

Performance of cowpea Recombinant Inbred lines for grain yield and stability under low soil moisture conditions in Northern Ghana

Sanatu Mustapha Alidu

Department of Crop Science, Faculty of Agriculture, University for Development Studies,
P. O. Box TL 1882, Nyankpala, Tamale, Ghana

Correspondence email: msanatu@uds.edu.gh

ABSTRACT

Drought is more prevalent in the semi-arid areas where cowpea has the greatest potential. Drought is unpredictable in occurrence. Drought affects both the vegetative and reproductive phase resulting in significant yield losses. The objective of this study was to determine grain yield and stability of cowpea inbred lines under low soil moisture conditions. Four hundred and fifty (450) $F_{2:6}$ inbred lines were developed through single seed decent from drought parent (IT 93K-503-1) and susceptible parent (IT 97K-279-3). Populations were initially screened, and further evaluation was done under field conditions using split plot design. Inbred lines were completely randomized in three replications. Moisture stress was imposed 10 days after planting and watering resumed at 40 days after planting. Physiological and agronomic data were collected during moisture stress at vegetative, flowering and at harvest. Analysis of variance was performed using GenStat discovery edition 12. Quantitative indices of stress tolerance were calculated using grain yield data. The results obtained indicated that, water stress caused percentage yield reduction for most of the inbred lines and the parental checks. The highest percentage yield reduction was observed for inbred line F20 (57.83%). Inbred lines F142, F408, F398, and F38, had their yields reduced under stress conditions whilst inbred lines F131, F186, F406 and F255 were less affected. Inbred line F84 rather had yield increase under drought (10.57%). The use of two contrasting parents in generating the inbred lines for the study revealed classes of maturity groups for drought tolerance. This aided the test of superiority for the inbred lines in relation to the parental performance across the environments used for the study. Further research using quantitative trait loci analysis will be required to enable the identification of loci responsible for drought and its relationship to maturity period.

Key words: Cowpea, drought tolerance, quantitative indices, Stability analysis, Recombinant inbred lines

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical or subtropical warm season crop that plays a vital role in the cropping systems of West Africa (Singh, and Tarawali, 1997) where, it is produced mainly in the semi-arid savannah and Sahelian zones for its grain and hay (Padi, 2004).

Soil moisture is a principal environmental factor limiting legume productivity in the tropics and sub-tropics (Carranca *et al.*, 1999; da Costa *et al.*, 2010). The lack of adequate soil moisture affects both vegetative (Ahmed and Suliman, 2010) and reproductive growth of food legumes, resulting in significant yield losses (Ramirez-Vallejo and Kelly, 1998). Although, cowpea is said to be relatively

drought tolerant, it has been shown that water stress leads to a decrease in plant water content, turgor reduction and consequently a decrease in cellular expansion and alteration of various essential physiological and biochemical processes that can affect growth and productivity (da Costa *et al.*, 2010; Lobato *et al.*, 2008; Pimentel *et al.*, 2004).

Early maturing varieties are often preferred by farmers and are becoming increasingly important in an era of climate change and unpredictable droughts, especially for farmers who farm along the hydromorphic lowland areas and around the irrigation facilities during the dry season (Alidu *et al.*, 2013; SARI 2002, 2007). Farmers often use residual moisture for crop establishment and harvest early before the main cereal crop production. However, some farmers during the participatory rural appraisal indicated their preference for long duration cultivars because of high biomass to feed their animals, and this characteristic is very common for the long duration cowpea line (T. Batieno, 2014a; B. B. Singh, 1997). Therefore, selection for both early and late maturing cowpea genotypes would contribute to increased production and yields in these production zones.

The objective of this study was to identify drought tolerant cowpea inbred lines from a RIL population using quantitative indices and physiological traits for grain yield under low soil moisture conditions in the field.

MATERIALS AND METHODS

Below is the breeding and selection methods used as well as the outline of the generations of the materials used for the study

Germplasm for the study

Four hundred and fifty (450) $F_{2:6}$ inbred lines were developed through single seed decent from drought tolerant and susceptible parents and were advanced to

the F6 stage. IT93K-503-1 is a well-recognized drought tolerant line and has been used by many scientists for drought studies (Batieno *et al.*, 2016; Muchero *et al.*, 2010; Muchero *et al.*, 2009; Muchero *et al.*, 2008).

The second parent IT97K-279-3 is a drought susceptible but early maturing advanced breeding line, obtained from IITA as well. Inbred lines development was carried out at the University for development studies experimental fields in Nyankpala. The lines were screened for seedling drought in wooden boxes and selection were done for field drought study.

Experimental design for drought evaluation under field conditions

A split plot design was used for the experiment. The watering regimes at two levels were the main plots and the 22 recombinant inbred lines selected from the seedling drought screening in wooden boxes plus two parental checks were the subplot factor. The land was prepared by disc ploughing, harrowing and ridging 75 cm apart. The net plot size was 3 m x 2 m consisting of five ridges of two-meter in length. Thus, an experimental unit consisted of five row plots of two-meter-long, and 10 plants per row giving a plot stand of 50 plants per plot. Spacing between and within plants were 60 cm x 20 cm. The inner three ridges were used for sampling and data collection, while the two outer ridges were left as guard ridges. Blocks and plots in both experiments were separated by a spacing of 2 m.

Dry season evaluation was done in February and December 2016 and 2017 at Golinga and Libga irrigation sites respectively in the Guinea Savanna ecology. Planting was done at a rate of two seeds per hole. The seeds were later thinned to one plant per hill.

Watering Regime

The plants were subjected to two watering regimes: well-watered and water stressed at the vegetative phase (10 days after planting,

DAP), until the beginning of flowering (40 DAP)). Both fields were watered to field capacity after planting and the stress field was thereafter left until flowering. Soil samples were taken for physical and chemical analysis prior to planting.

Data Collection

Agronomic data

Data were recorded on plot basis on both water-stressed and fully irrigated plots at both locations. Days from planting to first flowering for each plot were recorded, the days to 50% flowering was recorded when half of the plants per plot produced flowers. Based on this information, the days to 50% flowering were estimated. At harvest, data on number of pods per plant, number of seeds per pod and hundred seed weight were taken as average of five randomly selected plants within the plot excluding the border plants. The weight of hundred seeds (g) for each treatment was determined using an electronic scale. Data on grain yield was recorded on plot basis using three middle rows of 10 plants (30 plants per plot) in grams extrapolated to Kg/ha and t/ha.

Grain yield was calculated as
$$\frac{\text{grain weight} \times \text{per plot}}{\text{plot area harvested}} \times 10000$$

Biomass yield per plot was estimated by a random sample of five plants per plot and uprooted carefully. They were put in labelled envelopes and sun-dried.

