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Full Length Research Paper

Morpho-physiological parameters used in selecting drought tolerant cowpea varieties using drought index

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Drought stress represents one of the most important abiotic constraints limiting cowpea production in the arid and semi-arid zones of Africa. Since drought susceptibility index (S) represents drought tolerance at whole plant level regardless of drought tolerance mechanism in operation, a potted experiment was conducted in a plant house (August to October 2011) to identify morpho-physiological parameters and develop drought susceptibility index for selecting drought tolerant cowpea varieties. These parameters included relative water content (RWC), plant height (PHT), number of leaves per plant (NL), stem diameter (SD) and root dry mass (RDM). A 2×6 factorial arranged in completely randomized design with three replications was used. The results showed highly significant effects among the cowpea genotypes, as regards to the water regime treatments and their interaction for all the morpho-physiological parameters used in this study (RWC, PHT, NL, SD and RDM). Significant positive relationship was found between NL and RDM with r = 0.97, p<0.001. With relatively better performance under water-stressed condition, as indicated by the drought susceptibility index, variety Dan illa was the best genotype recommended to be used as source for drought tolerance in a cowpea breeding programme.

Key words: Drought tolerance, morpho-physiological parameters, drought intensity, drought susceptibility index.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important food and forage legumes in the semi-arid tropics that includes parts of Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh, 2005; Timko et al., 2007). The seed, or grain as it is sometimes referred to, is the most important part of the cowpea plant for human consumption. The seeds are most often harvested and dried for storage and consumption at a later time, either after cooking whole or after being milled like a flour product and used in various recipes (Nielsen et al., 1997; Ahenkora et al., 1998). In addition to human consumption, cowpea leaves and stems (stover) are also an important source of high-quality hay for livestock feed (Tarawali et al., 1997; 2002). Cowpea fodder plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali, 1997; Tarawali et al., 1997; 2002). It is also a valuable component of farming systems in areas where soil fertility is limiting. This is because cowpea has a high rate of nitrogen fixation (Elawad and Hall, 1987), forms effective symbiosis with mycorrhizae (Kwapata and Hall, 1985), and has the ability to better tolerate a wide range of soil pH when compared to other grain legumes (Fery, 1990). While cowpea is inherently more drought-tolerant than other crops, water availability is still among the most significant abiotic constraints to growth and yield. Significant differences exist among cowpea genotypes in drought tolerance (Turk et al., 1980; Watanabe et al., 1997; Mai-Kodomi et al., 1999; Singh et al., 1999) and several studies have shown the genotypic responses to water stress in wheat (Sadiq et al., 1994, Trethowan et al., 2002, Moinuddin et al., 2005), maize (Kamara et al., 2003), triticale (Ozkan et al., 1999), common beans

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Table 1. Physico-chemical properties of the soil used in the plant house experiment.

Soil property	Kwadaso
Soil depth (cm)	0 - 20
pH (1:1 soil: $H_2 0$)	5.59
Organic carbon (%)	2.21
Organic matter (%)	3.82
Total N (%)	0.21
Available P (cmol/kg)	8.94
Exchangeable cations (cmol/kg)	
Ca^+	2.28
Mg^+	1.39
K^+	2.77
Na^+	0.55
Total exchangeable bases	7.19
Exchangeable acidity (Al + H)	0.75
Effective cation exchange capacity (mol/kg)	7.93
Base saturation (%)	90.58
Bulk density (g/cm3)	1.64
Particle size	
Sand (%)	26.69
Silt (%)	62.91
Clay (%)	10.40

(Teran and Singh, 2002), barley (Rizza et al., 2004), peanut (Upadhyaya, 2005) and soybean (Hufstetler et al., 2007).

Progress in cowpea breeding for dry environments has been achieved by yield testing large collections over several locations and years (Hall et al., 1997). This empirical approach is slow, laborious, and expensive because of the need to assess yield of large number of lines across several locations and years, and the substantial variation from the effects of environment, error, and genotype x environment interactions (Blum, 1988). The approach of Blum (1983), which combines selection for yield potential in favorable conditions with selection under controlled, repeatable stress environment for the expression of traits thought to be associated with drought tolerance is most effective (Fussell et al., 1991). This requires therefore, the identification of specific traits under adequate moisture that are easy to measure and are associated with drought tolerance (Fischer and Wood, 1979). Furthermore, in plants, a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Nam et al., 2001; Martinez et al., 2007). Therefore, the objective of the present study is to identify useful, reliable, cheaper and rapid morpho - physiological parameters that could be used for selecting drought tolerant cowpea varieties using drought index.

