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Assessment of cowpea (*Vigna unguiculata* (L.) Walp) F_1 lines response to drought tolerance

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ABSTRACT

The objective of this study was to develop cowpea lines and assess them for drought tolerance using the pot screening approach. F1 lines were developed between September and November 2019 at University for Development Studies' plant house. The drought experiment was conducted between February to March 2020. The F1 lines were developed by an artificial hybridization between the genotype ITK93-503-1 used as a male parent, obtained from the International Institute of Tropical Agriculture. This was crossed to 4 local genotypes used as female parents. The populations raised from the crosses were used to conduct the drought experiment. The experiment was Complete Randomized Design in; *a 2 * 5 factorial experiment with 10 experimental units replicated three times, giving a total of 30 experimental units. The factors were; AbF1, GoF1, MF1, SaF1, ITK93-503-1 by Stress and Non-stress conditions. Plants were subjected to water stress for a period of 25 days and various drought related parameters were measured and analyzed. Results showed that water stress significantly (P<0.05) affected the chlorophyll content, leaf area and stem lodging for all F1 lines, however, water stress did not significantly (P>0.05) affect relative leaf water content of the F1 lines. The study showed that F1 lines (AbF1 and GoF1) performed better in chlorophyll content, leaf area, seedling recovery rate and plant main stem lodging. SaF1 recorded the highest relative leaf water content. Identified lines with drought tolerance potential should be further evaluated in subsequent generations to confirm their tolerance to drought for further selection and improvement.*

Keywords: *Chlorophyll content; Cowpea; Hybridization; Relative water content; Seedling recovery rate*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is cultivated widely across the world because of its social, economic, and nutritional importance. It is a basic source of protein to significant number of the population in sub-Saharan Africa (Muchero et al., 2008). The fresh leaves and green pods are consumed by people in Africa, Asia and the Caribbean, as delicious food source (Cui et al., 2019)., The leaves and stems are also used as high value hay to feed livestock in Africa (Timko & Singh, 2008). Cowpea cultivation is of high importance to sustainable farming systems in

Ghana because of its ability to fix nitrogen amounting to 240 kg/ha into the soil and 60 to 70 kg is left for the crop to be planted in the next cropping season on the same field (Quaye et al., 2009). It is an important component of the cropping system in most of the drought prone zones in Africa where it serves as source of income to farmers and seed traders (Fatokun et al., 2002). Cowpea is one of the older grain legume crops cultivated in the partially drier areas of West Africa where rainfall is characteristically low (mean annual range of 300-600 mm), which varies in time and places and also unreliable (Alidu et al., 2013). Singh, (1999) reported that cowpea yield in the semi-arid areas is always low due to drought as the primary abiotic constraint. Drought is a condition caused by unavailability or insufficient rainfall, which occurs whenever the distribution, amount or duration of precipitation deviates from the normal (Acquaah, 2007). In the northern savanna zones in Africa, cowpea improved cultivars with grain yield potential of 3 t/ha have been released for cultivation, low grain yields ranging between 0.3 to 0.65 t/ha are recorded at the farm level, which is far below the potential yield of 3 to/ha (Alidu et al., 2013). Drought is increasingly becoming the most important abiotic stress affecting cowpea production in Ghana (Padi, 2004) due to high variability in amount and distribution of rainfall which is common during the cropping season and can result to yield loss of about (15-100%), depending on the level of susceptibility (Horn et al., 2015). Both intermittent and terminal droughts occur in Guinea Agro ecologies but terminal drought is the most important because it impacts directly on grain yield (MoFA, 2016). Several studies have showed that cowpea cultivars which recover from seedling-stage drought has a big chance to thrive through water stress environment in their whole life cycle with appreciable level of yield (Muchero et al., 2013). Farmers over the years have resorted to using their local germplasm, some of which are very promising, and the traits can be improved on through breeding and selection. Therefore, the study sought to identify and develop drought tolerant cowpea lines by crossing a reputable drought tolerant advanced breeding line with local lines that have been selected from already existing famer germplasm in this ecology for drought tolerance assessment at the seedling stage.

