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Article

Building Resilient Crop Production Systems for Drought-Prone Areas—A Case for Bambara Groundnut (*Vigna subterranea* L. Verdc) and Groundnut (*Arachis hypogaea* L.)

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Abstract: Drought is a major crop production constraint worldwide. Some legume crops are known for their ability to resist water deficit stress. This study evaluated the responses of bambara groundnut (*Vigna subterranea* (L.) Verdc) and groundnut (*Arachis hypogaea* (L.) to soil water deficit stress. The experiment was set as a split-plot randomized complete block design. Three bambara groundnut landraces: viz DodR, NALBAM 4 and S19-3, and one groundnut variety, MNANJE, were assigned to subplots with three water regimes assigned to main plots (regime one: irrigated throughout the growing period, regime two: water deficit stress was imposed at the start of flowering to the end of first flush flowering, regime three: water was withheld during the pod development). Water deficit stress increased proline content by 123% in stressed plots. The highest (174%) and lowest (89%) proline increases were evident in the genotypes MNANJE and NALBAM 4, respectively. Water deficit decreased stomatal conductance, transpiration rate and photosynthetic rate, with MNANJE and S19-3 showing the smallest percentage decrease in most of the traits. This suggests that the two genotypes are drought resistant. The variations observed among landraces could be exploited to breed resilient varieties for cultivation in drought-prone areas, ultimately improving food security.

Keywords: water deficit; bambara groundnut; groundnut; gaseous exchange; proline; flowering stage; food security

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1. Introduction

Drought is a major crop production constraint worldwide, occurring on all continents with varying intensity and frequency, resulting in considerable crop yield reductions. It is estimated that crop cultivation on Earth is only possible on 16% of the potentially arable area due to limited availability of water [1]. Drought affects nearly all plant growth processes [2,3]; however, the stress response depends upon the intensity, rate and duration of exposure and the stage of crop growth [4]. The effect of water stress on plant growth and developmental processes and yield has been studied extensively. For example, water deficit stress reduces photosynthesis due to stomatal closure in sunflowers (*Helianthus*) [5], and affects growth, development and yield in bambara groundnut (*Vigna subterranea* (L.) Verdc) [6–9] and groundnut (*Arachis hypogaea* L.) [10,11]. Stomatal closure helps maintain high leaf water content and thereby a higher leaf water potential; however, this leads to a reduction in photosynthetic activity and, hence, a reduced crop biomass and yield. Under a variety of stresses, active solute accumulation of compatible solutes such as proline is claimed to be an effective stress resistance mechanism [12–14]. Proline works as both an osmoprotectant and as a redox-buffering agent, possessing an antioxidant property under conditions of stress [15–17]. Accumulation of proline under drought stress was found in several plants, for instance, in bambara groundnut [9,18–21] groundnut [22–24], black gram (*Vigna mungo*) and green gram (*Vigna radiata*) [25]. Legumes vary in their ability to resist drought depending on species and variety [26]. In a soil water deficit experiment, Ref. [27] revealed that lentil (*Lens culinaris* Medikus) and groundnut exhibited the lowest yield reduction compared to faba bean (*Vicia faba* L.). Ref. [28] showed that soil water deficit stress significantly reduced the growth of both cowpea (*Vigna unguiculata*) and bambara groundnut, but not of groundnut.

There are, therefore, contradictory statements about the comparative drought resistance capabilities of bambara groundnut and groundnut, two of the legumes grown in drought-prone regions. Bambara groundnut is considered to be the most drought resistant of the grain legumes [29,30], with a great potential as an alternative legume to groundnut and soybean (*Glycine max* L.). However, Ref. [31] reported groundnut to be a better performer than bambara groundnut under drought conditions. Ref. [18] reported that earliness and upright growth habit are key traits in a drought resistant groundnut line (LBM Branco), and the authors recommended the line for cultivation in semi-arid environments. Further drought related investigations need to be carried out to compare the two legume species. This will ascertain individual species' drought resistance ability and, hence, their possible utilization in climate change adaptation strategy to improve crop yields in drought-prone areas.

Bambara groundnut cultivation is mainly dependent on landraces, which are uncharacterized, under-researched and underutilized [32–34]. Additionally, although various drought related studies have been undertaken regarding bambara groundnut [6–9,25,26], the drought resistance mechanisms that permit its cultivation in dry areas have not been fully elucidated. Similarly, limited information is available to support its drought resistance superiority over its comparator, groundnut. The objective of the present study was to investigate the physiological effects of soil water deficit stress on bambara groundnut and groundnut as assessed by leaf proline accumulation, gas exchange and relative leaf water content. The outcome will not only further our understanding of drought response mechanisms in these two species but will also provide comparative evidence on the adaptability of the two species to semi-arid environments and their contribution to food security in drought-prone areas.