Quantitative indices of stress tolerance were calculated using grain yield data. These stress tolerance indices were:

- (i) Mean productivity (MP)
- (ii) Tolerance index (TOL)
- (iii) Stress susceptibility index (SSI)
- (iv) Geometric mean productivity (GMP)
- (v) Stress tolerance index (STI)
- (vi) Stress intensity (SI)

The selection indices of stress tolerance for mean productivity (MP), tolerance index (TOL), stress susceptibility index (SSI), stress intensity (SI), geometric mean

productivity (GMP), and stress tolerance index (STI) were calculated based on yield data in the two contrasting environments using the following formulae:

$$MP = \frac{(Y_s + Y_w)}{2} \text{-----} \quad (\text{Fernandez, 1992a})$$

$$TOL = Y_w - Y_s$$

$$SSI = \frac{1 - \frac{Y_s}{Y_w}}{1 - \frac{\bar{Y}_s}{\bar{Y}_w}} \text{-----} \quad (\text{Fernandez, 1992a})$$

$$SI = 1 - \frac{\bar{Y}_s}{\bar{Y}_w}$$

$$GMP = \sqrt{(Y_s \times Y_w)}$$

$$STI = \frac{Y_s \times Y_w}{\bar{Y}_w^2}$$

Where Y_s and Y_w (known as Y_p (Fernandez, 1992b) are the yields of each genotype under drought-stressed and non-stressed conditions. \bar{Y}_s and \bar{Y}_w are respectively the mean yields of all genotypes under drought-stressed and non-stressed conditions.

The stress intensity (SI) score was classified into mild, moderate and severe. Stress intensity was mild when yield reduction was between 0 and 25%, moderate when yield reduction was between 25 and 50% and severe when yield reduction was between 50 and 100% (B. J. Batiemo et al., 2016a; Chiulele, 2010).

Harvest index was computed using the formula by Donald and Hamblin (1976).

$$\text{Harvest index (\%)} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100$$

The percent reduction due to moisture stress and drought susceptibility index was computed using the formula suggested by Fischer and Maurer (1978) as:

$$\text{Percent reduction} = \frac{\text{Yield under non-stress} - \text{Yield under stress}}{\text{Yield under non-stress}} \times 100$$

Data Analysis

An initial analysis of variance was performed for each environment to verify the existence of differences between inbred

lines. After these analyses, the homogeneity between residual variances was determined, and a combined analysis of variance was used to test the genotype and environment effects and the magnitude of the genotype by environment (G×E) interaction. The additive main effects and multiplicative interaction (AMMI) analysis was used to adjust the main or additive genotype and environmental effects by analysis of variance, in addition to the adjustment of the multiplicative effects for the G×E interaction by principal component analysis.

The AMMI model as in Cornelius *et al.* (1992) follows:

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^n \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the cowpea yield of the i^{th} variety in the j^{th} environment, μ is the overall mean, g_i and e_j are the fixed varietal effects and environmental deviations, respectively, λ_k is a singular value of the K axis in the principal component analysis, γ_{ik} and δ_{jk} are genotype and environmental factors, respectively, of the singular vectors associated with λ_k from the interaction matrix, N is the number of principal components retained in the model,

ρ_{ij} is the residual G×E interaction, and ε_{ij} is the average independently assumed error $\varepsilon_{ij} \sim N(0, \sigma^2)$. The sum of squares of the G×E interaction was divided into an n singular axis or Interaction principal component axis (IPCA), which reflects the standard portion in which each axis corresponded to a particular AMMI model. The selection of a model that best describes the G×E

interaction as proposed by Cornelius *et al.* (1992). Once the additive main effects and multiplicative interaction (AMMI) model was selected. The adaptability and phenotypic stability using biplot graphs was then determined. Biplot graph interpretation is based on the variation of the additive main effects (genotype and environment) and the multiplier effect of the G×E interaction.

The abscissa represents the main effects (average of inbred lines evaluated), and the ordinate the interaction among the axes (IPCA). In this case, the lower the IPCA value (absolute value) the lower the contribution of the G×E interaction and the greater the genotype stability.

An ideal genotype is one with a high yield and IPCA values close to zero. An undesirable genotype is one with low stability, which is associated with low yields. The average predictions were estimated according to the AMMI model selected. All statistical analyses were performed using the AMMI procedure in R (R Development Core Team, version 3.0.1

RESULTS

The description of the soil physical and chemical properties and composition for the trial locations are presented in Table 1.0

The relevance of the initial soil analysis (Table 1.0) was to enable us to understand the compositions of the soil in study locations to understand the influence of photosynthesis, and reaction of inbred lines to drought under field conditions with its associated yield penalties

Table 1.0: Soil physical and chemical properties and composition for the trial locations

Location	Soil Sample depth	pH (1:1)	% OC	% N	Mg/kg P	EC ($\mu\text{S}/\text{cm}$)	Ca (cmol +/kg)	Mg (cmol +/kg)	K (cmol+/kg)	Texture		
										% Sand	% Silt	% Clay
Gollinga 2016	0-20cm	5.6	0.58	0.72	2.72	320.7	1.78	3.38	37	61.16	34	4.84
Gollinga 2017	0-20cm	5.5	0.41	0.71	2.81	322.5	1.94	2.72	38	60.87	35.5	3.625
Liba 2017	0-20cm	6.4	0.63	0.8	3.4	343.2	2.01	4.01	41	62.5	37	2.93

Climatic data for stress experiment

No rainfall was recorded for January and February 2016; the total rainfall recorded in March was 21.7 mm with the highest total rainfall occurring in July of about 365.6 mm. The total evaporation for January, February and March were 213.26, 232.92, and 219.44 respectively, while the lowest total evaporation was recorded in July with a total of 81.43. The mean relative humidity

(%) for the experiment period were 32, 26, 52, and 65 for, January February, March and April respectively (Fig. 5.2). The total evaporation (mm) for January, February, March, and April were 213.26, 232.92, 219.44 and 190.08 respectively. Fig. 5.2 show climatic data for Gollinga during the 2016 stress experiments.