MATERIALS AND METHODS

Study area

The experiment was conducted in the horticulture garden of the University for Development Studies (UDS) plant house, Nyankpala Campus. The place is located in the Guinea Savanna Agro-ecological zone, at longitude 1.00’ W, latitude 090 25’ N at altitude 183 m (Kombiok, 2013). The area has a unimodal rainfall with an average annual rainfall of about 1200 mm and distributed fairly from May to October. The average daily temperature ranges from a minimum of 26 ^{0}c to a maximum of 39 ^{0}c with a mean temperature of 32 ^{0}c Kombiok *et al*., 2000). Relative humidity is at its maximum during the rainy season with a monthly value of 80% which declines to 54% during the dry season as minimum monthly value (Imoro et al., 2012).

Materials use for development of F1 lines

Five cowpea genotypes with differences in seed and plant characteristics were used for the study. The genotypes used were; IT93K-503-1 is a drought tolerant variety obtained from the International Institute for Tropical Agriculture (IITA), Nigeria. Sanzi, Gorigori, ablaiyijagadow and Milo, were local germplasm obtained from farmers in the Guinea ecologies of Northern Ghana.

Methodology

The F1 lines were developed between September to November 2019. It was single cross between the genotype IT93K-503-1 (male parent) to each of the four local genotypes (Sanzi, Gorigori, ablaiyijagadow and Milo) as female parents under optimum moisture condition. The drought experiment was conducted between February to March 2020. The population raised from the crosses was used to conduct experiment under moisture stress and optimum moisture condition at the vegetative stage to evaluate their tolerance to drought. For both the crosses and the drought experiment, seeds were planted in plastic buckets. The buckets (pots) were perforated at the bottom to allow excess water, filled with top loam soil 10 kg per bucket and then watered and left for the soil to drain before planting.

Development of F1 Lines

Forty-five plastic pots of diameter 30 cm and height of 50 cm were used to plant the parental genotypes (IT93K-503-1, ablagbajadow, Gorigori, Milo and Sanzi). Three seeds were planted per pot and later thinned to two stands per pot at a spacing 50 cm by 20 cm. All the necessary agronomic practices were done to raise healthy seedlings for the crossing. At flowering, matured (non-opened) flowers from the male parent (IT93K-503-1) were collected in the morning (before 8:0 am) and stored in a refrigerator and crossing was carried out in the evening. Emasculation was done by using forceps to cut-opened the top of the female parent flower and all the pollen were removed leaving only the stigma. The male parent flower was bisected horizontally and the part containing the pollen was placed (cup) on the emasculated female flower after 4:0 pm. Each cross was tagged for easy identification and successful matured pods (F1) were harvested separately and dried**.**

A B C D

Plate 1: Development of F1 Seeds

A=Bisection of male flower, B=Female flower cup with pollen, C=Successful pod from cross and D=F1 seeds obtained from crosses.

Drought Experiment

In the drought experiment, IT93K-503-1 was planted as a standard check alongside the F1 inbred lines in pots in a Complete Randomized Design (CRD) with each pot containing five seedlings planted for the treatments. The plants were watered for the first two weeks until the emergence of the first trifoliate leaves. The plants were then subjected to water stress for the water stress treatments for a period of 25 days to evaluate drought tolerance and the control (non-stress) was irrigated with a volume of 0.5 l/10 kg of soil for every 48 hours. After the end of the drought period for the water stress treatment, watering was resumed throughout the experiment.

Design and Treatment

F1 lines were developed by crossing a parental line to 4 locally obtained germplasm. Several crosses were done to obtain enough F1 lines for the drought experiment. The design that was used for the drought study was Complete Randomized Design with three replications. The drought experiment was 2 * 5 factorial experiments with 10 experimental units replicated three times to give a total of thirty (30) experimental units. The factors were; Stress (S) and Non-stress (NS) by AbF1, GoF1, MF1, SaF1, ITK93-503-1

Table 1: Design Structure of the Crosses

Female parents (local lines)	Male (IT93K-503-1)
Ablagbajadow (Ab)	Ab x 503=(AbF1)
Gorigori (Go)	Go x 503=(GoF1)
Milo (M)	M x 503=(MF1)
Sanzi (Sa)	Sa x 503=(SaF1)

