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Variation in Canopy Temperature and Its Relationship with Drought Tolerance in Cowpea [*Vigna unguiculata* (L.) Walp] Recombinant Inbred Lines

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of the study was to develop drought tolerant cowpea inbred lines using leaf canopy temperature and grain yield under contrasting soil moisture conditions in the field.
Study Design: Split plot design was used for the experiment.
Place and Duration of the Study: The study was carried out in February and December 2016 and 2017 at Golinga and Libga irrigation sites respectively in the Guinea Savanna ecology of Ghana.
Methodology: The watering regimes at two levels were the main plots and the 22 recombinant inbred lines, with 2 parental checks, were the subplot factor. Treatment was completely randomized and in 3 replications given a total of 144 plots. Various agronomic data were taken and statistical analysis was done using Genstat edition 12. Leaf canopy temperature was used to calculate stress susceptibility index during the period of stress imposition.
Results: The genotypic and phenotypic correlations between yield and chlorophyll were r = -0.69 and r = -0.528 respectively. Negative correlations indicate that moisture stress delayed the onset

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and time to flowering and consequently reduction in yield. Under well-watered conditions, the susceptible lines had yields of 1.69t ha-^{1} whereas the low temperature inbred lines had mean yields of 1.9 t ha-$^{1.}$ The mean yields of drought susceptible inbred lines (high temperature) lines had 1.1t ha-^{1}, while that of the drought tolerant (low temperature) lines had mean yields of 1.24t ha-^{1}.
Conclusion: The study revealed that genotypes exhibited variation in mean canopy temperature across the two watering regimes. Watering regimes for canopy temperature were significant for days 39, 45, 48 and 54 days after planting. Leaf canopy temperature has proven to be a useful physiological index for selecting drought tolerant cowpea under field conditions.

Keywords: Cowpea; drought tolerance; leaf canopy temperature; recombinant inbred lines.

1. 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 [1] where it is produced mainly in the semi-arid savannah and Sahelian zones for its grain and hay [2].

Soil moisture is a principal environmental factor limiting legume productivity in the tropics and sub- tropics [3,4]. Lack of adequate soil moisture affects both vegetative [5] and reproductive growth of food legumes, resulting in significant yield losses [6]. 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 [4,7,8].

Early maturing varieties are often now 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 [9–11]. 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. Therefore, selection for both early and late maturing cowpea genotypes using leaf canopy temperature would contribute to increased production and yields in these production zones.

The objective of this study was to develop drought tolerant cowpea inbred lines similar to the drought tolerant parent IT93K-503-1 using quantitative indices and physiological traits for grain yield under low soil moisture conditions in the field.

2. MATERIALS AND METHODS

2.1 Germplasm for the Study

Four hundred and fifty (450) Recombinant Inbred Lines (RILS) of an $F_{2:6}$ population of cowpea were developed through single seed descent from drought tolerant and susceptible parents; which were advanced breeding lines obtained from the International Institute of Tropical Agriculture (IITA), Kano station, Nigeria. IT93K-503-1 is a well-recognized drought tolerant genotypes used by many researchers for drought studies [12–15].

The second parent IT97K-279-3 is a drought susceptible but early maturing advanced breeding line, obtained from IITA as well.

2.2 Population Development

Seeds of the parental lines were planted in plastic buckets measuring 32cm in diameter, filled with black, loamy top soil in a screen house facility at SARI. At flowering, the male parent was crossed with the female parent to generate F1 seeds. Series of plantings of the parents were carried out between the period of June and December 2010 to synchronize flowering and several crosses were done with the aim of obtaining a minimum of 400 F1 seeds.

A total 450 F1 seeds were generated from a cross between the two parents that contrast for drought tolerance between June to December 2010. In the following season, in July 2011, the F1 seeds were individually planted at a spacing of 60 cm x 60 cm. The F2 population was obtained by harvesting seeds from each F1 plant separately. The F2 seeds were planted in a field

at a spacing of 60 cm x 60 cm in July 2012 and allowed to self. Seeds from each plant were harvested and kept separately to obtain an F3 population. In July 2013, the F3 seeds were planted in the field in progeny rows to obtain an F4 population. Single plants harvested from each line in each of the F4 and F5 populations led to the development of an F6 population of recombinant inbred lines. Field drought screening started in December and February 2016 and 2017 respectively.