2. Materials and Methods

2.1. Experimental Site

Two experiments were conducted in a rainout shelter, at the University of Nottingham Malaysia (UNM) (GPS: 2°56′42.00″ N 101°52′26.40″ E and 66 m above sea level), in Semenyih, Malaysia. The experiments were carried out during two consecutive cropping seasons (2016/2017 and 2017/2018).

2.2. Plant Materials and Methods

Three landraces of bambara groundnut (DodR, NALBAM 4, S19-3) and one groundnut variety (MNANJE) were used. MNANJE, the groundnut, was included to observe the general response of the two species against water deficit stress. S19-3 was selected based on its known ability to withstand drought [6], while selection of the other landraces was based on their cultivation in the semi-arid areas of Tanzania. A mixture of 1:2 clay soil to river sand was sun-dried, followed by laboratory analysis to determine its physical and chemical proprieties. The textural class of the mixture was loamy sand. The mixture was put in phenyl vinyl chloride (PVC) columns (20 cm diameter and 100 cm height) [35], followed by thorough compaction. Based on the soil textural class of the soil mixture, loamy sand, the bulk density of 1.6 g cm^{-3} was optimized. Calculation of amount of soil followed the equation adopted from [36].

$$Dry\ soil\ weight\ (g) = \left(Bulk\ density\ \left(g\ cm^{-3}\right) * \left(Soil\ volume\ \left(cm^{3}\right)\right)\right) \tag{1}$$

Each PVC column contained 50 kg of the mixed soil. Before sowing, soil moisture at field capacity was determined as a basis for controlling the amount of water to be irrigated. The soil-filled columns were saturated with water and left overnight. After that, the soil moisture at field capacity was measured using a PR2 theta probe (Delta T Devices, UK, 1971).

2.2.1. Experimental Design and Set Up

The experimental design used was a split-plot in a randomized complete block design (RCBD) replicated thrice. Watering regimes were assigned to the main plots, whereas landraces were assigned in sub plots. Seeds were soaked in water overnight before being sown. Four seeds were directly sown per column at a depth of 3 cm using a dibbling method. Thinning to one seedling per column was conducted ten days after sowing. Before imposing water stress, all plants were well watered regularly until the start of flowering. Irrigation was conducted manually using a measuring cylinder to ensure the same amount of moisture loss was replenished. Each column was supplied with 500 ml at early stages of growth and then increased up to 1000 ml, depending on landrace water usage and growth stage. Physiological data collection started 25 days after emergence (DAE) with reference to crop growth stages (R1 = flowering, R2 = beginning peg, R3 = beginning pod, R4 = full pod, R5 = beginning seed, R6 = full seed) [37]. Soil moisture measurements were conducted on a weekly basis at 30 cm, 60 cm and 90 cm depths in one specific column per plot. The plants were subjected into three water regimes with three replicates per water regime. Water regime 1 (control) was well irrigated throughout the growing period and maintained at 100% field capacity, while in water regime 2, water deficit stress was imposed at the start of flowering until the end of first flush flowering. In water regime 3, water was withheld during the pod development stage (first pod appearance until the full seed (R6 = reproductive stage six)) [38]. At the end of each stress period, watering was resumed until maturity.

Weeding and gentle tilling of the soil was conducted by hand and hand fork to control weeds and improve soil aeration. Fertilizer was applied at the rate of 20:60:40 kg nitrogen, phosphorus and potassium (NPK) per hectare using NPK 15:15:15, triple super phosphate [46% phosphorus pentoxide (P_2O_5)] and muriate of potash [60% potassium oxide (K_2O)] as nutrient sources. All the fertilizers were applied and incorporated into the soil at sowing. A combination of fungicide (Mancozeb 80% w/w at the rate of 2.5 g l^{-1} of water) and insecticide (Cypermethrin 5.5% at the rate of 2.5 ml l^{-1} of water) was applied at the start of flowering, followed by two applications in two-week intervals (4, 6 and 8 weeks after sowing), for the control of insects and diseases.

2.2.2. Measurements

Relative Leaf Water Content

Leaf water status was estimated by relative water content (RWC). RWC measurements were carried out before drought, at mid-treatment of drought, at the end of drought and after recovery. This was carried out as described by [24]. The leaf relative water content was calculated from the relationship as described by [39,40].

$$\mathrm{RW}(\%) = \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right) * 100 \tag{2}$$

where: FW = fresh weight of leaf samples; TW = turgid weight of leaf samples; DW = dry weight of leaf samples.

Gas Exchange

Gas exchange measurements (photosynthesis, stomatal conductance and transpiration) were conducted weekly at 400 µmol m^{-2} s^{-1} PAR, reference CO_2 concentration of 390 µmol m^{-2} s^{-1} and leaf temperature of 29 °C using a Li-6400XT Photosynthesis System (Li-Cor Biosciences, Lincoln, NE, USA).

