Screening cowpea (*Vigna unguiculata*) germplasm for canopy maintenance under water stress

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Abstract

Cowpea provides a cheap source of proteins, vitamins and other important nutritive elements to smallholder farmers in Malawi, but moisture stress remains a big production challenge in drought prone areas. With the aim of identifying locally adapted cowpea germplasm with drought tolerance, 36 accessions were characterised for canopy maintenance in a glasshouse at Massey University, Palmerston North. Seedlings were adequately watered until the third week after germination, and then received no water for four weeks before being re-watered for the final two weeks. Canopy responses were scored using leaf wilting scales, leaf wilting index (LWI), relative water content, re-growth and stem greenness. The accessions showed highly significant variations ($P<0.0001$) for all the measured parameters. Accessions 479, 601, 645, 2226 and 3254 showed apical re-growth, high relative water content, stem greenness and lower scores for both leaf wilting scales and LWI at a soil moisture content of 2.9%. In contrast, accessions 517, 2231, 2232, 2883 and 3215 showed high levels of drought susceptibility. Multivariate analysis identified 5 distinct clusters with accessions in cluster 4 being drought tolerant and accession in cluster 5 being the most susceptible. The accessions in these contrasting clusters could provide genotypes for further genetic and crop improvement studies.

Additional keywords: drought tolerance, leaf wilting index, stem greenness, RWC, Malawi

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a common legume grown by subsistence farmers in Malawi and other tropical countries for its multiple uses. The crop is well known for its nutritional benefits from grains rich in protein, and fresh pods and vegetative parts rich in minerals and vitamins. In addition to the culinary benefits, cowpea improves soil fertility through nitrogen fixation. Cowpea is also a potential source of income through sales (Timko *et al*., 2007; Nkongolo *et al*., 2009). The crop is adapted to drought prone areas in comparison with other grain legumes (Singh *et al*., 1999; Hall, 2004); however, current climate change threatens its production in drought prone areas as frequency and intensity of droughts have increased (Lobell *et al*., 2008). Changes in the current climate pattern require responsive crop varieties that can adapt to reduced water availability. Such varieties will be developed through systematic evaluation of locally adapted genotypes that have been selected by farmers for their desirable attributes like yield, taste and both biotic and abiotic stresses (Burke *et al*., 2009).

Cowpea drought tolerance can be explored through either canopy or root characteristics due to their direct influence on water loss through transpiration or water absorption from the soil. For instance, drought tolerant cowpea genotypes have been identified through pot evaluation, wooden boxes, pin boards and field evaluation (Watanabe *et al*., 1997; Mai-Kodomi *et al*., 1999; Matsui and Singh, 2003; Muchero *et al*., 2008; Agbicodo, 2009). Although research work on drought tolerance has been conducted elsewhere using different methods, limited information exists on drought tolerance in locally adapted cowpea germplasm from Malawi. In this research, cowpea germplasm from Malawi was evaluated for drought tolerance using canopy maintenance. The results will contribute to a cowpea breeding programme.

Materials and Methods

Thirty six cowpea genotypes randomly selected from the Malawi Plant Genetic Resources Centre were evaluated in the glasshouse at the Plant Growth Unit (PGU), (40° 23’ 5” S, 175° 36’ 50” E) Massey University, Palmerston North, New Zealand. These genotypes are local landraces collected from farmers in Malawi. The experiment was laid out in a randomised complete block design (RCBD) with genotypes (accessions) as treatments replicated four times. Four healthy looking seeds were planted in 10 litre pots and the seedlings were thinned to two plants per pot after eight days. Each pot was filled with growth media which was prepared by mixing 100 litres of pot mix with 150g of short term release fertiliser (3-4 months) and 150g of Dolomite. Moisture stress was applied as described by Muchero *et al*. (2008). The plants were watered to field capacity (moisture content 30%) until the first trifoliate leaves were fully expanded (three weeks after germination) and then water was withdrawn for four weeks for drought response measurements. After a period of stress, plants were re-watered twice a week for two weeks. During the period of stress, day and night temperatures were maintained at 22-27°C and 15-19°C respectively. The soil moisture content during the water stress was monitored at 20cm depth using a Time Domain Reflectrometer (TDR) twice weekly.