MATERIALS AND METHODS

In this study, a potted experiment was conducted in the plant house of Soil Research Institute Kumasi, Ghana (Latitudes 6° 39' and 6° 43' North and longitudes 1° 39' and 1° 42' West of West of the Greenwich meridian, during the period of 15th August to 30th October, 2011. It is located in the semi-deciduous forest zone of Ghana (Taylor, 1952). Temperature and relative humidity in the plant house were 28.6±2°C and 85.21±3%, respectively. Six cowpea varieties and two water regime treatments (water-stressed and well-watered conditions) were used in the experiment; they are arranged in the plant house in a completely randomized design with three replications. Thirty-six plastic pots, each measuring 7857cm^3 with a perforated opening at their basal parts were used in this experiment. The pots were filled with 7 kg of top soil. The physico-chemical properties of the soil used are shown in Table 1. The pots were irrigated with water to field capacity. SSP fertilizer was applied at 5 g/pot and cowpea seeds were sown at 3 seeds/pot (August 26th, 2011) and later thinned to one plant/pot seven days after planting (7 DAP).

The crop received equal amount of water at four days interval for establishment. Watering treatments were introduced 10 days after planting. Two levels of water regimes were imposed and these include: treatment 1 (T1) known as well-watered, and treatment 2 (T2) known as water-stressed conditions. Before water withdrawal, all the pots were irrigated to field capacity. Both under well-watered and water-stressed conditions, continuous watering was maintained by weighing the pots prior to every irrigation and at the same time adding the amounts of water that equal to the loss in weight from the pot till end of the experiment (35 DAP).

Data collection

Relative water content

During the period of moisture stress, ten discs of leaf tissue of each genotype were taken using a cork borer with a diameter of 1.5 cm. The fresh weight was quickly measured, followed by flotation on distilled water for up to 4 h. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 50°C. Relative water content was then calculated according to Barrs (1968) as follows:

$$RWC (\%) = (FW - DW)/(TW - DW) \times 100$$

Where: FW - fresh weight, TW - turgid weight, and DW - dry weight.

Plant height

This was measured at four days interval until 35 DAP in order to assess plant growth. The first measurement was taken 17 DAP and at each sampling date, the height of each genotype was taken. Plant heights were measured from the base of the plant to the tip using a metallic measuring tape. Then, the average for each cowpea genotype was determined.

Number of leaves per plant

The number of leaves was recorded for each genotype one week (17 DAP) after imposing the water stress. Then the average number was determined for each cowpea varieties.

Stem diameter

The stem diameter of each genotype was measured with a digital caliper at 1.5 cm above soil surface to the nearest millimeter. The first measurement was taken 17 DAP and at each sampling date, the diameter of the plants of each genotype was taken. After then, the average diameter of each genotype was calculated.

Root dry mass

At harvest, roots were separated from the shoots and gently removed from the soil mass. The roots were gently washed to remove all soil, and then dried at 72°C to constant mass in order to get the dry mass.

The drought susceptibility index (*S*) based on relative water content, plant height, number of leaves per plant, stem diameter and root dry mass of water-stressed to well-watered conditions were estimated. The following relations proposed by Fischer and Maurer (1978) were used:

$$D = 1 - X_S/Xw \quad \text{[Equation 1]}$$

Then the drought susceptibility index (*S*) of individual varieties was calculated:

$$Y_S = Y_W (1 - SD) \quad \text{[Equation 2]}$$

$$S = \frac{X_W (Y_W - Y_S)}{(X_W - X_S) Y_W} \quad \text{[Equation 3]}$$

[Equation 3]

Where: S = drought susceptibility index; X_S = respective average yield under water stress condition; X_W = respective average yield under well-watered condition; Y_S = individual yield under water stress condition; Y_W = individual yield under well-watered condition.

A genotype with higher susceptibility index was considered as a susceptible genotype, while a genotype with low index was considered tolerant.

Data were subjected to ANOVA (Analysis of Variance) using Genstat statistical package 10th edition, while XLSTAT was used for the PCA (Principal Component Analysis). The least significant difference (LSD) was used to determine differences in treatment means at 5% probability level.