Proline Content

Proline content was determined according to the method described by [41]. The proline concentration in the samples was obtained from a standard curve obtained by linear regression of the absorbance of standards using the concentrations. Proline in each micromole per gram of the leaf fresh weight was calculated using the equation below [41]:

$$\text{Proline in } \mu\text{mole g}^{-1} \text{ tissue} = \frac{[(\mu\text{g proline/ml}) * \text{ml toluene} * \text{ml salycylic acid}]}{(115.5\ \mu\text{mole} * \text{sample (g)})} \quad (3)$$

where: 115.5 is the molecular weight of proline.

Environmental Data

The environmental conditions in the rainout shelter, which included relative humidity, photosynthetically active radiation (PAR) and atmospheric temperature, were recorded throughout the experimental period using a mini weather station: Watchdog 2000 Series, Spectrum Technologies, Plainfield, IL, USA.

Statistical Analysis

Statistical analyses were performed using Genstat statistical software version 18 (VSN International Ltd., Hemel Hempstead, UK). To avoid differences that might be caused by species differences, bambara groundnut and groundnut were analyzed separately. Multiple comparisons were carried out when necessary, using Duncan's Multiple Range Test at 5% level of significance.

3. Results

3.1. Leaf Proline

Results showed highly significant differences ($p < 0.001$) among bambara groundnut landraces and between water regimes (Figure 1), as well as in the interaction between water regimes and bambara groundnut landraces (Table A1). Proline accumulated under both water deficit treatments (Figure 1) resulted in the highest percentage increase in proline content (water stressed plants against control) observed during the flowering period. Among bambara groundnut landraces, S19–3 had the highest leaf proline increase (150%) against the control, followed by DodR (120%) and NALBAM 4 (89%). Among water regimes, water stressed plants had a 121% proline increase against control during flowering and a 103% increase against control during pod development. Although groundnut (MNANJE) was analyzed separately, it showed the same trend as bambara groundnut landraces but with higher proline content values (Figure 1). A higher percentage increase in proline content (water stressed plants against control) was found in MNANJE plants during flowering (174%), compared to percentage proline increase during pod development (113%).