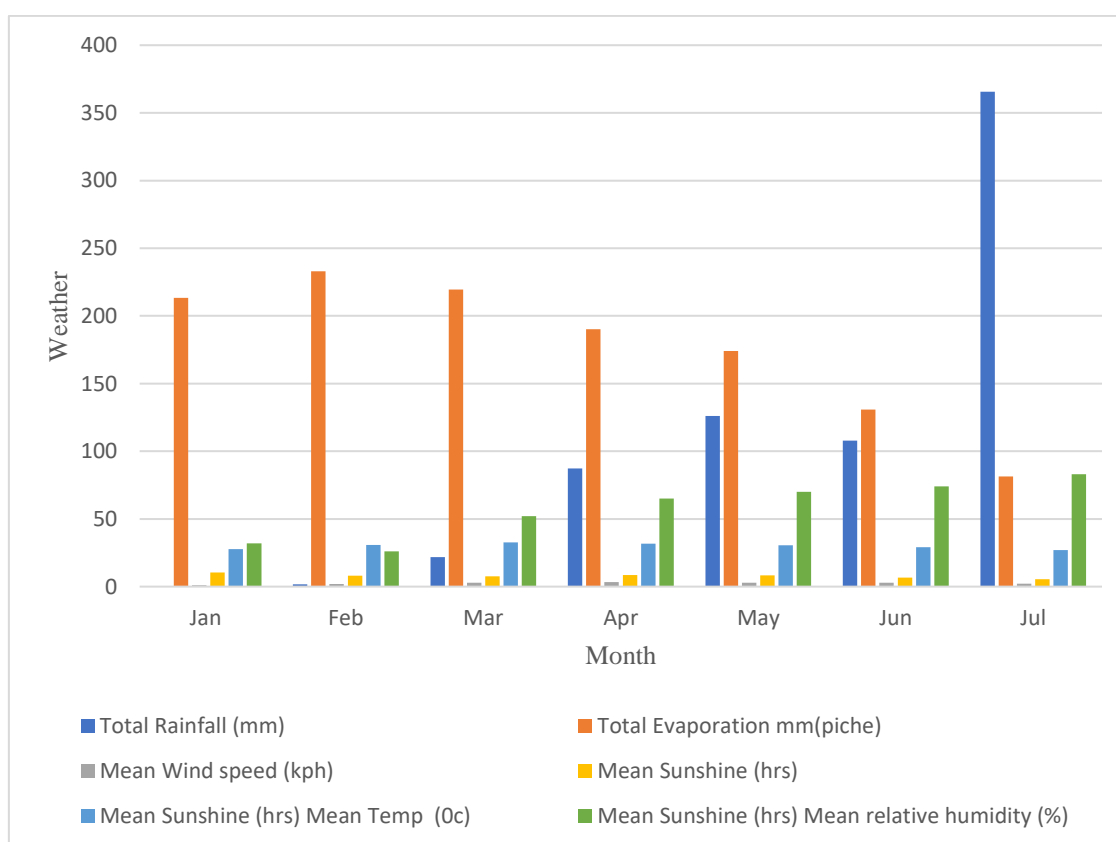


Fig. 1: Climatic data for Gollinga drought experiment

Performance of twenty-two (22) inbred lines and parental checks for grain yield.

There were significant ($P < 0.001$) genotypic differences for days to 50% flowering, number of seeds per pod, hundred seed weight, grain yield, biomass, and harvest index. Some inbred lines maintained their days to 50% flowering under moisture stress and non-stress conditions; inbred lines with numbers 116, 186, 189, 223, 353, 55, and 38 were able to maintain their days to flowering and 50% flowering without change. Others had very distinct differences of about 1 - 4 days by either reduction or an increase in days to flowering and 50% flowering. Those inbreds that were found within the range of 1-4 days were inbred lines with numbers 189, 230, 325, 396, 398, 406, 408, 57, 78, 255, 28, 75, 20 and 84 respectively. For the parental checks IT97k-279-3 which is a susceptible check maintained its days to 50% flowering (56) days; whereas the tolerant check; IT 93k-503-1 had extended days from 62 to 64 days (Table 1.3). Also, the number of pods per plant for each inbred

line was significantly ($P < 0.001$) different for each treatment (Table 1.2). The interactive effects on genotype and location were significant ($p < 0.01$), except for seeds per pod. Watering regime had significant ($P < 0.001$) effects on days to 50% flowering, seeds per pod, hundred seed weight, grain yield and harvest index; however, no significant differences were observed for pods per plant and biomass for all the environments. Also, the interactive effects of the inbred lines and the watering regimes were significant ($p < 0.001$) for days to 50% flowering, biomass and harvest index, for number of seeds per pod, but there were no significant differences for number of pods per plant, hundred seed weight and grain yield across locations and environments. The interactive effects of the genotype, location and watering regime were highly significant ($p < 0.001$) for days to 50% flowering, biomass and harvest index but no significant differences were observed among the inbred lines for pods per plant, seeds per pod, hundred seed weight and grain yield (Table 1.2).

Table: 1.2: Mean squares for combined analysis of variance for grain yield and its components of twenty-two (22) inbred lines and parental checks under water stress and non-stress conditions for the three environments

Source	df	DFF	PPP	SPP	HSW	Yield	Biomass	HI
G	23	276.49***	2.20**	5.56***	6.77***	8.94***	14.27***	9.57***
L	2	1395.07***	10.58**	47.01***	29.93***	60.16***	2.50 ^{ns}	51.4***
G x L	46	31.79***	1.59*	1.09 ^{ns}	2.19***	4.43***	2.62***	3.56***
WR	1	9.87**	3.62 ^{ns}	6.66*	17.51***	7.24**	0.00 ^{ns}	6.18*
G x WR	23	5.34***	1.04 ^{ns}	1.32*	1.18 ^{ns}	0.62 ^{ns}	3.11***	3.09***
Lx WR	2	10.70***	8.58**	2.89 ^{ns}	13.68***	7.86**	11.35***	2.97 ^{ns}
GxLxWR	46	8.97***	1.26 ^{ns}	1.0 ^{ns}	0.83 ^{ns}	1.13 ^{ns}	3.97***	1.95**

df = degree of freedom; DFF = days to 50%; flowering, ppp = pods per plant; SPP = seeds per pod; HSW = hundred seed weight; HI = harvest index, G= genotype, WR= watering regime, L= Location, * $p < 0.05$; $p < 0.01$; $p < 0.001$; ns= not significant

Performance of inbred lines for days to 50% flowering and pods per plant under water stress non-stress conditions for 2016 and 2017

A comparison of the performance of inbred lines and the parental checks under well-water and water stress conditions indicate visibly sharp differences for drought under field conditions for all the traits studied (Tables 1.3, 1.4, and 1.5). Some inbred lines maintained their days to 50% flowering under moisture stress and non-stress conditions; inbred lines with numbers F116, F186, F189, F223, F353, F55, and F38 were able to maintain their days to first flowering and 50% flowering without change. Others had very distinct differences of about 1 - 4 days by either reduction or an increase in days to flowering and 50% flowering. Those inbred lines that were found within the range of 1 and 4 days were inbred lines with numbers F189, F230, F325, F396, F398, F406, F408, F57, F78, F255, F28, F75, F20 and F84 respectively. The parental

check IT97K-279-3 which is a susceptible check maintained its days to 50% flowering (56) days; whereas the tolerant check; IT93K-503-1 had extended days from 62 to 64 days (Table 1.3). The pods per plant were also generally reduced under water stress conditions. The highest pod count was recorded for inbred line F186 of 17 pods under well water conditions, which eventually reduced to 14 pods under stress conditions. Inbred line F142 had the lowest pod count in the water stress category. Again, water-stressed inbred line F186 still had the highest pod count of 14 pods whilst F396 had the lowest of 10 pods per plant under water stress conditions. The parental checks under well water conditions for IT93K-503-1 and IT 97K-279-3 were 13 pods for each, but under water stress, IT93K-503-1 had a pod count of 14 and IT97K-279-3 had 11. Some inbred lines however, maintained their pod count under both conditions; notable among them is inbred line 223 (Table 1.3).