RESULTS AND DISCUSSION

The results from the analysis of variance (ANOVA) showed (Table 2) that there was significant genotypic differences in relative water content, plant height, number of leaves per plant, stem diameter and root dry mass. Under water-stressed condition variety, TN5-78 recorded the highest percentage of relative water content (66.5%) which was not significantly different from that of Dan illa (65.5%), IT96D-610 (65.5%) and TN88-63 (65%), while Dan illa (86 cm) was next to variety TN88-63 (110 cm) for mean plant height. Also, variety TN88-63 recorded highest values of mean number of leaves per plant (51), stem diameter (0.5 cm) and root dry mass (1.34 g) which were significantly different from the other counterparts (Table 2).

Highly significant differences were observed due to water treatments among cowpea varieties for all these parameters measured. As expected, cowpea genotypes performed well under the optimum than the stressed-conditions. Table 2 shows also, significant interactions

Table 2. Mean effect of cowpea genotypes, water treatment and their interaction on relative water content, plant height, number of leaves per plant, stem diameter and root dry mass.

Variety	Parameter				
	RWC (%)	PHT (cm)	NL	SD (cm)	RDM (g)
Asontem	62±2.53	83.5±2	24±11	0.45±0.2	0.56±0.32
Dan illa	65.5±0.9	86±1.7	20±1.4	0.4±0.02	0.45±0.10
IT96D-610	65.5±5	81±7	25±13	0.4±0.05	1.06±0.90
Nhyira	61.0±3	73±16	22±7	0.45±0.2	0.90±0.80
TN5-78	66.5±5	72±1.7	27±14	0.4±0.12	0.38±0.16
TN88-63	65.0±7	110±4	51±22	0.5±0.11	1.34±0.90
Grand mean	64.25	84.25	27.83	0.43	0.79
V [LSD(0.05)]	1.46	2.01	1.72	0.02	0.04
Water stress	61.00	79.83	18.00	0.34	0.32
Well-watered	67.50	88.67	38.00	0.53	1.25
WT[LSD(0.05)]	0.84	1.16	0.99	0.01	0.03
V × WT [LSD(0.05)]	2.07	2.84	2.43	0.03	0.07

RWC = relative water content, PHT = plant height, NL = number of leaves, SD = stem diameter, RDM = root dry mass, V = variety, WT = water treatments, V × WT = interaction ± standard deviation.

Table 3. Pairwise comparison of means for relative water content, plant height, number of leaves per plant, stem diameter and root dry mass of the water-stressed and non-stressed cowpea varieties.

Variety	Relative water content (%)		Plant height (cm)		Number of leaves		Stem diameter (cm)		Root dry mass (g)	
	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1
Asontem	60±2.0	64±2	83±1	84±2.0	14±1.0	34±11	0.30±0.03	0.60±0.20	0.26±0.01	0.86±0.3
Dan illa	65±0.5	66±1	85±2	87±2.0	19±2.0	20±1	0.40±0.02	0.41±0.02	0.36±0.02	0.54±0.1
IT96D-610	61±0.25	70±5	75±3	87±7.0	13±1.0	36±13	0.35±0.02	0.45±0.05	0.27±0.04	1.84±1.0
Nhyira	59±0.0	63±3	58±1	88±17	15±1.0	28±7	0.30±0.02	0.60±0.20	0.22±0.01	1.66±1.0
TN5-78	62±1.0	71±5	71±2	73±1.0	14±1.0	39±14	0.30±0.0	0.50±0.10	0.24±0.02	0.52±0.2
TN88-63	59±2.0	71±6	107±1	113±4.0	31±0.0	71±22	0.40±0.01	0.60±0.10	0.59±0.02	2.08±1.0
LSD (0.05)	2.07		2.84		2.43		0.03		0.07	
CV (%)	21		19		5.2		7.50		4.90	

effect due to varieties and water treatments on relative water content, plant height, number of leaves per plant, stem diameter and root dry mass.

The result indicated that there were significant differences among the varieties for relative water content, plant height, number of leaves per plant, stem diameter and root dry mass twenty five days after imposing water-stressed condition and varieties TN88-63 and TN5-78 recorded equally the same value of 71% which was the highest percentage under the control, while under the water-stressed condition, Dan illa (65%) significantly conserve much more water in its leaves than the other counterparts (Table 3). High percentage recorded under water stress gives an indication that Dan illa was relatively able to maintain better plant water status within the water deficit period (osmotic adjustment), to extract deep soil moisture (root capacity) and to reduce transpiration via stomata closure, as a water-saving mechanism.