Table 2: Treatment structure of Drought Experiment

Cowpea Inbred lines	Stress Condition (S)	Non-Stress Condition (NS)
IT93K-503-1	503+S	503+NS
AbF1	AbF1+S	AbF1+NS
GoF1	GoF1+S	GoF1+NS
MF1	MF1+S	MF1+NS
SaF1	SaF1+S	SaF1+NS

Data Collected

Percentage Seedlings Emergence

The number of seeds germinated from each treatment was counted a week after planting and the number expressed as percentage of the total number of seeds planted.

Leaf Chlorophyll Content

Leaf chlorophyll content was measured using the Minolta SPAD-502 plus Chlorophyll Meter (nmol/cm) (Tokyo, Japan). For each plant, three types of leaves: unifoliate, first trifoliate, and new (second) trifoliate were measured separately (three times in each leaf) for all the three leaves and the average of the measurements were kept as the final chlorophyll content value (Cui et al., 2019)**.** The chlorophyll content was measured at day 10, 15, 20 and 25 after the application of treatment.

Plate 2: Measuring chlorophyll content at 10 days after water-stress

Plant Leaf Area

A manual and non-destructive method was used to determine the leaf area at the end of the drought period (25 days after stress). Three leaflets from the tagged plants in each pot were used. The length [L (cm)] was taken along the midrib of the leaf from the point of attachment to the petiole to the tip of the leaf and the breadth [B (cm)] was taken by measuring the maximum width of the leaf (Wallace & Nelson, 1978). The leaf area [LA (cm^2)] was estimated and their means from the tagged plants was used to represent the leaf area.

Relative Water Content Measurements (RWC)

Relative water content (RWC) was calculated using new fully expanded leaflets, as stated by (Bogale et al., 2011) at day 15 and 20 after water-stress. The leaves were detached from the plants between the hours of 10 am and 2 pm during bright days, in order to avoid the effects of weather conditions on water loss from the detached leaves for RWC. As soon as after cutting at the base of the lamina, the leaves were weighed to obtain the fresh weight (FW). After weighing, the leaves were soaked in deionized water for 48 hours at room temperature for rehydration and then reweighed for turgid weight (TW). The leaves were then dried in an oven at 70 °C for 72 hours before dry weight (DW) measurements were taken. The RWC was calculated as follows: $\text{RWC}=\frac{FW-DW}{TW-DW} \times 100\%$ (M. S. Alidu et al., 2019).

Plant Main Stem Lodging Score

A scale of 1 to 3 was used to measure plant main stem lodging with 1=vigorous, that is main stem green, 2=mildly wilting and light green main stem and 3=completely lodged and yellow main stem. This was done on per plant basis on the 25^{th} day of water-stress. The average score of the plants from each water stressed treatment was recorded as the final main stem lodging score (Cui et al., 2019).

Seedling Recovery Rate

After the period of 25 days of drought, the plants were re-watered twice every five days with 0.5 l/10 kg of soil for a period of 10 days before taking recovery measurement. This was computed as; Recovery Rate (RR) = $\frac{Proportion\ of survived\ plants}{Total\ number\ of emerged\ plants} \times 100$ (Alidu et al., 2019).

Soil Moisture Content

Soil moisture meter (FIELDSCOUT), TDR 100, Spectrum Technologies, Inc.) was used to monitor the soil moisture content every five days, starting from 10 to 25 days after water stress. The moisture meter probe was inserted into the soil of each pot at a depth of 20 cm and the reading on the meter was recorded and the means were calculated as percent soil moisture content.