Table 1.3: Comparison of trait means for inbred lines under stress and non-stress conditions for 2016 and 2017 for days to 50% flowering and number of pods per plant

Genotype	DFF				PPP			
	NS		WS		NS		WS	
	Mean	SE	mean	SE	mean	SE	mean	SE
F 116	47.92	0.42	47.74	0.47	12.21	1.25	11.95	1.42
F 131	60.42	0.42	59.46	0.47	13.46	1.25	11.51	1.42
F 142	47.33	0.42	46.41	0.47	10.27	1.25	11.4	1.42
F 186	58.42	0.42	58.02	0.47	17.35	1.25	14.4	1.42
F 189	52.25	0.44	51.58	0.44	11.48	1.31	12.46	1.31
F 20	55.7	0.44	59.03	0.44	11.92	1.31	10.23	1.31
F 223	55.7	0.44	55.81	0.44	11.59	1.31	11.46	1.31
F 230	57.14	0.44	56.25	0.44	14.25	1.31	12.57	1.31
F 255	53.14	0.44	51.81	0.44	15.25	1.31	11.9	1.31
F 28	51.14	0.44	49.03	0.44	12.37	1.31	11.12	1.31
F 325	48.7	0.44	46.58	0.44	13.7	1.31	10.9	1.31
F 353	53.14	0.44	53.47	0.44	14.03	1.31	13.68	1.31
F 38	46.14	0.44	46.03	0.44	11.03	1.31	12.9	1.31
F 396	46.25	0.44	45.58	0.44	11.14	1.31	10.23	1.31
F 398	57.36	0.44	56.69	0.44	16.59	1.31	12.35	1.31
F 406	57.14	0.44	56.14	0.44	12.92	1.31	11.46	1.31
F 408	53.92	0.44	52.81	0.44	13.03	1.31	12.23	1.31
F 55	60.92	0.44	60.03	0.44	16.03	1.31	12.46	1.31
F 57	47.14	0.44	46.81	0.44	13.92	1.31	11.68	1.31
F 75	52.03	0.44	49.25	0.44	15.92	1.31	12.01	1.31
F 78	49.58	0.44	48.81	0.44	12.59	1.31	11.79	1.31
F 84	57.36	0.44	54.47	0.44	16.25	1.31	11.57	1.31

IT97K-279-3*	56.03	0.44	57.36	0.44	12.92	1.31	11.01	1.31
IT93K-503-1*	62.36	0.44	64.58	0.44	13.03	1.31	13.01	1.31

DFP = days to 50% flowering; PPP = pods per plant; NS = non-stress; WS = water stress.
SE = standard error.

Mean performance of inbred lines for seeds per pod and hundred seed weight under water stress and non-stress conditions for 2016 and 2017

Number of seeds per pod also generally reduced under water stress conditions (Table 1.4). Inbred line F408 had the highest mean seed of 13 per pod and inbred line 142 had 8 seeds per pod. The parental checks IT93K-503-1 and IT97K-2793- had 13 and 9 seeds per pod respectively. Under water stress conditions, inbred lines F255, F353, F131, F406 among others had the highest mean seeds per pod (12 seeds) respectively whereas inbred line F142 had the lowest seed count of 8. The highest

mean weight for one hundred seeds under well-watered conditions was obtained in inbred line F396 with a mean weight of 22.08g; whereas inbred line F186 had a mean weight of 17.45g. The parental checks IT93K-503-1 and IT97K-279-3 had seed weights of 22.09g and 18.35g respectively. Most inbred lines, however, performed better under moisture stress condition; inbred lines F396 and F38 among others had the highest seed weight of 21.94g and 21.71g respectively. Whereas inbred line F186 had the lowest seed weight of 17.77g. The parental checks IT93K-503-1 and IT97K-279-3 had seed weights of 21.45g, and 18.61g, respectively (Table 1.4).

Table 1.4: Comparison of Trait means for inbred lines under stress and non-stress conditions for 2016 and 2017 for seeds per pod and hundred seed weight

Genotype	SPP				HSW			
	NS		WS		NS		WS	
	Mean	SE	mean	SE	Mean	SE	mean	SE
F 116	11.08	0.48	10.78	0.55	18.88	0.54	18.95	0.61
F 131	11.58	0.48	11.94	0.55	19.70	0.54	19.51	0.61
F 142	9.61	0.48	8.78	0.55	20.88	0.54	21.6	0.61
F 186	11.75	0.48	11.22	0.55	17.45	0.54	17.77	0.61
F 189	11.22	0.51	11.11	0.51	18.16	0.56	19.78	0.56
F 20	11.00	0.51	10.78	0.51	19.9	0.56	20.32	0.56
F 223	12.56	0.51	10.11	0.51	18.37	0.56	19.88	0.56
F 230	10.44	0.51	10.78	0.51	19.93	0.56	20.34	0.56
F 255	11.67	0.51	12.33	0.51	18.45	0.56	19.36	0.56
F 28	11.22	0.51	10.11	0.51	19.45	0.56	18.35	0.56
F 325	10.67	0.51	10.33	0.51	19.7	0.56	19.26	0.56
F 353	11.00	0.51	12.22	0.51	18.97	0.56	20.11	0.56
F 38	10.56	0.51	10.33	0.51	19.09	0.56	21.71	0.56
F 396	10.67	0.51	9.56	0.51	22.08	0.56	21.94	0.56
F 398	12.44	0.51	11.22	0.51	18.65	0.56	19.05	0.56
F 406	10.78	0.51	11.78	0.51	18.58	0.56	20.37	0.56
F 408	12.89	0.51	11.00	0.51	17.96	0.56	18.63	0.56
F 55	11.44	0.51	11.22	0.51	17.89	0.56	19.58	0.56
F 57	12.22	0.51	11.67	0.51	19.27	0.56	20.03	0.56
F 75	11.44	0.51	10.78	0.51	18.93	0.56	20.3	0.56
F 78	11.78	0.51	11.56	0.51	19.38	0.56	20.56	0.56
F 84	11.33	0.51	11.33	0.51	19.03	0.56	20.03	0.56
IT97K-279-3*	9.11	0.51	8.89	0.51	22.09	0.56	21.45	0.56
IT93K-503-1*	12.78	0.51	12.33	0.51	18.35	0.56	18.61	0.56

SPP= seeds per pod, HSW= hundred seed weight, NS= non-stress, WS= water stress, SE=standard error.

Mean Performance of inbred lines for grain yield biomass and harvest index under water stress non-stress conditions for 2016 and 2017

Most of the inbred lines performed better under normal irrigated conditions compared to moisture stressed conditions; inbred lines F255, F353, F186, had mean grain yields of 1.83, 1.74, and 1.67 t/ha (t/ha). The lowest mean grain yields were obtained in inbred line F28 with grain yield of 0.89t/ha. The parental checks IT93K-503-1and IT97K-279-3 had mean grain yields of 1.07 and 1.60 t/ha respectively. The highest mean grain yield under water stress conditions was obtained in inbred lines F186 and F255 with mean yields of 1.48 t/ha, whereas the lowest mean yield was obtained for inbred line 38 with grain yield of 0.68 t/ha (Table 1.5). However, the parental checks IT93K-503-1and IT97K-279-3 had mean grain yields of 1.47 and 0.70 t/ha respectively. For biomass yields, inbred line F223 had the

highest mean biomass of 5.23t/ha whereas inbred line F142 had the lowest with biomass yields of 1.51t/ha, under well-watered conditions. The parental biomass (IT93K-503-1 and IT97K-279-3) were 5.31 and 5.25 respectively. Inbred line F230 had the highest mean biomass yields of 4.4 under water stress conditions whereas inbred line F142 again recorded the lowest biomass. The highest mean was obtained in inbred line F255 with an index of 40.32 under normal conditions whereas inbred line F223 had the lowest index of 13.50. The parental checks (IT93K-503-1 and IT97K-279-3) had harvest indices of 35.39% and 22.01% respectively (Table 1.5). However, under moisture stress conditions, inbred line F116 and F186 had the highest harvest index of 32.81 and 31.56 whereas the lowest was obtained for inbred line F223 with an index of 17.63. The parental checks under moisture stress had 21.94 and 11.80 for IT93K-503-1 and IT97K-279-3 respectively (Table 1.5).