This result confirms those reported earlier by Hall and Patel (1985) who observed little osmotic adjustment in the leaves of cowpea and little differences in leaf osmotic potential among 100 cowpea genotypes. The result suggests that Dan illa might not have only tolerated the drought but also might have avoided the drought as defined by Fisher and Sanchez (1979) and also Otoole and Chang (1979) that avoidance of drought is the ability of a plant to maintain relatively high water status despite the low moisture condition within the entire environment.

The results agree with the findings of Kumar et al. (2008) while screening and selecting cowpea genotypes for drought tolerance at early stages of breeding reported that the differences among the genotypes in leaf water potential (LWP) and relative water content at 1330 h were substantially large and significant. At 1330 h, genotypes CP6, CP4 and CP5 maintained the highest (>90%) content, while genotypes CP12, CP14, CP16 and CP13 had the lowest relative water content (<80%). Higher relative water content may be maintained either by developing a leaf water potential gradient from the soil to plant as displayed by CP6, CP7, CP8, CP9, CP11 and CP19 or by reduced water loss from the plant organs as displayed by genotypes CP5, CP10 and CP4. The former genotypes had higher ability to extract moisture at low soil water content due to reduced leaf water potential which contributed to the maintenance of higher relative water content (Omae et al., 2005). In cowpea also, osmotic adjustment had been found to be responsible in preventing the detrimental effects of drought in leaves (Sumithra et al., 2007). Plant height observed for the six cowpea varieties in this study was significantly higher in TN88-63 (113 cm), Nhyira (88 cm), IT96D-610 (87 cm) and Dan illa (87 cm) for the non-stressed plants, whereas TN88-63 (107 cm) followed by Dan illa (85 cm) and Asontem (83 cm) recorded the highest values under water-stressed condition. The results agreed with the findings of Onuh and Donald (2009) who reported that the highest mean plant height (117 cm) was observed from the cowpea plants that received 500 ml of water treatment, which was significantly different from the 47 cm; mean plant height observed from plants grown under rained condition. Water stress had the highest depressive effect on mean number of leaves per plant in IT96D-610 and TN5-78 with an equally relative reduction of 64% while Dan illa had the least reduction of 5%. These results confirm those of Wu et al. (2008) who reported that the plant height was reduced up to 25% in water stressed citrus seedlings. Under both the optimum and water-stressed conditions, the highest number of leaves recorded by TN88-63 could be attributed to its creeping and semi erect habit. Samson and Helmut (2007) reported in cowpea that water deficit reduced significantly the total leaf area and total dry matter. Variety Dan illa did not show any significant difference from the control compared to that of the water-stressed condition. This implies that this variety had the characteristics of plant adapted to water-limited environments, reduced plant size, leaf area and leaf area index (LAI) which are major mechanisms for moderating water use and reducing injury under drought stress (Mitchell et al., 1998). Stem diameter was relatively significantly decreased by 50% in Asontem and Nhyira varieties, 40% in TN5-78, 33% in TN88-63, 22% in IT96D-610 and lastly 2.43% in Dan illa variety under the water-stressed condition, compared to the control. Variety Dan illa, which recorded the least reduction in stem diameter exhibits a relatively tolerance to drought. This perhaps may be due, to its initially ability to survive under extreme drought conditions and can respond against the latter drought, and this was mainly achieved by slowing growth and reducing transpiration, as stated by Vianello and Sobrado (1991) that drought stress during vegetative stage provides diminution of the growth in maize crop leaves and stems. This result matches with the findings of Omae et al. (2007) who reported that by the dry treatment, cowpea plants reduced their stem diameter and fresh plant weight by 32 and 81%, respectively.

Significant variations were observed among cowpea varieties for root dry mass. Water stress relatively reduced root dry mass form 87 to 33% under drought stress, as compared to the control. The highest values of root dry mass were recorded by TN88-63 in both the control and water-stressed conditions with 2.08 and 0.59 g respectively. The genotypic variation among cowpea varieties for root dry mass subjected to water deficit may be attributed to the differences in root morphology and growth. TN88-63 had the ability to develop deep and extensive rooting system, in order to enhance water and nutrient uptake under water-stressed condition. These results concur with that of Alyemeny (1998) in *Vigna ambacensis L.* that water stress results in significant reduction in stem dry weight and increased root length. Increase in root biomass in water-stressed genotypes may be due to ability of the cowpea to divert assimilates to enhance the growth of the roots so as to exploit deeper parts of the soil water. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Anjum et al., 2003; Bhatt and Rao, 2005; Kusaka et al., 2005; Shao et al., 2008). In soybean, the stem length was decreased under water deficit conditions (Specht et al., 2001). Stem length was significantly affected under water stress in potato (Heuer and Nadler, 1995), *Abelmoschus esculentus* (Sankar et al., 2007; 2008), *Vigna unguiculata* (Manivannan et al., 2007), soybean (Zhang et al., 2004) and parsley (*Petroselinum crispum*) (Petropoulos et al., 2008). Significant strong positive relationship was found between number of leaves and root dry mass ($r = 0.96$, $p<0.001$). A co-efficient of determination (r^2) of 0.95 was observed among the varieties implying that about 95% of the variation in number of leaves was explained by its association with root dry mass (Figure 1). Variety with high number of leaves may therefore have high root dry mass production and vice-versa. This result is supported by the findings of Kage et al. (2004) who reported that productivity of crops under drought stress condition is strongly related to the dry matter partitioning in the plant and the spatial and temporal root distribution. Drought stress mostly reduced leaf growth and increases dry matter allocation into root fraction, leading to a declining shoot/root ratio (Wilson, 1998).