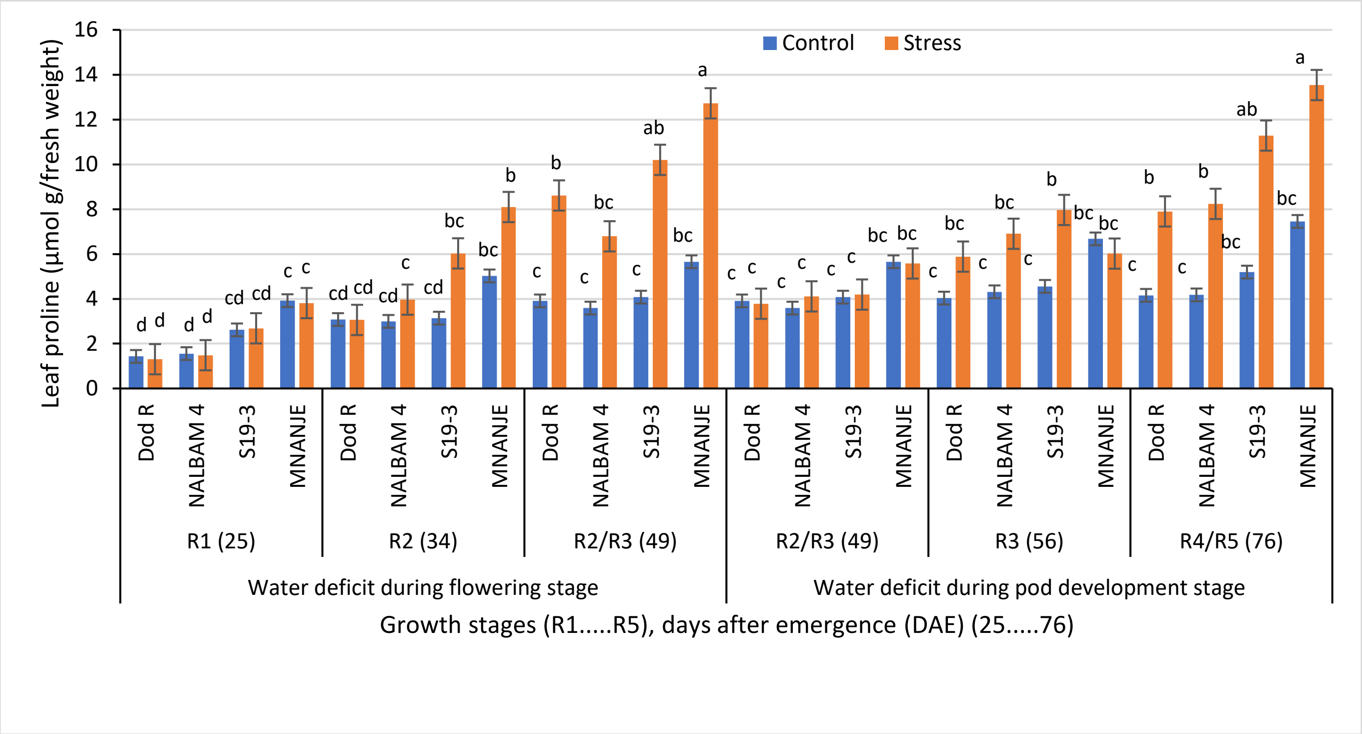

Figure 1. Combined mean leaf proline content (µmol g/fresh weight) of well watered and drought stress treated bambara groundnut (Dod R, NALBAM 4 and S19-3) and groundnut (MNANJE) landraces during flowering and pod development stages. Groundnut variety (MNANJE) was analyzed separately, R2 = beginning peg, R3 = beginning pod, R4 = full pod, R5 = beginning seed. (n = 12).

3.2. Gas Exchange

As shown in Table 1, photosynthesis rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were reduced under water deficit conditions in both the bambara groundnut and groundnut landraces. The P_N, g_s and *E* were significantly lower ($p < 0.001$) in water stressed plants both during flowering and pod development. The effect of water deficit stress was higher in plants stressed during flowering. Bambara groundnut landraces differed significantly ($p < 0.001$) in P_N, g_s and E. The interaction between water regimes and landraces revealed significant ($p < 0.001$) differences, as did the interactions between water regimes and years, and landraces and years ($p < 0.05$), in P_N and g_s (Table A2). Similarly, the interaction between the three parameters, viz., water regimes, landraces and years, was significant ($p < 0.05$) for P_N and g_s. Among bambara groundnut landraces, S19-3 had the highest gas exchange values, as well as the lowest percentage change values, compared to other landraces (Table 1). Nevertheless, groundnut (MNANJE) had the highest values of P_N, g_s and E, coupled with lowest percentage change values between the control and the stressed plants.

Table 1. The mean changes in photosynthetic rate (PN -µmol $C0_2$ m^{-2} s^{-1}), stomatal conductance (gs -H_2O mol m^{-2} s^{-1}) and transpiration (E-H_2O mol m^{-2} s^{-1}) of bambara groundnut (DodR, NALBAM 4, S19-3) and groundnut (MNANJE) landraces under water deficit stress during flowering and pod development stages.

		Photosynthesis Rate				**Stomatal Conductance**				**Transpiration Rate**			
		S1	**% Δ**	**S2**	**% Δ**	**S1**	**% Δ**	**S2**	**% Δ**	**S1**	**% Δ**	**S2**	**% Δ**
Water regimes	Control	70.41 ^{a}		45.87 ^{a}		1.97 ^{a}		0.38 ^{a}		18.68 ^{b}		4.46 ^{a}	
	Stressed	13.30 ^{b}	−81	11.80 ^{c}	−74	0.20 ^{b}	−90	0.14 ^{c}	−64	3.37 ^{c}	−82	1.88 ^{b}	−58
	p-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
	LSD	3.437		0.39		0.091		0.012		0.821		0.375	
Landraces	DodR	53.44 ^{b}		31.41 ^{b}		1.39 ^{b}		0.20 b		11.65 ^{c}		2.71 ^{c}	
	NALBAM 4	39.60 ^{c}		16.57 ^{c}		0.67 ^{c}		0.17 ^{c}		14.95 ^{b}		3.13 ^{b}	
	S19-3	60.86 ^{a}		43.97 ^{a}		2.03 ^{a}		0.36 ^{a}		16.02 ^{a}		4.89 ^{a}	
	p-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
	LSD	1.762		1.053		0.068		0.013		0.601		0.18	
	MNANJE	108		32.88		2.8		0.29		18.1		4.6	
Landrace x Water regime													
DodR	Control	74.72 ^{bc}		39.03 ^{c}		1.99 ^{b}		0.43 ^{a}		14.38 ^{c}		3.91 ^{bc}	
	Stressed	11.07 ^{f}	−85	12.17 ^{e}	−69	0.19 ^{d}	−90	0.04 ^{e}	−90	2.94 ^{d}	−84	1.04 ^{e}	−73
NALBAM 4	Control	54.89 ^{d}		23.34 ^{d}		0.99 ^{c}		0.27 ^{c}		18.73 ^{b}		3.80 ^{bc}	
	Stressed	9.48 ^{f}	−83	4.08 ^{f}	−83	0.11 ^{d}	−89	0.08 ^{e}	−69	2.04 ^{d}	−88	1.64 ^{e}	−57
S19-3	Control	81.64 ^{a}		45.23 ^{c}		2.96 ^{a}		0.45 ^{a}		21.93 ^{a}		5.68 ^{a}	
	Stressed	19.35 ^{e}	−76	17.15 ^{e}	−62	0.37 ^{d}	−88	0.29 ^{c}	−37	4.13 ^{d}	−81	2.97 ^{d}	−48
	p-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
	LSD	1.169		0.449		0.033		0.014		0.763		0.153	
MNANJE	Control	147.00 ^{a}		49.93 ^{a}		3.03 ^{a}		0.37 ^{a}		23.06 ^{a}		5.57 ^{a}	
	Stressed	35.10 ^{b}	−76	20.15 ^{c}	−60	0.44 ^{b}	−86	0.24 ^{b}	−34	4.95 ^{b}	−79	3.02 ^{b}	−46
	p-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
	LSD	1.678		1.53		0.156		0.029		1.529		0.369	

