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Screening for Drought Tolerance in Cowpea (*Vigna unguiculata* L. Walp) at Seedling Stage under Screen House Condition

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ABSTRACT:
One of the challenges limiting the productivity of cowpea is drought. Ten accessions of cowpea (*Vigna unguiculata*) were received from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and were evaluated for their drought tolerance at seedling stage between February and April, 2016. The experiment was laid out in a Completely Randomized Design (CRD) with three replications at the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. Drought was imposed at 16th day of sowing for 21 days when about 90% of seedlings of the most susceptible accession have completely wilted or died. Parameters measured were drought susceptibility scores (DSS), percentage permanent wilting (PPW), and plant height, number of leaves, terminal leaflet length, terminal leaflet width, stem girth, stomata conductance and stomata resistance. Twenty one (21) days after imposition of drought stress, watering then resumed, and 14 days after, percentage plant recovery (PREC), stem regrowth (STR) and stem greenness (STG), were also recorded. Analysis of variance (ANOVA) revealed significant genotypic differences among accessions for DSS and PPW; morphological traits and physiological parameters during the 21 days of drought stress. Analysis also revealed significant differences among accessions for PREC, STR and STG. Among the traits studied, drought susceptibility scores, percentage permanent wilting, stem greenness and regrowth, number of leaves and stem girth were the best traits useful for the study of seedling drought tolerance in cowpea under a controlled condition.
Keywords: cowpea, *Vigna unguiculata*, drought tolerance, wilting scales, recovery parameters

INTRODUCTION

The role of cowpea as an essential component of cropping systems in the tropical and subtropical regions of the world and its importance being a food legume can not be over emphasized [1]. Cowpea contains about 20 – 25% protein; this makes the crop attractive as a source of quality nourishment for both the rural and urban poor people [1], [2], [3]. Cowpea grows fast, curbs erosion by providing cover for ground surface, fixes atmospheric nitrogen and fertilizes the soil with its decaying residues after harvest [4].

Cowpea production has faced numerous challenges in different areas of the world. Few of the stresses encountered by cowpea include drought, heat and cold. Drought is a condition of sustained deficiency in soil moisture to prevent efficient crop growth and development and this has been shown to reduce yield significantly in cowpea [5], [6], [7]. The fact that upcoming drought may be predicted using modern climatological techniques does not rule out its devastating effect on the economy [7]. Drought can occur at mid-season or close to the end of the planting season, and its occurrence regardless of its time has been found to be devastating on yield of cowpea. In the Sahelian and dry savanna zones of West Africa cowpea can experience both heat and drought stress [8]; with the early flowering types escaping drought in some locations and years to end up producing useful grain yields. Unfortunately, the early flowering cowpea cultivars are found to be very susceptible to droughts that occur at the commencement of reproductive development [9]. Meanwhile, delayed – leaf – senescence (DLS) traits have been proved to be beneficial to cowpea as regards adaptation to drought, both in the dry savanna and wetter part of the Sahelian zone, in allowing the quick recovery (and production of substantial pods) of early flowering cowpea after early drought stress, and enhancing the production of second flush of pods [10].

Breeding for drought tolerance in cowpea has not been as pronounced as for other traits [11]; this is partly due to lack of simple, cheap and reliable screening techniques to select for drought tolerant plants and also because of the complexity of the factors involved in drought tolerance. There has been considerable progress at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, on breeding for enhanced drought tolerance in cowpea adopting simple, cheap and non-destructive screening methods. These screening methods have been developed by cowpea scientists and their effectiveness at identifying drought tolerant cowpea varieties have been confirmed [4]. Still, it is necessary to identify more efficient methods for evaluating the levels of tolerance in germplasm for crossing and selection of segregated breeding materials [12]. There is also a need to identify new sources of longer drought tolerance among different selected cowpea genotypes and the level of heritability of such traits under drought condition.

The first report of monogenic inheritance of drought tolerance in cowpea was achieved by using a box screening method. Two types of shoot drought tolerance in cowpea were identified [13] and this simple inheritance of drought tolerance in cowpea was reported as type 1 and type 2 tolerance. Type 1 plants were found to stay green for a long time after withholding water and the whole plant died with continued dry conditions, while type 2 plants stayed alive for a much longer period, but the whole plant did not die with continued drought conditions. Moisture was mobilised from the lower leaves to keep the growing tips alive for longer periods, hence the plants dropped the lower leaves first and senescence progressed upward slowly such that when watering was resumed, the plants recovered. Both type 1 and 2 drought tolerance are inherited as monogenic dominant traits; F1 crosses between them showed dominance of type 1 and F2 giving rise to three type 1: one type 2, suggesting that they are alleles at the same locus.

Several methods have been adopted to measure the level of drought tolerance in plants. These include traits like root characteristics [14], [15]; leaf rolling [16]; stomata behaviour and conductance [12], [17], [18]; osmotic adjustment [19]; leaf membrane stability [17]; molecular markers [18], [20], [21]; and leaf wilting scales [6], [12], [22]. Percentage wilting of seedlings at the onset of drought treatment has also been assessed too and a simple screening technique for drought tolerance in cowpea developed [7] based on seedling survival under water stress. This technique revealed heritable differences among the tested genotypes as regards their reaction to drought stress. Multiple traits adoption of evaluating drought tolerance in cowpea confirms the complexity of the trait in cowpea and other crop species [23]. Leaf wilting remains one of the best indicators of drought stress in plants, as it reduces the complexities associated with drought evaluation in crops [23].

Different scales of wilting have been used for assessing drought tolerance in cowpea; the International Board on Plant Genetic Resources [24] developed a scale of 1 – 9 (1 represents normal and 9, dead under moisture stress) and this has been adopted [12]. Another scale of 1 – 5 (1, normal and turgid leaves; 5, completely dead plants) has been designed [25]. Other researchers have adopted these scales while screening for drought tolerance in cowpea [18], [20], [22], [26]. Watanabe [6] developed a scale (1 – 5) which operates in reverse (1, susceptible and 5, highly tolerant) to the one developed by [25].

Despite the successes that have been associated with wilting scales, it still does not exist without certain drawbacks or limitations [23], [27]. First, the existing scales are associated with biased scoring, due to visual and qualitative assessment. Secondly, experience is needed to effectively score for leaf wilting in plants. Fortunately, Lawrent *et al.* [23] has addressed these issues by improving on the scoring procedure for wilting in cowpea to cater for the challenges associated with existing scales.

The pot screening of genotypes at seedling stage has also been found to be a reliable technique for identifying drought tolerance, for its practicality as being an easy set-up under a controlled environment; and adaptable for screening a large number of genotypes [12]. This has been confirmed by Watanabe *et al.* [6] who concluded that the phenomenon conferring seedling drought tolerance is also manifested at the flowering stage.

