Original Research Article

Morpho-Physiological Adaptations of Sorghum to Drought: Implications for Biomass Production and Grain Yield

ABSTRACT

Drought is a significant environmental stress affecting crop productivity, particularly, in semi-arid regions where sorghum [*Sorghum bicolor* (L.) Moench] serves as an essential crop for food and fodder. Therefore, it is paramount to evaluate such crop varieties with potential for use in the development of climate-resilient ones through breeding and selection. A greenhouse pot experiment was carried out to determine the morpho-physiological responses of seven sorghum varieties, focusing on the stay-green trait under drought-stressed (DS) and well-watered (WW) conditions. Parameters measured included green leaf area (GLA), relative water content (RWC), chlorophyll content, and grain yield. The results showed that the Dorado and Kapaala varieties exhibited greater drought resilience, maintaining higher GLA, RWC, chlorophyll levels and grain yield under drought stress conditions. Strong positive correlations between RWC, GLA, chlorophyll level, and grain yield parameters under DS highlighted these metrics as potential indicators for selecting drought-tolerant sorghum varieties. This study underscores the importance of genetic diversity in crop resilience and provides valuable insights for breeding programs aimed at enhancing drought tolerance in sorghum.

Keywords: Drought, Green leaf area, Relative water content, grain yield, sorghum, Chlorophyll content.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal grain crop that is grown in the arid and semi-arid tropics. It is the fifth most important cereal crop grown worldwide after wheat, barley, rice and maize, based on yield; and it is an important source of food, feed, fodder, fiber and fuel (Marshall et al., 2013). It is known to be particularly well adapted to conditions of low water availability. In some countries, sorghum is used as a fuel source, construction material, leather dye and/or as a physical support crop for vine crops, such as yams (Nair, 2023).

Global population is expected to rise to 9 billion by 2050 with most of the increase occurring in sub-Saharan Africa (Warner & Jones, 2018), thus, increasing the risk of food insecurity in this region (Sasson, 2012). Heat and drought are the two most important environmental stresses imposing huge impact on crop growth, development, grain yield and biomass productivity (Badigannavar et al., 2018). With the increasing expectations of losses in crop yields due to global climate change and the exponential population growth, there is an urgent need to accelerate plant breeding and mining of novel traits for increased yield potential and better adaptation to abiotic stresses to ensure food availability and meet the future demand for agricultural production (Kamal et al., 2019).

Reduction in grain quality negatively impacts on market value, nutrient content and processing appropriateness (Henry & Kettlewell, 2012). Plants choose water conservation over growth in times of water scarcity, putting less energy into production of above-ground biomass like leaves and stems (Pereira, 2017). During periods of natural and environmental stress, leaves gradually lose their greenness as chlorophyll is lost. Leaf yellowing, the visible sign of leaf senescence, is the loss of chlorophyll, however, in some stay-green plants, chlorophyll catabolism is impaired or postponed (Munaiz et al., 2020). Therefore, staying green is a crucial agronomic characteristic that enables plants to keep their leaves actively engaged in photosynthetic processes, improving the grain-filling process even in adverse environmental conditions (Munaiz et al., 2020). The stay-green trait is the ability of the plant to retain greenness during grain ripening under water-limited conditions (Thomas & Ougham, 2014). In the long history of sorghum breeding for drought adaptation, stay-green is the best-characterized trait contributing to drought adaptation in sorghum (Abebe et al., 2021).

Genetic variation for tolerance to stress factors in the environment has been found among plant species as well as in cultivars within the same species, which could be exploited to improve yields (Gambetta et al., 2020). An effective and economically feasible method to alleviate problems of crop production associated with drought is the development of crops that can withstand low soil moisture conditions (Parry et al., 2005). Sorghum genotypes with the stay-green trait continue to fill their grains normally even under limited water or drought stress conditions (Borrell et al., 2000a). Therefore, studying the morpho-physiological characteristics of sorghum varieties which potentially have the stay-green trait has become prudent for identifying climate-smart sorghum cultivars for further improvement. The primary objective of this study was to evaluate the physiological responses of seven sorghum varieties to drought stress (DS) in comparison to well-watered (WW) conditions, focusing on key parameters such as green leaf area retention, leaf relative water content, chlorophyll content and grain yield.

MATERIALS AND METHODS

Seed material

Seeds of seven sorghum cultivars were obtained from Savannah Agricultural Research Institute (SARI) – Nyankpala and Manga stations as shown in Table 1.

Table 1. Sorghum accessions used in the study

ACC No.	Local name	Station	Desired trait

SARSORG 16	Dorado (DO)	Nyankpala	Early maturing and drought tolerant
SARSORG 153	Naga White (NW)	Manga	Early maturing
SARSORG 40	Kapaala (KP)	Nyankpala	High yielding and early maturing
SARSORG 06	Kadaga (KD)	Nyankpala	Medium maturing
SARSORG 186	Bawku Red (BR)	Manga	High yielding
SARSORG 187	GO-1	Manga	
	GO-2	Manga	