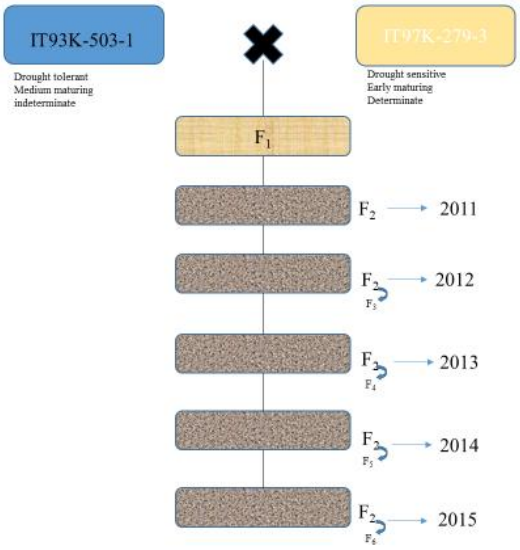

Fig. 1. Schematic representation of the population development process

2.3 Experimental Design for Drought Evaluation under Field Conditions

Split plot design was used for the experiment. The main plots were allotted for the watering treatments and the sub-plots to the test genotypes and completely randomized with three replicates. The watering regimes at two levels were the main plots and the 24 recombinant inbred lines were the subplot to give a total of 144 plots. The land was prepared by disc ploughing, harrowing and ridging 75 cm apart. The net plot size was 3m x 2m 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 2m.

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.

The fields were weeded twice during the growing period of the crop. Plants were sprayed twice with lambda cyhalothrin (product K- Optimal) at the rate of 20g active ingredient per liter of water, first at three weeks after planting, at the beginning of floral bud initiation, and during flowering to control insect pests. All field observations and plant samples were obtained from the central three rows of each five-row plot. In addition, the central three rows were harvested for seed yield. Both experiments were harvested manually three to four times as soon as they reached a stage of physiological maturity.

2.4 Watering Regime

The plants were subjected to two watering regimes: well-watered and water stressed at the vegetative phase (10 days after planting), until the beginning of flowering (40 days after planting). 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.

2.5 Data Collection

Weekly chlorophyll meter readings: Soil Plant Analytical Development (SPAD) chlorophyll meter reading was taken at a week interval from the seedling stage until the end of the second week of flowering. This was to estimate the leaf nitrogen status for each of the inbred lines for the period of the experiment. The second leaf from terminal bud of the main stem of each plant was measured for Specific chlorophyll meter readings (SCMR) by a Minolta handheld portable SCMR meter (SPAD- 502 Minolta, Tokyo, Japan), using four leaflets per sample. In recording the SCMR, care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from veins and midribs could be avoided.

Leaf surface temperature: Leaf surface temperature was measured using, a hand-held infrared thermometer (Everest Inter-science Inc., Fullerton, CA) to measure canopy temperature

depression (CTD). The thermo gear is an image or visual temperature equipment, the photo camera of the plant was taken and then uploaded into analyzing software on a computer and the surface temperature was determined.

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 data 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 t/ha and t/ha.

Grain yield was calculated as

$$\frac{\text{grain weight x per plot}}{\text{plot area harvested}} \text{x } 10000$$

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.

Leaf canopy temperature was used to calculate stress susceptibility index as:

SSI =[1-(LTs/LTw)]/SI

Where

SSI = stress susceptibility index
LTs = leaf temperature under stress conditions
SI = Stress intensity

Weather data: The temperature, relative humidity, rainfall and solar radiation at the experimental locations were obtained from the meteorological department of the Savanna agricultural research institute and the meteorological division of the Ministry of food and agriculture in northern Ghana.

Soil sampling: Soil samples were taken before and after land preparation diagonally to cover all sections across the trial field before planting from a depth of 0-20 cm and bulked together. The samples for 2016 trial were analysed by the Chemistry Department of CSIR-Savanna Agricultural Research Institute, Tamale. The soil samples for 2017 stress experiment and main season evaluation were however, analysed by the Ecological laboratory of the University of Ghana, Legon.

2.6 Data Analysis

An initial analysis of variance was performed for each environment to verify the existence of differences between inbred lines using GenStat edition 12. 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.