In total fourteen variables were recorded after stressing the plants to assess drought tolerance of the 36 accessions as described in Table 1.

Table 1: Variables used to categorise the drought tolerance of the 36 cowpea accessions assessed.

Variable Identifier	Description
LWI 1	Leaf wilting index after the first week of stress
LWI 2	Leaf wilting index after the second week of stress
LWI 3	Leaf wilting index after the third week of stress
LWI 4	Leaf wilting index after the fourth week of stress
IB 2	International Board on Plant Genetic Resources scale after the second week
IB 3	International Board on Plant Genetic Resources scale after the third week
IB 4	International Board on Plant Genetic Resources scale after the fourth week
MAIK 2	Mai-Kodomi *et al*. (1999)scale after the second week
MAIK 3	Mai-Kodomi *et al*. (1999) scale after the third week
MAIK 4	Mai-Kodomi *et al*. (1999) scale after the fourth week
RWC 2	Relative water content after the second week
RWC 4	Relative water content after the fourth week
STG	Stem greenness after 2 weeks of re-watering
Re-growth	Resumption of growth after 2 weeks of re-watering

Leaf Wilting Index (LWI) was calculated using the ratio of leaves showing wilting signs and total number of leaves per plant. Two different wilting scales were used in assessing wilting in cowpeas. International Board on Plant Genetic Resources currently known as Bioversity International developed a 1-9 scale where 1 represents normal and 9 dead and dry plants under moisture stress (IBPGR, 1983). Mai-Kodomi *et al*. (1999) used a 1-5 scale with 1 representing green turgid leaves and 5 completely dead plants. Stem greenness was scored using a scale of 1-5 where 1 was brown and 5 completely green. Re-growth was scored using three categories as; 1 with no recovery, 3 recovery from axillary buds and 5 recovery from the apical stem. Relative Water Content (RWC) was calculated on new fully expanded leaflets after the second and fourth weeks of stress. Leaves were cut at the base of lamina, and weighed for fresh weight (FW). After soaking leaves for 24 hours, leaves were weighed for turgid weight (TW). After TW measurement, leaves were dried with tissue paper and then oven dried at 70°C for 72 hours followed by dry weight (DW) measurements. RWC was calculated as follows:

$$RWC = \frac{FW - DW}{TW - DW}$$

Data was analysed by analysis of variance using the General Linear Model (GLM) procedure in SAS (version 9.2, SAS Inc., North Carolina, USA). Mean separation was done using the least significant difference (LSD) at $P<0.05$. Means for all variables were calculated and standardized for cluster analysis in Minitab 16 statistical package (Minitab, Inc., Pennsylvania, USA) to group the accessions according to their similarities. Standardization was done to minimize the dominance of variables with higher numerical values. The standardization was implemented by subtracting the mean of each variable from each individual entry and dividing each value with a standard deviation so that variance is zero and standard deviation is one (Endresen, 2010). Euclidean distances and ward linkage were used in the cluster analysis (Jeffers, 1967).

Results

Volumetric soil water content at 20cm depth showed no significant differences between genotypes. The initial average soil moisture content was 26.2% and the final was 2.9% representing field capacity and the driest moisture content respectively (Figure 1).

Analysis of variance showed highly significant variation ($P<0.0001$) for all the variables measured. The variation was clearer with the advancement of moisture stress (Table 2).

