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Physiological Resilience of Bambara Groundnut (*Vigna subterranea* L. Verdc) Genotypes to Intermittent Periods of Drought Stress at Different Growth Stages

Kennedy Agyeman[1]*, Felix Frimpong[1], Patricia Amankwaa-Yeboah[1], Isaac Osei-Bonsu[1], Stephen Yeboah[1], Agbesi Keteku[1], Paul Marno[1], Mavis Brempong Brempong[1], Eric Owusu Danquah[1], Asamoah Emmanuel Adjei[1], Michael Odenkey Quaye[2], Joseph Nketiah Berchie[1]

[1]CSIR-Crops Research Institute, Fumesua-Kumasi, Ghana
[2]Department of Agricultural Science Education, University of Education, Winneba, Ghana
Email: *agyemanken@yahoo.com

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Abstract

Different genotypes of Bambara groundnut (*Vigna subterranea* L. Verdc) grow well under conducive environmental conditions, provided that adequate soil moisture is available during vegetative and reproductive phases. However, drought stress is the major limiting factor to bambara production, which accounts for up to 40% of yield losses. This situation could worsen due to drastic and rapid changes in the global climate. Landraces grown by farmers are low-yielding. Understanding the physiological response of different genotypes to drought stress is key to achieving food security through crop improvement and diversification. This study focused on variations in the response of Bambara groundnut genotypes to intermittent drought stress during the crop's critical growth (vegetative and reproductive) stages. The experiment was undertaken at CSIR-Crops Research Institute Screen-house. The treatments were used in a factorial experiment with three replications in a randomized complete block design. The Bambara genotypes showed considerable variability in tolerance to drought stress. Drought stress during vegetative and reproductive stages significantly reduced crop growth indices, the leaf relative water content, chlorophyll content and leaf area. Drought stress during vegetative and reproductive stages had a more severe impact on the seed yield of genotype Nav Red, reducing it by 69% and 13%, respectively. Farmers should pay more attention to adopting drought-tolerant and high-yielding varieties for improved Bambara groundnut productivity and livelihoods.

Keywords

Drought Stress, Bambara Groundnut, Genotypes, Seed Yield

1. Introduction

Bambara groundnut has historically been part of inexpensive food security crops throughout Sub-Saharan Africa as they play a major role in the fight against hunger, drought, soil fertility decline and malnutrition. Regarded as the third most important legume crop after groundnut (*Arachis hypogea*) and Cowpea (*Vigna unguiculata*) in Africa [1], its growth and development are challenged by several climatic and edaphic constraints among which drought stress and nutrient deficiencies are increasingly important. Recent evidence on strategies for the adaption of Bambara groundnut to drought stress [2], the morphological and physiological response of Bambara to drought [3], and the identification of traits influenced yield under water limitation.

Although Bambara groundnut's growth and yield potential have been documented [1] [4], among the questions yet to be addressed is how alteration in water availability during vegetative and reproductive growth stages may be a constraint to the growth and yield of different cultivars. Most importantly, the response and sensitivity to the time of drought stress vary significantly among different species and varieties and are linked to the intensity and length of the stress [5].

Drought stress negatively influences crop growth and development and has been described as the most damaging climate hazard affecting two-thirds of the global population [6] and threatening food security [7]. The frequency and severity of drought are projected to increase due to decreased precipitation and increased evaporation due to global climate change [8]. Based on its negative implication on crop growth and development, plants have advanced various molecular strategies to reduce their use of resources and adjust their growth to adapt to hostile environmental conditions [9].

Usually, crop responses to water stress are measured using selected physiological parameters such as water potential, leaf relative water content (LRWC), stomatal conductance, photosynthesis, or osmotic adjustment, which have been shown in several research findings to be good indicators of drought tolerance [10]. Crop responses to drought stress, on the other hand, vary significantly and are dependent on the severity and duration of the drought stress [11].

The complexities of such responses whether physiological or morphological are often regarded as multifaceted and understanding crop responses to water stress is important and fundamental to the selection and breeding of drought-tolerant crops, especially in the case of neglected underutilized species (NUS) such as Bambara groundnut where there is a lack of such information.

In this study, drought stress was imposed at different growth stages to explore the effects on the growth, development and yield of Bambara groundnut genotypes. The study aimed to 1) evaluate the effects of water deficit on morphological and physiological responses of eight Bambara groundnut genotypes and 2) ascertain the growth during which water deficit exerts the most significant negative effect on the morphology and phenology of Bambara groundnut. The results of this study could help farmers make the best use of limited water resources in areas that suffer water shortages, increase yield and provide strong theoretical support for water-saving agricultural methods.

2. Materials and Methods

2.1. Experimental Site

This experiment was conducted in a screen-house at the experimental station of CSIR-Crops Research Institute (6˚42'4.0728"N, 1˚31'53.364"W) in Fumesua, Ejisu municipality, which falls within the Forest agro-ecological zone in Ghana. Annual temperatures range from a minimum of 21.1˚C to a maximum of 32.7˚C and a mean of 31.6˚C. The average annual rainfall is 1550 mm, and the seasonal distribution of rainfall is uneven; there is less precipitation in the third quarter of the year.

2.2. Planting Materials

Eight (8) Bambara groundnut landraces (Table 1) were evaluated in this study to determine the effect of drought stress on agro-morphology and phenology. Seeds of the genotypes were provided by the seed bank of Crops Research Institute, Ghana. Seeds were surface sterilized in 0.58% sodium hypochlorite solution for one minute with frequent shaking and subsequently rinsed under running water. Two seeds were sown per pot in March 2020, and seedlings thinned to one per pot 14 days after seedling emergence.