Plant culture and treatment

All plants were raised from seeds. Three seeds were sown per bucket of 21-cm diameter and 26-cm depth filled to about ¾ full with a mixture of black soil, peat and pebble in a ratio of 4:4:2, respectively. Urea, TSP, MP and Gypsum were applied @ 190, 90, 90 & 50 kg ha^{-1}, respectively. The weight of 1 hectare soil at the depth of 15 cm was considered 2 x 10^6 kg soil. According to the above rate, fertilizers were calculated as per kg of soil (Hossain et al., 2016). Plants for each genotype were grouped but randomly positioned on either side of the glasshouse to prevent the tall plants from shading the dwarf ones. At 14 days after emergence (DAE), they were thinned to one per bucket. At flowering, when anthers were visible on 50% of main shoot panicles, half of the plants of each cultivar were either well-watered or subjected to drought stress. Plants were subjected to water deficit stress (DS) by withholding irrigation (RWC 65-70%), while in well-watered (WW) plants RWC ranged from 80-85% as described by Shivramakrishnan et al. (2016). For the stay-green trait to be expressed sufficiently to be used for selection, a prolonged drought period is required during grain-filling enough to accelerate senescence but not sufficient to cause premature death of plants (Galyuon et al., 2019). The drought treatment used in this study was to prevent premature plant death and enable the trait to be expressed, if present. The experiments were conducted between 25th July and 15th November in 2022, and between 25th January and 14th May in 2023.

Collection of data

Data were collected at flowering (F), 21 days after flowering (DAF) and shortly before the final harvest, when the seeds were physiologically mature (PM).

Determination of leaf production, size and area

Leaves with the collar exposed were counted at weekly intervals. The length of the leaf (from the collar to the tip) along the midrib was determined using a thread and metre rule. The width of the leaf was determined in the middle, where the widest part in ensiform leaves occurs (Tsukaya, 2018). The green leaf area (GLA) per plant at F, 21DAF, and PM was then determined and the product of leaf length, breadth and the shape factor 0.747 was expressed as leaf area in cm^2 per plant (Nagarjuna, 2007), viz;

$$\text{GLA } (cm^2) = \text{Breadth x Length x shape factor } (0.747).$$

Determination of leaf relative water content (RWC)

Leaf sections were cut from the middle on one side of the midrib, the fresh weight (FW) was immediately taken and length and width measured using a ruler. The sections were placed in labelled vials, covered with distilled water, placed in a container and incubated in the dark (cupboard) for 24 hrs. On removal from the container, the sections were immediately blotted between tissue paper and the turgid weight (TW) determined using an electronic balance (Mettler Toledo-PG203). They were then transferred into labelled drying bags and dried in an oven at 80 ºC (Genlab E4S-96K110) for 72 hrs and the dry weight (DW) also recorded as per the method of Colom & Vazzana, (2001). The percent RWC was then calculated using the method described by Teulat et al. (2003), viz;

$$\text{RWC}(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \text{x100}$$

Where FW is the fresh weight, TW is the turgid weight, and DW is the dry weight.

Determination of leaf chlorophyll levels

A leave chlorophyll tester (Portable Plant Chlorophyll Analyzer Meter GYJ-A, GOYOJO, China) was used to measure the flag leaf chlorophyll levels at flowering. SPAD values for sorghum have been found to be highly correlated with total leaf chlorophyll determined by spectrophotometry (Xu et al., 2000). Each leaf was divided into three sections, base, middle and tip. Within each section six readings were taken, three on each side of the midrib. In all, eighteen readings were taken on each leaf, nine on each side of the midrib and the average recorded.

Determination of grain yield

The panicles were cut off from the main stems, fresh weights measured and then placed in drying bags and allowed to sun-dry in the glasshouse for two weeks and then transferred into an oven set at 60 ºC to further dry for 48 hrs. The dry weights of the heads were determined on a Mettler Toledo Electronic Balance (PG-203) beforehand-threshing. The seeds were separated from the chaff and the seed weight per panicle and seed size determined from 100-seed weight measured using the Mettler Toledo electronic balance. The 100-seed weight and total seed weight per panicle were used to determine the number of seeds per head as described by Borrell et al. (2000b).

$$\text{Total number of seeds} = \frac{\text{TSW (g)}}{\text{HSW (g)}} \text{ x } 100$$

Where, TSW is total seed weight and HSW is 100-seed weight.

Data analysis

The data were analysed using R software. Descriptive statistics were first employed to summarize key metrics, such as green leaf area (GLA), seed weight, relative water content of the leaf (RWC) and chlorophyll content.

To further explore the relationships among these variables, scatter plots by using ggplot2 packages in r studio were generated. Additionally, correlation analysis, including heatmap figures were used to analyse the strength of relationships under both DS and WW conditions at 5% level of significance. LSD was determined for all traits measured if there were significant

Commented [SA1]: If available possibly add version of r studio and packages particularly used like Agricole etc. However well written contents.

differences (alpha value (α) = 0.05). This multifaceted approach allowed for a nuanced understanding of how the different sorghum varieties responded to water availability, revealing inherent genetic resilience and performance variations across treatments. Strong correlational associations could also be used for selection of certain traits indirectly.