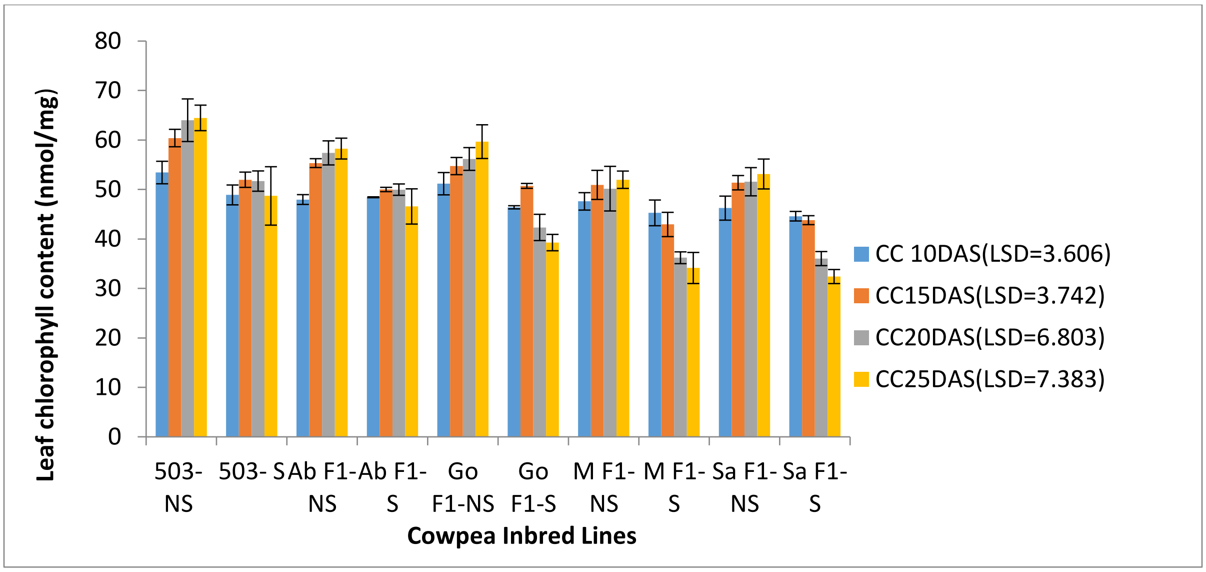
Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) using GenStat statistical package edition 12. . Means were separated using least significance difference (LSD) at 5% significance level.

Results and Discussion

Leaf chlorophyll content

The result of the leaf chlorophyll content (nmol/mg) is presented in figure 1. The chlorophyll content measured on the parental check and the F1 lines were significantly (P<0.05) different under both watering regimes. At 10 days of water stress, the chlorophyll content (nmol/mg) of the genotypes ranges from 48.70 to 44.60 with the parental check (IT93K-503-1) recording the highest value of 48.70 and the least chlorophyll content was recorded by SaF1-S (44.60). At 15 Days After Stress (DAS), the chlorophyll content in the water stress condition ranged from 51.97 to 42.93 with the parental check (IT93K-503-1) recording the highest value of 51.97 and SaF1-S obtained the least value of 42.93. Similar trends were recorded at the 20 and 25 DAS.