The temperature in the screen house was maintained at a daily mean of 32˚C (with a ±5˚C diurnal variation). Watering of the plants was done twice a week.

Table 1. Attributes of selected Genotypes.

	Genotype	Key traits	Seed colour	Origin
1	Kenya Capstone	Dual purpose; drought tolerant	Mottled Black	Kenya
2	Nav red	High yielding; dual purpose	Red	Ghana
3	Tiga Necuru	High-yielding	Cream	Mali
4	Burkina	High-yielding, farmer preference	White	Burkina Faso
5	Bolga Red	High-yielding	Red	Ghana
6	S19-3	High-yielding	Black	Namibia
7	Mottled Cream	Earliness	Cream	Ghana
8	Tom	Moderate yielding	Brownish cream	Ghana

The plants were sown in plastic pots measuring 18 cm in diameter and 20 cm in height with holes created at the bottom to allow for drainage. These were laid out with water-permeable fleece to reduce soil loss, restrict root growth, and prevent waterlogging. The plastic pots were filled with 5.0 kg of air-dried and steam-sterilized natural sandy soil.

2.3. Experimental Design and Treatment

The experiment was set up as an 8 × 3 factorial with treatments arranged in a randomized complete block design (RCBD) and replicated three times. The factors were Bambara groundnut landraces (**Table 1**) and the timing of water stress. Water deficit treatments were:

T1: Control (plants were watered throughout with 600 ml of water per plant, twice a week);

T2: Vegetative stage (water stress imposed starting 25 DAS for 21 days);

T3: Reproductive stage (water stress imposed starting 40 DAS for 21 days).

Each plant in each pot for each landrace was treated as a replicate.

2.4. Soil Preparation, Potting and Treatment Imposition

Soil collected from the Crops Research Institute's fields at 0 - 15 cm depth was sieved through a one cm mesh to remove clods and stones. Each plastic pot was filled with five (5) kg of soil and the weight of the pot and soil were determined and recorded. Clean water was added until full saturation, allowed to drain for 48 h until field capacity, and then re-weighed to ensure uniformity in soil moisture levels. Preliminary studies based on pot capacity determined the amount of water added to each pot. Nitrogen (N): Phosphorus (P): Potassium (K) fertilizer was applied to the soil at 60 kg/ha, based on soil analysis.

Plants were watered until the formation of three fully expanded trifoliate leaves. Irrigation was withheld to simulate drought stress conditions during the vegetative and reproductive growth phases.

2.5. Plant Measurements

Relative water content (RWC) was measured using the entire second lateral leaflet of the trifoliate leaf (Shackel and Hall, 1983). Four pairs of leaflets were excised and weighed immediately and recorded as fresh weight (FW). They were then placed in pre-weighed airtight plastic bags containing water and placed in a picnic cooler (around 10˚C - 15˚C) but not frozen on ice. The turgid weights (TW) and dry weight (DW) were measured after drying the leaves in the oven for 24 hours at 80˚C. Relative water content was determined according to Shackel and Hall (1983) using the formula:

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100\text{; according to [12]}$$

The total leaf surface area per plant was measured using an LI-3000C (LICOR, USA) Portable Leaf Area Meter.

The chlorophyll content index (CCI) was measured at 40 DAS using the SPAD-502 Plus Chlorophyll Meter (Konica Minolta, USA) on the adaxial surface of fully expanded, fully exposed and actively photosynthesizing leaves.

At maturity, the plants were harvested to measure yield components (number of pods per plant, number of seeds per pod, 100 seed weight) and seed yield per plant. Ten plants were sampled and used for growth analysis, and the other samples were used to measure the shoot weight and calculate harvest index (HI) as the fraction of pod weight to total biomass, excluding root weight.

2.6. Data Analysis

The data were processed using Microsoft Excel 2013. The data were fitted and figures were generated using Origin 8.5 (OriginLab, MA, USA). Data were subjected to analysis of variance (ANOVA) using GenStat® (Version 14, VSN International, UK). Standard error of difference (SED) was used to separate means at a 95% level of probability.

3. Results

3.1. The Percentage Relative Leaf Water Content of Bambara Groundnut Genotypes to Vegetative and Reproductive Stage Drought Stress

Table 2 shows the relative leaf water content percentage during water deficit and recovery at different growth stages for the Bambara groundnut landraces. The leaf-relative water content decreased significantly ($P < 0.001$) among all genotypes

Table 2. Percentage of leaf relative water content, (LRWC %) after 21 days of drought imposition for hydrated and drought-stressed leaves of Bambara groundnut.