3. RESULTS

3.1 Mean Yield Performance of Cowpea Recombinant Inbred Lines across All the Six Environments

The overall mean yields for all the six environments (Golinga 2016, Golinga 2017, and Libga 2017 for well-watered and water-stressed experiments) were computed and presented in Table 1. Environment 1 had a mean range of 2.045t ha-^{1} and 0.64t ha-^{1} for inbred line 131 and 396 respectively. The mean range for environment two were 2.43 for inbred line 255 and 1.37 for 38. That of environment three ranged between 3.98t ha-^{1} and 1.06t ha-^{1} for inbred line 84 and 255 respectively. Inbred line 255 recorded the highest mean yield of 2.45 t ha-^{1} for environment five whereas inbred line 38 had the lowest mean yield of 1.1t ha-^{1}. The mean yield for environment six ranged between 2.45t ha-^{1} and 0.95t ha-^{1} for inbred lines 255 and 28, respectively. The grand mean ranged between 2.56 and 1.35; with their interaction principal components for one ranging between 0.75 and -0.62, while that of component two ranged between 0.56 and -0.038 (Table 1). The parental checks however had mean ranges of 3.49 and 1.59 for environment four and one respectively for IT93K-503-1 and 3.022 and 1.085 for IT97K-279-3 with their grand mean range of 2.86 - 2.22. However, the Principal components for their interactions ranged between 0.1446 and -0.629 and 0.028 and -0.322 respectively, (Table 1).

Table 1. Mean yields in t/ha, of inbred lines across all the six environments

Genotype	Test Environment						Grand mean	IPCAg	
	1	2	3	4	5	6		1	2
F116	1.1	1.433	2.414	1.445	1.144	1.125	2.11	-0.502	-0.344
F131	2.045	2.108	2.735	2.511	1.378	1.129	2.318	0.3919	-0.937
F142	0.731	1.388	1.132	1.841	1.074	0.99	1.193	0.7497	0.1338
F186	1.452	2.29	3.031	2.558	2.31	2.403	2.507	-0.011	0.546
F189	1.209	1.67	2.019	3.362	1.522	1.571	2.392	-0.639	-0.092
F20	0.794	1.634	2.154	2.038	1.726	1.864	2.035	-0.31	0.5784
F223	1.337	1.549	1.71	3.959	1.175	1.123	2.309	-0.575	-0.562
F230	1.395	1.885	1.989	1.449	1.737	1.782	2.539	-0.544	-0.049
F255	1.525	2.427	1.006	2.294	2.393	2.445	2.348	0.4367	0.6195
F28	0.895	1.401	2.068	2.475	1.045	0.959	1.474	0.3908	-0.112
F325	0.909	1.418	2.527	2.742	1.197	1.193	1.831	-0.162	-0.05
F353	1.857	2.371	2.927	3.399	2.013	1.923	2.415	0.4336	-0.099
F38	0.774	1.369	1.772	2.127	1.063	0.994	1.35	0.4947	0.0439
F 396	0.636	1.379	1.822	1.699	1.2	1.187	1.321	0.484	0.32
F 398	1.47	2.01	2.922	3.025	1.705	1.645	2.129	0.2977	-0.038
F 406	1.286	1.92	3.116	2.887	810	1.855	2.312	-0.192	0.1829
F 408	0.844	1.598	1.239	2.281	1.629	1.743	2.056	-0.359	0.4241
F 55	1.438	1.858	3.569	3.562	1.616	1.614	2.443	-0.396	-0.194
F 57	1.507	2.167	2.659	2.746	1.924	1.883	2.148	0.4626	0.1677
F75	2.171	2.347	3.129	4.437	1.725	1.527	2.556	0.3362	-0.721
F 78	1.578	2.082	2.282	3.08	1.681	1.567	2.045	0.5696	-0.134
F 84	1.132	1.744	3.976	2.783	1.619	1.659	2.152	-0.206	0.1442
Standard									
IT93K-503-1	1.596	2.185	5.517	3.49	2.142	2.236	2.861	-0.629	0.1446
IT97K-279-3	1.065	1.597	4.65	3.022	1.481	1.539	2.226	-0.523	0.0286

F= families, Environment 1=Golinga 2016 stress, environment 2= Golinga 2016 watered, environment 3=Golinga 2017 stress, environment 4= Golinga 2017 watered, environment 5=Libga 2017 stress, environment 6=Libga 2017 watered. IPCA= Interaction Principal Component Axis

3.2 Phenotypic and Genotypic Correlation Analysis for Single and Combined Locations

Phenotypic and genotypic associations between the traits measured across all the six environments was carried out (Table 2). There were significant associations between days to 50% flowering and harvest index, yield also correlated significantly with pods per plant and seeds per pod, biomass and harvest index. Genotypic correlations were only significant between biomass and days to flowering, biomass and yield. However, under well-watered conditions, phenotypic correlations showed highly significant positive associations between days to 50% flowering and grain yield, biomass, and harvest index. Hundred seed weight, harvest index, days to flowering and biomass as well as hundred seed weight and harvest index were positively correlated. (Table 2).