Table 1.5: Comparison of genotypic trait means for inbred lines under stress and non-stress conditions for 2016 and 2017 for grain yield, biomass and harvest index

Genotype	GY				Biomass				HI			
	NS		WS		NS		WS		NS		WS	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	mean	SE
F116	0.95	0.13	0.86	0.15	2.74	0.34	2.38	0.39	27.10	2.28	32.81	2.58
F131	1.32	0.13	1.12	0.15	3.64	0.34	3.22	0.39	27.92	2.28	24.71	2.58
F142	0.98	0.13	0.72	0.15	1.51	0.34	1.90	0.39	39.03	2.28	30.59	2.58
F186	1.67	0.13	1.48	0.15	4.02	0.34	3.31	0.39	27.70	2.28	31.56	2.58
F189	1.08	0.13	1.17	0.13	4.07	0.36	4.40	0.36	21.97	2.38	21.24	2.39
F20	1.31	0.13	0.83	0.13	4.31	0.36	3.16	0.36	24.60	2.38	21.26	2.39
F223	0.91	0.13	0.95	0.13	5.24	0.36	4.29	0.36	13.51	2.38	17.63	2.39
F230	1.39	0.13	1.30	0.13	3.64	0.36	4.41	0.36	27.74	2.38	22.99	2.39
F255	1.83	0.13	1.48	0.13	2.68	0.36	3.36	0.36	40.33	2.38	29.34	2.39
F28	0.89	0.13	0.77	0.13	1.83	0.36	2.22	0.36	36.68	2.38	28.03	2.39
F325	1.04	0.13	0.79	0.13	2.42	0.36	2.72	0.36	29.60	2.38	27.08	2.39
F353	1.74	0.13	1.41	0.13	4.01	0.36	3.72	0.36	29.68	2.38	26.37	2.39
F38	0.91	0.13	0.69	0.13	1.66	0.36	1.92	0.36	37.25	2.38	26.92	2.39
F396	0.97	0.13	0.77	0.13	1.93	0.36	1.97	0.36	35.48	2.38	29.39	2.39
F398	1.37	0.13	1.22	0.13	3.45	0.36	3.02	0.36	28.70	2.38	28.54	2.39
F406	1.31	0.13	1.21	0.13	3.54	0.36	2.92	0.36	26.32	2.38	30.51	2.39
F408	1.33	0.13	1.06	0.13	2.76	0.36	3.13	0.36	33.57	2.38	26.81	2.39
F55	1.38	0.13	1.13	0.13	4.12	0.36	3.41	0.36	24.79	2.38	26.79	2.39
F57	1.58	0.13	1.30	0.13	3.71	0.36	4.04	0.36	30.03	2.38	27.39	2.39
F75	1.52	0.13	1.31	0.13	4.48	0.36	3.32	0.36	24.81	2.38	28.31	2.39
F78	1.37	0.13	1.20	0.13	3.52	0.36	3.54	0.36	28.16	2.38	25.67	2.39

F84	1.10	0.13	1.23	0.13	4.60	0.36	3.95	0.36	20.19	2.38	23.58	2.39
IT97K-279-3*	1.07	0.13	0.71	0.13	3.89	0.36	5.25	0.36	22.02	2.38	11.81	2.39
IT93K-503-1*	1.61	0.13	1.47	0.13	2.90	0.36	5.31	0.36	35.39	2.38	21.95	2.39

GY= grain yield, HI= harvest index, NS= non-stress, WS= water stress, SE=standard error

Mean grain yield, percentage yield reduction, and stress tolerance indices computed for all the six environments (Golinga 2016, Golinga 2017 and Libga 2017)

In order to assess the yield penalties associated with drought tolerance on field conditions, the percentage yield reduction, mean productivity, stress tolerance, stress susceptibility index, geometric mean productivity and the extent of stress (stress intensity), were computed for all the six environments (Table 1.6). This was followed by correlation analysis for the two moisture regimes (Table 1.7), using stress tolerance indices for all the six environments.

The percentage mean yield reduction ranged between 57.83% and -10.57% for inbred line 20 and 84, respectively. Parental checks: the drought tolerant line (IT93K-503-1) had a yield reduction of 9.52% while the second parent a drought susceptible check (IT97K-279-3) had a mean yield

reduction of 50.70%. The mean productivity also ranged between 1.58 t/ha and 0.80 t/ha for inbred lines 186 and 38, respectively while the checks were 1.54 t/ha for IT93K-503-1 and 0.89 t/ha for IT97K-279-3. Almost all the inbred lines had their mean yields reduced under stress conditions with various rates of magnitudes. Inbred line F84 rather had mean yield of 1.10 t/ha under well-watered conditions and increase to 1.23 t/ha (Table 1.7) under water stress conditions. The tolerance index ranged between 0.48 for inbred line F20 and 10.98 for inbred line F84. The parental checks had tolerance indices of 0.36 and 0.14 for IT97K-279-3, and IT93K-503-1, respectively. The stress intensity was 0.98. The stress susceptibility index ranged between -0.49 and 0.98. The geometric mean productivity also ranged between 1.65 and 0.79 for inbred lines F255 and F38, respectively. The stress tolerance index also ranged between 2.02 and 0.59. Correlation analysis for the stress tolerance indices revealed significant association for all the estimated indices (Table 1.7).

Table 1.6: Means yields, percentage reduction and tolerance Indices for contrasting moisture conditions for 2016 and 2017 irrigation experiments for six environments

Genotype	Yw	Ys	Reduction (%)	MP	TOL	SSI	GMP	STI
F116	0.95	0.86	10.47	0.91	0.09	-0.77	0.90	0.69
F131	1.32	1.12	17.86	1.22	0.20	-0.71	1.22	1.24
F142	0.98	0.72	36.11	0.85	0.26	-0.59	0.84	0.59
F186	1.67	1.48	12.84	1.58	0.19	-0.75	1.57	2.08
F189	1.08	1.17	-7.69	1.13	-0.09	-0.94	1.12	1.06
F20	1.31	0.83	57.83	1.07	0.48	-0.49	1.04	0.91
F223	0.91	0.95	-4.21	0.93	-0.04	-0.90	0.93	0.73
F230	1.39	1.30	6.92	1.35	0.09	-0.80	1.34	1.52
F255	1.83	1.48	23.65	1.66	0.35	-0.67	1.65	2.28
F28	0.89	0.77	15.58	0.83	0.12	-0.73	0.83	0.58
F325	1.04	0.79	31.65	0.92	0.25	-0.62	0.91	0.69
F353	1.70	1.41	20.57	1.56	0.29	-0.69	1.55	2.02
F38	0.91	0.69	31.88	0.80	0.22	-0.62	0.79	0.53
F396	0.97	0.77	25.97	0.87	0.20	-0.65	0.86	0.63
F398	1.37	1.22	12.30	1.30	0.15	-0.75	1.29	1.41
F406	1.31	1.21	8.26	1.26	0.10	-0.78	1.26	1.33
F408	1.33	1.06	25.47	1.20	0.27	-0.66	1.19	1.19
F55	1.38	1.13	22.12	1.26	0.25	-0.68	1.25	1.31
F57	1.58	1.30	21.54	1.44	0.28	-0.68	1.43	1.73