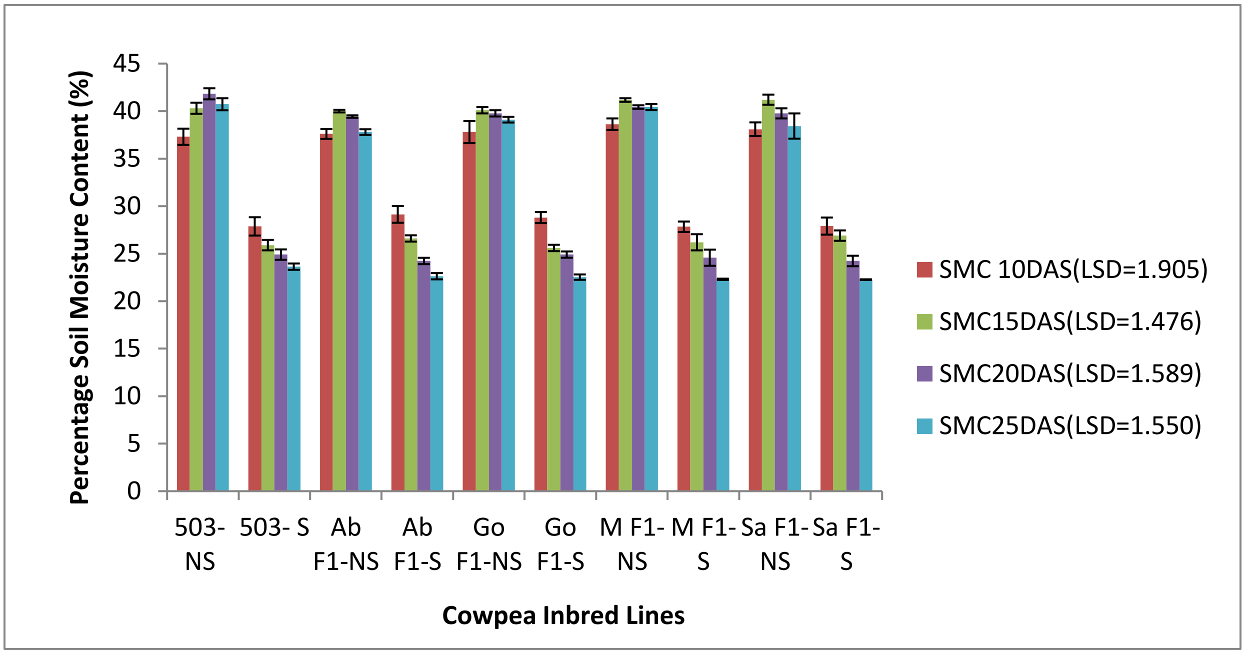
LSD=least significant difference; DAS= days after stress, CC= Chlorophyll Content

Figure 1: Leaf chlorophyll content under water stress and non-stress condition (Error bars represent Standard Error of Means). Interaction between cowpea inbred lines and watering regimes were not significant.

The four cowpea F1 lines and the parental check were subjected to drought for 25 days. In the water stress condition, the chlorophyll content of cowpea lines had significantly decreased as the drought period increases from 10 to 25 days after water stress. This corroborates Cui et al. (2018) who reported that, increasing drought period slows down cell division which affect photosynthetic ability, due to decrease in CO_2 in the plant leaves. Alidu et al. (2019) and Bastos et al. (2011) also reported similar decline in chlorophyll content among the cowpea lines as the period of drought increases. However, under the optimum water condition, the chlorophyll content of the parental check and the F1 lines increased as the plants grew older from 10, 15, 20 and 25 days due to moisture availability which enhances plant metabolic activities. This findings supports (Abed, 2014) who stated that Chlorophyll content is a trait that is responsible for keeping plant greenness for a photosynthetically active leaves which subsequently leads to delay in leaf senescence and therefore increases the product of photosynthesis from source to sink

Soil moisture content

Interaction between inbred lines and watering regimes were not significant, but the parental check and the F1 lines were significantly (P<0.05) influenced by soil moisture content in both watering regimes. The soil moisture content was similar among the genotypes in the water stress condition throughout the water stress period at a range of 22.27 to 29.13%. A similar trend was recorded among the parental line and the F1 lines in non-water stress condition at day 10 and 15, except 20 and 25 DAS, where differences in moisture content were recorded among genotypes. In the non-stress condition, IT93K-503-1-NS recorded the highest moisture content of (41.83%) and the least recorded by AbF1-NS (36.97%) (Figure 2).



LSD=least significant difference; DAS= days after stress; SMC= soil moisture content

Figure 2: Soil moisture content of the five (5) genotypes under water-stress and non-stress condition (Error bars represent Standard Error of Means)

Soil moisture content differences were recorded among the two different watering conditions but similarities were observed within the same condition among the parental line and the inbred lines. This agrees with (Bastos et al. (2011) who in a similar water-stress experiment on cowpea genotypes, reported soil moisture differences among two different irrigation regime and reduction in soil moisture content from the beginning of drought till the end of the drought period.