GENOTYPES	FULLY IRRIGATED		VEGETATIVE STAGE		REPRODUCTIVE STAGE	
	Initial (LRWC %)	Final (LRWC %)	Stressed (LRWC %)	Recovered (LRWC %)	Stressed (LRWC %)	Recovered (LRWC %)
BOLGA RED	89.8	91.4	79.0	88.7	74.7	90.0
BURKINA	87.2	91.6	81.7	87.3	69.0	91.8
KENYA CAPSTONE	95.9	96.9	82.3	89.9	76.2	93.1
MOTTLED CREAM	92.0	95.0	81.1	90.2	75.1	93.1
NAV RED	95.8	96.8	81.6	93.3	79.6	92.3
S19-3	90.4	93.1	81.2	92.1	79.8	90.5
TOM	87.8	90.1	81.1	88.8	73.8	93.1
UNISWA RED	93.9	95.5	86.2	93.6	80.9	96.1
CV (%)	**3.6**	**2.7**	**5.7**	**3.7**	**5.7**	**3.4**
SED	**2.6**	**2.1**	**3.8**	**2.5**	**3.5**	**2.6**
MEAN	**91.6**	**93.8**	**81.8**	**90.5**	**76.1**	**92.5**

when drought was imposed at the vegetative and reproductive phases relative to fully irrigated (Table 2). The reduction in RWC was more pronounced with water deficit treatment at the reproductive stage of growth for all genotypes. However, recovery of RWC % for the stressed plants at the vegetative stage failed to reach the values for control and had the lowest recovery rates compared to their control plants (Table 2).

3.2. Chlorophyll Content

The effect of water stress and genotype was significant ($P < 0.01$) on leaf chlorophyll content as estimated by the SPAD chlorophyll meter readings (SCMR) (Figure 1). Water deficit caused significant reductions in the chlorophyll levels among all genotypes. Reduction in chlorophyll level was more severe at both the vegetative and the reproductive stages.

The reduction in chlorophyll levels was 57.1% lower in Nav Red when stressed during the vegetative stage as compared to control plants. SPAD chlorophyll content of Nav Red was also reduced under drought stress at the reproductive stages. Under water-stressed conditions, the Kenya Capstone landrace had the highest SCMR compared to the other landraces, suggesting the presence of a stay-green train in the Kenya Capstone genotype.

3.3. Leaf Area

Leaf area measurement was significantly higher for non-stressed plants compared to water-stressed plants during reproductive and vegetative growth stages (Figure 2). For the water-stressed plants, leaf area reduction was pronounced at the reproductive stage compared to the vegetative stages (Figure 2). The results indicate that all the genotypes had a smaller leaf area under water stress than the control (Figure 2), with the highest reduction in leaf area noted in the Mottled

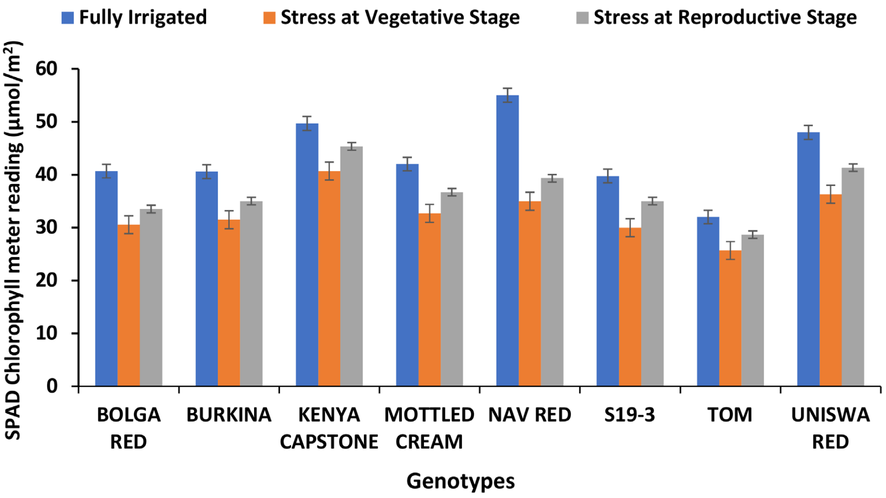

Figure 1. Variations in SPAD chlorophyll meter readings of Bambara groundnut landraces. Data shown are the means of three replicates with standard error of means indicated by vertical bars.

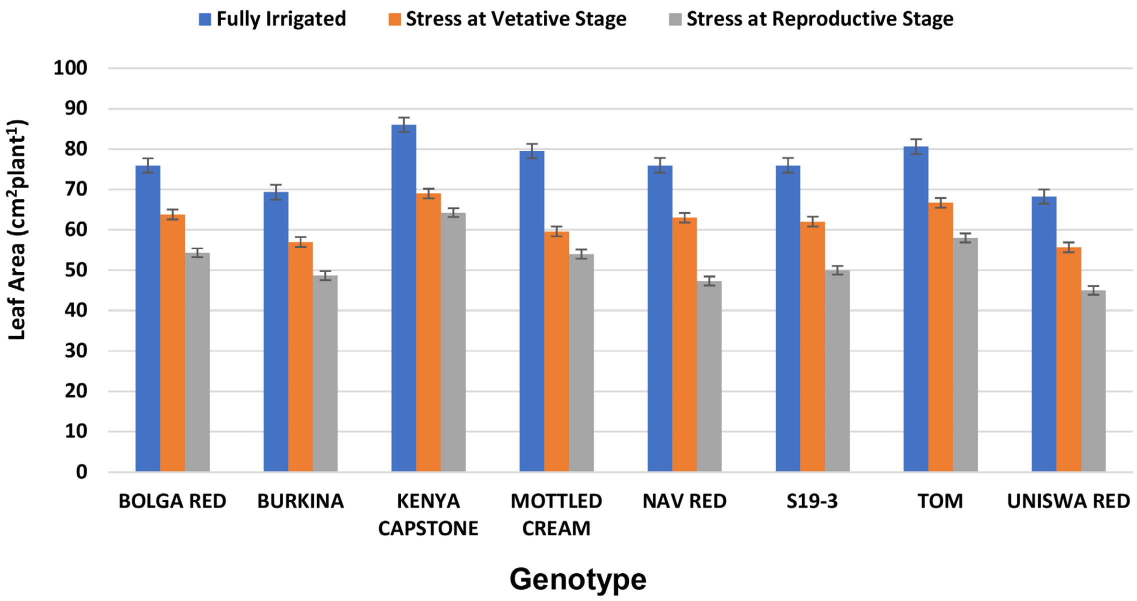

Figure 2. Variations among Bambara groundnut genotypes for Leaf Area. Data shown are the means of three replicates with standard error of means indicated by vertical bars.

