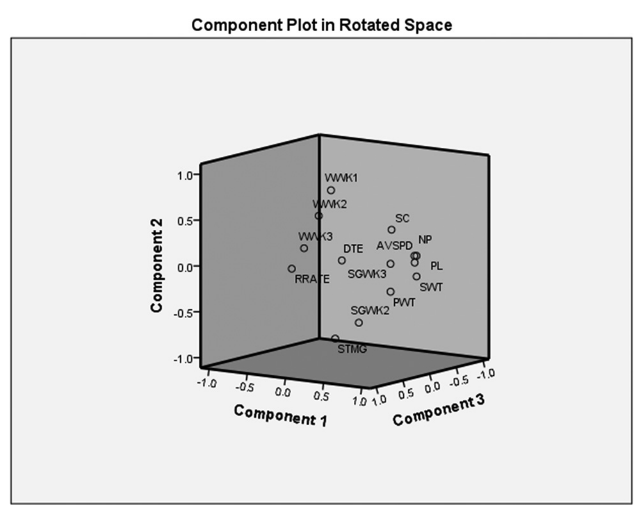

Figure 2: Component plot in rotated space showing contributions of principal component variables. SGK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK 3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; and SWT – seed weight.

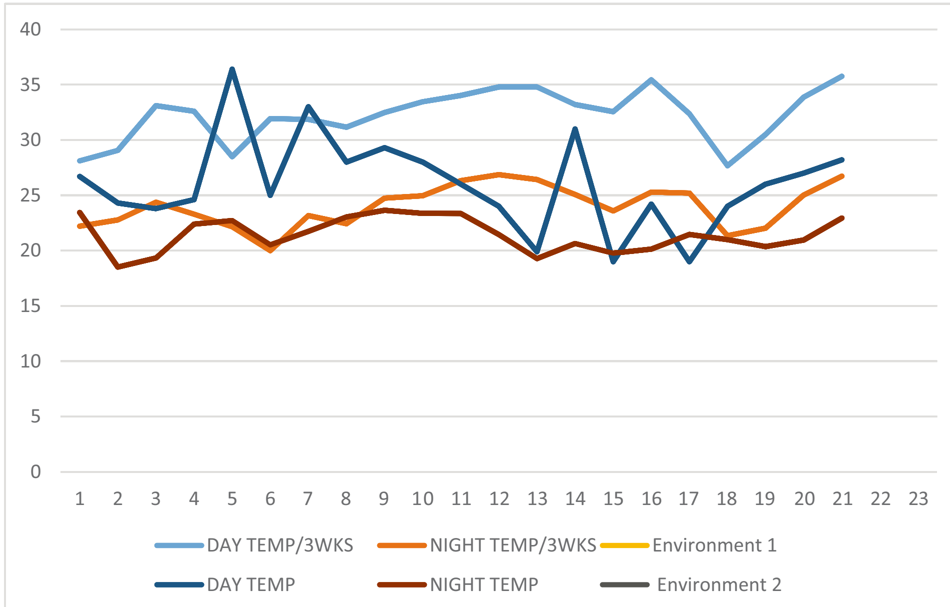

Figure 3: Graph showing day and night temperature ranges for 3 weeks.

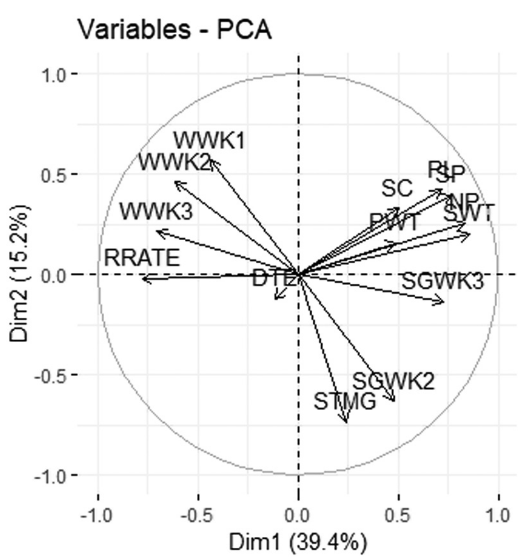

Figure 4: The contribution of various variables among 60 cowpea accessions screened for drought tolerance. SGK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; SWT – seed weight.