Relative leave water content and seedlings recovery rate

Water stress (S) and Non-Water stress (NS) conditions did not significantly (P>0.05) affect relative water content (RWC) of the F1 lines and the parental check. The trend of the response was similar at 15 and 20 Days After Water Stress (DAS) for both conditions. GoF1-NS recorded the highest RWC of 68.4% and 70.9% between the ranges of 68.4 to 60.15% and 70.9 to 65.519 at day 15 and 20, respectively. However, in the water stress condition, SaF1-S obtained the highest RWC of 61.28% and 59.48% at day 15 and 20 of stress, respectively (Table 3). The general decrease in relative water content (RWC) under the water-stress condition was observed among the parental check and the F1 lines at 15 and 20 DAS as the drought period progressed. However, RWC in the optimum water condition increased from 15 to 20 days. This result is in accordance with (Tuberosa, 2012) who reported that there was decrease in the leaf water content of potato cultivars imposed for three weeks drought as compared to the control. In this study there was a general high RWC recorded among the parental check and the F1 lines. Similar work done by (Abed, 2014) confirmed that high RWC was due to low transpiration rate and water absorption from the soil, which leads to increase ribulose1, 5-biphosphate (,ATP) synthetase activity in phosphorylation. While decreasing of RWC in plant was due to high transpiration rate from stomata and high absorption of water from soil.

However, the overall performance of the genotypes indicated that AbF1-S, GoF1-S and IT93K-503-1 had higher mean percentage recovery values above the average mean recovery value of 73.33% therefore, these three F1 lines are rated to be drought-tolerant lines. This result confirmed the result of previous studies by (Ravelombola et al., 2018) on a similar research on drought-tolerance in cowpea genotypes, reported that the percentage of dead plants per genotype varied from 0% to 100%, with an average of 54.26%. Seedlings recovery rate was not statistically significant (*P*>0.05) among the parental line and the F1 lines at 10 days after resumption of watering. The highest mean recovery rate was 93% for AbF1-S and the least was recorded by MF1-S (53%).

Table 3: Relative Leaf Water Content under Water Stress and Non-Stress Condition

	Relative Leaf Water Content (RWC) (%)			
	10 DAS		**15 DAS**	
	Watering Regime		**Watering Regime**	
Cowpea inbred lines	NS	S	NS	S
ITK93K503-1	67.04 ab	58.29 ab	69.49 ab	56.44 a
AbF1	62.35 ab	57.46 ab	68.83 ab	55.61 a
GoF1	68.4 b	58.09 ab	70.9 b	58.11 ab
MF1	60.15 ab	56.36 a	65.51 ab	57.69 ab
SaF1	66.95 ab	61.28 ab	69.09 ab	59.48 ab
LSD	**10.16**		**12.47**	

Means followed by the same letter(s) are not significantly (p>0.05) different. NS=Non-Stress, S=Stress, DAS=Days After Stress. Interaction between inbred lines and watering regimes were not significantly different.

Table 4: Seedlings Recovery Rate

Cowpea Inbred Lines	Seedling Recovery Rate (%)
MF1-S	53 a
SaF1-S	67 ab
IT93K-503-S	75 ab
GoF1-S	78 ab
AbF1-S	93 b
LSD	**36.1**

Means followed by the same letter (s) are not significantly ($p>0.05$) different.

Leaf Area

Interactions were significant between cowpea lines and watering regimes at 20th day of water stress. Leaf Area (LA) (cm^2) significantly varied ($P<0.05$) among the parental check and the F1 lines, as affected by both watering regimes on the 20th day after water stress. However, in the water stress condition the LA ranges from 26 to 33.33 cm^2 with GoF1-S having the smallest leaf area of 26 cm^2 and the parental check (IT93K503-1-S) (28.0 cm^2), while MF1-S recorded the largest leaf area of 33.33 cm^2. In the non-stress condition, the LA ranges from 43.17 to 39.90cm^2. MF1-NS recorded the largest leaf area and the least LA was recorded by GoF1-NS (Figure 3).