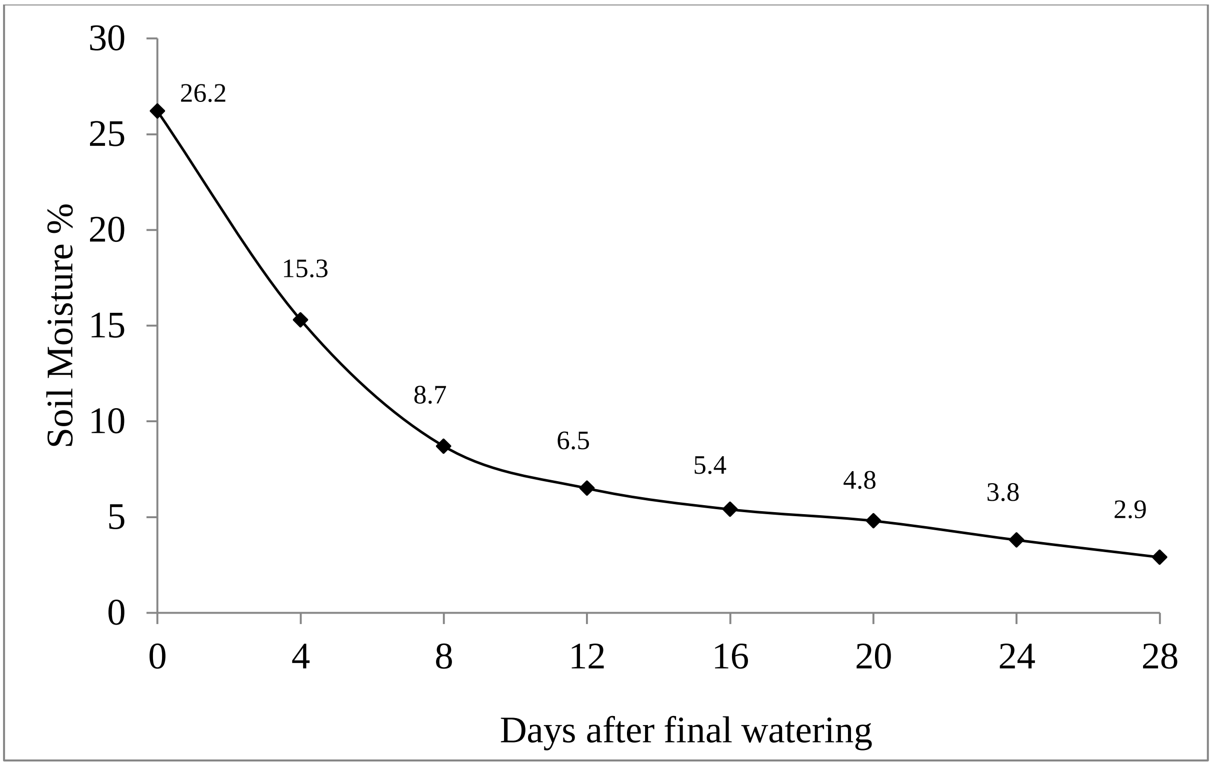

Figure 1: Variation in volumetric moisture content of the growth media during the water stress period.

Table 2: Variation among 36 cowpea accessions for leaf wilting and relative leaf water content during four the weeks of water stress, and re-growth and stem greenness after two weeks of re-watering.

	LWI 1	LWI 2	LWI 3	LWI 4	IB 2	IB 3	IB 4	MAIK 2	MAIK 3	MAIK 4	Re-growth	STG	RWC 2	RWC 4
Mean	0.09	0.25	0.50	0.78	2.82	3.1	4.8	1.6	2.0	2.7	2.2	2.87	0.69	0.39
Minimum	0.00	0.00	0.00	0.23	1.00	1.0	1.2	1.0	1.0	1.2	1.0	1.00	0.55	0.20
Maximum	0.72	0.77	0.89	1.00	6.00	7.0	7.5	3.7	4.0	4.0	5.0	0.57	0.81	0.57
P-value	***	***	***	***	***	***	***	***	***	***	***	***	***	***

Note: all the variables measured are unitless because they are either ratios or scales (refer to methodology for details).

Cluster analysis assigned the 36 accessions into five significantly different (P<0.0001) clusters (Figure 2; Tables 3 and 4). Each cluster showed particular attributes regarding response to moisture stress. Clusters 3 and 4 showed similar pattern of variables (sign) towards each cluster (Table 4). However, clusters 4 with five accessions was characterised by low LWI and wilting scales but with high values of regrowth, RWC2, RWC4 and stem greenness. In contrast to clusters 3 and 4, clusters 1 and 5 showed similar pattern of variables towards each cluster. Cluster 5 displayed high values of LWI and wilting scales but low values of regrowth, RWC2, RWC4 and stem greenness. Cluster 2 displayed its own pattern of characteristics with reference to factor (variable) loadings (Table 4).

Table 3: Means and standard deviations for five clusters based on variables measured during moisture stress and after re-watering periods averaged over the accessions in each cluster.