3.3 Mean Squares, Correlation Matrix Estimations for Chlorophyll and Leaf Temperature for Traits across Locations

A further analysis of variance across the locations with the study traits indicated significant differences for all the traits and yield (Tables 3 and 4). Significant differences were observed among the genotypes for days to 50% flowering and watering regimes across all the locations. Genotype and watering regime was only significant for days to 50% flowering at Libga. The mean squares for the other locations also followed a similar pattern of significance (Table 5).

Table 2. Genotypic (below diagonal) and phenotypic (above diagonal) correlations between yield and yield related traits among 22 cowpea inbred lines and parents for yield and related traits for the dry season

Traits	DFF	Pods_plant	Seeds_pod	HSW	Yieldt_ha	Biomass	HI
DFF	1	0.505**	0.470*	-0.220*	0.744ns	0.692***	0.462*
Pods_plant	0.836***	1	0.604**	-0.465*	0.674***	0.444*	0.177
Seeds_pod	0.596***	0.999***	1	-0.370	0.671***	0.497*	0.254
HSW	-0.191	0.939***	-0.495**	1	-0.312	-0.147	0.4523*
Yieldt_ha	0.923***	0.999***	0.999***	-0.485**	1	0.826*	0.395*
Biomass	0.999***	0.999***	0.946***	-0.197ns	0.999***	1	0.435*
HI	0.707***	0.602***	0.563***	0.709***	0.269ns	0.829***	1

(, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively), DFF = days to 50% flowering; ppp = pods per plat; SPP = seeds per pod; HSW= hundred seed weight; HI = harvest index*

Table 3. Mean squares for Chlorophyll content at Golinga in 2016

Source	df	Mean Squares			
		14 DAP	21 DAP	28 DAP	35 DAP
Genotypes	23	31.30*	66.88	34.06*	39.79*
Irrigation	1	654.51**	2652.25**	1660.56**	9587.67**
G x I	23	20.71	77.22	17.53	22.72
Rep	2	18.84	162.97	35.08	211.72
Residual	94	17.87	58.23	17.05	22.14
Total	143				
CV %		7.2	12.0	6.6	7.9

*** P < 0.01; * P < 0.05, Rep = replications, G= genotype, I= irrigation, DAP= days after planting, CV= coefficient of variation. DAP = Days after planting; Genetic correlation between yield and chlorophyll 17/03/2016 Rg = -0.690; Rp = -0.528***

Table 4. Mean squares for Chlorophyll content at Golinga in 2017

Source	df	Mean Squares						
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP
Genotypes	23	33.59**	57.36**	60.09**	74.93**	67.43**	76.92**	43.12**
Irrigation	1	66.29*	40.11	225.00**	817.01**	1018.67**	19.51	10.56
G x I	23	15.82	27.76	43.32**	23.66	20.04	17.96	26.24
Rep	2	44.05	69.26	56.33	14.76	81.38	24.51	7.09
Residual	94	12.98	25.40	19.17	26.86	18.14	19.54	18.33
Total	143							
CV %		6.8	8.1	6.8	8.1	6.6	6.9	6.7

** P < 0.05; ** P < 0.01*

DAP = days after planting; G = genotype; I = irrigation; Rep = replication; CV = coefficient of variation; Df = degree of freedom

Table 5. Mean squares for days to flowering from the analyses of variance of 24 cowpea families evaluated under two irrigation regimes at Golinga and Libga in 2017

Source	Df	Mean squares			
		Days to first flower (DFF)		Days to 50% flower (D50%F)	
		Golinga	Libga	Golinga	Libga
Genotypes	23	65.04**	96.72**	154.01**	237.19**
Irrigation	1	121.00**	11.11	342.25**	7.11
G x I	23	8.28	15.04	16.77	27.89*
Rep	2	33.13	46.03	121.05	58.58
Residual	94	7.93	12.06	19.14	14.07
Total	143				
CV %		5.2	7.1	8.1	6.4