In the PCA plot, NP, SP, SC, PW, and WWK1 had the most significant contributions to genetic variability in the drought tolerance cowpea accessions, as well as to yield after stress imposition (Figure 4).

Cowpea accessions 835-911, IT 07-292-10, RV 344, *Kangorongondo*, and IT 90K-59 contributed the most to both DIM 1 and DIM 2 (Figure 5). The accessions IT 89KD288, *Chibundi mavara*, and TVU12746 contributed the least.

The main contributors to DIM 3 were PWT, SGWK3, and RR (Figure 6). The variables that contributed the least were SWT, PL, and SPD.

Figure 7 shows the relationships among traits in DIM 1 to DIM 5. SWT, NP, and SGWK3 dominated DIM 1. DIM 2 was dominated by SGWK1 and SGWK2 after drought imposition. PWT was the dominant trait in DIM 3, while that in DIM 4 was DTE.

The cluster plot analysis showed that the cowpea accessions could be grouped into three distinct clusters; red, blue, and green (Figure 8). Most accessions were grouped into the red and blue clusters. However, there was an overlap of accessions in the green and red clusters. As such, some accessions (TVU 13004, ITOOK 1263, IT89 KD 288, RV 588, Bechuana White, TVU 12746, IT07-318-33, and TVU 9671) managed to withstand water stress and went on to flower and produce pods when irrigation was resumed after 3 weeks of water stress.

The relationship of cowpea traits was studied using correlation coefficients. The correlation coefficient was statistically significant between SWT and SGWK3, WWK2, WWK3, SC, NP, SP, PL, as well as PWT. Also, the correlation coefficient was statistically significant in SGWK2 compared to those of other traits. This means that there was an inverse relationship between stem greenness and weeks. This was due to the fact that as the cowpea accessions were subjected to more time under drought stress, they showed different