cream genotype (47%) during the vegetative stages phenophases. The leaf area of stressed plants had the lowest leaf area, with the leaf area decreasing from the mean value of 62.2 to 52.6 cm^{-2} (**Figure 2**).

3.4. Plant Height at Flowering

The analysis of variance showed a significant ($P < 0.05$) main effect of genotype and the interaction of genotype and water stress on plant height. Under non-stressed conditions, genotypic variations existed, with Uniswa Red (25.0 cm) and Burkina (18.7 cm) recording the highest and lowest plant height (**Table 3**). However, under water-stressed conditions, Kenya Capstone was the tallest (plant height = 27.0 cm) whereas Nav Red was the shortest at vegetative (23.0 cm) and reproductive (17.0 cm) stages, respectively.

The interaction between water stress and landrace was highly significant ($P < 0.001$). Although in most genotypes plant height increased under non-stressed conditions, Kenya Capstone (vegetative and reproductive stages) and Bolga Red (reproductive stage), genotypes grew taller under water-stressed relative to irrigated conditions.

3.5. Days to Flowering

Results of crop phenology, observed as days to flowering, showed a significant ($P < 0.001$) interaction effect between genotypes and water stress at different growth stages of Bambara groundnut. Differences in the days to 50% flowering between water-stressed and well-watered plants were particularly visible after the imposition of stress at the vegetative stage, resulting in a consistent and significant prolonging in the days to flowering of the tested genotypes (**Table 2**).

Table 3. Interactive effect of water stress and genotypes on Plant height, days to 50% flowering, and Days to maturity of Bambara groundnut.

Genotype	Plant Height (cm)			Days to 50% Flowering			Days to Maturity		
	Fully Irrigated	Vegetative Stress	Reproductive Stress	Fully Irrigated	Vegetative Stress	Reproductive Stress	Fully Irrigated	Vegetative Stress	Reproductive Stress
BOLGA RED	24.0	25.3	22.0	32.0	36.0	33.0	109.7	108.7	109.2
BURKINA	18.7	25.7	23.3	35.3	34.5	34.0	111.3	110.3	110.8
KENYA CAPSTONE	23.7	27.0	26.7	39.3	39.7	39.3	125.0	127.7	126.3
MOTTLED CREAM	24.7	24.3	20.7	36.3	35.7	34.7	109.7	108.7	109.2
NAV RED	24.7	23.0	17.0	37.0	35.3	35.0	114.0	113.3	113.7
S19-3	24.7	24.7	24.0	36.3	34.7	33.7	121.0	119.0	120.0
TOM	19.7	26.7	21.3	40.0	38.0	37.0	116.3	114.3	115.3
UNISWA RED	25.0	25.7	23.3	37.7	35.7	34.7	127.0	124.0	125.5
CV (%)		7.7			2.5			1.3	
SED		1.5			0.7			NS	
MEAN		23.5			36.1			116.3	

The pattern of flowering showed large variability among genotypes across all watering regimes, Bolga Red had a shorter flowering duration (32 days) under fully irrigated conditions, whereas TOM took the highest number of days to 50% flowering also under well-watered treatment. Drought stress imposed at both vegetative and reproductive stages, significantly ($P \leq 0.001$) decreased the number of days to 50% flowering.

3.6. Number of Pods

The yield-related parameters were significantly affected by genotypes, water stress and genotype x water-stress interactions. The average number of pods per plant in Bambara groundnut was maximum (35 pods per plant) in well-watered plants, which significantly declined to 25 and 26 pods per plant when plants were exposed to water stress at vegetative and reproductive stages, respectively. Among the water-stressed treatments, Nav Red had the highest number of pods during both vegetative and reproductive stages (33 and 39, respectively). On the other hand, Tom had the least number of pods (20) at the vegetative stage, while it produced no pods during the reproductive stage under water-stressed conditions.

The interaction between water stress and landrace concerning pod number per plant was highly significant ($P < 0.001$, Table 4). Among the genotypes, pod number was reduced by 11.8% and 25.1% for the highest yielding Nav Red when stressed during the reproductive and vegetative stages, respectively.

Table 4. Interactive effect of water stress and genotype on the number of pods and Seed Yield of Bambara groundnut.