Drought susceptibility index (*S*)

Selection and ranking of the six cowpea varieties were

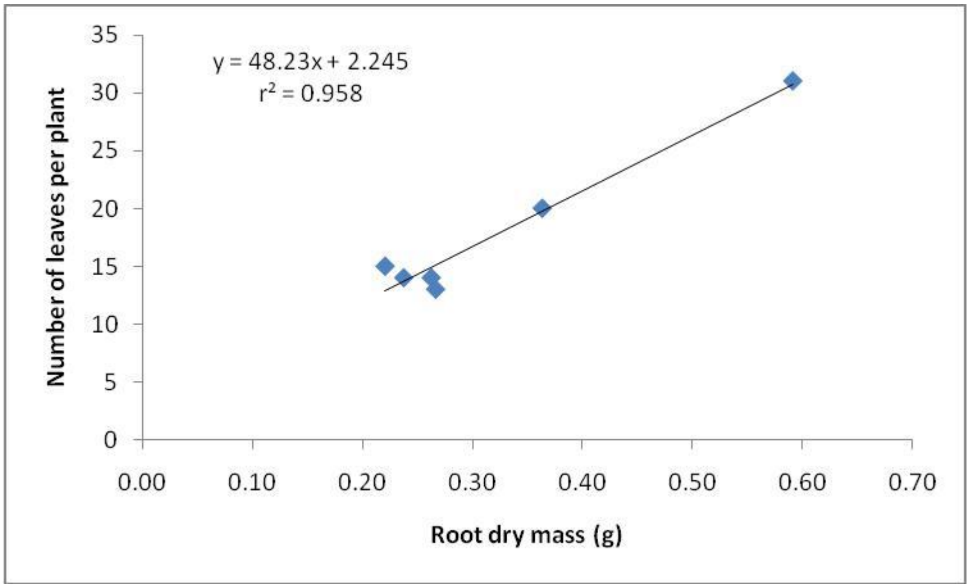

Figure 1. Relationship between number of leaves per plant and root dry mass.

Figure 1: Relationship between number of leaves per plant and root dry mass

Table 4. Scoring and ranking of cowpea genotypes based on drought susceptibility index of the six morpho-physiological parameters under water-stressed condition.

Variety	RWC	PHT	NL	SD	RDM	Total score	Ranking
Asontem	5	3	6	2	4	20	2
Dan illa	6	6	5	6	6	29	1
IT96D-610	2	2	2	5	2	13	5
Nhyira	4	5	1	2	1	13	5
TN5-78	3	2	4	3	5	17	3
TN88-63	1	4	3	4	3	15	4

RWC: relative water content, PHT: plant height, NL: number of leaves, SD = stem diameter, RDM: root dry mass.

based on the drought susceptibility index calculated from Equation 3. The scoring was done in such a way that the genotype with the lowest value of drought susceptibility index was scored number six (6), while the following genotype was scored five (5), till it got to the highest index which was scored 1, because the lower the index the more tolerance to drought. The following ranking was therefore obtained for the six cowpea varieties in decreasing order of drought tolerance: Dan illa > Asontem > TN5-78 > TN88-63 > IT96D-610 = Nhyira. Dan illa was relatively the most tolerant variety, while Asontem showed relatively moderate drought tolerance. Varieties TN5-78, TN88-63 and Nhyira showed apparent susceptibility to drought (Table 4).