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Pooled Std Dev.
LWI 1	0.16±0.08b	0.02±0.02c	0.03±0.7c	0.00±0.00c	0.72a	0.08
LWI 2	0.34±0.08b	0.21±0.06c	0.14±0.12cd	0.05±0.07d	0.77a	0.06
LWI 3	0.57±0.07b	0.57±0.09b	0.33±0.07c	0.17±0.14c	0.89a	0.09
LWI 4	0.86±0.08a	0.85±0.05 a	0.70±0.08b	0.43±0.13c	1.00a	0.08
IB 2	3.40±0.24b	2.71±0.23c	2.38±0.32cd	2.00±0.61d	6.00a	0.32
IB 3	4.11±0.44b	2.67±0.62c	2.50±0.71c	1.30±0.45d	7.00a	0.54
IB 4	5.25±0.98ab	5.46±0.47b	4.31±1.24b	1.90±0.45c	7.50a	0.81
MAIK 2	1.98±0.21b	1.42±0.17c	1.28±0.24cd	1.10±0.14d	3.75a	0.19
MAIK 3	2.43±0.21b	1.85±0.23c	1.56±0.47cd	1.20±0.27d	4.00a	0.26
MAIK 4	2.89±0.38ab	3.02±0.25ab	2.50±0.35b	1.55±0.33c	4.00a	0.33
Re-growth	1.57±0.63c	1.94±0.36c	2.88±0.97b	4.65±0.22a	1.00c	0.55
RWC 2	0.67±0.04c	0.68±0.03bc	0.72±0.05ab	0.77±0.03a	0.55d	0.03
RWC 4	0.36±0.07b	0.35±0.04bc	0.48±0.05a	0.53±0.03a	0.20c	0.05
STG	2.38±0.66bc	2.63±0.25bc	3.25±0.57b	4.73±0.10a	1.88c	0.49

Means with the same letter are not significantly different based on the Tukey procedure at a 95% confidence interval.