** P < 0.05; ** P < 0.01*

DFF= days to 50% flowering, Df= degree of freedom, G= genotype, I= irrigation

Secondary Climatic Data for Golinga 2017 for Leaf Surface Temperature: The relative humidity and temperature recorded for the period of the leaf surface temperature measurement is shown in Fig. 2. The highest temperature was recorded on 17th of January and on the 10th of February, with readings of 39.12^{0}C, and 39.13^{0}C whereas the highest relative humidity values were recorded on the 31st of January and 10th of February with readings of 43.5% and 19.32%, respectively.

3.4 Mean Squares, Correlation Matrix Estimations for Leaf Temperature for Traits across Locations for Traits

Analysis of variance for leaf temperature was significant for all the genotypes as well as the watering regimes (Table 6). Genotype and watering regime interaction was only significant for 45 days after stress imposition and 51 days after stress imposition. The average mean leaf temperature, standard errors and coefficient of variation are presented in Table 7.

3.5 Correlation Analysis for Days to Flowering, Yield and between Leaf Canopy Temperature

Correlation of leaf temperature for days to 50% flowering and yield showed negative associations (Table 8). However, significant associations were observed for 39, 45, 48, 52, 62 and 66 days. The leaf temperature taken at different times during the flowering stage were used to calculate stress index (Table 9) as SSI = [1-(LTs/LTw)]/SI for each of the 11 days of leaf temperature measurement to confirm the quantitative index estimation. Leaf canopy temperature was used to classify the lines as high (tolerant genotypes) or low (sensitive genotypes) temperature lines (Table 10). Analysis of variance was further carried out based on High canopy temperature or Low canopy temperature lines to see whether for the various traits, what role leaf temperature played. The more negative values indicate higher temperatures (more stress); hence the negative indices were an indication that, the higher the temperature, the more intense the stress level for the inbred lines.

Table 6. Mean squares for Leaf temperature at Golinga in 2017

Source	df	Mean Squares									
		36DAP	39 DAP	42 DAP	45DAS	48 DAP	52 DAP	55 DAP	59 DAP	62 DAP	66DAP
Genotypes	23	7.095**	2.095*	7.638**	3.548**	1.71	2.575**	1.8301**	3.319*	3.6**	6.556**
Irrigation	1	1044.442**	1911.861**	886.471**	504.1**	2149.095*	8.033**	76.8048**	185.023**	14.973**	94.327**
G x I	23	3.11	1.303	6.709**	2.746	2.682	0.569	1.781**	1.317	0.731	1.779
Rep	2	0.395	3.762	4.143	17.968	3.154	1.48	17.5065	15.71	8.338	21.893
Residual	94	2.815	1.283	2.046	1.311	1.944	1.23	0.8533	1.749	1.127	1.641
Total	143										
CV %		6.7	4.2	5.5	4.0	5.2	4.0	3.6	5.3	3.9	4.2

*** P < 0.01; * P < 0.05; DAP = Days after planting, G= genotype, I= irrigation Rep= replication, CV= coefficient of variation.*