RESULTS AND DISCUSSION

Heading

Depending on the cultivar, heading was visible between 41- and 60-days following emergence. Naga White (NW) headed first at 41 days after emergence (DAE) followed by Kapaala (KP) (51 DAE), Kadaga (KD) (53 DAE), Bawku Red (BR) (56 DAE), Dorado (DO) (57 DAE), GO-2 (58 DAE) and GO-1 (60 DAE). Flowering followed a similar trend being earliest in NW (46 DAE), followed by KP (55 DAE), KD (57 DAE), DO and BR (61 DAE), GO-2(63 DAE) and GO-1(65 DAE) (Table 2). Under WW conditions, the genotypes exhibited consistent growth with variation in timing and height, with GO-1 having the tallest plants (341.9 cm) and last to reach physiological maturity (116 days). Under DS conditions, plant height was slightly reduced compared to WW, and there was minimal drought stress impact on swelling, heading, flowering and physiological maturity as shown in Table 2, indicating that the variations in responses were likely ~~mainly~~ due to genotypic differences rather than environmental influences~~.~~

Table 2: Developmental Responses of Sorghum Genotypes to Well-Watered and Drought-Stressed Conditions

		Developmental Data				
Line	**Treatment**	**Swelling**	**Heading**	**Flowering**	**PM**	**Height (cm)**
NW	WW	37	41	46	71	169.4
	DS					164.7
DO	WW	53	57	61	92	133
	DS					132.5
KP	WW	48	51	55	87	232.5
	DS					232.4
KD	WW	49	53	57	107	297.6

	DS					287
BR	WW	53	56	61	103	331.1
	DS					333.3
GO-1	WW	56	60	65	116	341.9
	DS					338.8
GO-2	WW	53	58	63	111	335.7
	DS					334.6

Table 3: Leaf descriptors at flowering (F), 21 DAF and physiological maturity (PM)

Variety	**Treatment**	**GLA (F) (cm^2)**	**GLA (21 DAF) (cm^2)**	**GLA (PM) (cm^2)**	**RWC (%) (21DAF)**	**RWC (%) (PM)**	**CL (21DAF)**	**CL (PM)**
BR	DS	624.30±143.66	386.09±134.37	56.27±67.80	71.07 ± 3.39	58.4 ± 5.13	17.72 ± 2.64	10.57±2.69
BR	WW	618.13±138.47	568.40±119.39	309.15±214.98	81.25 ± 6.05	64.95 ± 2.09	16.28 ± 2.26	10.12±2.26
DO	DS	957.73±199.34	879.88±161.40	481.10±276.83	86.47 ± 3.3	77.03 ± 8.94	20.29 ± 2.09	21.64±3.40
DO	WW	957.38±193.11	913.17±171.47	821.87±136.29	93.2 ± 2.91	85.38 ± 6.74	22.42 ± 3.4	20.75±2.09
GO-1	DS	593.38±171.84	290.89±192.45	159.58±130.32	69.08 ± 10.27	64.6 ± 12.44	15.33 ± 1.29	10.64±0.99
GO-1	WW	578.82±163.63	430.07±176.30	281.97±183.82	73.58 ± 10.68	72.4 ± 10.05	18.97 ± 0.99	7.64±1.29
GO-2	DS	618.13±144.31	225.62±205.20	112.61±133.29	72.55 ± 2.88	59.95 ± 2.92	24.55 ± 2.98	16.72±2.98
GO-2	WW	632.49±164.66	456.59±134.23	385.32±170.11	76.62 ± 9	63.85 ± 4.95	20.05 ± 0.77	17.80±0.77
KD	DS	574.79±165.27	188.29±106.70	79.03±94.71	73.35 ± 9.08	56.33 ± 4.48	28.98 ± 3.04	15.33±3.04
KD	WW	575.71±161.93	501.40±116.02	276.25±41.30	83.3 ± 7.93	81.4 ± 8.66	23.90 ± 1.05	29.26±1.05
KP	DS	743.21±241.61	620.06±176.83	405.56±164.62	91.35 ± 5.21	78.72 ± 7.71	32.1 ± 6.32	27.64±1.36
KP	WW	742.60±239.95	679.15±208.04	644.51±193.38	92.08 ± 5.59	88.1 ± 2.21	33.63 ± 1.36	24.36±6.32
NW	DS	785.58±173.80	263.09±234.01	130.91±159.63	74.6 ± 2.77	64.08 ± 8.46	23.79 ± 1.48	22.04±3.65
NW	WW	790.68±176.72	678.84±190.93	643.93±184.67	90.15 ± 7.68	82.12 ± 9	26.73 ± 3.65	16.66±1.48
LSD		**94.19**	**172.12**	**114.65**	**5.77**	**6.06**	**4.07**	**3.86**

GLA = Green leaf area, RWC = Relative water content, CL = Chlorophyll Level/SPAD reading

Green leaf area retention and chlorophyll content

Across all varieties, GLA was reduced in the drought-stressed plants compared to the respective well-watered at all sampling dates. At flowering, GLA in DO was the largest under well-watered conditions followed by NW, KP, GO-2, BR and then GO-1 and KD, which had similar sizes of GLA. These values also indicate that sorghum varieties with small GLA values produced smaller leaves. By 21DAF DO still maintain the largest GLA followed by NW and KP, which had similar GLA values, then BR, KD, GO-2 and GO-1 with the smallest GLA. Further variations occurred at physiological maturity, but Dorado (DO) maintained the largest GLA followed by NW and KP (with similar values). GLA in GO-2 was the fourth position largest followed by BR (which had the fourth largest GLA at 21 DAF). GO-1, which had the least GLA at 21 DAF had more GLA than the KD variety, which maintained the smallest GLA at PM. This indicates phenological differences among the seven sorghum varieties exposed to drought stress.