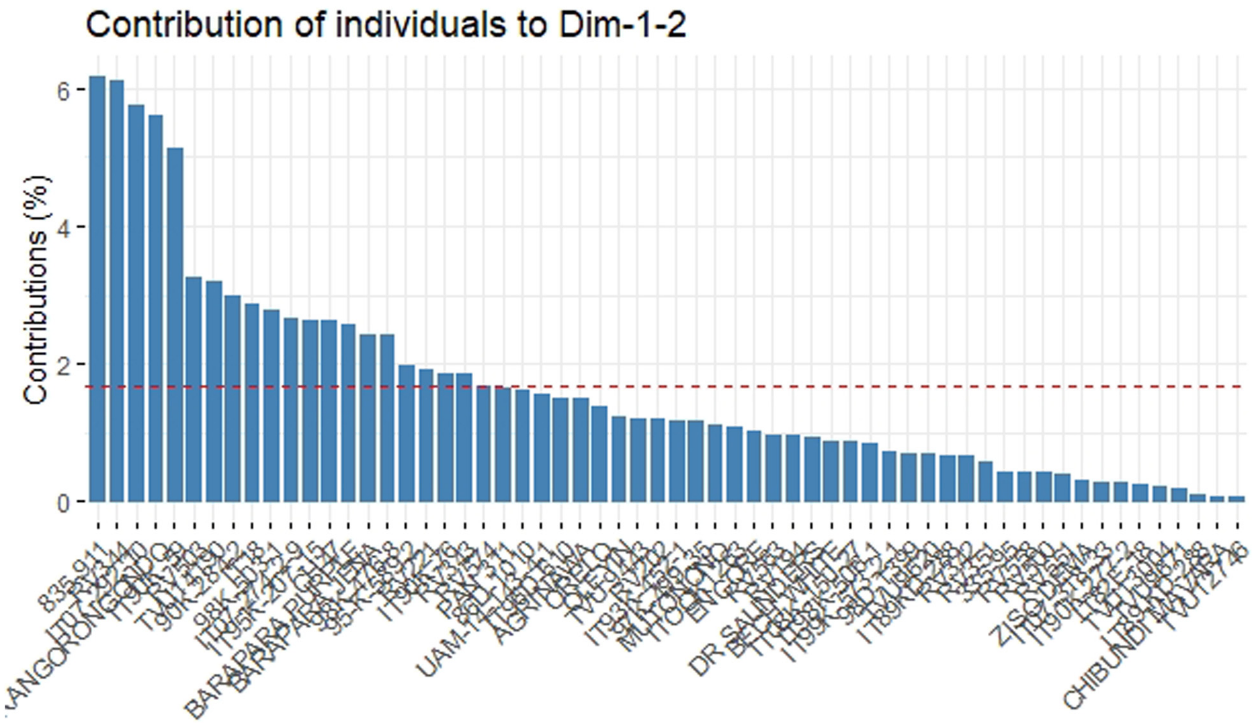

Figure 5: The contribution of 60 cowpea accessions screened to DIM 1 and 2.

responses. Some wilted earlier, while others were gradual. All of the significant correlation coefficients were positive and were mainly between DTE, SGWK1, SGWK3, SC, NP, SP, PL, PWT, and SWT (Table 5). Pearson correlation analysis showed that the most significant relationships were observed from SGWK2 up to SWT. In addition, DTE had mostly weak negative correlations with most of the measured attributes. Most positive correlations were observed in SWT, PWT, and PL.

The 60-cowpea accessions used in this study varied in their response to drought imposition. Thirty-six cowpea accessions from both screen houses were tolerant to drought, 15 were moderately tolerant, while 23 were susceptible, based on the 14 traits measured (Table 6).

The biplot highlights the relationship of each traits and accessions to drought tolerance at the seedling stage (Figure 9).

The neighbour-joined cluster analysis generated by UPGMA divided the 60-cowpea accessions into two main clusters (Figure 10). The cluster analysis showed that the 60 accessions were grouped into two major clusters and other subclusters with their respective distances.

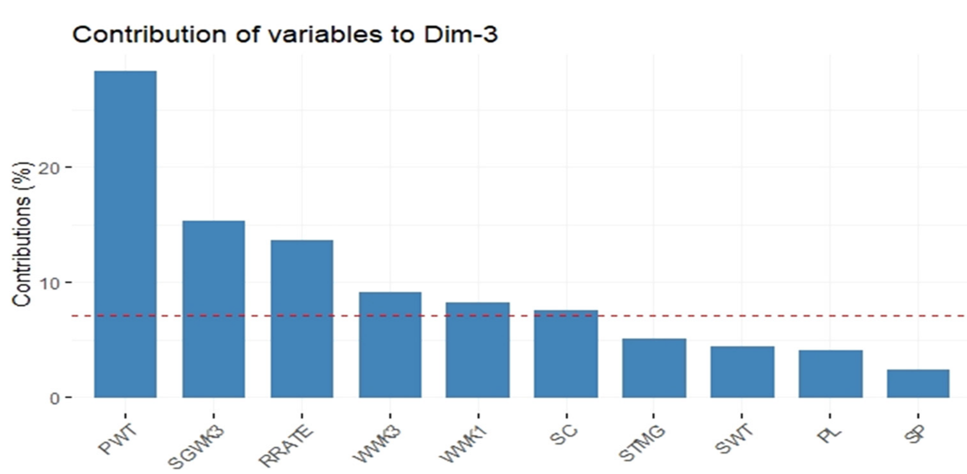

Figure 6: The contribution of various variables among 60 cowpea accessions screened for drought tolerance. SGK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; SWT – seed weight.