Table 7. Means for leaf temperature measurements across the locations

Families	36 DAP	39 DAP	42 DAP	45 DAP	48 DAP	52 DAP	55 DAP	59 DAP	62 DAP	66 DAP	70 DAP
279 -3	30.44	27.71	31.1	31.1	30.87	28.31	28.08	23.83	27.99	27	25.86
503 – 1	32.87	29.14	32.59	32.59	32.66	30	30.15	25.64	29.82	28	28.17
F 116	30.53	28.72	30.08	30.08	30.63	28.02	27.32	23.76	28.41	26.06	27.09
F 142	31.92	28.16	31.38	31.38	30.56	28.17	28.45	23.93	28.27	25.62	27.23
F 186	30.54	26.39	31.02	31.02	29.48	26.73	25.99	23.23	27.63	25.08	25.73
F 189	31.66	27.34	30.28	30.28	30.13	26.57	24.65	23.42	27.29	25.56	24.87
F 20	29.92	27.6	30.78	30.78	31.72	28.46	29.08	24.11	28.82	27.14	25.89
F 223	30.51	27.2	30.73	30.73	29.84	26.94	25.76	23.64	27.24	24.91	25.73
F 230	29.8	27	30.01	30.01	30.13	27.12	25.57	23.81	27.24	25.92	24.55
F 255	30.32	26.38	30.12	30.12	29.24	26.5	26.71	23.83	26.49	25.04	24.98
F 28	30.65	27.36	32.18	32.18	31.35	28.74	29.11	23.64	27.7	26.78	26.28
F 325	29.52	27.73	30.87	30.87	31.31	28.05	27.94	24.08	27.09	26.1	27.31
F 353	30.41	28.19	31.66	31.66	31.31	27.66	27.44	23.99	27.32	26.77	26.6
F 38	32.63	29.95	32.47	32.47	32.22	29.42	30.47	23.38	29.29	27.1	26.59
F 396	30.73	26.51	32.36	32.36	32.14	29.12	29.35	24.27	28.31	26.67	28.31
F 398	29.92	26.54	30.96	30.96	30.17	27.07	26.92	24.44	27.64	26.4	25.19
F 406	30.97	27.65	31.7	31.7	31.75	28.01	27.84	24.44	27.58	26.45	26.87
F 408	29.73	28.1	30.45	30.45	30.69	27.71	27.1	24.5	28.77	26.38	27.06
F 55	29.27	26.21	30.7	30.7	29.56	26.68	26.09	23.56	27.56	25.84	25.68

Families	36 DAP	39 DAP	42 DAP	45 DAP	48 DAP	52 DAP	55 DAP	59 DAP	62 DAP	66 DAP	70 DAP
F 57	29.41	26.61	29.54	29.54	29.85	27.51	26.88	23.82	27.72	25.76	25.99
F 75	31.02	27.56	30.36	30.36	31.32	28.11	27.56	23.34	28	26.37	26.64
F 78	30.18	27.82	30.09	30.09	30.97	27.68	27.5	23.24	27.28	25.59	26.36
F 84	30.13	28.28	30.34	30.34	30.33	27.89	27.37	24.17	28.6	26.16	26.64
F131	29.74	27.96	29.91	29.91	29.61	27.45	25.98	23.71	27.37	25.07	25.28
Average	**30.53**	**27.59**	**30.9**	**30.9**	**30.74**	**27.83**	**27.47**	**23.91**	**27.89**	**26.16**	**26.29**
SED	**1.075**	**1.182**	**1.26**	**1.26**	**1.141**	**0.988**	**1.249**	**0.755**	**1.062**	**0.775**	**1.2**
CV	**1.6**	**1.2**	**1.3**	**1.3**	**1.7**	**1.2**	**2.6**	**0.7**	**1.1**	**1.6**	**1.8**
P<0.05	0.108	0.279	0.536	0.536	0.173	0.068	0.002	0.53	0.429	0.53	0.204

Table 8. Correlation matrix between leaf temperature at different times, and with yield and days to 50% flowering in 2017 at Golinga

36 DAP	1											
39 DAP	0.543**	1										
42 DAP	0.6**	0.524**	1									
45 DAP	0.505*	0.686**	0.491**	1								
48 DAP	0.633**	0.643**	0.441*	0.773**	1							
52 DAP	0.725**	0.653**	0.41**	0.714**	0.863**	1						
55 DAP	0.568**	0.681**	0.407**	0.688**	0.681*	0.622**	1					
59 DAP	0.626**	0.519**	0.567**	0.699**	0.782**	0.658**	0.601**	1				
62 DAP	0.642**	0.557**	0.516**	0.64**	0.756**	0.77*	0.762**	0.797*	1			
66 DAP	0.617**	0.544**	0.435**	0.751**	0.825**	0.761*	0.754*	0.764*	0.889*	1		
Yield	-0.311	-0.446*	-0.095ns	-0.47	-0.559	-0.482*	-0.31*8	-0.364*	-0.248*	-0.397*	1	
Dff	-0.359*	-0.166	-0.399*	-0.389*	-0.4*	-0.456*	-0.157*	-0.416*	-0.511*	-0.604*	0.312*	1
	36 DAP	39 DAP	42 DAP	45 DAP	48 DAP	52 DAP	55 DAP	59 DAP	62 DAP	66 DAP	Yield	Dff