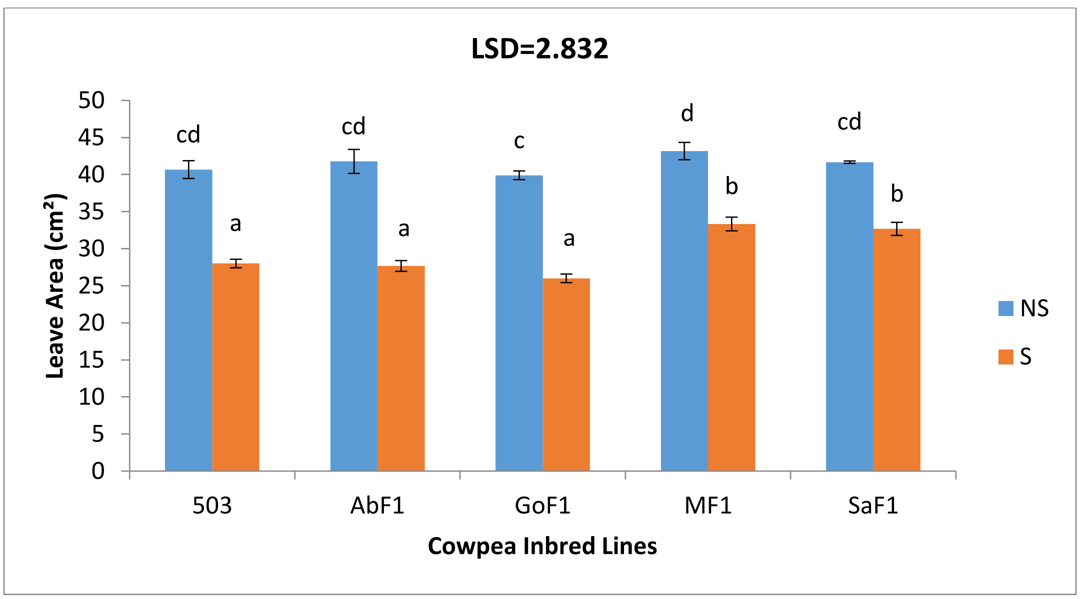
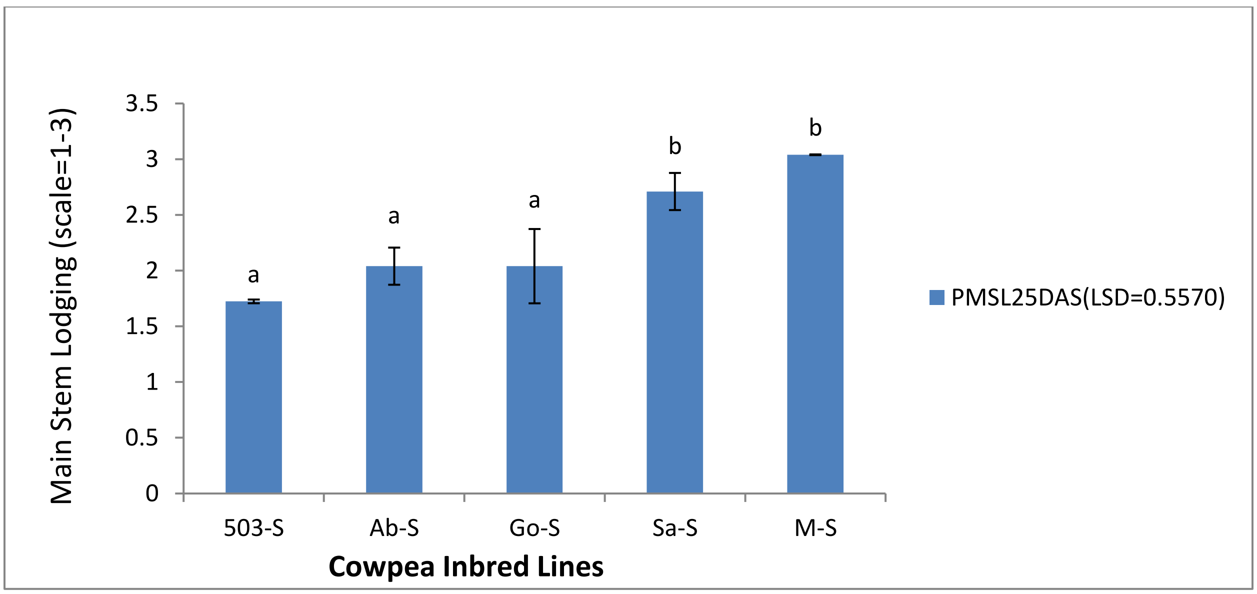