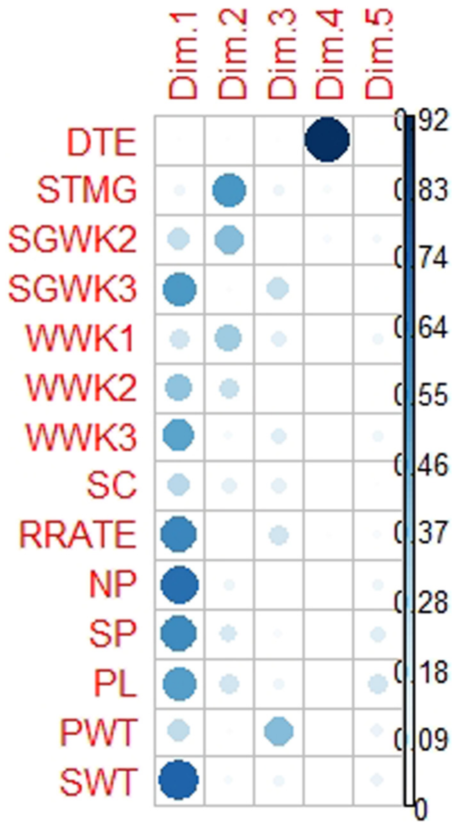

Figure 7: The contribution of various variables to DIM 1 to DIM 5. SGK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; SWT – seed weight.

The phenotypic height index based on morphological traits ranged from 1 (IT 89KD-288 from IITA) to 50 (TVU 13004 and IT96D-610 from IITA). The phenotypic height index of other accessions in other subclusters was less than 20.

4 Discussion

This study revealed that moisture is a very important component in plant growth and reproduction. According to ref. [15], when moisture stress is imposed during the vegetative stage, it has the most effect on shoot and dry weight reduction in cowpea. It is also during the vegetative stage that plants set up their architecture for reproduction. Author of ref. [16] observed that moisture stress imposed after the pod-filling stage in determinate accessions has a limited reduction in the shoot and root biomass.

Most of the cowpea accessions showed differences in their response to drought imposition in their stem greenness from week 1 to week 3 after drought imposition. A similar variation was also observed when wilting was recorded from week 1 to week 3 after drought imposition. In both environments, the temperature had a significant effect on the performance of the accessions. In the greenhouse (environment 1) experiment, the average day and night temperatures were 34.24°C and 23.98°C, respectively. In the glasshouse (environment 2) experiment, the mean day and night temperatures were 26.06°C and 21.42°C, respectively. According to ref. [17], the optimum

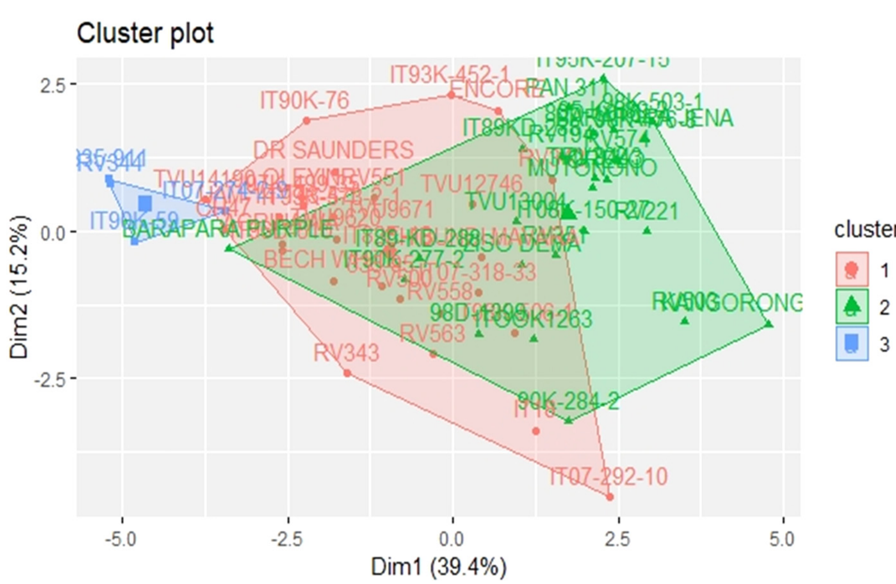

Figure 8: Cluster plot showing the three groups of cowpea accessions grouped according to their levels of drought tolerance. Cluster 1 – moderately tolerant; cluster 2 – susceptible; cluster 3 – tolerant.