Genotype	Number of pods/plant			Seed yield/plant(g)		
	Fully Irrigated	Vegetative Stress	Reproductive Stress	Fully Irrigated	Vegetative Stress	Reproductive Stress
BOLGA RED	33.0	25.3	29.0	17.8	13.7	15.7
BURKINA	36.0	21.0	25.7	20.6	12.0	14.7
KENYA CAPSTONE	32.7	24.3	28.7	19.7	10.1	11.7
MOTTLED CREAM	32.3	20.7	25.7	20.9	13.3	16.6
NAV RED	45.0	33.7	39.7	29.4	17.3	25.8
S19-3	38.3	26.3	32.0	25.1	14.5	21.0
TOM	28.3	20.0	0.0	12.2	5.6	0.0
UNISWA RED	37.0	28	32.7	22.5	17.0	19.9
CV (%)		4.7			19.1	
SED		1.03			2.34	
MEAN		27.18			15.03	

3.7. Dry pod Yield

Genotype x water-stress interaction was significant ($P \leq 0.001$) indicating that water stress influenced the pod yield of Bambara groundnut (Figure 3). The average dry pod weight was maximum (47 g/plant) under well-watered treatment and it declined significantly by 62 and 46% when plants were subjected to water stress at the vegetative and reproductive stages, respectively. Among the well-watered genotypes, Nav Red was the highest-yielding genotype (54.7 g/plant) followed by Uniswa Red (53.5 g/plant).

On the contrary, Mottled Cream (39.0 g/plant) and Kenya Capstone (41.0 g/plant) produced the highest number of pods per plant under stress at vegetative and reproductive stages of growth respectively (Figure 3). Drought stress reduced the pod dry yield, but, improved genotypes and landraces responded differently to the stress. Nav Red had pod dry yield reduction of 52.5% and 30.5% under water-stressed conditions at the vegetative and reproductive stages respectively.

3.8. Seed Yield

The seed yield of Bambara groundnut genotypes was significantly affected by water stress imposed at the vegetative and reproductive stages with significant interaction between genotype and water stress (Table 4). Under fully irrigated conditions, Nav Red had the highest seed yield (29.4 g/plant), which was significantly different from the rest (Table 4). Among the water-stressed treatment, the highest average seed yield per plant was 25.8 (Nav Red) at the reproductive stage which declined significantly by 49.1% when water-stressed was imposed at the

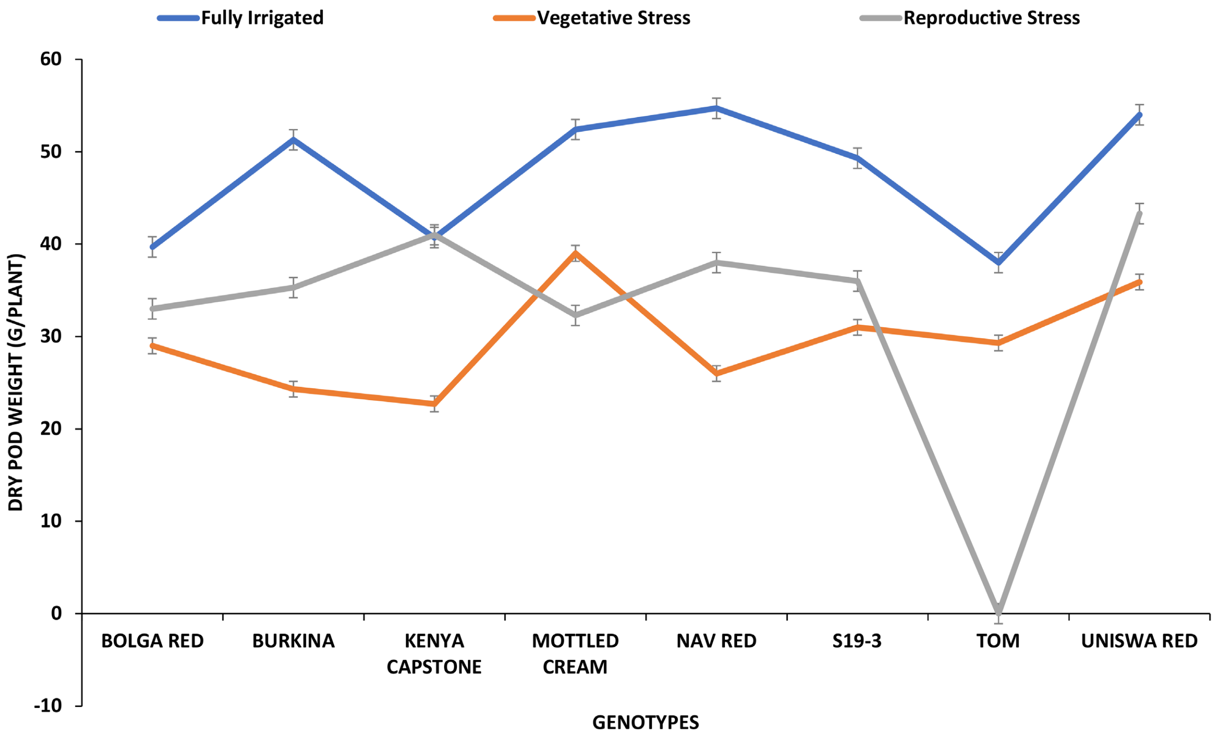

Figure 3. Dry Pod Weight of Bambara groundnut as affected by watering regime imposed at different growth stages. Error bars margins represent the standard error of means.

vegetative stage. Similarly, water-stressed at the reproductive stage decreased the seed yield per plant for all genotypes. Tom was the genotype most affected by water stress, with more severe effects observed when stress was imposed at the reproductive stage where it produced no pods or seeds.