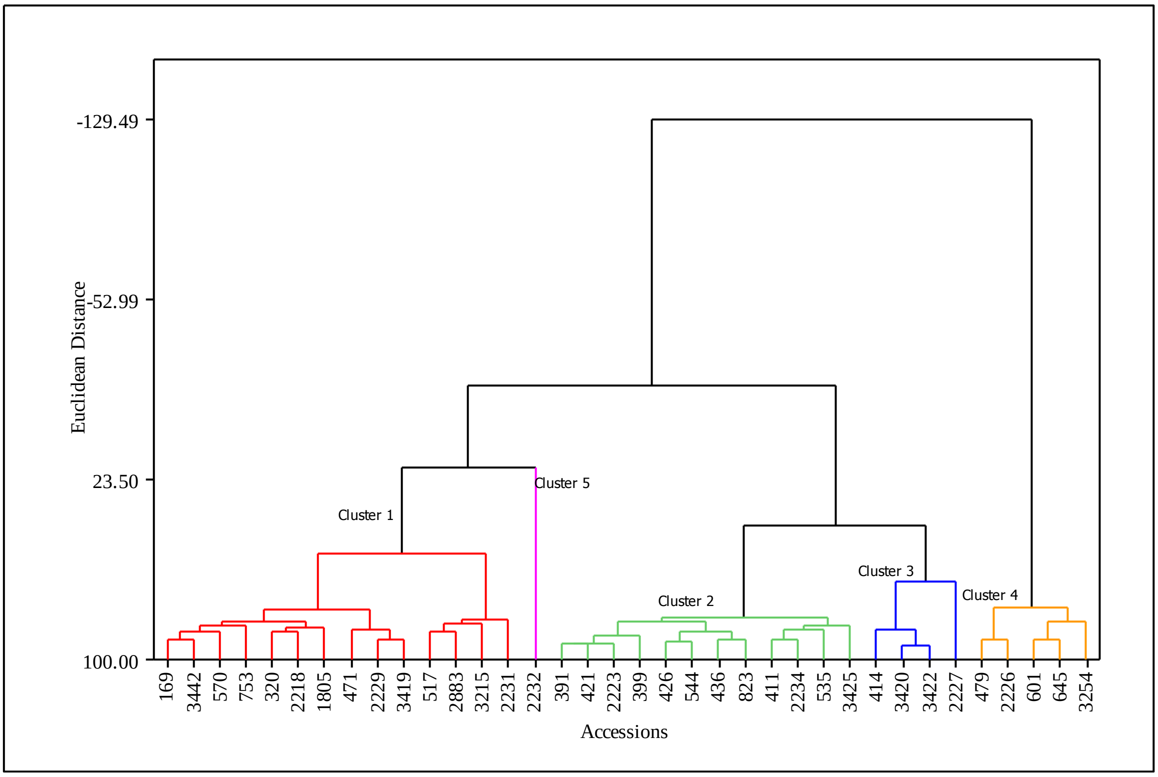

Figure 2: Dendrogram showing relationships among 36 accessions based on 14 drought response variables using Eclidean distance and Ward linkage analysis.

Table 4: Contribution of the measured variables towards clustering of the 36 accessions.

Variable	Cluster1	Cluster2	Cluster3	Cluster 4	Cluster5
LWI 1	0.47	-0.53	-0.40	-0.64	4.51
LWI 2	0.60	-0.22	-0.71	-1.26	3.39
LWI 3	0.41	0.37	-0.88	-1.76	2.10
LWI 4	0.43	0.41	-0.48	-2.04	1.26
IB 2	0.59	-0.29	-0.71	-1.18	3.88
IB 3	0.75	-0.37	-0.49	-1.42	3.00
IB 4	0.29	0.43	-0.33	-1.94	1.79
MAIK 2	0.59	-0.40	-0.67	-0.99	4.21
MAIK 3	0.68	-0.26	-0.74	-1.34	3.28
MAIK 4	0.27	0.48	-0.38	-1.94	1.68
Re-growth	-0.57	-0.26	0.53	2.03	-1.06
RWC 2	-0.40	-0.20	0.72	1.53	-2.56
RWC 4	-0.31	-0.45	0.97	1.60	-2.13
STG	-0.53	-0.27	0.41	2.00	-1.07

Discussion

Moisture content of 2.9% after four weeks of no water created sufficiently severe stress for the identification of tolerant and susceptible genotypes. Accessions 479, 601, 645, 2226 and 3254 of cluster 4 maintained high RWC, stem greenness, re-growth from apical buds, lower LWI and lower scores for wilting scales. This suggests that these accessions can survive under drought conditions. In contrast, at the same moisture content level, accessions 2232 (cluster 5), 517, 2231, 2883 and 3215 (part of cluster 1) did not survive indicating their susceptibility to drought. The 2.9% moisture content level for screening germplasm for drought tolerance is similar to that used in other studies. In a pot study on cowpeas, Watanabe *et al*. (1997) recommended 3% as the optimum moisture content for discriminating germplasm into tolerant and susceptible groups. Similarly, Abraham *et al*. (2004) studied drought tolerance of bluegrass and identified drought tolerant genotypes that survived at <3% moisture content after 35 days of water stress.

Wilting is the visible sign of drought stress in plants (Engelbrecht *et al*., 2007). Some accessions started wilting after the first week of stress by showing high values of LWI1 (Table 3). Accessions in clusters 1 and 5 showed high values of LWI and wilting scales in contrast to accessions in clusters 3 and 4. In other cowpea studies, (Mai-Kodomi *et al*., 1999; Muchero *et al*., 2008) wilting was observed after the first week of stress in drought susceptible genotypes which support our results. Sharma and Kumar (2008) identified stomata conductance, leaf water potential, and osmotic adjustment as key mechanisms preventing early wilting in cowpeas. Plants that do not close their stomata wilt within a short period of stress as water is lost into the air at a faster rate creating water imbalance in plant tissues. This may have been the case for accessions 517, 2231, 2232, 2883 and 3215 that wilted in early periods of stress and consequently dried.