Green leaf retention varied considerably among the sorghum varieties under drought stress, however, Dorado (DO) maintained the largest GLA at all sampling dates just as under the WW conditions but with a reduction of almost 50%. Compared to the well-watered plants, the DS plants of DO retain more than 96% GLA at 21 DAF and more than 58% at PM. Naga White (NW) which had the second largest GLA at flowering lost more than 83% at 21 DAF and placed fifth with 38.76% of GLA compared to the well-watered plants. Kapaala (KP) which had the third largest GLA at flowering retained the second largest GLA at 21 DAF and PM. GLA in KP under DS treatment was 91.3% and 62.9% at 21 DAF and PM, respectively, compared to the GLA in the WW plants. Thus, GLA in these plants had reduced by 45.5% between flowering and physiological maturity, the least among the seven sorghum varieties, followed by DO. Thus, KP and DO can be described stay-green since these plants retained greenness during grain filling under drought-stressed conditions (Thomas & Ougham, 2014). BR, which had similar GLA as GO-2 at flowering retained the third largest GLA at 21 DAF, which was 68% compared to the corresponding well-watered plants. By physiological maturity, GLA had reduced by 91%, which was just 18% of the GLA in the well-watered plants. GO-1 had the fourth GLA, while GO-2 had the sixth with KD with the least. However, at physiological maturity, GO-1 had the 3rd largest GLA with a reduction of 73% between flowering and PM. The reduction of GLA in the drought-stressed KD plants between flowering the physiological maturity was 86%. Overall, the best performers in green leaf area retention were KP (63%), DO (59%) and GO-1 (57%). GO-2 and KD, with GLA retention of 29% at physiological maturity, were intermediate while NW and BR with 20% and 18% retention of GLA, respectively, were the poorest performers. Thus, based on retention of GLA Kapaala, Dorado and GO-1 could be termed as stay-green while the rest are senescent varieties.

The loss of chlorophyll under drought stress and as a leaf ages is a normal physiological phenomenon, however, in stay-green sorghum plants, chlorophyll catabolism is impaired or postponed (Munaiz et al., 2020). Chlorophyll content is a critical indicator of plant health and photosynthetic efficiency, particularly in crops like sorghum, which are often subjected to varying water availability. The data in **Table 3** reveal significant genotypic differences in chlorophyll content under well-watered (WW) and drought-stressed (DS) conditions, highlighting the impact of water availability on plant physiology. Indeed, chlorophyll levels in the drought-stressed KD were significantly ($P < 0.05$) reduced compared to the well-watered. At physiological maturity, Kadaga and Bawku Red were most affected by DS resulting in reductions of 47% and 40% respectively, in chlorophyll content from 21 DAF. In the other sorghum varieties, there were no significant differences between the well-watered and the drought-stressed plants at physiological maturity. However, chlorophyll levels in Dorado (DO) and Kapaala were the least affected by drought-stress. Indeed, chlorophyll catabolism was seemingly absent in the DO plants since chlorophyll levels were slightly higher in the drought-stressed plants compared to the well-watered ones. Kapaala (KP) also demonstrated remarkable resilience under drought stress, maintaining a chlorophyll levels comparable to the well-watered plants. Indeed, KP plants maintained the highest chlorophyll levels under drought stress conditions. Stay-green sorghum plants are known to maintain photosynthetically active leaves under limiting water conditions which enables them to continue to fill their grains. Plants under WW conditions had higher chlorophyll levels, consistent with existing research, indicating that adequate water supply enhances photosynthetic activity (Nemeskéri & Helyes, 2019). The maintenance of high levels of chlorophyll in the Kapaala and Dorado varieties under drought stress supports the maintenance of GLA and clearly shows their superior adaptability and resilience under water-limiting conditions. This characteristic is crucial for sustaining photosynthetic processes during periods of water deficit, which can lead to maintenance of high grain yields under such conditions.

Relative water content

Relative water content, an indicator of plant water status, was reduced in drought-stressed plants compared to their respective well-watered plants at 21 DAF and PM, suggesting that the leaves of plants under drought stress contained less water. KP and DO varieties had highest RWC under both well-watered and drought-stressed conditions at 21 DAF and PM, showing a consistent ability to retain water. GO-1 and GO-2 plants had the lowest RWC at 21 DAF. However, the reductions of RWC in BR, DO and NW plants due to drought stress were significant ($P<0.05$). At physiological maturity, drought stress significantly ($P<0.05$) resulted in further significant reductions of RWC in all varieties except in GO-2 plants. Kadaga and Naga White were most affected by DS resulting in reductions of 31% and 22% in RWC compared to their respective well-watered plants. The rest retained 89% (Kapaala) to 94% (GO-2) RWC values compared to the respective well-watered plants at physiological maturity. It is clear here that the stay-green sorghum varieties (KP and DO) again maintained higher leaf water contents compared to the others. The maintenance of leaf water could enable physiological activities, such as photosynthesis, thereby leading to higher grain yields.