3.9. Dry Matter of Different Bambara Groundnut Genotypes to Water Stress

As shown in Figure 4(a), genotypes significantly varied in their response to water stress conditions. Under the stressed conditions at the reproductive stage, the decline in dry matter was gradual for most of the genotypes. The average dry matter yield was significantly highest with Kenya Capstone (51.7 g/plant) (Figure 4(a)). The interaction between water stress and genotype was not significant, although dry matter yield was generally greater under fully irrigated treatment relative to the stressed plants at both vegetative and reproductive stages (Figure 4(b)).

Furthermore, no significant difference was observed between water stress at the vegetative and reproductive stages (Figure 4(b)).

4. Discussion

The Bambara groundnut has been integrated into the natural ecosystem, making it a key model for studying the genetic differences in growth patterns and responses to water constraints. In the context of current and future climatic difficulties, the

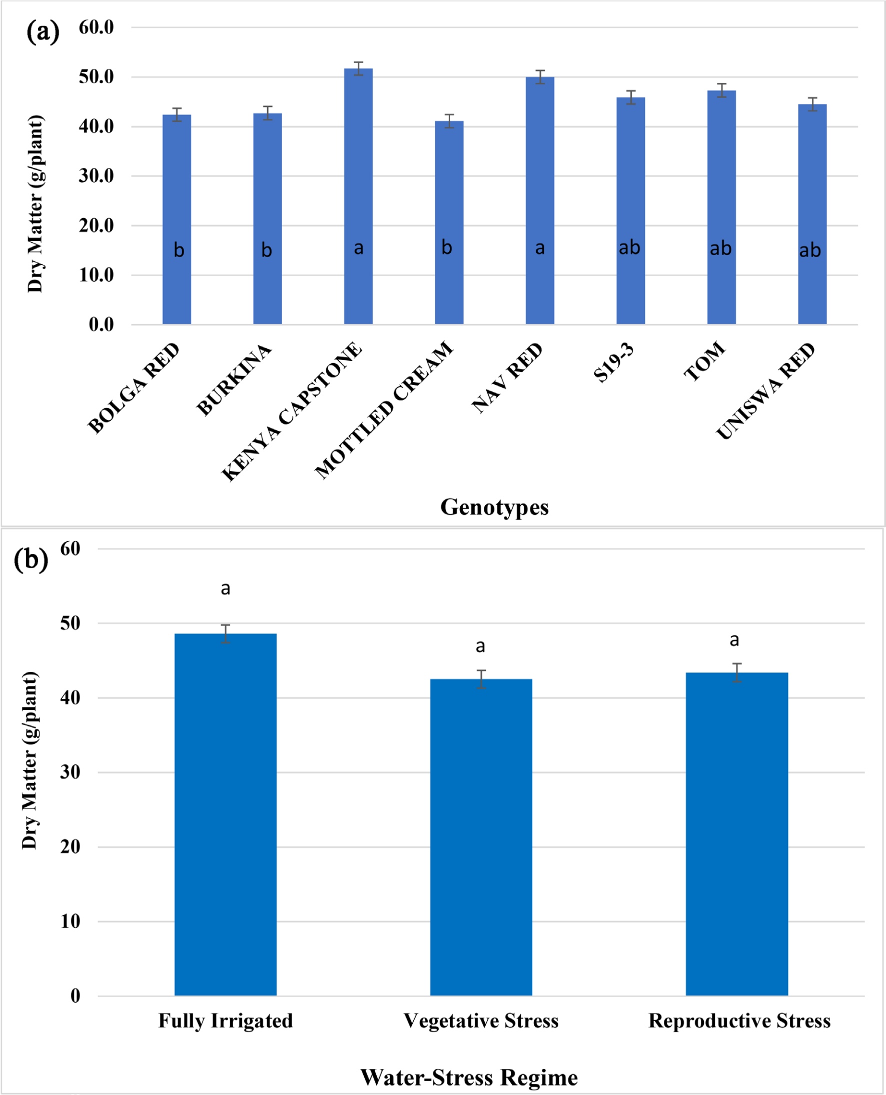

Figure 4. Dry matter of Bambara groundnut genotypes affected by water-stress imposition at different growth stages (a); effect of watering regime on dry matter on Bambara groundnut (b). Error bars margins represent standard errors of the means.

physiological adaptation that allows this plant to flourish under drought and other extreme stress conditions represents an invaluable opportunity and offers great promise. Plant tolerance to drought stress varies depending on plant species and even among genotypes of the same species [13]. This study showed that the growth and development of Bambara groundnut were influenced by periods of drought stress as well as the stage of plant development.

4.1. Relative Water Content (RWC)

Physiological traits (Table 2) of Bambara groundnut genotypes deceased under drought stress regimes, compared to well-watered conditions. A useful tool for determining a plant's water status and its ability to withstand drought is relative water content [14]. It is clear from this study that the relative water content of all genotypes declined in response to water stress at a varying rate. It has been suggested that Bambara's extensive root system and the presence of leaf vesicles, which may lessen transpiration, are responsible for the plant's ability to withstand drought [15] [16]. Mostly, plant metabolism is generally affected by leaf water status, which was assessed by RWC [14]. Uniswa Red showed a gradual decline in RWC, maintaining a high value (80.9%) at the reproductive stage as compared to Burkina which showed a rapid decline in RWC (69.0%) at the reproductive stage.