Table 5: Pearson correlation coefficients of traits assessed for 60 cowpea accessions evaluated under two moisture regimes

	DTE	STMG	SGWK2	SGWK3	WWK1	WWK2	WWK3	SC	RRATE	NP	SP	PL	PWT	SWT
DTE	1	−0.062	0.132	0.047	−0.012	0.042	0	−0.034	0.07	−0.204	−0.102	−0.118	−0.216	−0.179
STMG		1	0.460**	0.11	−0.476**	−0.362**	−0.256*	−0.104	−0.079	−0.008	0.003	−0.028	0.068	0.064
SGWK2			1	0.372**	−0.356**	−0.475**	−0.266*	0.091	−0.24	0.178	0.141	0.096	0.169	0.214
SGWK3				1	−0.255*	−0.360**	−0.729**	0.285*	−0.696**	0.516**	0.402**	0.349**	0.439**	0.472**
WWK1					1	0.371**	0.349**	0.108	0.162	−0.19	−0.184	−0.203	−0.246	−0.258*
WWK2						1	0.456**	−0.218	0.449**	−0.313*	−0.286*	−0.195	−0.324*	−0.378**
WWK3							1	−0.324*	0.667**	−.460**	−.396**	−0.358**	−.397**	−0.446**
SC								1	−0.506**	0.374**	0.382**	0.367**	0.421**	0.416**
RRATE									1	−0.687**	−0.550**	−0.456**	−0.602**	−0.640**
NP										1	0.781**	0.723**	0.912**	0.939**
SP											1	0.933**	0.813**	0.839**
PL												1	0.778**	0.795**
PWT													1	0.954**
SWT														1

**– significant at $p < 0.01$; * – not significant; DTE – date to emergence; SGWK1 – stem greenness in week 1; SGWK2 – stem greenness in week 2; SGWK3 – stem greenness in week 3; WWK1 – wilting in week 1; WWK2 – wilting in week 2; WWK3 – wilting in week 3; SC – survival count; RR – recovery rate; NP – number of pods; SP – seeds per pod; PL – pod length; PWT – pod weight; SWT – seed weight.

Table 6: The response to drought stress of 60-cowpea accessions based on 14 traits measured

Entry	Name	Source	Origin	Response to drought
1	Dr Saunders	ARC-GC	South Africa	Tolerant
2	IT96D-610	IITA	Nigeria	Tolerant
3	RV 574	ARC-GC	South Africa	Moderate
4	RV 342	ARC-GC	South Africa	Moderate
5	Pan 311	ARC-GC	South Africa	Moderate
6	Bechuana white	ARC-GC	South Africa	Tolerant
7	Barapara jena	Buhera	Zimbabwe	Susceptible
8	TVU 9443	IITA	Nigeria	Moderate
9	95K-589-2	IITA	Nigeria	Susceptible
10	RV 344	ARC-GC	South Africa	Susceptible
11	Agrinawa	ARC-GC	South Africa	Tolerant
12	IT 95K-207-15	IITA	Nigeria	Susceptible
13	Orelo	IITA	Nigeria	Moderate
14	TVU 9671	IITA	Nigeria	Tolerant
15	Mutonono	Buhera	Zimbabwe	Moderate
16	UAM-14-143-4-1	IITA	Nigeria	Tolerant
17	98K-503-1	IITA	Nigeria	Susceptible
18	RV 503	ARC-GC	South Africa	Moderate
19	86 D 1010	IITA	Nigeria	Moderate
20	TVU 9620	IITA	Nigeria	Tolerant
21	RV 202	ARC-GC	South Africa	Moderate
22	RV 351	ARC-GC	South Africa	Moderate
23	Encore	ARC-GC	South Africa	Moderate
24	TVU 14190	IITA	Nigeria	Tolerant
25	IT 89KD-288	IITA	Nigeria	Moderate
26	RV 551	ARC-GC	South Africa	Tolerant
27	IT 82E-18	IITA	Nigeria	Tolerant
28	Barapara purple	Buhera	Zimbabwe	Susceptible
29	Kangorongondo	Buhera	Zimbabwe	Susceptible
30	835-911	IITA	Nigeria	Tolerant
31	ITOOK 76	IITA	Nigeria	Tolerant
32	98K-476-8	IITA	Nigeria	Susceptible
33	Ziso dema	Buhera	Zimbabwe	Tolerant
34	Chibundi mavara	Buhera	Zimbabwe	Tolerant
35	90K-284-2	IITA	Nigeria	Tolerant
36	RV 221	ARC-GC	South Africa	Susceptible
37	RV 343	ARC-GC	South Africa	Tolerant
38	IT 98K-506-1	IITA	Nigeria	Tolerant
39	Oleyin	IITA	Nigeria	Tolerant
40	IT 07-292-10	IITA	Nigeria	Tolerant
41	IT 08K-150-27	IITA	Nigeria	Moderate
42	RV500	ARC-GC	South Africa	Tolerant
43	IT 90K-277-2	IITA	Nigeria	Tolerant
44	98D-1399	IITA	Nigeria	Tolerant
45	ITOOK 1263	IITA	Nigeria	Tolerant
46	RV 563	ARC-GC	South Africa	Tolerant
47	IT 18	Buhera	Zimbabwe	Tolerant
48	RV 194	ARC-GC	South Africa	Moderate
49	335-95	IITA	Nigeria	Tolerant
50	TVU 12746	IITA	Nigeria	Tolerant
51	IT 07-274-2-9	IITA	Nigeria	Tolerant
52	97K-499-35	IITA	Nigeria	Tolerant
53	IT 07-318-33	IITA	Nigeria	Tolerant
54	IT89-KD-288	IITA	Nigeria	Tolerant
55	RV558	ARC-GC	South Africa	Tolerant
56	IT 99K-573-2-1	IITA	Nigeria	Tolerant
57	Mupengo dema	Buhera	Zimbabwe	Moderate
58	CH47	ARC-GC	South Africa	Tolerant
59	TVU 13004	IITA	Nigeria	Tolerant
60	IT 90K-59	IITA	Nigeria	Tolerant