Table 4: Yield determinants at 21 DAF and physiological maturity (PM)

Variety	Treatment	SW (21DAF) (g)	HSW (21DAF) (g)	NS (21DAF)	SW (PM) (g)	HSW(PM) (g)	NS (PM)	Panicle harvest index
BR	DS	6.4±0.72	1.90±0.18	747.25±13.76	7.76±0.97	2.72±0.28	737.75±10.50	60.6
BR	WW	7.98±0.53	2.00±18	758.00±29.36	9.16±0.85	2.89±0.28	744.00±12.41	51
DO	DS	13.80±0.32	1.50±0.18	1481.75±18.19	17.47±0.34	2.90±0.25	1465.75±218.39	59
DO	WW	13.43±0.62	1.85±0.24	1889.00±12.46	17.55±0.44	3.15±0.12	1872.50±83.24	56.7
GO-1	DS	4.35±0.53	1.60±0.18	702.00±8.98	4.92±0.50	2.38±0.37	714.25±17.54	64.6
GO-1	WW	5.88±0.50	2.08±0.25	681.75±11.03	6.35±1.66	3.01±0.20	673.75±24.45	58.6
GO-2	DS	9.23±0.52	1.83±0.22	683.75±7.63	10.13±1.98	2.52±0.22	691.00±7.75	53.2
GO-2	WW	10.28±0.43	2.00±0.18	672.75±13.94	12.06±0.58	2.70±0.54	661.25±7.46	40.1
KD	DS	9.63±0.85	1.71±0.17	1124.25±16.82	11.46±0.38	2.28±0.40	1140.00±54.60	29.5
KD	WW	10.25±0.77	1.75±0.24	1167.25±14.95	12.24±0.27	2.93±0.21	1161.50±56.62	31.9
KP	DS	13.58±0.41	1.65±0.13	1174.00±69.70	17.52±0.47	2.93±0.10	1178.25±98.73	25.2
KP	WW	13.95±0.53	1.76±0.13	1247.00±91.85	17.99±0.41	3.05±0.14	1239.25±196.64	18.4
NW	DS	6.28±0.93	0.85±0.21	935.50±14.84	7.36±0.58	1.20±0.18	924.00±27.60	42.9
NW	WW	8.00±0.83	1.06±0.13	967.75±18.06	9.60±1.20	1.91±0.55	950.00±47.96	39.7
LSD		**2.49**	**0.24**	**202.38**	**3.35**	**0.40**	**199.59**	**1.89**

BR = Bawku Red, DO = Dorado, KD = Kadaga, KP = Kapaala, NW = Naga White, DS = Drought-stressed, WW = Well-watered, SW = Seed weight, HSW = 100-seed weight, NS = Number of seeds.

Grain yield

Stay-green is a crucial agronomic characteristic that enables plants to keep their leaves actively engaged in photosynthetic processes, improving the grain-filling process even in adverse environmental conditions (Munaiz et al., 2020). Differences in mean seed weight at 21DAF were ~~was~~ mainly due to varietal differences with KP and DO having the greatest while GO-1 had least. For 100-seed weight, drought stressed caused significant reductions in DO and GO-1. Number of seeds were also reduced in DO plants under drought stress at 21 DAF. Therefore, seed size and seed set were affected in DO and GO-1 plants.

At physiological maturity, differences in seed weight were also due to genotypic differences. The greatest weed weight was produced by Kapaala and Dorado, while those in Kadaga and GO-2 were intermediate and GO-1, BR and NW produced the least seed weights. Seed weight was reduced by 23% in NW and GO-1, 16% in GO-2, 15% in BR, 6% in KD, 3% in KP and just 0.5% in Dorado. Seed size, estimated by 100-seed weight, was significantly ($P<0.05$) reduced by drought stress in GO-1, KD and NW and the reduction was severest in in NW. However, DO and KP produced the biggest seeds while the smallest seeds were produced by NW. The mean number of seeds was significantly ($P<0.05$) reduced in DO at physiological maturity while in the other plants seed production was not affected. However, DO, KD and KP produced the highest number of seeds followed by NW and BR with intermediate numbers while the lowest numbers were produced by GO-1 and GO-2 plants. Panicle harvest index (PHI) ranged from 18.4% in Kapaala to 58.6% in GO-1 under well-watered conditions. Under drought-stressed conditions, PHI ranged from 25% in KP to 61% in Bawku Red (BR). Apart from Kadaga, PHI was higher in the drought-stressed plants. Thus, in DO and Kapaala, grain yields were less affected by the stressed conditions. DO and KP plants, which retained greater GLA and higher chlorophyll levels under DS conditions had better grain yields compared to those which lost a significant portion of GLA and chlorophyll. The retention of GLA in DO and KP might have enabled photosynthesis to continue under stress conditions since chlorophyll catabolism was delayed, thus, making photosynthates available for grain filling to continue. Even though seed set was adversely affected by drought stress, seed size probably compensated for total gain yield (weight).

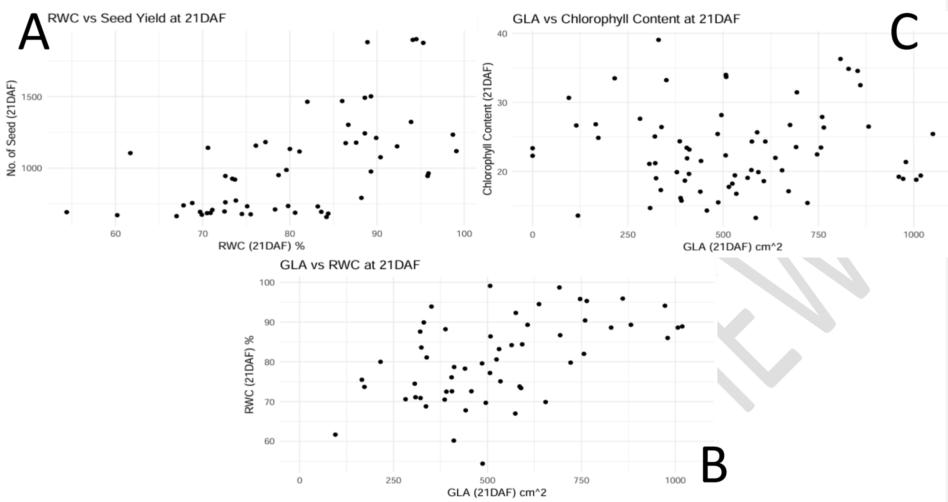

Figure 1: ~~Scatter plot depicting relationships among variables~~ Scatter plot showing the association between key variables at 21 DAF.