Values followed by the same superscript are not significantly different according to Duncan's Multiple Range Test (DMRT). % Δ = percentage change with respect to control. Groundnut (MNANJE) landrace was used only for comparison. Analysis of variance was conducted separately for each species. Bambara groundnut (n = 12).

3.3. Relative Water Content (RWC) (%)

Separate analysis of variance showed significant ($p < 0.05$) differences in the landraces, water regimes and their interactions for RWC in both years (there were no significant differences observed between the two years; hence, combined analysis of variance was carried out (Table A1)). Stressing bambara groundnut and groundnut significantly ($p < 0.001$) reduced RWC compared with the control plants at both flowering and pod development stages (Figure 2). Landraces significantly ($p < 0.001$) differed in maintenance of RWC. RWC did not significantly ($p > 0.05$) differ over the two years (Table A1). The interaction between landraces and watering regimes was also significant ($p < 0.001$). In both species, higher percentage changes were found in plants stressed during flowering than in plants stressed during pod development. The decrease was significant ($p < 0.001$) in all bambara groundnut and groundnut varieties, compared with the control. After seven days of recovery, there was a significant ($p < 0.05$) increase in RWC in all bambara groundnut landraces, with the highest levels observed in S19-3. MNANJE, the comparator, had the highest values in all water regimes compared to individual bambara groundnut landraces (Figure 2). In addition, the lowest percentage change values between stressed and control plants were observed in MNANJE.