F75	1.52	1.31	16.03	1.42	0.21	-0.72	1.41	1.68
F78	1.37	1.20	14.17	1.29	0.17	-0.74	1.28	1.38
F84	1.10	1.23	-10.57	1.17	-0.13	-0.98	1.16	1.14
Mean	1.03	1.05						
SI	0.98							
IT97K-279-3	1.07	0.71	50.70	0.89	0.36	-0.52	0.87	0.64
IT93K-503-1	1.61	1.47	9.52	1.54	0.14	-0.77	1.54	1.99

Yw = yield under well water, Ys = yield under water stress, Tol = tolerance index, Mp = mean productivity, SSI = stress susceptibility index, GMP = geometric mean productivity, STI = stress intensity, SI= Stress Intensity

Correlation analysis for the stress tolerance indices revealed significant association for all the estimated indices (Table 1.7), negatively correlated associations is an indication of how difficult to select

indirectly for related traits in a drought study and positively related indices is an indication for the possibility of selecting useful traits under drought conditions in cowpea breeding for crop improvement

Table 1.7: Correlation analysis for stress tolerance indices and yield for six environments.

	Yw	Ys	MP	TOL	SSI	GMP	STI
Yw	1						
Ys	0.87*	1					
MP	0.97*	0.97*	1				
TOL	0.35	-0.15	0.10	1			
SSI	0.05	-0.44*	-0.11	0.94*	1		
GMP	0.97*	0.97*	0.99*	0.09	-0.20	1	
STI	0.97*	0.96*	0.99*	0.12	-0.18	0.99*	1

P < 0.001)

Ys= yield under stress, Yield under well-watered, Mp = mean productivity, TOL= Tolerance index, SSI= stress susceptibility index, GMP= geometric mean productivity, STI= stress intensity.

GGE biplot and Stability analysis of twenty-two Cowpea inbred lines with their parental checks across the six environments.

Table 1.8 shows the AMMI analysis of variance for all traits across the six environments. Significant (P < 0.001)

differences were observed for all the traits measured. Across all the locations, genotypes (G), environment (E) and their (I) interactions (GEI), were highly significant, indicating the presence of variability for drought tolerance among the genotypes.

Table 1.8: Analysis of variance for yield across six environments Using AMMI

Source	df	SS	MS	F
Total	431	608.3	1.411	
Treatments	143	491.6	3.438***	8.98
Genotypes	23	74.1	3.221***	8.42
Environments	5	289.3	57.863***	62.39
Block	12	11.1	0.927***	2.42
Interactions	115	128.2	1.115***	2.91
IPCA	27	72.3	2.678***	7
IPCA	25	33.6	1.345***	3.51
Residuals	63	22.2	0.353	0.92
Error	276	105.6	0.383	

df=degree of freedom, SS=sum of squares, MS= mean squares, F=probability level

The GGE biplot of the grain yield of twenty-two inbred lines and the two parental checks across the six environments revealed that Axis 1 explained 61.98% of the total variation whereas Axis 2 explained 22.16% (Fig. 1.0). The two axes together explained in total 84.14% of the variation in

the grain yield performance of the inbred lines and the parental checks across the six environments. Across the six environments, Inbred lines F75, F353, F255, F406, F398, F186, and the parental check IT93K-503-1 were the best performing and stables lines (Fig. 1.0).

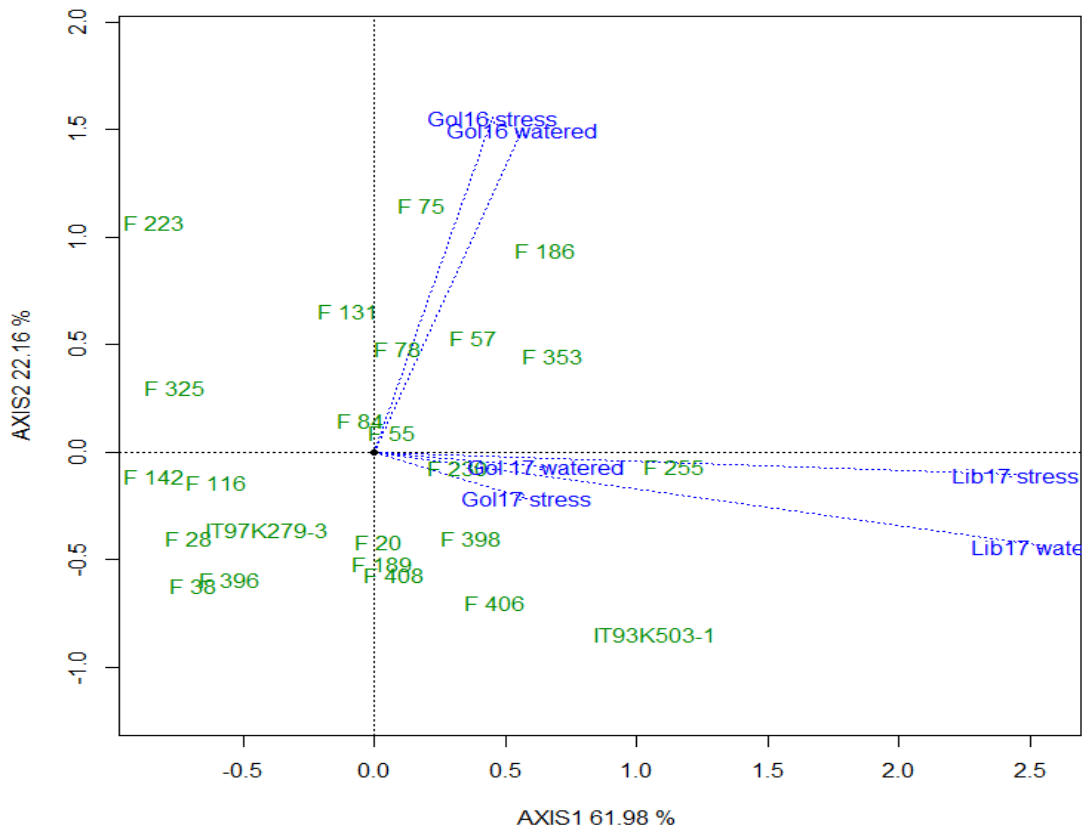


Fig. 1.0: GGE biplot yield for all six environments.

The GGE biplot-based on polygon view of the genotypes across the six environments explained 84.14% of the total variation for grain yield, with the PCI (X-axis) accounting for 61.98% and PC2 accounting for 22.16% of the variance, (Fig. 1.1). Across the six environments, the best mean grain yield performing inbred lines 75, 186, 255, 398, 142, 325, IT93K-503-1, IT97K-279-3, and 223 were located at the corners of the polygon. Inbred lines located at the origin of the polygon, have the same mean yield performance across all the six environments. For the three stress environments, inbred lines 398, 28, 38, 142,

186, and 255 (Fig 1.1) and 503 were the best in terms of yield, while inbred lines 131, 255, 223, 116, 142, and 38 were the best performing lines for three non-stress environments (Fig 5.8).