In view of the foregoing, this study was conducted with the following objectives:

i. to evaluate cowpea seedling's shoot and physiological traits that are associated with drought tolerance.
ii. to identify sources of longer drought tolerance in accessions of cowpea.

MATERIALS AND METHODS

Ten (10) cowpea accessions used in this study were collected from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The collected accessions are presented in Table 1.The ten (10) accessions were screened for drought tolerance at the seedling stage in the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, (latitude 7.2^{0} N, longitude 5.44$'$ E, Altitude 423M above sea level), Ondo State, Nigeria, between February and April, 2016.

Drought treatment: Seeds were planted in plastic bags filled with 3.5kg of sieved sandy loam soil without fertilizer in the screen house. After emergence, plants were thinned to two fairly uniform plants per pot with 10 pots per treatment and three replications for each accession in a completely randomized design (CRD); total number of plants in the screen house was 600. Each pot was watered with 250ml of water per day until the full expansion of the first trifoliate leaf (at day 16), after which watering was stopped.

Drought susceptibility scores (DSS) using wilting scales and percentage permanent wilting (PPW): Susceptibility was scored for plants using the International Board for Plant Genetic Resources (IBPGR) descriptors for cowpea. They were scored on a 1 to 7 scale, where 1 to 3.9 = low susceptibility (plant alive with green leaves); 4 to 5.9 = medium susceptibility (plant alive with most of the leaves yellow / or wilting); 6 to 7= high susceptibility (plant dead and dry). The scores of two plants in each pot were averaged, after which the mean of scores of the three replicates of each accession was calculated. Using the means of the evaluation, the tested accessions were then classified into the three categories of susceptibility: (1) Those with a mean ranging from 6 to 7, the accession was classified as highly susceptible; (2) Those with a mean ranging from 4 to 5.9, the accession was classified as medium; and (3) Those with a mean ranging from 1 to 3.9, the accession was classified as having low susceptibility. Percentage permanent wilting was recorded at various intervals (14 days and 21days) for each accession until 90 percent of the plants of the most susceptible accessions were completely wilted or died.

Morphological and physiological traits: Morphological traits like plant height, number of leaves per plant, terminal leaflet length, terminal leaflet width, stem girth and physiological parameters such as stomata conductance and stomata resistance at 14 days of drought stress were also used to assess the level of tolerance of the accessions to drought stress.

Plant height, terminal leaflet length and width: These were measured at the commencement of the drought stress (initial - 1day), 7 days, 14 days and 21 days of imposition of water stress. These were measured using a meter rule. Terminal leaflet length and width were only measured at the commencement of drought stress and day 7. The average of values of two plants in each pot was determined after which the mean value of the three replicates of each accession was calculated.

Number of leaves: Number of leaves was counted for each accession at the commencement of drought stress (initial), 7 days, and 14 days of drought stress and the mean of the three replicates was determined.

Stem girth: The stem girth of each accession was measured with a digital caliper at 2cm above the soil surface to the nearest millimeter. The measurement was taken at the commencement of drought stress (initial); 7 days and 14 days of imposition of drought stress. Then the average girth was determined for each accession per replicate and the mean for the three replicates determined.

Stomata conductance and stomata resistance: These were determined on the 14th day of imposition of drought stress with a steady state Leaf Porometer Model SC – 1, Decagon Devices, Inc. between 11.30 am and 5.00 pm utilizing the same leaflet used for leaflet length and width on three plants per accession per replicate. Average value of each parameter was determined for each accession per replicate and the mean for the three replicates were determined.

Recovery parameters: Twenty one (21) days after imposition of drought stress, watering then resumed, and 14 days after, percentage plant recovery in each accession was recorded. Based on permanent wilting and percent recovery, plants were grouped as susceptible or drought tolerant. Stem greenness and re-growth were scored as recovery parameters, after 14 days of re-watering. Stem greenness was scored using a scale of 1-5, where 1 was yellow (plant recovered) and 5 was completely green. Re-growth was scored using three categories: 1 with no re-growth (plant recovered); 3 with re-growth from auxiliary buds; and 5 with re-growth from the apical meristems.

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the Statistical Package for Social Science (SPSS) version 20 (SPSS Inc., Chicago IL). Means were separated using Duncan Multiple Range Test (DMRT) at P ≤ 0.01 level of significance. The data on ranking of changes in morphological characters under drought stress were subjected to Cluster Analysis and Dendrogram constructed with Paleontological Statistics Software Package for Education and Data Analysis (PAST).

RESULTS

Drought susceptibility scores (DSS) and percentage permanent wilting (PPW):

Results obtained revealed high significant genotypic differences among accessions for all traits under drought stress. At seven days of imposition of drought stress, the highest DSS was 2.00 for AC10, and the least was 1.00 for AC03, AC04, AC05, AC06, AC07 and AC09. AC10 and AC08 were already showing signs of wilting on the seventh day of imposition of water stress. On Day 14 of drought stress, highest DSS was still maintained by AC10 and the value had increased to 2.27 followed by AC08 with the value of 1.97 while the least value of 1.00 was maintained by AC03, AC04 and AC09. The level of stress on Day 21 of drought imposition on accessions dramatically worsened more in accession AC08 and AC03 with DSS of 6.97 and 6.00 respectively. Despite the level of resistance displayed by AC03 till Day 14 of drought stress but changed dramatically between Day 14 and 21 when the level of stress in AC03 surpassed all accessions with higher DSS as at Day 14. The least DSS (4.00) on Day 21 of drought stress was obtained in AC06 while the highest (6.97) was obtained in AC08. On Day 14 of drought imposition, PPW was highest (32.38%) in AC10 while the least (6.67%) was observed in AC04. PPW on Day 21 was highest (96.67%) in AC08 while the least (58.89%) was observed in AC06.

Based on DSS at 21 days, accessions were grouped into three categories; the first group consisted of accessions with DSS scores of 6 and above and accessions involved were AC03 (6.00) and AC08 (6.97). The second group consisted of accessions with DSS scores of between 5 and 5.9; and these comprised AC10 (5.30), AC09 (5.31), AC05 (5.33) and AC07 (5.37). The third group consisted of accessions with DSS scores of between 4 and 4.9; and these involved AC06 (4.00), AC02 (4.50), AC04 (4.63) and AC01 (4.83) (Table 2).