There was a positive relationship between RWC and number of seeds per panicle at 21 days after flowering (DAF) as shown in Figure 1(A). There appeared to be a trend where higher RWC values (> 80%) were associated with higher seed numbers, particularly in the range of 1000 - 1500 seeds. This is expected especially under drought stress conditions since leaf moisture content influences ~~phtosyntthesis~~photosynthesis and, consequently, seed set and grain filling. This supports our finding that sorghum varieties such as Dorado and Kapaala, which had higher RWC also produced greater number of seeds compared to the others. In plot B (Figure 1) a positive relationship between GLA and RWC at 21DAF is also demonstrated, indicating that plants which maintained larger green leaf areas also maintained higher relative water contents. However, since the relationship shows considerable scatter, other factors may also influence RWC, particularly the ability of roots to absorb water from deeper soil layers. In this study, the plants were raised in pots, hence, it was not possible for the roots to exploit other soil layers for water. A field study may on root characteristics and spread might provide some insights. GLA values between 0 and 500 cm² show greater variability in chlorophyll content, ranging from below 20 to above 35. This dispersion suggests that factors other than GLA may influence chlorophyll content, or the relationship may be non-linear (Poorter et al., 2009).

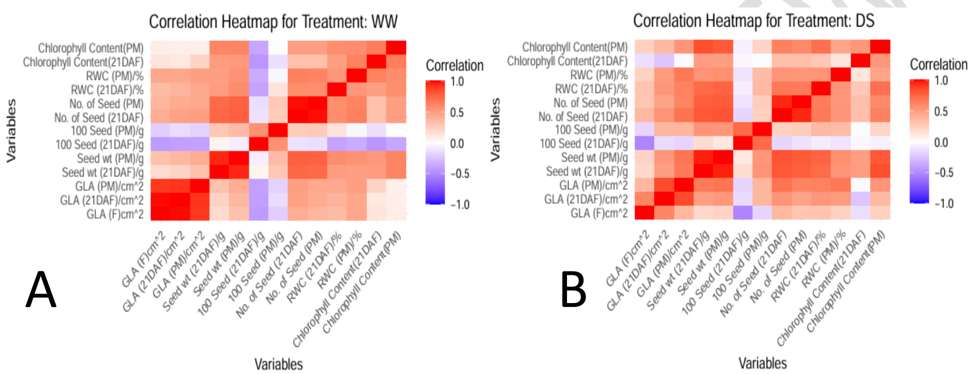

Figure 2: Correlation heatmap for treatments: WW & DS

The correlation heatmap for the drought-stressed (DS) plants revealed distinct patterns which differed from the well-watered conditions. Each heatmap displays pairwise correlation coefficients between traits, such as green leaf area (GLA), chlorophyll content (SPAD readings), seed weight, number of seeds, and relative water content (RWC). The correlations coefficients ranged between ±1.0 with red indicating positive correlations, blue representing negative correlations, and white reflecting no correlation. These patterns revealed the differences in plant responses under optimal and stressed environmental conditions.

In the WW plants (Figure 2 A), strong positive correlations dominate. GLA retentions at different stages were highly correlated. Similarly, seed weight traits, including individual and 100-seed weights, exhibit strong positive relationships, reflecting favorable growth conditions for reproductive traits. Therefore, under normal conditions, it is possible to use GLA at flowering to select for the other GLA traits.

In contrast, under the DS (Figure 2 B) a more complex pattern of correlations ~~were~~was observed. While positive correlations among GLA and SPAD readings remain, their strengths are slightly reduced, reflecting the impact of water limitation on growth consistency. There were also strong positive correlations among GLA measurements across various sampling dates (flowering, 21DAF and PM). 21DAF is a critical post-flowering stage where plants generally allocate resources for seed development. This timing is significant as drought stress during this period can influence both seed quantity and quality, making it ideal for examining correlations between growth, leaf area, and seed-related traits. Studies show that water availability at this stage can impact final yield, making it crucial for understanding responses to stress at key phenological points (Blum, 2010). The relationships appeared more pronounced, suggesting that early leaf area development could be a stronger predictor of later green leaf area maintenance under water limitation. This tight correlation in GLA measurements might indicate that plants struggling early with drought stress continue to show reduced leaf area throughout their development. Seed-related parameters showed interesting shifts under drought conditions. While seed weight measurements between 21 DAF and PM were positively correlated, the relationship appeared slightly weaker compared to well-watered conditions. The number of seeds at 21 DAF and PM showed strong positive correlation, but there were notable negative correlations (shown in purple) between 100-seed weight and other parameters, particularly at 21 DAF. This suggests that drought stress may force trade-offs between number of seeds and seed size, with plants potentially sacrificing individual seed weight to maintain overall seed numbers (Chaves et al., 2009).

Additionally, stronger negative correlations emerge, particularly between RWC and reproductive traits like seed weight and number of seeds, suggesting significant trade-offs under water stress. Negative relationships between chlorophyll content and seed traits are also more pronounced, highlighting the physiological adjustments plants make to cope with drought, such as prioritizing water retention or photosynthesis over reproductive output.