These findings suggest that Uniswa Red responded to water deficit by maintaining higher tissue water content than other genotypes. Even though there were differences among genotypes, their ability to maintain RWC above the critical level under stress is indicative of the ability of these Bambara groundnut genotypes to sustain metabolic processes through the maintenance of relatively higher leaf water status for their survival. [17] and [18] have similarly shown that the RWC of Bambara groundnut decreased significantly when exposed to limited water supply at different stages of development. Using RWC as a drought determinant parameter, Nav Red, Uniswa Red, IITA 686 and Kenya Capstone were the most promising drought-tolerant genotypes for improvement.

4.2. Chlorophyll Content

The SPAD Chlorophyll meter content was reduced by varying degrees in the genotypes. In this study, the maintenance of high chlorophyll content by some genotypes and slow decline when drought was imposed at the vegetative stage suggests that they are relatively drought-tolerant. These observations are in line with [19] who reported that drought-tolerant cultivars showed a lower reduction in chlorophyll content than susceptible ones. Various reports have also explained that water stress significantly reduces the chlorophyll content in other crops such as maize [20]. Reduced chlorophyll content under drought stress negatively impacts the photosynthetic rate, culminating in yield decline.

Other crops like cereals [21] and soybean [22] have demonstrated a continuous production of chlorophyll during drought periods, indicating the resilience of these crops under drought stress. In this study, Uniswa Red and Kenya Capstone all maintained chlorophyll content above mean chlorophyll content under water stress and exhibited a greater ability to recover from stress and clearly emphasized that variations in genotypes and severity of the stress affect the ability to sustain chlorophyll content.

4.3. Leaf Area

At several levels, from physiology to genetics, and the cellular to the whole-plant level for reproduction, it has been documented how plants respond to drought stress [23]. In this study, results of the leaf area provided important clues to the physiological role of the leaf in possibly improving plant performance under water stress conditions. Leaf area development, irrespective of the stage of drought imposition, decreased compared to non-stressed plants. This suggests that water stress not only limited the capacity of the source and sink tissues but also impaired the phloem loading, assimilate translocation and dry matter partitioning. According to [24] the amount of assimilates allocated to the leaves controls leaf area development and determines light interception and dry matter production.

A decrease in leaf area in all the genotypes was recorded when subjected to drought stress at the vegetative and reproductive stages (**Figure 2**). This corroborates the findings of [3] [18] and [25], who observed a decrease in growth and development of leaf area in response to drought. Reduction in leaf area under drought stress could be an adaption strategy to conserve moisture by limiting the surface area for transpiration [26]. However, it has a penalty on biomass production and yield in that it decreases the surface area for light interception and photosynthesis. On the other hand, decreased leaf area could also be a consequence of drought stress, whereby cell expansion is impaired as a result of low soil moisture. Therefore, leaf area data and its relationship with drought tolerance should be interpreted in light of other available data.

4.4. Plant Height

The growth and development of crops rely prominently on water availability. Physiological and biological processes that account for cell division and cell expansion are all driven by water availability. Thus, it was not surprising that some Bambara groundnut genotypes showed considerable differences in plant height under water-stressed conditions compared to non-stressed plants. When the impact of drought on several stages of soybean plant growth was examined, it was found that a lack of water decreased plant height, with the lowest plants being found under drought-induced conditions during the vegetative stage [27].

Nonetheless, genotypes may vary in their responses to water stress but this may not necessarily affect plant growth similarly. For instance, Kenya Capstone consistently performed above the mean plant height under both non-stressed and water-stressed conditions and hence may be selected as stable in terms of plant height. This study found that some drought-related physiological parameters, such as leaf area and chlorophyll content at the reproductive growth stage, significantly decreased during water-stress regimes across eight Bambara genotypes. However, an increase in plant height was observed in genotypes that were subjected to water stress during the vegetative stage relative to the well-watered plants. [28] obtained comparable results. These observations suggest that drought stress does not severely impact plant height in these genotypes. According to [26], the reduction in plant height and leaf area constitutes an adaptation mechanism aimed at minimizing water loss under drought stress. Such has been corroborated by the findings of [3] [18] and [25].

4.5. Days to 50% Flowering

Many phenological processes in plants respond to water stress. Key among the plant's response to water stress is the duration and timing of key phenological events such as flowering [29]. Water stress in many ways controls this phenological process. In this study, flowering days of the genotypes under water-stressed and non-stressed treatments were in line with the range of number of days to flowering reported by [30] for Bambara groundnut. Water stress caused more plants of most genotypes to flower earlier, suggesting that drought escape is a mechanism utilized by Bambara groundnuts to cope with drought. Most crops resort to early flowering as a major mechanism for ensuring survival under water stress [31] [32].

4.6. Number of Pods per Plant

There was considerable variation in pod number per plant among evaluated genotypes. The presence of wide genetic variations for pod number under drought showed the potential of the evaluated Bambara groundnut germplasm to develop high-yielding genotypes for specific and broad adaptation to water stress conditions.

The highest reduction in pod number per plant occurred when drought stress was imposed at vegetative and flowering stages on all genotypes of Bambara groundnut (**Table 4**). This was in line with previous work [33] [34]. In other reports, [35] and [36] reported that the reduction in pod number is mainly caused by the abscission of flowers and embryo abortions, or by failure of fertilization due to the production of unviable pollens. Likewise, [37], reported that seed development is incredibly susceptible to drought stress, mainly because it comprises multiple processes that are particularly sensitive to changes in plant water status. The findings from this study show that pod initiation, pod setting and seed filling stages were more sensitive to drought stress, which could even lead to pod failure in some genotypes as in the case of Tom.