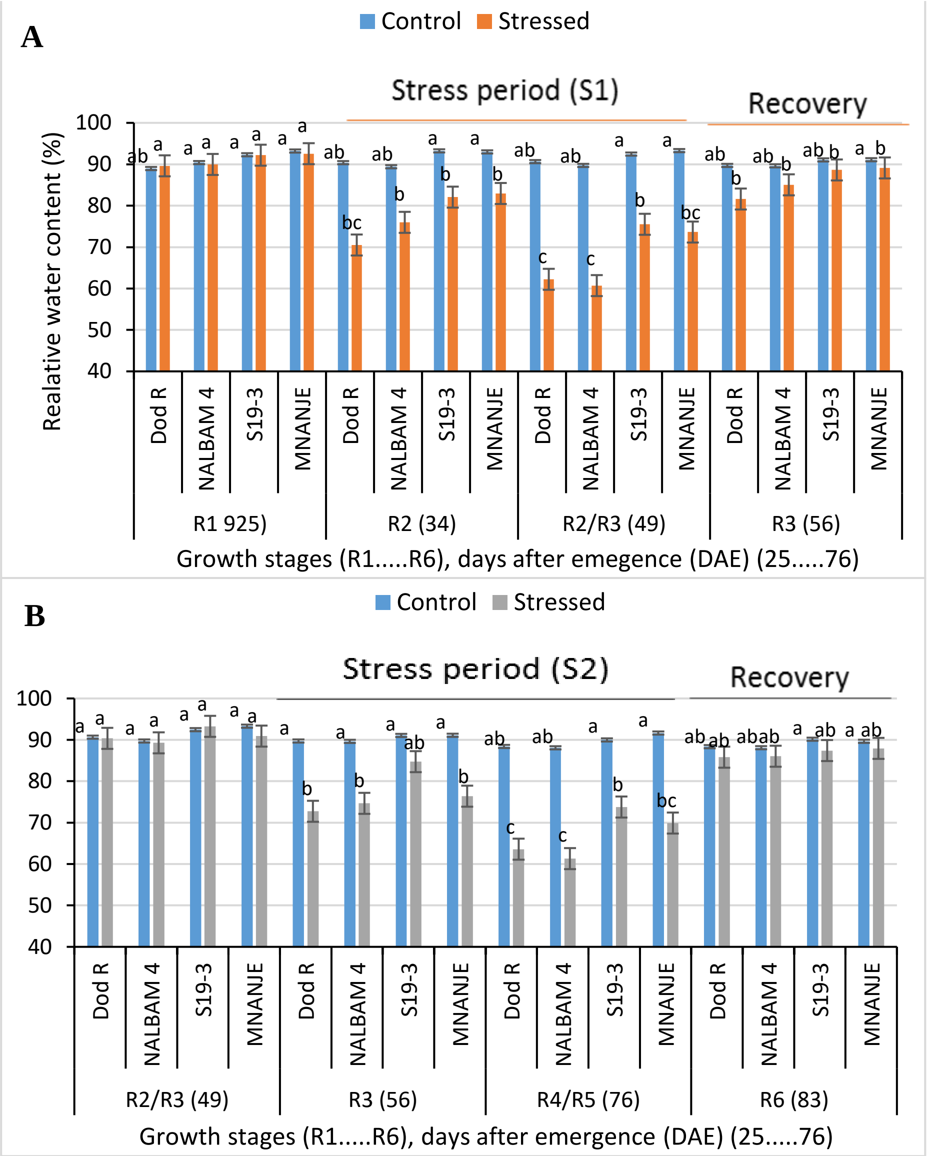

Figure 2. Combined mean relative water content (%) of bambara groundnut landraces (DodR, NALBAM 4, S19-3) and groundnut variety (MNANJE) under well irrigated (control) and water deficit conditions. (**A**), S1 = water deficit stress during flowering, (**B**), S2 = water deficit stress during pod development; R1 = flowering, R2 = beginning peg, R3 = beginning pod, R4 = full pod, R5 = beginning seed, R6 = full seed (n = 12).

4. Discussion

A potential strategy to minimize the impacts of drought stress derived from climate change is to introduce crop species and cropping systems which are capable of resisting and adapting to prevailing stress conditions while producing sufficient yields. Given bambara groundnut's ability to tolerate drought [7], its cultivation in drought-prone areas could potentially enhance food production resilience in these areas. Groundnut, on the other hand, which is more popular as both a protein and an oilseed crop [42], is more widely grown in the semi-arid tropics [43]. Both these crops occupy the same agroecological niche and have been referred to as 'Climate-Smart Crops' [33,44] because of their ability to tolerate drought and heat, among other stresses. The present study investigated the physiological effects of soil water deficit stress on bambara groundnut and groundnut to further our understanding of drought response mechanisms in these two species and to provide comparative evidence of their adaptation to semi-arid environments and potential contribution to food security in drought-prone areas.

4.1. Leaf Proline

Drought related studies have been conducted to characterize the accumulation of proline, a compound known to contribute to the osmotic adjustment and tolerance of plants exposed to unfavorable environmental conditions [45]. Proline is thought to contribute to osmotic adjustment, detoxification of ROS and protection of membrane integrity [46]. Refs. [22–24,44] also reported that proline accumulation in stressed plants is a tolerance mechanism against oxidative stress and it is the main strategy of plants in avoiding harmful effects of drought stress. In the present study, higher amounts of proline were mostly observed in water stressed plots, confirming proline's involvement in drought stress adaptation mechanisms [25,44,47,48]. The landraces which accumulated more proline during water stress are likely to be drought resistant [49,50]. Higher proline accumulation in plants during water stress might be osmoprotectant and help resistant landraces in maintaining a higher RWC and leaf water potential [51,52]. The present results agree with findings reported by [19,52] in bambara groundnut. Increased amounts of proline due to drought stress have been reported in other crops [53–61]. Most reports have linked proline accumulation with stress resistance and proposed that proline be used as one of the parameters for the selection of plant species resistant to drought and salinity [25,62,63]. Furthermore, proline increase, and metabolism are related to mechanisms of abiotic stress avoidance in plants [44]. In the present study, MNANJE (groundnut) accumulated the highest amount of proline during drought stress and therefore showed a superior capacity to withstand water stress compared with the three landraces of bambara groundnut (S19-3, DodR and NALBAM 4). Drought resistance superiority of groundnut over bambara groundnut has also been reported by [28]. S19-3, a drought resistant [64] landrace, was outperformed by MNANJE in terms of proline content and/or accumulation, implying that MNANJE is a superior drought resistant landrace considering this trait. This has a clear practical implication, both in terms of selecting parental materials for drought stress resistance breeding and matching crops to prevailing climatic conditions to build resilient crop production systems.