The heatmaps highlight the contrasting dynamics of plant trait interactions under well-watered and drought-stressed conditions. In the WW plants, the dominance of positive correlations underscores a harmonious environment where traits interact synergistically to maximize growth and yield. On the other hand, plants under DS were more variable in their responses to the stress, with a mix of positive and negative correlations. This highlights the physiological trade-offs plants face under stress, as they allocate limited resources to maintain critical functions like water retention and photosynthesis while sacrificing aspects like seed development. While WW conditions promote uniform growth and positive trait relationships, DS conditions result in a more variable and complex interplay, reflecting the plant's adaptive responses to water scarcity **(Figure 2)**. For example, the correlation between seed weight and relative water content under drought stress showed a strong positive correlation comparative with well-watered treatments. Such tightly linked physiological processes under drought stress may reflect mechanisms for managing limited resources, with physiological aspects like leaf area, water status, and tissue composition becoming increasingly interdependent. This indicates an evolved response where plants integrate various parameters to adapt to and survive in water-limited environments (Tardieu et al., 2018).

The correlation heatmap analysis further elucidates patterns among variables under both WW and DS treatments. Under WW conditions, strong positive correlations were observed among GLA, RWC, seed weight, and chlorophyll content across all varieties. In contrast, the DS treatment revealed distinct patterns where correlations weakened or became negative among certain variables. This divergence underscores the impact of drought stress on physiological relationships within plants. For example, while GLA may remain positively correlated with RWC under optimal conditions, this relationship may diminish under stress due to reduced photosynthetic capacity or impaired water retention mechanisms (Wassie et al., 2023).

Table 5: Correlation Analysis of GLA, Chlorophyll content and RWC with Seed Yield under Drought Conditions

Correlation_GLA_Seed_Yield				
	Estimate	**P_value**	**Conf_Lower**	**Conf_Upper**
	0.543794	0.00278033	0.21416621	0.762231517
Correlation_RWC_Seed_Yield				
	Estimate	**P_value**	**Conf_Lower**	**Conf_Upper**
	0.671931	9.01767E-05	0.398835088	0.835550621
Correlation_Chlorophyll_Content_Seed_Yield				
Statistic	**Estimate**	**P.value**	**Conf.Lower**	**Conf.Upper**
2.967962	0.503053	0.006361	0.160006	0.737683

GLA and seed yield were significantly positively correlated ($r = 0.54$, $P = 0.003$). This indicates that plants which are able to maintain larger green leaf area produce higher seed yields under drought conditions. Indeed, in stay-green sorghum varieties, maintenance of GLA under drought stress conditions results in higher grain yields (Borrell et al., 2000a). Under WW conditions, all varieties exhibited greater GLA and seed weight compared to those under DS conditions. The DO and KP varieties consistently had the highest GLA and seed weight values under both treatments, highlighting their genetic potential for biomass production. This suggests that DO and KP plants were able to obtain adequate water supply thereby enhancing photosynthetic activity, leading to increased growth rates and reproductive success (Cao & Qiu, 2024). Conversely, varieties such as GO-2 and NW showed substantial reductions in GLA and seed weight under DS conditions. The lower performance of these varieties can be attributed to their inherent genetic vulnerabilities to drought stress. It has been argued that the expression of the stay-green phenotype is the consequence of water conserving mechanisms operating earlier during crop development (Vadez et al., 2011, 2013; Borrell et al., 2014a, b). Among these mechanisms include the capacity to restrict transpiration under high evaporative demand (Choudhary et al., 2013), a smaller crop canopy (Kholová et al., 2014; Borrell et al., 2014a, b), a lower number of tillers that decreases the canopy size (Kim et al., 2010a, b; van Oosterom et al., 2011; Borrell et al., 2014a, b), and a smaller size of the upper leaves (Borrell et al., 2014b). Thus, the reductions in GLA could be an adaptive mechanism to conserve water for grain production. Research has shown that plants with deeper root systems are generally more capable of accessing soil moisture during drought periods (Khan et al., 2020). Indeed, the performance of sorghum is known to be influenced by deeper rooting under terminal water stress (Mace et al., 2012; Singh et al., 2012; Borrell et al., 2014b). The observed variability among these sorghum varieties could be exploited in breeding programmes to develop genotypes with resilience to the changing climate.

Similarly, the correlation between RWC and seed yield was even stronger ($r = 0.67$; $p < 0.001$). This suggests that RWC, which reflects better water retention in plants, is closely linked to higher seed yields under drought stress. RWC serves as a critical measure of a plant's ability to maintain hydration during periods of stress. In the current study, while most varieties experienced a decline in RWC under DS conditions, KP and DO maintain relatively high RWC levels. This ability could be attributed to enhanced water retention mechanisms such as osmotic adjustment and improved stomatal conductance. Osmotic adjustment allows plants to maintain turgor pressure by accumulating solutes within their cells, facilitating continued cellular function even when external water availability is limited (Munns & Tester, 2008). The positive correlation between RWC and number of seeds at 21 DAF suggests that higher RWC values are associated with increased reproductive success. This finding emphasizes the role of water availability not only in vegetative growth but also in reproductive outcomes. Additionally, the relationship between GLA and RWC further highlights that plants with larger leaf areas are better positioned to capture sunlight for photosynthesis while maintaining hydration. Plants with larger leaf areas capture more sunlight, enhancing photosynthesis through increased chlorophyll activity. High RWC supports this process by maintaining turgor pressure, which keeps leaves optimally oriented for light absorption and ensures stomata remain open for carbon dioxide (CO_2) uptake. Adequate hydration also facilitates transpiration, cooling the leaf surface and preventing heat stress, thus, sustaining photosynthetic efficiency and energy production for growth and reproductive success. This synergistic relationship underscores how water availability influences vegetative and reproductive outcomes, particularly under stress conditions (Blum, 2010).