Across the six environments, the best mean grain yield performing inbred lines F75, F186, F255, F398, F142, F325, IT93K-503-1, IT97K-279-3, and F223 were located at the corners of the polygon (Fig 1.1). Inbred lines located at the origin of the polygon, have the same mean yield performance across all the six environments.

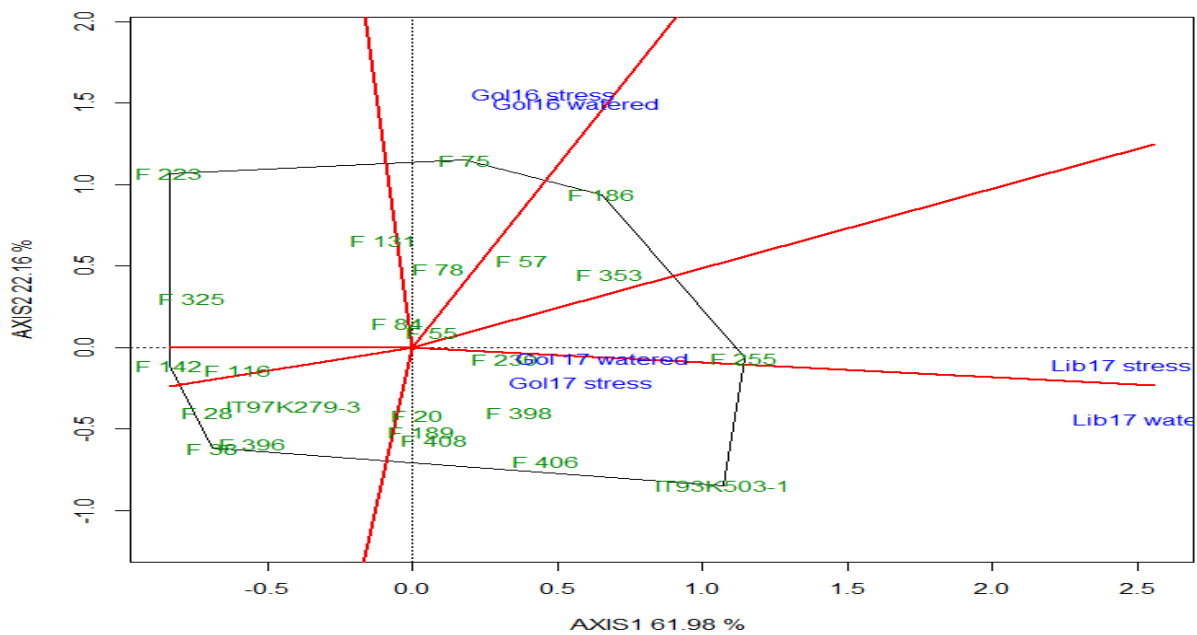


Fig. 1.1: GGE biplot of the “which- worn-where/what” view of genotypes based on genotypes by environment interaction on yield of twenty-two cowpea inbred lines and their two parental checks across six environments for three locations in the Guinea Savanna ecologies

The descriptiveness and representative view of the GGE biplot based on genotype by environment interaction on yield across six environments, showed a total variation of 84.14 for all the six environments (Fig.

1.2). The most stable inbred lines are the inbred with the longest vectors and they were inbred lines F131, F75, F353, F84, F406, and the parental check IT93K-503-1 (Fig. 1.2).

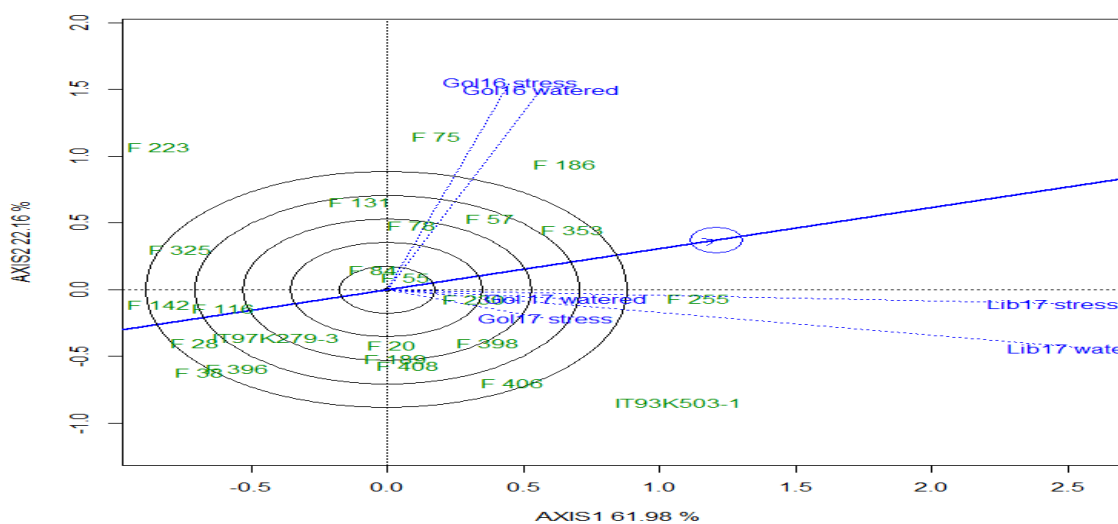
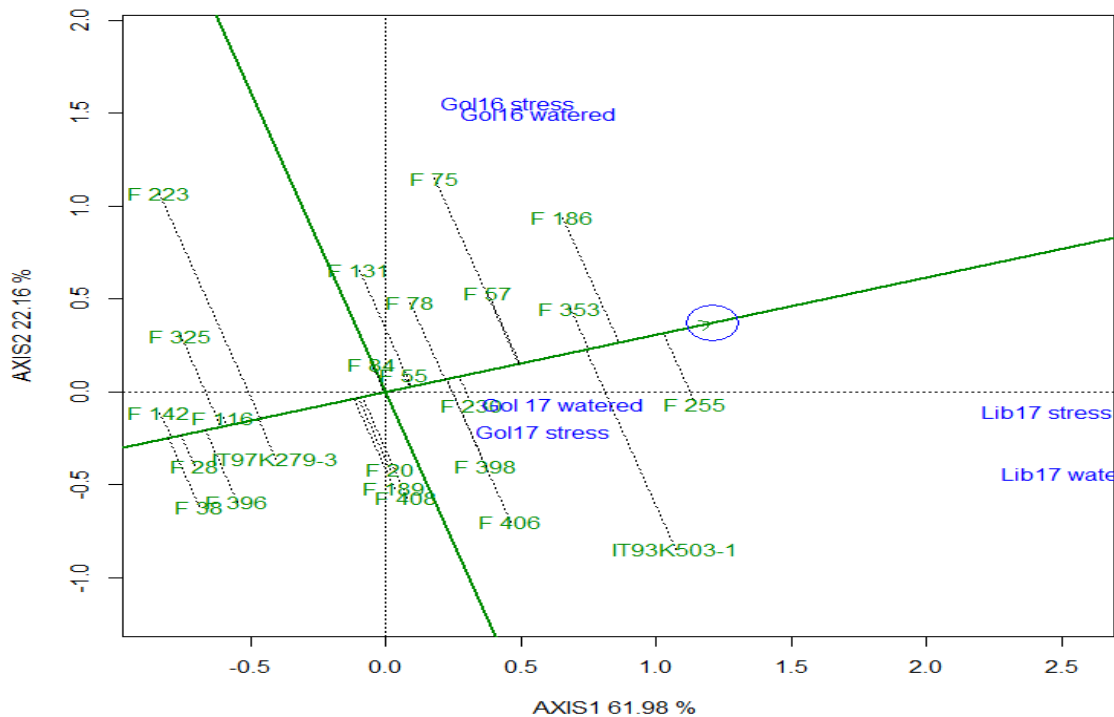