DAP = days after planting, dff = days to 50% flowering

Table 9. Stress tolerance estimation using leaf canopy temperature

LTSSI1	LTSSI2	LTSSI3	LTSSI4	LTSSI5	LTSSI6	LTSSI7	LTSSI8	LTSSI9	LTSSI10	LTSSI11
-0.24263	-0.31356	-0.20898	-0.13864	-0.33341	-0.01726	-0.05917	-0.09432	-0.02377	-0.06256	0.001497

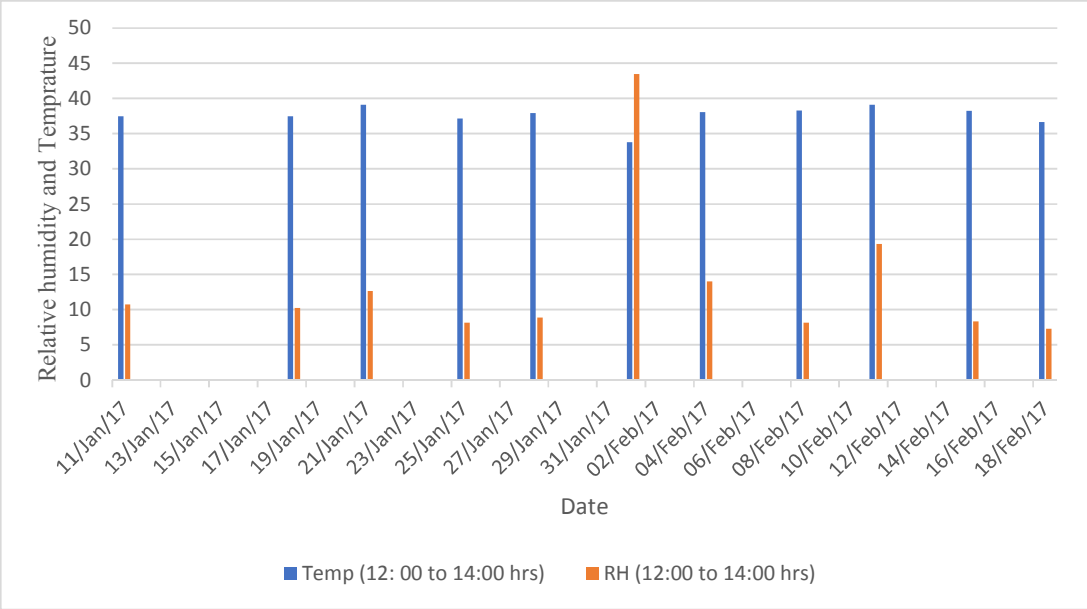

Fig. 2. Climatic data for 2017 drought experiment for leaf canopy temperature

Table 10. Ranking of inbred lines and parents based on average SSI over recording period

Low leaf canopy temperature	High leaf canopy temperature
F 230	F 57
F131	F 116
F 186	F 78
F 408	F 142
F 398	F 223
F 20	F 325
F 353	F 55
F 396	F 75
IT93k-503-1	F 255
F 406	F 189
F 84	**IT97K-279-3**
F 28	F 38

Based on this classification, there were no differences between high leaf temperature types (sensitive, high water extraction) and low leaf temperature types (tolerant, or low soil water extraction) under well-watered conditions. Traits for which differences were found under stress conditions are presented in Tables 11, 12 and 13 respectively.

Table 11. Performance of 24 cowpea lines under well-watered conditions based on leaf temperature

Trait	High temp	Low temp	Prob.	LSD	CV (%)
Yield (t/ha)	0.57	0.68	0.054	0.11	40.1
Biomass	2.97	3.98	0.005	0.686	42.7
DFF	51.22	55.58	0.001	2.5088	10.2
HSW	19.45	18.47	0.014	0.7546	8.6
SPP	9.58	10.78	0.009	0.87024	18.5
HI	0.1877	0.1573	0.122	0.038044	47.8
PPP	10.78	11.69	0.203	1.39748	26.9

DFF= days to 50% flowering, ppp= pods per plant, SPP=seeds per pod, HSW= hundred seed weight, HI= harvest index

Table 12. Relationship between leaf temperature at Golinga 2017 and agronomic traits in 2016 at Golinga

A. Well-watered conditions (class = either high temp or low temp)

Trait	High temp	Low temp	Prob.	LSD	CV (%)
Yield (t/ha)	1.696	1.908	0.170	0.3042	35.9
Biomass	21.0	20.5	0.852	5.41	55.4
DFF	48.1365	45.3950	0.004	1.821	8.2
SPP	12.11	12.83	0.066	0.770	13.1
PPP	14.14	16.42	0.044	2.220	30.9