4.2. Gas Exchange

Lower values of gas exchange parameters were observed in water deficit stressed plants, compared to control plants. In the initial stages of growth in all landraces, values of gas exchange parameters increased with plant growth; however, in later growth stages, exchange parameters decreased with plant growth. Progressive decline in stomatal conductance, photosynthetic rate and transpiration rate were evident in all soil water deficit stressed plants. This is consistent with findings reported by [8,19,65,66] in bambara groundnut, [67] in soybean and [68] in groundnut. This implies that the regulation of stomatal closure for the control of water loss could be one of the early events in bambara groundnut and groundnut in response to drought. The higher rate of percentage decrease in both species was observed during water deficit during flowering, compared to water deficit stress during pod development. Regardless of water regime, in later stages of plant growth there was a continuous decrease in stomatal conductance, photosynthetic rate and transpiration. This implies that these three parameters are not only affected by water stress but also influenced by plant age. These three parameters are positively related, stomatal conductance being the main driving force. Under drought, plants close their stomata to minimize water loss by transpiration, thereby conserving tissue moisture. Generally in plants, the overarching factor determining transpiration, photosynthesis and stomatal response to drought stress is soil water content [69]. Plants that tend to preserve water under drought are referred to as drought avoiding plants [9]. Ref. [70] reveals that plants subjected to drought stress conserve water by reducing transpiration and stomatal conductance. Decrease in transpiration and stomatal conductance points to the possibility of a drought avoidance strategy [71]. Preserving water during drought is associated with stomatal closure and limiting the amount of CO_2 required for photosynthesis. As a result, net photosynthesis is negatively affected, as is an economic yield. Drought avoidance mechanism traits alone must not be given the utmost priority in breeding programs, as their contribution to economic yield is less important. This is because a drought avoidance mechanism is achieved at the expense of crop yield [71,72].

4.3. Relative Water Content (RWC) (%)

Water stress during flowering (25–49 DAE) and pod development (49–76 DAE) stages of growth of bambara groundnut and groundnut decreased their RWC. Effects of decreased RWC include stomatal closure [73] and decreased CO_2 assimilation [74]. The reduction in RWC observed in this study could be associated with the decrease observed in gas exchange parameters. Water deficit in plants occurs when water loss due to transpiration exceeds water absorption by roots [75], hence, a reduced RWC. Subsequently, plants tend to reduce water loss through stomatal closure, which then leads to decreased stomatal conductance and CO_2 intake [76,77]. Plants stressed during pod development showed the lowest percent change in RWC compared to plants stressed during flowering. This may be because the plants were on their last stage of growth; hence, there was a decrease in metabolic activities [2,78] compared to those at the flowering stage. The differences that existed in RWC among the bambara groundnut landraces suggest the degree of drought resistance among the landraces [38] and, therefore, the useful candidates for breeding drought resilient varieties. RWC is an important trait which indicates drought resistance, as species which exhibit restricted changes in RWC per unit reduction of water potential are often considered to be relatively drought resistant. In the present study, there was a progressive reduction in leaf RWC in both species during water stress. The resistant bambara groundnut landrace S19-3 maintained a relatively higher leaf RWC than DodR and NALBAM 4. However, MNANJE, the groundnut, was found to maintain both higher levels of relative water content and lower percent decrease values than S19-3. The ability to maintain higher levels of RWC under limited soil water content implies the ability of plants to persist photosynthesis even under current negative environment changes. This is therefore a positive attribute of bambara groundnut and groundnut in combating food insecurity posed by the failure of major crops in coping with prevailing climate conditions.

5. Conclusions

The results obtained in this study showed that differences existed among bambara groundnut landraces as well as between the two species in response to water deficit stress. The degree of drought resistance depends on the interactions between the landraces, drought intensity and the stage of plant growth. Drought occurring during flowering is more harmful than that occurring during pod development. It was also noted that stomata are the main driver of most of physiological processes under either water deficit conditions or normal supply of water. Among the parameters studied, RWC, stomatal conductance and leaf proline content are important traits for selecting drought resistant plant species and/or genotypes. The results indicated that the negative effects of drought were greater in bambara groundnut landraces than in groundnut. Decrease in stomatal conductance, transpiration, photosynthesis and increase in proline content point to the possibility of a drought avoidance strategy. In addition, proline accumulation could be associated more with a drought tolerance mechanism than with drought avoidance. It was also found that the studied species mitigate water deficit conditions via more than one drought resistance mechanism. Drought avoidance (via stomatal closure and reduced transpiration) and tolerance (via accumulation of proline) were all shown in MNANJE and S19-3, although they varied in their response. The identified materials of bambara groundnut (S19-3) and groundnut (MNANJE) with multi-drought tolerant mechanisms can further be used in breeding programs to develop varieties which are resilient to changing climate, and hence, ensure food and nutritional security.

Author Contributions: A.C.K., S.M. and F.M. conceived and designed the research; A.C.K. performed all the experiments; A.C.K. carried out the data analysis; A.C.K. and F.M. wrote the manuscript; B.M., P.N. and S.M. read and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Sum of squares from the combined ANOVA for leaf proline content and relative leaf water content during flowering and pod development water deficit stress in 2017 and 2018 cropping seasons.