Recovery parameters: Twenty one (21) days after imposition of drought stress, watering then resumed, and 14 days after, percentage plant recovery in each accession was recorded. Percentage recovery (PREC), stem re-growth (STR) and stem greenness (STG), showed highly significant differences among the accessions. Based on permanent wilting and percent recovery, plants were grouped as susceptible or drought tolerant. Percentage of plants recovered ranged from 0.00 to 36.67%. The least percentage recovery (13.33%) was found in accession AC03 while the highest (36.67%) was found in accession AC02. Accessions AC08 and AC10 did not recover at all. In spite of the fact that only 76.19% of AC10 and 96.67% of AC08 wilted before re-watering, the rest of the plants of these accessions continued to die even as watering continued, and all plants were dead within two weeks of re-watering. The highest value for stem re-growth (4.33) was found in accession AC01 while the least (1.17) was found in AC03. Three groups were observed based on re-growth parameter. The first contained accession with above 4.00 score and this consisted of AC01, which was the only accessions that had plants which re-grew mainly from the apical meristems. Accessions AC06 and AC07 of the second group consisted of accessions with scores of 3.43 and 3.53 respectively. The plants of these accessions re-grew mainly from their axillary buds. Group three consisted of accessions with scores of 1.17, 1.73, 2.12, 2.53 and 2.83 and these involved accessions AC03, AC04, AC05, AC02 and AC09 respectively. These accessions consisted of plants which re-grew mainly from apical buds and some which did not re-grow but maintained their greenness. The fourth group consisted of accessions with scores of 0.00 which involved accessions AC08 and AC10; these accessions were completely dead. For stem greenness, accessions AC06, AC07 and AC04 had high scores of 4.82, 4.13 and 4.10 respectively. These were followed by AC01, AC02, AC09 and AC05 with scores of 3.57, 3.67, 3.67 and 3.81 respectively. The least score of 2.83 was obtained in AC03. Stems were completely dried in AC08 and AC10, hence their score of 1.00. Based on recovery parameters, plants were ranked as most susceptible (1), susceptible (2), moderately susceptible (3) and least susceptible (4) (Table 3).

Morphological and physiological traits: Morphological characters like length and width of terminal leaflet, plant height and stem girth, among the accessions under drought stress were only monitored till 14 days of drought stress as they either had wilted completely in many plants of some accessions before Day 14 (as found in leaf characters) or remained unchanged in values across all accessions after 14 days of drought stress (as found in plant height and stem girth). Statistical analysis revealed very high variations among the accessions for all the morphological and physiological parameters during the 21 days of drought stress.

Physiological parameters like stomata conductance (SCND) and stomata resistance (SREST) were only monitored on Day 14 of drought stress. Conductance was generally high in all accessions with the highest value (926.70 mmol $m^{-2}s^{-1}$) observed in accession AC03 while the lowest (70.19mmol $m^{-2}s^{-1}$) was observed in AC05. Stomata resistance was highest (14.45s/cm) in AC05 while the least (1.07s/cm) was found in AC03. Accessions with resistance of less than 5s/cm, were considered to be of low resistance while the ones with above 5s/cm, were considered to have high resistance. Accessions were ranked based on the value of stomata resistance and two groups were formed from this parameter. First group consisted of accessions (AC03, AC04 and AC10) with low stomata resistance and were ranked 2. The second group consisted accessions (AC01, AC02, AC05, AC06, AC07, AC08 and AC09) with high stomata resistance and ranked 1 (Table 4).

The highest mean value (18.89cm) for plant height at 14 days of drought stress was observed in accession AC02 while the lowest mean value (13.36cm) was observed in AC08. Plant height increased in all accessions from the day of stress imposition till 14 days after which the plant height became constant till Day 21 of stress. The highest increase in plant height (32.62%) was found in accession AC07 which was the second tallest among the cowpea accessions. All accessions were ranked 1 since they all increased in height at day 14 of drought stress (Table 5).

Mean number of leaves was found to be highest (2.73) in AC01 on day 14 of drought stress, while the least mean value (1.08) was found in AC08. Number of leaves increased from the first day of drought stress in all accessions till the 7th day of stress but reduced in AC02, AC08, AC09 and AC10 between Day 7 and Day 14 of drought stress with AC08 having the highest reduction in number of leaves (-60.44%) while the highest increase in number of leaves (52.94%) was found in AC04.

Accessions were ranked according to the reduction/increase in the number of leaves. Four groups were derived according to the reduction in number of leaves; the first group consisted of accessions (AC01, AC03, AC04, AC05, AC06, AC07) with increase in number of leaves and they were generally ranked as 0. The second group consisted of an accession (AC10) with less than 10% reduction in number of leaves and was ranked as 1 while the third and fourth groups consisted of accessions (AC02 and AC09) with 10 to 20% reduction were ranked as 2 and accession (AC08) with above 20% reduction was ranked as 3 (Table 6).

Terminal leaflet length and width were only observed on day 7 of drought stress as most of the tagged terminal leaflets across all accessions wilted before Day 14 of drought stress. The highest mean value (5.99cm) for terminal leaflet length was obtained in accession AC01 while the least (3.98cm) was observed in AC06. Percentage reduction in terminal leaflet length at 7 days of drought stress ranged from 1.95% in AC08 to 5.84% in AC03 (Table 7). Terminal leaflet mean width was highest (2.54 cm) in accession AC02 and least (1.88 cm) in AC06. The highest reduction in terminal leaflet width (8.88%) on 7th day of drought stress was found in AC07 while the least (3.42%) was found in AC02. Accessions were ranked according to the percentage reduction in terminal leaflet length and width as a result of drought stress. Based on terminal leaflet length, accessions were ranked as 1 for those which reduced in leaflet length by between 1.00 to 3.99%, and this comprised of AC01, AC02, AC04, AC06, AC07, AC08 and AC09 and termed group 1. Those with reduction of between 4.00 to 5.99% were ranked as 2 and these comprised of AC03, AC05 and AC10 and termed group 2 (Table 8). Based on percentage reduction in terminal leaflet width, four groups were recognized from all accessions. The first group consisted of accessions (AC02, AC03 and AC04) which reduced in leaflet width by between 1.00 to 3.99% and ranked as 1. The second group consisted of accessions (AC01, AC05, AC08, and AC09) which reduced in leaflet width by between 4.00 to 5.99% and ranked as 2. The third group consisted of accession (AC10) which reduced in leaflet width by between 6.00 to 7.99% and ranked as 3 while the forth group comprised of accessions (AC06 and AC07) which reduced in leaflet width by between 8.00 to 9.99% and ranked 4 (Table 8).

The highest mean value for stem girth (2.60mm) was found in accession AC01 while the lowest mean (1.72mm) was found in AC03. Stem girth increased only in accessions AC01, AC02, AC03 and AC05 between initial day of drought stress and day 7, while it reduced between day 7 and day 14 of drought stress. Other accessions experienced reduction of stem girth from the inception of drought until day 14 of drought stress after which girth now remained constant in all accessions till day 21. Percentage reduction in stem girth ranged from 1.59% in AC04 to 20.07% in AC08.