DFF= days to 50% flowering, ppp= pods per plant, SPP=seeds per pod, HSW= hundred seed weight, HI= harvest index

Table 13. Relationship between leaf temperature at Golinga 2017 and agronomic traits in 2016 at Golinga

B. Under water stress

Trait	High temp	Low temp	Prob.	LSD	CV (%)
Yield (t/ha)	1.071	1.235	0.227	0.2671	49.3
Biomass	17.5	19.9	0.390	5.66	64.4
DFF	51.36	45.19	0.001	3.549	15.6
HI	6.27	7.93	0.071	1.807	54.1

DFF= days to 50% flowering, ppp= pods per plant, SPP=seeds per pod, HSW= hundred seed weight, HI= harvest index

4. DISCUSSION

Analysis of variance for chlorophyll and leaf temperature indicates significant differences among genotypes and watering regimes. Days to 50% flowering varied significantly ($P < 0.001$) for both Golinga and Libga respectively. The genotypic and phenotypic correlations between yield and chlorophyll were r = -0.69 and r = -0.528 respectively. The negative correlations indicate that moisture stress delayed the onset and time to flowering, which would consequently affect the grain production and eventually would result in yield reduction. This is in line with results obtained by Abayomi and Abidoye [16].

Leaf temperature and chlorophyll contents showed highly significant differences for genotypes and days to flowering under stress and non-stress conditions. This corroborates with Blum et al. [17] who reported that canopy temperatures are related to plant water stress; he further on stated that lower canopy temperatures were indicative of higher leaf water potential. He went on further to conclude that identification of relevant physiological drought resistance mechanisms as a selection criterion would be helpful in selection of potential drought tolerant lines. Also, in related studies by Montago and Woo [18]; and Pirzard [19] revealed that, water stress significantly decreased leaf chlorophyll content.

Correlation for leaf temperature at different times with yield and days to 50% flowering for the dry season experiment across the six environments were strongly associated. Based on the strong associations for leaf temperature stress susceptibility were calculated for the second time using the physiological indices (leaf temperature and chlorophyll). the more negative values implied higher temperatures (more stress); hence the negative indices were an indication that, the higher the temperature, the more intense the stress level for the inbred lines. Belko et al. [20] also reported that tolerant genotypes are able to maintain higher transpiration rate and lower canopy temperature under severe water stress thus reducing the leaf temperature for tolerant genotypes compared to the sensitive ones.

Based on these leaf temperature ratings, the inbred lines were again categorized into low leaf temperature (tolerant) genotypes and high leaf temperature (susceptible) genotypes. Apparently, the two rankings (quantitative index ranking and leaf temperature ranking) of inbred lines for drought tolerance were similar. This corroborates related studies by Saba et al. [21]. Ramirez and Kelly [22] and Rashid et al. [23].

The relationship between leaf temperature verses the agronomic traits under well-watered conditions were evaluated. Under well-watered conditions, the susceptible lines had yields of 1.69t ha-^{1} whereas the low temperature lines (tolerant) inbred lines had mean yields of 1.9 t ha-$^{1.}$ The mean yields of drought susceptible inbred lines (high temperature) lines had 1.1tha-^{1}, while that of the drought tolerant (low temperature) lines had mean yields of 1.24t ha-^{1}. These significant correlations between canopy temperature and yield under stress conditions and drought susceptibility index revealed the potential for screening cowpea genotypes for drought under water stress and well-watered conditions [23].

5. CONCLUSIONS

This study revealed that genotypes exhibited variation in mean canopy temperature across the two watering regimes. Watering regimes were significant for days 39, 45, 48 and 54 but there were no significant differences between stress and non-stress inbred lines at other different times and days for leaf canopy temperature, this could be as a result of evaporative cooling especially for the tolerant lines. Leaf canopy temperature and chlorophyll content measurements taken during the onset of drought for both water stress and well-watered conditions can be used as an effective physiological parameter for identifying drought tolerant lines.

The use of leaf canopy temperature for classifying genotypes as “low temperature lines” or otherwise drought tolerant and “high-temperature lines” otherwise drought susceptible, based on their sensitivity to drought have been carried out in this study. This could be another selection strategy aside using quantitative indices for selection for drought tolerance under field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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