			Proline				Relative Water Content			
			S1		S2		S1		S2	
	SOV	**D.F.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**
Bambara groundnut landraces	Year (Y)	1	0.44932	0.026	0.19135	0.116	2.1947	0.097	0.172	0.785
	Replication	2	0.32291		0.01186		2.0999		2.371	
	Water regime (WR)	2	253.35351	<0.001	216.52585	<0.001	7380.9205	<0.001	6141.568	<0.001
	Landrace (L)	2	15.90017	<0.001	35.06486	<0.001	532.7802	<0.001	326.942	<0.001
	WR* L	4	20.38004	<0.001	26.13972	<0.001	336.9364	<0.001	249.417	<0.001
	WR * Y	2	1.25707	0.003	0.01989	0.869	4.5449	0.066	5.979	0.287
	L * Y	2	0.02289	0.861	0.03541	0.78	2.3038	0.228	1.045	0.794
	WR * L * Y	4	0.09944	0.856	0.28814	0.421	13.1433	0.01	8.521	0.456
	Pooled error	53	293.82658		280.17074		8322.2257		6811.878	
	SOV	**D.F.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**
MNANJE (Groundnut)	Year (Y)	1	5.7576	<0.001	0.15806	0.132	8.216	0.103	4.686	0.196
	Replication	2	0.4899		0.21365		4.558		3.293	
	Water regime (WR)	2	262.8715	<0.001	35.61232	<0.001	1379.84	<0.001	1876.284	<0.001
	WR * Y	2	0.8783	0.091	1.98602	0.002	45.464	0.012	12.89	0.131
	Pooled error	17	271.0491		38.53605		1453.449		1912.517	

SOV = Source of variation, D.F = Degrees of freedom, F pr. = Probability, S.S = Sum of squares, S1 = Stress during flowering, S2 = Stress during pod development.

Table A2. Sum of squares from the combined ANOVA for photosynthesis rate, stomatal conductance and transpiration rate during flowering and pod development water deficit stress and water use efficiency (WUE) at harvest in 2017 and 2018 cropping seasons.

			Photosynthesis Rate				Stomatal Conductance				Transpiration Rate				WUE At Harvest		Grain Yield (t ha-1)	
			S1		S2		S1		S2		S1		S2					
	SOV	**D.F.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**
Bambara groundnut landraces	Year (Y)	1	10.16	0.137	2.46	0.061	0.00019	0.817	0.004601	0.013	2.88	0.22	0.10254	0.249	0.00032	0.596	0.001552	0.632
	Replication	2	4.85		0.91		0.0204		0.000682		3.7		0.43269		0.19531		0.016025	
	Water regime (WR)	2	38985.35	<0.001	10801.76	<0.001	36.56537	<0.001	0.573558	<0.001	3202.15	<0.001	77.67934	<0.001	3.05943	<0.001	14.231746	<0.001
	Landrace (L)	2	4193.34	<0.001	6769.23	<0.001	16.64476	<0.001	0.349248	<0.001	186.26	<0.001	48.37738	<0.001	0.07069	<0.001	5.247252	<0.001
	WR* L	4	842.35	<0.001	3645.15	<0.001	7.92622	<0.001	0.109697	<0.001	112.02	<0.001	2.79888	<0.001	0.00009	<0.001	0.573331	<0.001
	WR * Y	2	41.02	0.02	7.58	0.009	0.02748	0.036	0.004235	0.051	1.94	0.589	0.19606	0.283	0.00168	0.092	0.003856	0.748
	L * Y	2	24.2	0.081	9.26	0.004	0.04723	0.006	0.000314	0.774	0.53	0.863	0.01628	0.894	0.00038	0.555	0.015566	0.327
	WR * L* Y	4	38.51	0.098	13.16	0.005	0.08037	0.003	0.000649	0.894	0.79	0.977	0.03945	0.967	0.0012	0.449	0.033327	0.317
	Pooled error	53	44340.86		21286.54		61.51605		1.0582		3553.66		132.33831		3.34184		20.319897	
	SOV	**D.F.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**	**S.S.**	**F pr.**
MNANJE (Groundnut)	Year (Y)	1	99.98	<0.001	0.57	0.588	0.00004	0.959	0.001037	0.352	0.21	0.625	0.1259	0.375	0.00269	0.806	0.000381	0.803
	Replication	2	4.54		3.25		0.0102		0.000026		0.01		0.0766		0.51825		0.011084	
	Water regime (WR)	2	47963.81	<0.001	3888.47	<0.001	59.17861	<0.001	0.205105	<0.001	1836.46	<0.001	22.8552	<0.001	0.00025	<0.001	13.244015	<0.001
	WR * Y	2	86.61	0.002	2.77	0.497	0.04697	0.291	0.000791	0.694	0.68	0.672	0.0055	0.98	0.00073	0.712	0.002835	0.784
	Pooled error	17	48197.98		3908.42		59.36584		0.21436		1845.79		24.1012		0.53556		13.306443	

SOV = Source of variation, D.F = Degrees of freedom, F pr. = Probability, S.S = Sum of squares, S1 = Stress during flowering, S2 = Stress during pod development.

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