Reduction of stem girth was generally high in AC03, AC06, AC07, AC08, AC09 and AC10. The percentage reduction of stem girth was used to rank accessions and this gave rise to four groups. Group one consisted of accession (AC04) which reduced in girth by between 1.00 to 3.99% and ranked as 1. Group two comprised of accessions (AC01, AC02 and AC05) which reduced in stem girth by between 6.00 to 7.99% and ranked 3. Group three comprised of accessions (AC03 and AC07) which reduced in stem girth by between 8.00 to 9.99 % and ranked 4. While those accessions (AC06, AC08, AC09 and AC10) which reduced by more than 10% in stem girth were ranked 5 and termed group four (Table 9). No accession was ranked 2 for this trait since no accession fell between the values of between 4.00 and 5.99% reduction of leaflet width (Table 9).

Cluster analysis based on ranking of accessions: Ranking based on changes in morphological characters under drought stress produced dendrogram that grouped the ten accessions into three major clusters; cluster I, cluster II and cluster III. Cluster I had three sub-clusters; A, B, C. Sub-cluster A consisted only AC04, a moderately susceptible accession with low stomata resistance. Sub-cluster B consisted of AC03, the only highly susceptible accession with low stomata resistance. Sub-cluster C consisted of AC01 and AC02 closely linked together as accessions with moderate susceptibility and high stomata resistance and distantly linked with accession AC05, a susceptible accession with high stomata resistance. Cluster II consisted of two sub clusters; A and B. Sub-cluster A consisted of AC10 and AC09, susceptible accessions with high stomata resistance and highest percentage reduction of stem girth. Sub-cluster B consisted of AC06 and AC07, moderately susceptible and accessions with high stomata resistance and high reduction of terminal leaflet width. Cluster III consisted of AC08, the most susceptible accession (Figure 1).

Table 1. List of screened accessions for drought tolerance

S/N	ACCESSION	COUNTRY OF ORIGIN	CODE
1	TVu-199	USA	AC01
2	TVu-207	USA	AC02
3	TVu-218	USA	AC03
4	TVu-235	Ghana	AC04
5	TVu-236	Ghana	AC05
6	TVu-241	USA	AC06
7	IT98K-205-8	Nigeria	AC07
8	IT98K-555-1	Nigeria	AC08
9	TVu-4886	Niger	AC09
10	TVu-9256	Burkina Faso	AC10

Table 2. Drought Susceptibility Scores (DSS) and Percentage Permanent Wilting (PPW %) of cowpea seedlings under drought stress

ACCESSION	DSS7	DSS14	DSS21	PPW14	PPW21	RANK
AC01	1.33±0.33^{ab}	1.53±0.32^{ab}	4.83±0.33^{ab}	20.00±5.77^{abc}	73.33±3.33^{ab}	1
AC02	1.33±0.33^{ab}	1.41±0.36^{ab}	4.50±0.58^{a}	20.19±5.19^{abc}	66.67±8.82^{ab}	1
AC03	1.00±0.00^{a}	1.00±0.00^{a}	6.00±0.78^{ab}	10.00±0.00^{ab}	83.33±6.67^{ab}	2
AC04	1.00±0.00^{a}	1.00±0.00^{a}	4.63±0.43^{a}	6.67±1.67^{a}	66.67±6.67^{ab}	1
AC05	1.00±0.00^{a}	1.13±0.13^{ab}	5.33±0.61^{ab}	13.33±3.33^{abc}	76.67±8.82^{ab}	1
AC06	1.00±0.00^{a}	1.13±0.13^{ab}	4.00±0.60^{a}	13.67±3.18^{abc}	58.89±9.49^{a}	1
AC07	1.00±0.00^{a}	1.35±0.33^{ab}	5.37±0.12^{ab}	16.19±6.83^{abc}	77.78±2.22^{ab}	1
AC08	1.70±0.21^{ab}	1.97±0.44^{ab}	6.97±0.03^{b}	27.86±6.35^{bc}	96.67±3.33^{b}	2
AC09	1.00±0.00^{a}	1.00±0.00^{a}	5.31±0.46^{ab}	8.09±1.56^{a}	76.35±6.71^{ab}	1
AC10	2.00±0.00^{b}	2.27±0.27^{b}	5.30±0.47^{ab}	32.38±0.47^{ab}	76.19±6.87^{ab}	1

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). DSS7: Drought susceptibility score at day 7; DSS14: Drought susceptibility score at day 14; DSS21: Drought susceptibility score at day 21. PPW14: Percentage permanent wilting at day 14; PPW21: Percentage permanent wilting at day 21.

Table 3. Recovery parameters after two weeks of re-watering

ACCESSION	PREC (%)	STR	STG	RANK
AC01	23.33±3.33^{bc}	4.33±0.67^{c}	3.57±0.73^{ab}	3
AC02	36.67±3.33^{d}	2.53±0.79^{abc}	3.67±1.33^{ab}	4
AC03	13.33±3.33^{b}	1.17±0.72^{ab}	2.83±0.93^{ab}	2
AC04	33.33±1.66^{cd}	1.73±0.13^{abc}	4.10±0.59^{ab}	4
AC05	30.00±0.00^{cd}	2.12±0.44^{abc}	3.81±0.42^{ab}	4
AC06	33.33±3.33^{cd}	3.43±0.80^{bc}	4.82±0.09^{b}	4
AC07	33.33±3.33^{cd}	3.53±0.29^{bc}	4.13±0.45^{ab}	4
AC08	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{a}	1
AC09	33.00±3.33^{cd}	2.83±1.40^{abc}	3.67±1.33^{ab}	4
AC10	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{a}	1

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). PREC: Percentage recovery; STR: Stem re-growth; STG: Stem greenness.

Table 4. Physiological parameters at 14 days of drought stress

ACCESSION	SCND (mmol $m^{-2}s^{-1}$)	SREST (s/cm)	RANK
AC01	146.37±15.34^{a}	6.98±0.73^{b}	1
AC02	131.90±24.21^{a}	8.15±1.57^{b}	1
AC03	926.70±38.74^{d}	1.07±0.08^{a}	2
AC04	368.64±50.32^{b}	2.82±0.39^{a}	2
AC05	70.19±5.56^{a}	14.42±1.06^{c}	1
AC06	108.41±12.41^{a}	9.45±1.03^{b}	1
AC07	93.14±8.11^{a}	10.91±1.02^{bc}	1
AC08	122.99±1.84^{a}	8.13±0.11^{b}	1
AC09	76.11±8.39^{a}	13.50±1.65^{c}	1
AC10	554.43±21.12^{c}	1.80±0.07^{a}	2

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). SCND: Stomata conductance; SREST: Stomata resistance.