ARC-GC – Agriculture Research Council-Grain Crops; IITA – International Institute of Tropical Agriculture.

temperature for growth and development of crops is around 30°C; hence, 27 out of the 37 tolerant accessions were located in the glasshouse (environment 2). Authors of ref. [18] also confirmed that temperatures above 30°C increase the intensity of stress levels in cowpea, thus fewer accessions were found in the greenhouse.

The PC plot highlighted the importance of the distance of variables to PCs and their ultimate contributions to the drought tolerance of accessions, as well as to the yield after stress imposition. The PC plot showed that the NP, SP, SC, PWT, and WWK1 had the most significant contributions to genetic variability in drought tolerance in cowpea accessions, as well as to the yield after stress imposition. In the PC plot, accessions placed far from each other were more diverse. Based on the PC, and scatter plot, the accessions IT 07-292-10, RV 343, and IT 95K-2017-15 had the maximum variability for the NP, SP, SC, PWT, and WWK1, and could be used in future breeding programmes. Authors of ref. [19] used the PC plot to reveal the large variation among 60-cowpea accessions in terms of seed length and width, 100-seed weight, and seed colour.

In DIM 1 and DIM 2, SWT, NP, and SG were the major determinants. Both groups had accessions 835-911, IT07-292-10, IT90-59, IT89KD288, *Chibundi mavara*, and TVU12746, which were tolerant to drought, while RV344 and *Kangorongondo* were susceptible to drought during the first week of drought imposition.

Authors of ref. [20] observed significant and positive correlations among the number of pods per peduncle and number of seeds per pod, PWT, seed length, seed thickness, SW, 100-seed weight, biomass, and harvest index at the genotypic and phenotypic levels. Authors of ref. [21] revealed that at the genotypic and phenotypic levels, a significant and positive correlation was shown by pod yield in quintal per hectare with pod yield per plant and pod length. Authors of ref. [22] observed that