4.7. Dry Pod Yield of Different Bambara Groundnut Genotypes to Water Stress

Pod yield production is the result of the interaction between the genetic potential of the crop and the conditions of the environment the crop is growing. For maximum pod yield, the genetic potential of the crop must be high and the environmental factors must be conducive [38]. Unfortunately, pod yield can be poor when environmental factors are limiting despite the genetic potential of the crop and vice versa. Limited water availability is a hindrance to the expression of the full genetic potential of Bambara groundnut. The trend observed showing lower pod yield under water stress conditions was consistent with reports of [3] and [18] who observed reduced pod yield in Bambara genotypes under limited water availability.

At those stages where pegs were developing pods, water stress-related conditions could translate into seed abortion. In this study, water stress during vegetative and reproductive stages decreased plant growth, which eventually delayed and reduced node appearance, leading to plants that had fewer inflorescences, pods, and seeds per plant following re-watering. This is in line with the findings of [18] and [39]. This explains why pod yields achieved under water stress during the reproductive stage were lower than yields achieved during water stress in the vegetative stage. The reproductive stage appears to be the most sensitive stage to water stress for the Bambara groundnut genotypes used in the study as is the case for many other crops.

4.8. Seed Yield of Different Bambara Groundnut Genotypes Water Stress

Primarily, the ability of a crop to produce seeds under water stress is a key feature for crop selection in any breeding effort. [40] reported that different morphological and physiological characteristics significantly contribute to grain yield and the knowledge of the genetic relationship between grain yield and its components enhances the selection efficiency in a breeding program. These results are similar to other reports in the literature [3] [39] who all reported reduced seed yield in Bambara groundnut genotypes in response to limited water availability.

There are several reports that drought stress reduces seed yield [33] [34]. We attributed the reduction in seed yield under drought stress to the decrease in yield components such as the number of pods per plant and the number of seeds per pod. However, these yield components are reportedly influenced by their sensitivity to water stress, especially at flowering and pod-filling stages of growth [40]. Compared to plants fully irrigated, water stress imposed at vegetative and flowering phases caused decreased leaf area, chlorophyll content and relative water content. In effect, fully irrigated plants achieved the best growth and development and were superior to drought-stressed plants. This is in line with previous studies [41] which show that the influence of water stress on the growth of Bambara is most severe on yield parameters (number of pods per plant, number of seeds per pod and seed weight).

Interestingly, drought stress at the vegetative stage had a more severe impact on seed yield for most genotypes compared to water stress at the reproductive stage. A possible explanation is that the imposition of water stress was initiated at the reproductive state when some flowers had already formed and since the onset of the drought was gradual, these flowers were likely able to be pollinated and formed before water stress became severe. On the other, water stress at the vegetative stage could have reduced stomatal opening [42], which limits CO_2 uptake and hence reduces photosynthetic activity [43]. These impacted the overall growth, thereby affecting reproductive development, culminating in a more severe reduction in yield. Previous studies have shown this relationship between water stress and crop growth [44]. Genotype Tom had the greatest decline in yield at the vegetative stage (54.1%), which was lower than the value obtained by

[18] who reported a significant seed loss of 82% due to water stress at the vegetative growth stage, albeit for a different genotype. Contrary to what was observed in other genotypes, Tom was the only genotype that had the lowest yield (no seeds) when drought was imposed at the reproductive stage. This could be because Tom was late maturing as evidenced by its higher number of days to 50% flowering and thus likely had slower floral initiation and pod formation which was overwhelmed by water stress.

4.9. Dry Matter (DM)

Results of dry matter production showed that, despite much variability between the genotypes, there was a trend of declining dry matter under water-stressed relative to non-water stress conditions. Such a trend was consistent with the trend observed for seed yield, number of pods and plant height. Differences in DM as a result of the stress imposed were not statistically significant, however, yield components evaluated were significantly different—an indication of some landraces being able to divert a higher percentage of dry matter to yield and yield component. The reduction in dry matter in response to water stress was consistent with the findings of [31], who reported that drought avoidance mechanisms had the downside of reduced biomass production. This according to [29] is because for the plant to avoid drought, it would require to minimize water losses through stomatal closure and reduced canopy size, both of which ultimately reduce the amount of biomass produced by the plant. However, the findings of this study are contrary to reports of [45] who recorded an increase in dry matter among stressed genotypes, with stressed plants showing higher gain than well-watered plants after re-watering. Reduction in leaf area and dry matter production in Bambara groundnut can vary between genotypes and in this study, the magnitude of the reduction in dry matter appeared to vary according to the age of the plant and soil moisture availability.

5. Conclusion

The study sought to determine the impact of water stress imposed at the vegetative and reproductive growth stages on the physiology and yield of Bambara groundnut genotypes. The findings have revealed a variety of responses to water deficit, most of which are represented by changes in plant development and yield. Some of these effects could be attributed to the reduced leaf water status under drought in addition to other effects such as photosynthesis which were not directly measured in this study. The results have shown significant yield reduction due to water-stress imposition during vegetative and reproductive phases, with a more severe decline when drought was imposed at the vegetative stage. The negative impact of drought observed when water stress was imposed for only 21 days suggests that a longer duration of drought exposure to Bambara could prove devastating to growth as well as crop yield. Furthermore, variations observed in the responses of the different genotypes to drought imply that some of the genotypes could be used in breeding programs to improve the drought tolerance of the crop.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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