Table 5. Effect of drought stress on Plant Height (cm)

ACCESSION	IPH	PH7	PH14	INCREASE (%)	RANK
AC01	12.55±0.34^{ab}	12.59±0.33^{ab}	15.09±0.58^{a}	20.24	1
AC02	16.49±0.29^{c}	16.55±0.29^{b}	18.89±1.06^{a}	14.55	1
AC03	12.54±0.83^{ab}	12.56±0.82^{ab}	16.63±1.91^{a}	32.62	1
AC04	13.96±0.01^{bc}	13.98±0.62^{ab}	17.73±1.79^{a}	27.00	1
AC05	12.05±0.52^{ab}	12.07±0.51^{ab}	16.41±2.07^{a}	36.18	1
AC06	13.05±0.53^{ab}	13.08±0.52^{ab}	14.87±1.09^{a}	13.95	1
AC07	12.68±1.31^{ab}	12.69±1.30^{ab}	17.34±0.29^{a}	36.75	1
AC08	11.69±0.59^{ab}	11.70±0.60^{ab}	13.36±0.88^{a}	14.29	1
AC09	11.42±0.48^{ab}	11.46±0.47^{ab}	13.92±0.43^{a}	21.89	1
AC10	10.72±0.41^{a}	10.74±0.42^{a}	13.72±0.91^{a}	27.99	1

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). IPH: Initial plant height; PH7; PH14: Plant height at day 7; Plant height at day 14.

Table 6. Effect of drought stress on Number of Leaves

ACCESSION	INL	NL7	NL14	INCREASE (%)	RANK
AC01	2.10±0.10[ab]	2.87±0.34[ab]	2.73±0.37[a]	30.00	0
AC02	1.80±0.10[a]	2.56±0.18[a]	1.50±0.87[a]	-16.67	2
AC03	1.64±0.27[a]	2.77±0.20[ab]	2.50±0.87[a]	52.44	0
AC04	1.70±0.12[a]	2.43±0.08[a]	2.60±0.50[a]	52.94	0
AC05	1.63±0.82[a]	2.57±0.08[a]	2.40±0.58[a]	47.24	0
AC06	1.97±0.33[a]	2.86±0.09[ab]	2.13±0.37[a]	8.12	0
AC07	1.92±0.22[b]	2.47±0.11[a]	2.17±0.44[a]	13.02	0
AC08	2.73±0.16[b]	3.65±0.29[b]	1.08±0.65[a]	-60.44	3
AC09	2.31±0.21[ab]	3.58±0.24[b]	1.98±0.31[a]	-14.29	2
AC10	1.80±0.07[a]	3.06±0.94[ab]	1.65±0.37[a]	-8.33	1

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). INL: Initial number of leaves. NL7; NL14: Number of leaves at day 7; Number of leaves at day 14.

Table 7. Effect of drought stress on Terminal Leaflet Length (cm)

ACCESSION	ITLL	TLL7	REDUCTION (%)	RANK
AC01	6.15±0.35[b]	5.99±0.39[b]	2.60	1
AC02	5.02±0.49[ab]	4.86±0.53[ab]	3.19	1
AC03	4.45±0.56[ab]	4.19±0.64[ab]	5.84	2
AC04	4.53±0.25[ab]	4.35±0.27[ab]	3.97	1
AC05	4.19±0.31[a]	4.01±0.19[a]	4.29	2
AC06	4.12±0.68[a]	3.98±0.36[a]	3.39	1
AC07	5.01±0.29[ab]	4.81±0.12[ab]	3.99	1
AC08	5.14±0.13[ab]	5.04±0.09[ab]	1.95	1
AC09	5.41±0.35[ab]	5.20±0.35[ab]	3.88	1
AC10	4.84±0.79[ab]	4.61±0.76[ab]	4.75	2

Means followed by the same alphabet within a column are not significantly different from one another at P ≤ 0.01 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ITLL: Initial terminal leaflet length; TLL7: Terminal leaflet length at day 7.

Table 8. Effect of drought stress on Terminal Leaflet Width (cm)

ACCESSION	ITLW	TLW7	REDUCTION (%)	RANK
AC01	2.59±0.23^{a}	2.44±0.27^{a}	5.79	2
AC02	2.63±0.29^{a}	2.54±0.26^{a}	3.42	1
AC03	1.96±0.26^{a}	1.89±0.23^{a}	3.57	1
AC04	2.47±0.29^{a}	2.38±0.31^{a}	3.64	1
AC05	2.23±0.17^{a}	2.11±0.13^{a}	5.38	2
AC06	2.06±0.20^{a}	1.88±0.18^{a}	8.74	4
AC07	2.70±0.12^{a}	2.46±0.04^{a}	8.88	4
AC08	2.40±0.09^{a}	2.27±0.43^{a}	5.42	2
AC09	2.60±0.14^{a}	2.49±0.22^{a}	4.23	2
AC10	2.32±0.07^{a}	2.32±0.07^{a}	6.47	3

Means followed by the same alphabet within a column are not significantly different from one another at $P \leq 0.01$ using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ITLW: Initial terminal leaflet width; TLW7: Terminal leaflet width at day 7.

Table 9. Effect of drought stress on Stem Girth (mm)

ACCESSION	ISG	SG7	SG14	REDUCTION (%)	RANK
AC01	2.79±0.05^{e}	2.83±0.06^{d}	2.60±0.17^{b}	6.81	3
AC02	2.36±0.09^{bcd}	2.46±0.00^{bcd}	2.21±0.14^{ab}	6.36	3
AC03	1.90±0.10^{a}	1.95±0.06^{a}	1.72±0.24^{a}	9.47	4
AC04	2.52±0.02^{cde}	2.50±0.03^{bcd}	2.48±0.18^{ab}	1.59	1
AC05	2.14±0.05^{abc}	2.17±0.09^{ab}	2.00±0.37^{ab}	6.54	3
AC06	2.40±0.07^{bcd}	2.31±0.15^{abc}	1.94±0.31^{ab}	19.16	5
AC07	2.49±0.04^{bcd}	2.47±0.05^{bcd}	2.25±0.33^{ab}	9.64	4
AC08	2.69±0.06^{de}	2.67±0.06^{cd}	2.15±0.08^{ab}	20.07	5
AC09	2.67±0.15^{de}	2.63±0.16^{cd}	2.37±0.24^{ab}	11.23	5
AC10	2.11±0.06^{ab}	2.07±0.16^{ab}	1.88±0.44^{ab}	10.9	5

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.01$ using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ISG: Initial stem girth; SG7: Stem girth at day 7; SG14: Stem girth at day 14.