RWC is associated with maintenance of water in leaf tissues. Accessions in cluster 4 maintained RWC of 0.77 (Table 3) after being stressed for two weeks. Such high RWC under stress signifies the ability of the accessions to sustain metabolic processes for their survival. Under well watered conditions, cowpea can maintain leaf water contents between 0.88-0.91 (Lobato *et al*., 2008) a level which is comparable with accessions in cluster 4. Abraham *et al*. (2004), identified drought tolerant genotypes of bluegrass which were able to maintain a RWC of greater than 0.50 after 35 days of drought stress. Taiz and Zeiger (1998) reported that most plants maintain physiological processes at RWC>0.50. After four weeks of stress, accessions in cluster 4 preserved water well above 0.5 signifying their ability to maintain metabolic processes during extremely low moisture conditions. In contrast to accessions in cluster 4, early wilting accessions registered a RWC of between 0.20 and 0.32. RWC of 0.20-0.32 is low enough to cause physiological injury to most plant species. Kaiser (1987) reported that when RWC falls below 0.30 the chances of recovery are very low due to reduced photosynthetic capacity. Maintenance of high RWC in some genotypes may be attributed to their ability to minimise water loss from the leaves or extraction of water from the deep layers or dry soils (Oliver *et al*., 2010; Taiz and Zeiger, 2010). In this study, extraction of water from deep layers of soil was not possible as the plants were grown in 10 litre pots. The maintenance of high RWC by some genotypes suggests that the moisture content after four weeks of stress was appropriate to screen cowpea for drought tolerance as there were clear differences due to either death or recovery of some accessions after re-watering for two weeks.

Cluster analysis provides useful information to breeders through visual presentation of different groupings (dendrograms) and it also explains the contribution of each variable towards different clusters (Mohammadi and Prasanna, 2003). Significance of each variable towards clustering is expressed by magnitude and sign of the particular variable against particular cluster (Table 4). The higher the absolute value, the more important a particular variable is towards a cluster. Positive and negative signs indicate whether the value of a particular variable is above or below average respectively (Hopke *et al*., 1976). In this study, five clusters were identified and further analysed to summarise their characteristics (Tables 3 and 4, Figure 2). Cluster 4 with five accessions exhibited drought tolerance characteristics. The most important variables which define cluster 4 in order of importance were LWI 4, re-growth, stem greenness, IB 4 and MAIK 4 (Table 4). The strong relationship of cluster 4 with LWI 4 and regrowth indicates the ability of the accessions to withstand water stress till the end of stress period, and recover after re-watering. A similar pattern of variable loadings (positive and negative signs) between clusters 4 and 3 could indicate moderate tolerance of accessions in cluster 3.

In contrast to cluster 4, cluster 5 exhibited drought susceptibility characteristics. Variables in order of importance describing cluster 5 are LWI 1, MAIK 2, IB 2, LWI 2 and MAIK 3 (Table 4). Cluster 5 can be described as early wilting since variables measured during early weeks of stress contributed significantly towards the cluster. These factors are associated with early wilting of leaves and the most important variable is LWI 1 which indicates that wilting started in the first week of stress. Similarities in the pattern of variable loadings between cluster 5 and 1 show that accessions in cluster 1 are moderately drought susceptible.

Cluster 2 followed its own unique pattern in terms of response to drought with reference to variable loadings (Table 4). One of the possible reasons for this unique response could be the availability of genotypic mixtures in the accessions belonging to this cluster. Landraces are characterised by within accession variability due to local seed exchanges and intercropping of crops by subsistence farmers (Thomas *et al*., 2011). Accessions in this group may have been collected as mixtures. The other possibility could be the presence of hybrids in the mixture due to natural crossing in the field before collection although cowpea is a predominantly self-pollinated crop (Timko *et al*., 2007).

Conclusions

In conclusion, this study has revealed potential genotypes of cowpea for drought tolerance which could be included in the Malawi National Cowpea Improvement Program as part of climate change adaptation. Also, the results will help to strengthen on farm conservation of drought tolerant genotypes in areas where such genotypes were collected. Accessions 479, 601, 645, 2226 and 3254, have shown desirable attributes for maintenance of canopy at moisture level of 2.9% in contrast with accessions 517, 2231, 2232, 2883 and 3215 which completely dried. Further work needs to be conducted to explore yield potentials and physiological mechanisms controlling canopy maintenance of the tolerant genotypes before genetic and crop improvement studies. This study has identified potential genotypes for drought tolerance based on canopy characteristics only, which limits application of the results in the absence of detailed root characteristics.

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