Fig. 1.2: GGE biplot view of genotypes by environment discriminativeness versus representativeness of genotypes by environment interactions across six environments in the 2016 and 2017 drought evaluations at Golinga and Libga respectively

The mean stability across the six environments showed a total variation of 84.14% (Fig. 1.3),

The highest mean yielding inbred lines across the six environments (Fig 5.11) in the direction pointed by the arrow were F255,

F186, F353, F57, F75, F230, F398, F406, F78, F131, F55, F84, F20, and the parental check IT93K-503-1. environments were F255, F353, F186, F75, F230, F57, F398, F84, F406 and the parental check was IT93K-503-1.



1.3: GGE biplot -based view of means versus stability of the genotype by environment interaction on yield of twenty-two inbred lines of cowpea and their parental checks across the six environments.

DISCUSSION

In the current study, water stress resulted in yield reduction (Tables 1.6 and 1.7) for both the inbred lines and the parental checks. Similar study by Shanko *et al.* (2014) also reported that, grain yield and its components were significantly reduced under water stress conditions.

The strong association between grain yield, harvest index and biomass were reported by Kamai *et al.* (2014) who in their study on the phenotypic basis for yield differences among cowpea cultivars in semi-arid regions of Nigeria concluded that water

stress resulted in reduction of yield and its components. In the current study, the average mean performance for yield under well-watered conditions, was generally better than yields under drought. In a related study, Batiemo *et al.* (2016b) and Padi (2004) reported that, pod and grain yield of cowpea genotypes were reduced under stress conditions compared to yield under well-watered conditions.

A comparison in terms of rank of mean genotype performance or potentially tolerant and susceptible inbred lines based on quantitative indices estimates indicated that inbred lines that combined lower

tolerance index and susceptibility index, proposed by Rosielle and Hamblin (1981), and high mean productivity proposed by Fernandez (1992c) and stress tolerance were said to be potentially drought tolerant; whereas the susceptible inbred lines were those that combined higher tolerance index, higher susceptibility index, with lower mean productivity index were said to be potentially susceptible inbred lines.

The selection index as proposed by Batierno *et al.* (2016c) was 38% with an interval of 25% to 50%. On basis of this estimate, inbred lines F255, F186, F353, F84, F406 and F131 ranked the best. However, inbred line F84 performed best with a percentage yield gain under drought stress conditions. However, the potentially susceptible ones were the following inbred lines: with numbers F142, F396, F38, and F28 among others. The parental checks however, maintained their inherent yield potential for both contrasting moisture conditions. This was earlier reported by Dadson *et al.* (2005) who stated that water stress affects the seed yield of cowpea. The ranking of genotypes or inbred lines based on either GMP, MP and stress susceptibility indices, took similar patterns, this has been reported by (Ramirez-Vallejo & Kelly, 1998; Saba, Moghadam, Ghassemi, & Nishabouri, 2010).

Correlation analysis performed for the tolerance indices revealed that yield under well-watered conditions correlated positively with yield under stress conditions ($r = 0.87$), mean productivity ($r = 0.96$), and finally geometric mean productivity and stress tolerance index with ($r = 0.96$). However, yield under stress conditions correlated positively with mean productivity ($r = 0.87$), geometric mean productivity ($r = 0.96$) but negatively with tolerance index and stress susceptibility index. This corroborates with drought tolerance studies by Anwar *et al.* (2011) and Batierno (2014b).

The GGE biplot analysis of the genotypes and environment interaction reflects the response of the inbred lines in six environments for this study. In the current study the highest yielding across all the six environments were inbred line F75, F186, F353, F57, F255, F131, and IT93K-503-1 among others. Under the three stress environments, inbred lines F131, F75, F353, F255, and the parental drought tolerant check; IT93K-503-1 were the best performing inbred lines. Those that performed well under non-stress conditions were F396, F28, F189, F116, F38, F325, F131, F84, and IT97K-279-3. This corroborates many related studies by (Khan *et al.*, 2016; Khan and Kabir, 2014; Khan and Iqbal, 2011; Padi, 2007; Padi, 2004), who reported that genotype by environment interactions in cowpea and in wheat revealed significant variability in terms of performance and stability among the genotypes. Ahmed *et al.* (1993) also reported that genotypes showed wide adaptations and stability over a wide range of environments, while others exhibited specific adaptation to specific environments (Sabaghnia, *et al.*, 2012b).

The GGE-biplot polygon view as described by Yan (2001), of the mean yield performance of inbred lines for the six environments, for stress and non-stress environments showed distinct variation in terms of their response to the various environments. The “vertex cultivars” which were those located at the corners of the polygon include inbred lines F186, F75, F255, IT93K-503-1, F223, F142, and F396 for all the six environments. The convex cultivars (Yan and Tinker, 2006) are those close to the origin (F398, F55, F255, F78, F84, and F230), would have almost the same mean yields across all the environments. The ideal genotype is usually a projection on the average tester coordinate (X-axis) is designed to be the longest vector of all the genotypes (Mohammadi, *et al.*, 2010; Nwangburuka and Denton, 2011; Padi, 2007). It is the highest performing inbred

line, because its projection on the average tester coordinate on the Y-axis is zero. In this case, the best fit is inbred line F255 under the six environments. The most stable (Becker and Leon, 1988; Goyal *et al.*, 2011; Lin *et al.*, 1986; Purchase, 1997) inbred line was F255. Consequently, the combination of quantitative, physiological as well as stability analysis of genotypic reaction to contrasting moisture regimes has been helpful in this study.

CONCLUSION AND RECOMMENDATION

The inbred lines generated for the study reacted differently to water stress, indicating the existence of genetic variability for drought tolerance among the tested inbred lines; for instance, inbred lines

F142, F408, F398, F38, had their yields reduced under stress conditions whilst inbred lines F84, F131, F186, F406 and F255 were less affected. The use of two contrasting parents; drought tolerant and drought susceptible advance breeding lines in generating the inbred lines for the study revealed classes of maturity groups for drought tolerance. This resulted in identifying the inbred lines based on the performance of the parental lines and thus enabling the test of superiority for the inbred lines in relation to the parental performance across the environments used for the study. Further study of the inbred lines using quantitative trait loci analysis will be required to enable the identification of loci responsible for drought and its relationship to maturity period.

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