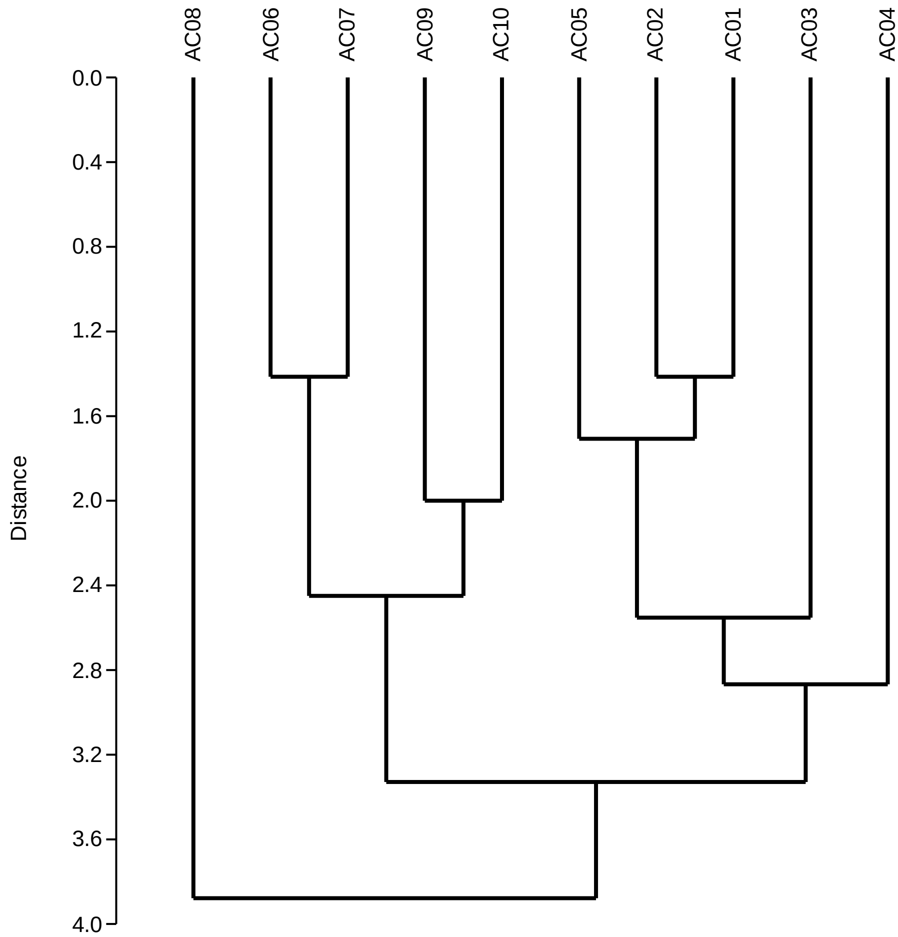

Figure 1. Dendrogram (Euclidean Distance) based on the ranking of accessions using DSS and changes in morphological characters under drought stress.

DISCUSSION

Wilting in plants, a consequence of drought stress can be visually observed, allowing different wilting intensities to be recorded on cowpea seedlings. Results from this study show that cowpea accessions responded differently to drought stress and the response is time dependent.

Utilising drought susceptibility score which was based on the level of wilting in plants, accessions AC01, AC02, AC08 and AC10 were already showing wilting within 7 days of drought stress in agreement with the reports of several authors [13], [18], [20], [22], [28] who had reported wilting within the first week of drought stress in cowpea. This suggests that in conducting assessments, susceptible accessions can easily be identified within the first week of drought stress [23]. Other accessions however remained unchanged till day 7 of drought stress. At Day 21 of drought stress, AC08 became the most susceptible with the highest number of plants that were either dead or completely wilted (Table 2). The percentage of completely dead or wilted plants for each accession was also in accordance to the level of susceptibility of each accession as earlier reported [12].

After two weeks of re-watering, seedlings of some accessions completely recovered while some continued to die; among these are accessions AC08 and AC10 in which death of seedlings reached 100% at two weeks of re-watering (Table 3). Among accessions, those with slow wilting from the commencement of drought stress and those with lower drought susceptibility scores and percentage permanent wilting at the end of drought stress had high recovery rates while the accession with the highest susceptibility never recovered. Most of them also maintained high re-growth and stem greenness. These accessions apparently possess an inherent mechanism to slow their moisture loss by minimizing transpiration rate much unlike those with high wilting scores. This contributed to their reduced susceptibility and maintenance of greenness.
Physiological and morphological characteristics promoting water loss from leaf tissues might be a key factor responsible for early wilting among the accessions showing wilting within one week of drought stress [23]. Stomatal behaviour at the initiation of drought stress [18], [23] might be a crucial factor since late wilting accessions were able to regulate stomatal opening to prevent water loss. As drought stress continued, most of the seedlings of the early wilting accessions died while those of the least susceptible accessions survived through stomata closure, a first line of defense against drought or as a result of osmotic adjustment, the second line of defense during intense drought stress [29], [30].

Generally, the accessions had high stomatal conductance. Seedlings of two of the accessions with the slowest rate of wilting (AC03 and AC04), had stomatal conductance among the highest recorded at day 14 of drought stress (Table 4). These results show that these accessions have the ability to maintain photosynthesis under drought stress. Early drought could therefore be survived by plants with low susceptibility as a result of their ability to resist wilting and maintain photosynthesis and growth at low soil moisture levels. These traits have been found to be very important when drought occurs early during the growing seasons of plants [31].

Osmotic adjustment in leaves has also been found to be a mechanism of minimising water loss during drought stress [32]. This might play a significant role in reduced wilting observed among the least susceptible accessions of cowpea. Osmotic adjustment allows plants to maintain turgor and ensure survival as water deficits develop in the soil, encouraging plant recovery on re-watering after severe drought stress [31], [32].

Significant differences were observed among the accessions for morphological traits such as number of leaves, plant height and terminal leaflet length but not for terminal leaflet width under drought stress at day 7, whereas no significant differences were observed among all accessions at day 14 which marked the end of any increase in these traits for all the accessions involved. The significant reduction in number of leaves and terminal leaflet length among the most susceptible accessions at day 7 indicate that drought stress leads to reduced leaf initiation and area of existing leaves which in turn lead to leaf shedding on the plants. Two of the most susceptible accessions (AC08 and AC10) had their number of leaves reduced by 60.44% and 8.33% respectively, while most of the susceptible and least susceptible accessions had increased number of leaves. The highest reduction in terminal leaflet length also occurred among the highly susceptible accessions (AC03 and AC10) except in AC08. Even though their differences were not significant at day 14, the most susceptible accessions were among accessions with the lowest values for number of leaves, terminal leaflet length and plant height (Table 5, 6, 7 and 8).

Stem girth however had significant differences among the accessions till day 14 of drought stress. Number of leaves was reduced in accessions AC10, AC09, AC02 and AC08 while increment of number of leaves was witnessed in other accessions. Number of leaves was mostly reduced by drought stress in accession AC08 followed by accession AC02 on day 14 of drought stress, while the least reduction in number of leaves was recorded in accession AC10 (Table 6). Drought stress also significantly reduced terminal leaflet length and terminal leaflet width among the accessions during the drought stress period (Table 7 & 8). These are similar to earlier findings [33]. Lack of significant differences for these traits among the seedlings at day 14 of drought stress suggests that they all had a level of adaptation to drought conditions and also that these traits were similarly affected by drought among accessions [34].