The positive correlation coefficient of 0.503 obtained from the correlation analysis indicates a moderate positive relationship between chlorophyll content and seed weight, with a statistically significant p-value of 0.006. The 95% confidence interval further confirms this relationship, as it does not include zero, suggesting robustness in the observed correlation. This positive correlation underscores the critical role of chlorophyll content in determining seed weight. Higher chlorophyll levels during the early reproductive stage (21 DAF) may enhance photosynthetic activity, leading to increased production and translocation of assimilates to developing seeds. The relationship is consistent with the understanding that chlorophyll content during critical growth stages directly influences grain yield components, including seed weight (Wang et al., 2016).

Table 6: Regression analysis of GLA, Chlorophyll content and RWC on seed yield at 21 DAF

Regression_GLA_Seed_Yield				
Term	**estimate**	**std.error**	**statistic**	**p.value**
(Intercept)	662.9331718	105.7709785	6.267628	1.24E-06
GLA_21DAF	0.684678463	0.207224463	3.304043	0.00278
Regression_RWC_Seed_Yield				
Term	**estimate**	**std.error**	**statistic**	**p.value**
(Intercept)	-538.118149	330.2591785	-1.62938	0.11529
RWC_21DAF	19.71368595	4.261357698	4.626151	9.02E-05
Regression Seed_Yield_Chlorophyll				
Term	**estimate**	**std.error**	**statistic**	**p.value**
(Intercept)	15.21233	2.931559	5.18916	2.04E-05
Seed_Yield_21DAF	0.900217	0.303311	2.967962	0.006361

The regression analysis indicates that GLA significantly contributes to seed yield under drought conditions, with a positive estimate of 0.68 ($p = 0.0028$). This result implies that for every unit increase in GLA measured at 21 days after flowering, the seed yield is expected to increase by 0.68 units. The significant intercept (estimate = 662.93, $p < 0.0001$) suggests a baseline yield value, emphasizing the role of GLA as a contributing factor to seed productivity.

For RWC, the positive regression estimates of 19.71 ($p < 0.0001$) indicates a strong association with seed yield, suggesting that a unit increase in RWC at 21 days after flowering corresponds to a 19.71 unit increase in seed yield. Although the intercept for RWC (-538.12) is not statistically significant ($p = 0.115$), the strong positive effect of RWC on seed yield demonstrates its potential as an important factor for enhancing drought resilience in sorghum.

In the regression analysis between seed weight and chlorophyll content at 21 DAF, the intercept of 15.212 indicates the predicted chlorophyll content when seed weight is zero. While this value provides a baseline, it may not be biologically significant since seed weight cannot realistically be zero. The slope estimate of 0.90 suggests that for every unit increase in seed weight, chlorophyll content increases by 0.90 units. This positive relationship is statistically significant ($p = 0.006$). This result suggests that the plant with higher chlorphyll levels produce heavier seeds, likely reflecting the ability of plants to allocate more resources to photosynthetic processes, which in turn enhance seed development. This aligns with findings that chlorophyll content, an indicator of photosynthetic capacity, directly contributes to assimilate production, which is critical for seed filling and overall grain yield (Nestler et al., 2016).

Inherent Genetic Differences

The observed performance variability among sorghum varieties under DS conditions points to inherent genetic differences in drought tolerance as observed by Danquah et al. (2019). Varieties like DO and KP maintained relatively high GLA and seed weight even under DS conditions, indicating strong drought tolerance mechanisms. In contrast, varieties such as GO-2 and NW exhibited significant reductions in both GLA and seed weight under DS conditions, highlighting their vulnerability to water stress. Genetic resilience in crops can be attributed to various traits including root architecture, stomatal regulation, and metabolic adjustments that enhance water use efficiency (Jogawat et al., 2021). For example, deeper root systems enable plants to access moisture from lower soil layers during drought periods, while efficient stomatal regulation minimizes water loss through transpiration. The findings from this study emphasize the importance of selecting diverse genetic backgrounds when developing new sorghum cultivars capable of thriving in variable climatic conditions.

CONCLUSION

In conclusion, varieties such as DO and KP showcase resilience through their ability to maintain higher GLA, seed weight, chlorophyll levels and RWC even under drought stress. Conversely, other varieties demonstrated vulnerabilities due to lower inherent drought tolerance. The strong positive correlations observed between GLA, RWC, chlorophyll levels and seed yield underscore the importance of these parameters as reliable indicators of drought resilience. In particular, RWC's role in preserving hydration appears crucial for sustaining grain production under water-limited conditions. Higher chlorophyll levels typically correlated with increased photosynthetic efficiency, enabling plants to better utilize available light energy for growth, particularly under optimal water conditions. In the context of drought stress, maintaining chlorophyll levels become essential for sustaining photosynthesis and mitigating the adverse effects of water scarcity on plant development. These findings suggest that the stay-green trait and efficient water-use strategies are vital targets for breeding programmes aiming to enhance sorghum's drought tolerance.

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