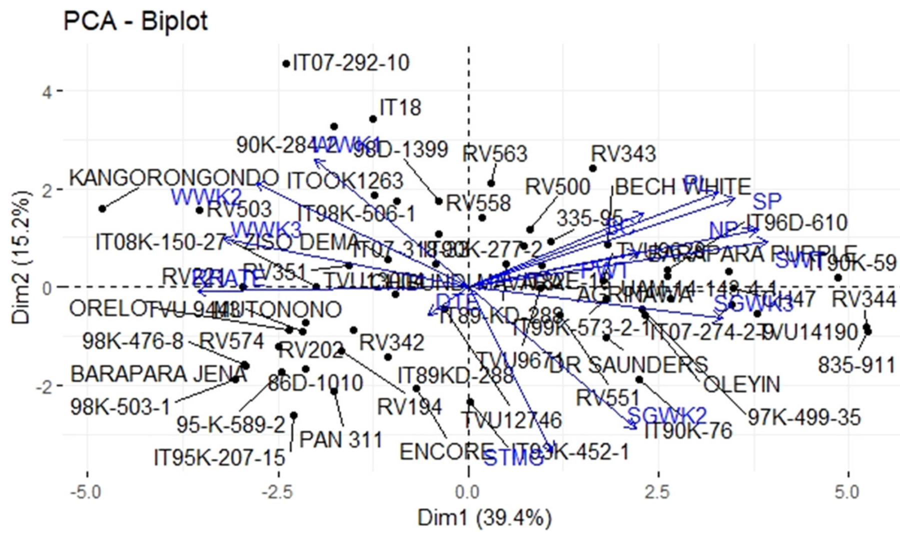

Figure 9: The contribution of various traits and accessions to drought tolerance at the seedling stage.

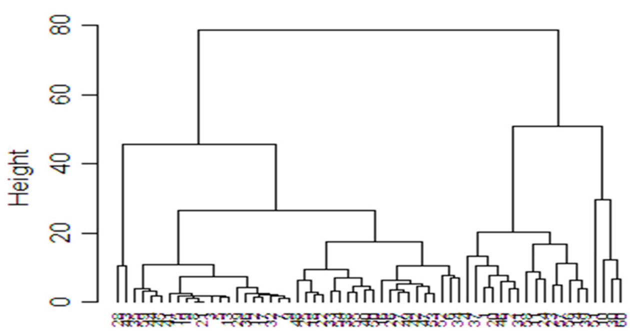

Figure 10: Clustering patterns of the 60-cowpea accessions constructed based on the neighbour-joining algorithm using the unweighted pair group method (UPGMA) according to drought tolerance and yield-related traits.

the genotypic coefficient of variation (GCV) was lower than the phenotypic coefficient of variation (PCV) for all studied traits. They observed that both the GCV and PCV were reduced as drought stress went beyond 21 days among the wilting parameters and morphological traits, because of the influence of the environment on these traits.

The main traits that accounted for variability from PC1 to PC4 in the screen houses were PWT, PL, SP, SWT, NP, SGWK, SC, and DTE. This implies that accessions that emerged earlier and withstood the imposition of drought had higher chances of podding and producing seeds. Thus, it is imperative to consider these traits in further enhancing cowpea accessions' tolerance to drought at the seedling stage. Authors of ref. [11] recommend the drought susceptibility score, percentage of permanent wilting, stem greenness and regrowth, number of leaves, and stem girth as the ideal traits for use in the study of drought tolerance in cowpea seedlings. However, authors of [10] recommend a wide collection of cowpea lines in order to select the most tolerant genotypes for various growth stages as parents in a hybridisation programme.

On the cluster plot analysis, accessions in cluster 1 had higher values compared to all other clusters for all traits investigated in this study except for SGWK1, SGWK2, and SGWK3 after drought imposition and wilting in WWK1 after drought imposition. In both greenhouse and glasshouse experiments, this cluster had early maturing and high yielding accessions that can be used in future cowpea-breeding programmes for drought tolerance at the seedling stage. The accessions used in this study, however, showed very little variation as was highlighted by UPGMA. This supports the findings of the principal component analysis. The differences and similarities in accessions on some clusters because of their locations indicate the extent of accession exchange among farmers from different regions [9].

5 Conclusion

The findings of this study provided a useful tool for screening and determining drought-tolerant and -susceptible accessions at the seedling stage. The results of the investigation were also useful in selecting accessions especially for AVSD, NS, PL, PWDTH, and PWT for further breeding programmes. Some accessions were able to perform well in both screen houses, under different temperature conditions. While the experiments were done in screen houses, it is necessary to evaluate these accessions under different field conditions in different agro- ecological regions. This would further help in screening for the stability of the high tolerant accessions to drought. This stability of accessions with minimal variation in any environment or location can serve as a genetic pool or germplasm collection for the breeding of drought-tolerant cowpea accessions.

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