Plant height was found to be more affected by drought stress in accessions AC06 as the least increased in height followed by AC08 and least affected by AC07 as the accession with highest increment in height. All seedlings increased in height during the drought stress, with clear significant differences among them at day 7 but without any significant differences at day 14. Overall, the accession with the lowest height at day 14 of drought stress was AC08 (Table 5). These results are similar to the findings of Abdou Razakou [34] and Onuh and Donald [35]; who reported different plant height of cowpea under different level of drought conditions.

Most morphological and physiological traits have been found to be negatively affected in seedlings of sorghum under drought stress [37], [38]. Shoot growth is normally suppressed by drought stress and; much reduction in seedling growth as shown by some accessions can be linked to restricted cell division and enlargement due to drought stress [38], [39]. Highly significant differences in plant height were also found among maize plants under different drought stages in different locations. Reduction in height as a result of drought stress at vegetative stage implies that water stress retards plant height [40]. Similar conclusion has been reached by Salami et al. [41] and Hajibabaee et al. [42].

Stem girth was significantly reduced among the accessions up to day 14 of drought stress with most reduction of stem girth occurring in accession AC08 and least reduction found in accession AC04. Accession AC08 which exhibited the highest reduction of stem girth happened to be the most susceptible among the accessions while accession AC04 with the least reduction suggests the level of tolerance as exhibited by this accession (Table 9). These results are in agreement with the findings of [43] who reported that drought stress in cowpea plants reduced their stem girth by 32%. Reduction of plant size and leaf area have been found to be the major mechanisms for controlling water use and reducing injury under drought stress [34], [44]. Genotypes of maize also differed significantly for stem diameter at different drought stages [40].

The dendrograms based on ranking of accessions showed the distribution of accessions into three groups (Fig. 1) which indicate the existence of much variability among them for drought tolerance. Susceptible accessions of cowpea were clearly separated by the dendrogram. Cluster analysis has also been employed in cowpea [45], and also to group seedlings of tomatoes to classes under drought stress [46].

CONCLUSIONS

The need for fast, easy and effective selection criteria for drought tolerant cowpea accessions under controlled environments can not be overemphasized. Integration of such techniques in breeding programmes will allow the screening of numerous varieties of cowpea at seedling stage in a relatively short time at low cost. Among all the traits studied, drought susceptibility scores, percentage permanent wilting, stem greenness and regrowth, number of leaves and stem girth were the best traits for use in the study of drought tolerance in seedlings of cowpea. Based on wilting scales and recovery parameters, the following classifications of cowpea accessions were made:

1. Highly Susceptible (AC03, AC08 and AC10)
2. Susceptible (AC09, AC05 and AC07) and
3. Moderately Susceptible (AC06, AC02, AC04 and AC01).

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REFERENCES

[1] Fatokun, C. A., Tarawali, S. A., Singh, B. B., Kormawa, P. & Tamo, M. (2002). Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, 4 – 8 September, 2000.

[2] Uarrota, V. G. (2010). Response of cowpea (*Vigna unguiculata* L.) to water stress and phosphorus fertilization. Journal of Agronomy, 9: 87 – 91.

[3] Ajayi, A. T., Adekola, M. O., Taiwo, B. H. & Azuh, V. O. (2014). Character expression and differences in yield potential of ten genotypes of cowpea (*Vigna unguiculata* L. Walp). International Journal of Plant Research, 4 (3): 63 – 71.

[4] Singh, B. B., Elhers, J. D., Sharma, B. & Freire Filho, F. R. (2002). Recent progress in cowpea breeding. In: challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

[5] Blum, A. (1985). Breeding crop varieties for stress environments. CRC Critical Reviews in Plant Science, 2: 199 – 238.
[6] Watanabe, I. S., Hakoyama, T., Terao, T., Singh B. B. (1997). Evaluation methods for drought tolerance in cowpea. In: Advances in cowpea research Eds. B. B. Singh, D. R. Mohan Raj, K. E. Dashiell, Jackai L. E N. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS), Ibadan, Nigeria, Pp 142 – 146.
[7] Ishiyaku, M. F. & Yilwa, V. M. (2009). New source of drought tolerance in cowpea (*Vigna unguiculata* (L.) Walp) from irradiation – induced mutation. Nigerian Journal of Botany, 22 (1): 53 – 60.
[8] Hall, A. E., Singh, B. B. & Ehlers, J. D. (1997a). Cowpea breeding. Plant Breeding Reviews, 15: 215 – 274.
[9] Thiaw, S., Hall, A. E. & Parker, D. R. (1993). Varietal intercropping and the yields and stability of cowpea production in semi-arid Senegal. Fields Crops Research, 33: 217 – 233.
[10] Hall, A. E., Ismail, A. M., Ehlers, J. D., Marfo, K. O., Cisse, N., Thiaw, S. & Close, T. J. (2002). Breeding cowpea for tolerance to temperature extremes and adaptation to drought. In: challenges and opportunities for enhancing sustainable cowpea production. Eds. C. A. Fatokun, S. A. Tarawali, B. B. Singh, P. M. Kormawa, M. Tamo. Proceedings of the World Cowpea Conference III held at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4 – 8 September, 2000. Pp. 14 – 21.
[11] Singh, B. B. & Mai–kodomi, Y. & Terao, T. (1999). A simple screening method for drought tolerance in cowpea. Indian Journal of Genetics, 59: 211 – 220.
[12] Nkouannessi, M. (2005). The genetic, morphological and physiological evaluation of African cowpea genotypes. University of Free State, SA
[13] Mai–kodomi, Y., Singh, B. B., Myers, O., Yopp, J. H., Gibson, P. J. & Terao, T. (1999). Two mechanisms of drought tolerance in cowpea. Indian Journal of Genetics, 59 (3): 309 – 316.
[14] Watanabe, I. S. (1993). Roles of tops and roots in drought tolerance of cowpea. Japanese Journal of Tropical Agriculture, 37: 7 – 8.
[15] Matsui, T. & Singh, B. B. (2003). Root characteristics in cowpea related to drought tolerance at seedling stage. Experimental Agriculture, 39: 29 – 38.
[16] Mathews, R. B., Azam-Ali, S. N. & Peacock, J. M. (1990). Response of four sorghum lines to mid-season drought. Field Crops Responses, 5: 297 – 308.
[17] Labuschagne, M. T., Verhoeven, R. & Nkouanessi, M. (2008). Drought tolerance assessment of African cowpea accessions based on stomata behavior and cell membrane stability. Journal of Agricultural Science, 146: 689 – 694.
[18] Agbicodo, E. M. (2009). Genetic analysis of abiotic and biotic resistance in cowpea (*Vigna unguiculata* (L.) Walp). Dissertation, University of Wageningen, the Netherlands
[19] Laurie, R. N. (1999). Determination of drought tolerance in nineteen *Vigna unguiculata* cultivars and breeding lines using four reliable screening methods. M.Sc. Dissertation, University of Witwatersrand, South Africa
[20] Muchero, W., Ehlers, J. D., Roberts & P. A. (2008). Seedling stage induced phenotypes and drought – responsive genes in diverse cowpea genotypes. Crop Science, 48: 541 – 552.
[21] Muchero, W., Ehlers, J. D. & Roberts, P. A. (2010). Restriction site polymorphism – based candidate gene mapping for seedling drought tolerance in cowpea (*Vigna unguiculata* (L.) Walp). Theoretical and Applied Genetics, 120: 509 – 518.
[22] Mai-kodomi, Y., Singh, B. B., Terao, T., Myers, O. & Gibson, P. J. (1999). Inheritance

of drought tolerance in cowpea. Indian Journal of Genetics, 59 (3): 317 – 323.