Figure 3: Leaf Area at the 20th Day of water-stress and non-stress condition (Error bars represent Standard Error of Means). NS=Non-Stress, S=Stress

The results of LA showed significant variation among the F1 lines and the parental check under both watering conditions. The results obtained indicated a decreased in LA among the parental check and the inbred lines under water-stress as compared to the results of the same genotypes under optimum moisture condition. This result supported the findings of (Bastos et al., 2011) and (Matsui & Singh, 2003) who reported that there was a reduction of LA of cowpea genotypes under water stress. (Taiz & Zeiger, 2004) in similar studies reported that, reduction in soil moisture result in lower leaf growth showing its sensitivity to water deficit. With smaller LA, there is decrease in transpiration keeping the water in the soil for a longer period. Considering the current result, the highest LA reduction was obtained by AbF1, GoF1 and IT93K-503-1 (parental line) with LA reduction values of 14.1 cm^2, 13.9 cm^2 and 12.67 cm^2, respectively. SaF1 and MF1 recorded the smallest LA reduction with values of 9 cm^2 and 9.84 cm^2 respectively, indicating they are drought susceptible lines. A similar observation made by Bastos et al.(2011) indicated that drought-tolerant cultivars showed greater maintenance of leaf area under water stress condition. The reduction in LA in plants under water stress condition is a survival strategy in order to reduce the area available for transpiration (Correia & Nogueira, 2004).

Plant main stem lodging

Water stress significantly ($P<0.05$) affected plant main stem lodging among the parental line and the F1 lines at 25 DAS. The least main stem lodging was recorded by the parental line IT93K-503-1-S (1.707). The highest main stem lodging was recorded by MF1-S with a score of (3.040) followed by SaF1-S (Figure 4).



PMSL= plant main stem lodging. (Error bars represent Standard Error of Means)

Figure 2: Plant Main Stem Lodging After 25 Days of Stress

There was variation among the parental check and the F1 lines for the number of plants main stem lodged. The mean stem lodging score ranged between 1.71- 3.04, with an average score of 2.31. The parental check (IT93K-503-1), AbF1 and GoF1 had the least number of stems lodged below the average score, while MF1 and SaF1 had the highest score above the average score. This result shows that AbF1 and GoF1 are the most drought tolerance F1 lines. This is also in agreement with the result of previous studies by Cui et al. (2019), who, in their drought study reported that cowpea lines that had stem lodging score above the average score were completely drought susceptible lines, while those that scored below the average score were most drought-tolerant lines. Similar result obtained by Ravelombola et al. (2020) showed that the percentage of lodged plants varied from 0%-100% with an average of 44.28%. They reported that those genotypes that scored stem lodged above the average were suggested to be drought susceptible genotypes.

CONCLUSION AND RECOMMENDATION

In conclusion, two F1 lines (AbF1 and GoF1) consistently performed better in chlorophyll content, leaf area, seedlings recovery rate and plant main stem lodging. SaF1 had the highest value for RWC, but all the lines performed better above 50% average. AbF1, GoF1, MF1 and SaF1 should be further evaluated in the subsequent generations to confirm their drought tolerance ability. This is because Segregation for various traits relating to drought tolerance will occur in later generations and will offer the opportunity to develop varieties that meet local preferences for crop improvement

Acknowledgement

We would like to acknowledge the support of farmers in obtaining local germplasm that have potential for drought and grain yield. Also, we would like to thank the University for Development Studies for giving us working space and some basic equipment's to enable us carry out the research

Declaration of interest statement

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Data Availability statement

Data supporting the analyses and results presented in this paper has been deposited in Dryad with the title "Data for assessment of First Filial (F1) generation of cowpea (Vigna unguiculata (L.) Walp) F1 lines variability to drought tolerance in the guinea savanna agro-ecologies of Ghana" with doi: 10.5061/dryad.h44j0zpmf

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