Drought susceptibility index (S) represents drought tolerance at whole plant level regardless of drought tolerance mechanism in operation (Grzesiak et al., 1996; Ramírez-Vallejo and Kelly, 1998). In order to further select cowpea drought tolerant varieties, principal components analysis was performed based on drought index (S). Figure 2A gives the most variations between data expressed by two components (83.70%). The first vector shows 57.31% of variations and root dry mass (RDM), number of leaves (NL) and stem diameter (SD) had the highest drought indices with positive correlation with the first component. If genotypes are selected for high drought index, therefore, we can call this component as drought susceptible component. The second component had 26.39% of these variations. This component has high and positive correlation with the plant height (PHT), so it called the drought tolerant component. Based on this study, the five morpho-physiological parameters (relative water content, plant height, number of leaves per plant, stem diameter and root dry mass) were the most appropriate indicators for screening cowpea genotypes. According to Biplot (Figure 2B), genotype Dan illa had large PC2 and its PC1 is almost small, so it is more drought tolerant than the other genotypes. The results suggest that variety Dan illa selected for its lower drought susceptibility index may

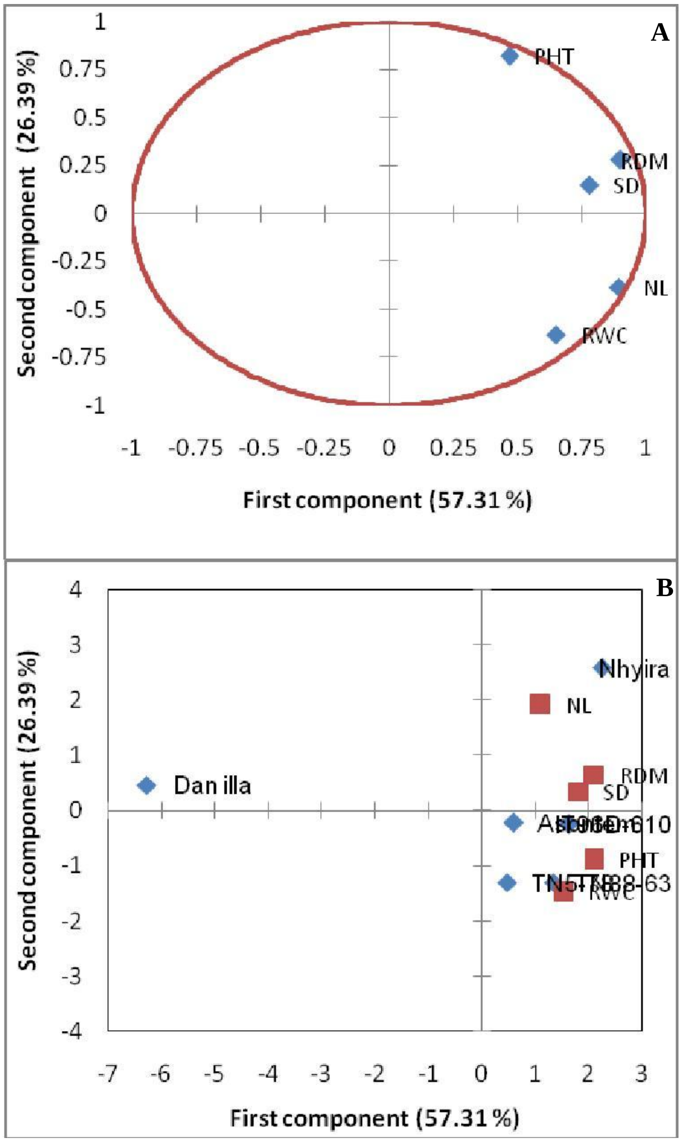

Figure 2. Contribution of variables (A) and biplot of cowpea genotypes based on drought susceptibility index (B).

Figure 2: Contribution of variables (A) and biplot of cowpea genotypes based on drought

have diverse tolerance mechanisms rather than based on single drought tolerant traits. Therefore, such type of genotype may successfully cope with drought under range of environments. Significant negative correlation was observed between the second component and relative water content (r = -0.63) (Figure 2A). Similar relationships were observed in other crops (Abdul, 2008; Falconer, 1990; Fereres et al., 1989).

Conclusion

Drought stress significantly affected growth and development of the six cowpea varieties used in this study. Relative water content, plant height, number of leaves per plant, stem diameter and root dry mass are useful, reliable, cheaper and rapid indicators to identify and select drought tolerant cowpea genotypes using drought intensity and index. Variety Dan illa is recommended for use as source for improving drought tolerance in cowpea breeding programme.

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