[23] Lawrent, L. M., James, P. M., Warren, M. W. & Mackson, B. (2013). Improvement of leaf wilting scoring system in cowpea (*Vigna unguiculata* L. Walp): from qualitative scale to quantitative index. Australian Journal of Crop Science, 7 (9): 1262 – 1269.

[24] Cowpea Descriptors, IBPGR, International Board for Plant Genetic Resources (1983). Rome, Italy. Pp. 30

[25] Singh, B. B., Mai-kodomi, Y. & Terao, T. (1999b). Relative drought tolerance of major rain fed crops of semi-arid tropics. Indian Journal of Genetics, 59: 1 – 8.

[26] Belko, N., Zama-Allah, M., Cisse, N., Diop, N. N., Zombre, G., Ehlers, J. D. & Vadez, V. (2012). Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. Functional Plant Biology, 39 (4): 306 – 322.

[27] Xu, W., Rosenow, D. T. & Nguyen, H. T. (2000). Stay green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration. Plant Breeding, 119 (4): 365 – 367.

[28] Fatokun, C. A., Boukar, O., Muranaka, S. & Chikoye, D. (2009). Enhancing drought tolerance in cowpea. Paper presented at the 9th African Crop Science, Cape Town, South Africa, and 28th September - 1st October, 2009.

[29] Jaleel, C. A., Manivannan, P., Wahid, A., Farook, M., Al-Juburi, J., Sumasundaran, T. & Panneerselvam, R. (2009). Drought stress in plants: A review on morphological characteristics and pigments composition. International Journal of Agriculture and Biology, 11: 100 – 105.

[30] Singh, S. K. & Reddy, R. K. (2011). Regulation of photosynthesis fluorescence, stomatal conductance and water use efficiency of cowpea (*Vigna unguiculata* L. Walp) under drought. Journal of Photochemistry and Photobiology B, 105 (1): 40 – 50.

[31] Mukeshimana, G. (2013). Dissecting the genetic complexity of drought tolerance mechanisms in common bean (*Phaseolus vulgaris* L.). Dissertation, Michigan State University, U.S.A

[32] White, R. H., Engelke, M. C., Morton, S. J. & Ruemmele, B. A. (1992). Competitive turgor maintenance in tall fescue. Crop Science, 32: 251 – 256.

[33] Samson, H. & Helmut, H. (2007). Drought effect on yield, leaf parameters and evapotranspiration efficiency of cowpea. Conference of International Agricultural Research for Development, University of Kassel-Witzenhense and University of Gotteingen

[34] Abdou Razakou, I. B. Y., Mensah, B., Addam, K. S. & Akromah, R. (2013). Using morpho-physiological parameters to evaluate cowpea varieties for drought tolerance. International Journal of Agricultural Science Research, 2 (5): 153 – 162.

[35] Onuh, M. O. & Donald, K. M. (2009). Effect of water stress on the rooting, nodulation potentials and growth of cowpea (*Vigna unguiculata* L. Walp). Science Journal, 4 (3): 31 – 34.

[36] Wu, Q. S., Xia, R. X. & Zou, Y. N. (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. European Journal of Soil Biology, 44: 122 – 128.

[37] Bibi, A., Sadaqat, H. A., Akram, H. M. & Mohammed, M. I. (2010). Physiological markers for screening sorghum (*Sorghum bicolor*) germplasm under water stress condition. International Journal of Agricultural Biology, 12: 451 – 455.

[38] Bibi, A., Sadaqat, H. A., Tahir, H. N. & Akram, H. M. (2012). Screening of sorghum (*Sorghum bicolor* Var. Moench) for drought tolerance at seedling stage in polyethylene glycol. The Journal of Animal and Plant Sciences, 22 (3): 671 – 678.

ISSN: 0974 – 3987
IJBST (2018), 11(1):1-19

[39] Kramer, P. J. (1983). Water relations of plants. Academic Press, Inc., New York, USA.
[40] Sabiel, S. A. I., Abdumula, A. A., Bashir, E. M. A., Khan, S., Yinying, S., Yank, Y., Baloch, S. U. & Bashir, W. (2014). Genetic variation of plant height and stem diameter traits in maize (*Zea mays* L.) under drought stress at different growth stages. Journal of Natural Sciences Research, 4 (23): 116 – 122.
[41] Salami, A. E., Adegoke, S. A. O. & Adegbite, O. A. (2007). Genetic variability among maize cultivars grown in Ekiti State, Nigeria. Middle-East Journal of Scientific Research, 2 (1): 9 – 13.
[42] Hajibabaee, M., Azizi, F. & Zargari, K. (2012). Effect of drought stress on some morphological, physiological and agronomic traits in various foliage corn hybrids. Journal of Agriculture and Environmental Sciences, 12 (7): 890 – 896.
[43] Omae, H., Kashiwaba, K. & Shono, M. (2007). Evaluation of drought and high temperature resistance in cowpea (*Vigna unguiculata* L. Walp) for Sahel, Africa. African Crop Science Conference Proceedings, 8: 1969 – 1974.
[44] Mitchell, J. H., Saimhan, D., Wamala, M. H., Risimeri, J. B., Chinyamakobru, E., Henderson, S. A. & Fukai, S. (1998). The use of seedling leaf death score for evaluation of drought resistance in rice. Field Crops Research, 55: 129 – 139.
[45] Pungulani, L. L. M., Miller, J. P. & Williams, W. M. (2012). Screening cowpea (*Vigna unguiculata*) germplasm for canopy maintenance under water stress. Agronomy New Zealand, 42: 23 – 32.
[46] Zdravkovic, J., Jovanovic, Z., Djordjevic, M., Girek, Z., Zdravkovic, M. & Stikic, R. (2013). Application of stress susceptibility index for drought tolerance screening of tomato populations. Genetika, 45 (3): 679 – 689.