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Physiological, developmental and growth responses to desiccation induced stress in four seed coat colour varieties of *Vigna unguiculata* (L. Walp).

Kwadwo Owusu Ayeh*, Anning Kwame Peter, Adoteye Emmanuella Grace and Lewis Enu-Kwesi

Department of Plant and Environmental Biology, School of Biological Sciences, University of Ghana, P. O. Box LG 55 Legon, Ghana.

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Cowpea (*Vigna unguiculata* L. Walp) is a widely grown leguminous crop in Ghana. This study was conducted using four varieties of cowpea based on the colour of the seed coat namely; *red*, *brown*, *black* and *cream* cowpea varieties, and were subjected to three different watering regimes, namely: Normal, moderate and severe water stress regimes. These different watering regimes were used as potential screening criteria to determine their drought tolerance ability. We monitored changes in physiological, morphological and yield traits due to the effects of four seed coat colour varieties and imposed water regimes. The effects of seed coat colour on relative water content (RWC) was significant ($F = 13.15; p \leq 0.05$). *Red* variety recorded the highest RWC (60.14%) with the lowest RWC (50.71%) in the *Black* seed colour, all under severe water stress. The *Red* and *Cream* seed coats had a soil moisture content (SMC) of 62.61 and 48.70% respectively when the severe water stress treatment was considered. There was a decreasing trend in chlorophyll content for all four cowpea varieties. The *Brown* and *Red* varieties recorded the highest chlorophyll contents under moderate and severe water stress respectively. Proline concentrations were high with increasing drought severity. The *Red* cowpea variety recorded the maximum concentration of free proline (5.7 µg/g) under severe water stress. Mean seed weight in Red seed coat, subjected to severe water stress, was 0.48 g. Results in this experiment revealed that the *Red* seed coat colour appeared to be tolerant to severe water stress compared to *Cream*, *Black* and *Brown* seed coats.

Key words: Cowpea, seed coat colour, water stress, relative water content, soil moisture content

INTRODUCTION

Climate change adversely affects the agricultural sector through increased water stresses, increased erratic

*Corresponding author E-mail: koayeh@ug.edu.gh.

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patterns of rainfall, seasonality fluctuation and temperature variations (Mihiretu et al., 2020). These precipitation irregularities, resulting in low water availability is a major environmental factor limiting growth, development, and the agricultural production of plants worldwide (Chaves et al., 2002; Gómez-Guerrero et al*.,* 2013; Tack et al*.,* 2015). Effects of desiccation stress on plant development occur through osmotic stress and other responses such as cell turgidity, stomatal conductance, transpiration, photosynthesis, respiration, antioxidant activity, light absorption and capture, resulting in reduced crop production (Gómez-Guerrero et al*.,* 2013; Velázquez-Márquez et al*.,* 2015). Drought impacts on plants include growth, yield, pigment content, membrane integrity, osmotic adjustment, water relations, and photosynthetic activity (Praba et al*.,* 2009). Susceptibility of plants to drought stress varies according to the degree of stress, different accompanying stress factors, plant genetic diversity, and their developmental stages (Demirevska et al*.,* 2009).

Cowpea [*Vigna unguiculata* (L) Walp.] is a dicotyledonous crop in the order Fabaceae, subfamily Faboideae (Syn. Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus Vigna and section Catiang (Timko and Singh, 2008). The genus *Vigna* is made up of seven subgenera and over 80 known species (Pasquet, 2001). Cowpea contains four subspecies and one of the widely cultivated species in the genus *Vigna* (Panella and Gepts, 1992). Among the four subspecies, the subspecies *unguiculata* is the most widely cultivated worldwide. Cowpea is both used as food and animal feed, containing high levels of protein (25%), carbohydrates (64%), vitamins, fiber and folic acid (Hall, 2012; Timko and Singh, 2008). It is generally considered to be drought resistant compared to other leguminous crops (Belko et al*.,* 2013; Fatokun et al*.,* 2016). However, the ability to cope with low water potential varies with the particular genotype (Timko and Singh, 2008).

The cowpea seed coat acts as a barrier between the internal structures of the seed and the external environment and this further contributes to the protection of the embryo from mechanical injuries and attacks of pest diseases (Tiryaki et al*.,* 2016; Weber et al*.,* 1996). In addition, the seed coat ensures that seeds survive in the presence of harsh environmental conditions (de Souza and Marcos-Filho, 2001). Research findings have proven that seed size and coat colour are important characteristics for distinguishing between hard-seeded and soft-seeded varieties of *Vicia sativa* (Buyukkartal et al., 2013). Moreover, it appears that there is the implication of seed coat colour in drought tolerance in a Zimbabwean *cream* bambara groundnut landrace due to the seed's ability to maintain leaf turgor pressure through a combination of osmotic adjustment, reduction in leaf area and effective stomatal control (Collinson et al*.,* 1997). In addition, varying growth, phenological and yield responses have been observed in brown, red and light brown seeds of Bambara groundnut (*Vigna subterranean* L.) under imposed water stress (Mabhaudhi et al., 2013). In an unrelated finding, it has been shown that seed coat colour has a profound effect on viability and vigour in Bambara groundnut (Chibarabada et al*.,* 2014). Further, varying response in mineral reserves in seeds of Bambara groundnut has been shown to relate to seed coat colour (Mandizvo and Odindo, 2019).

Some adverse effects of abiotic stresses such as drought, triggers the generation of reactive oxygen species (ROS) in plant tissues and this causes oxidative damage through protein degradation and reduces membrane stability (Abd El-Mageed et al*.,* 2020; Yadav et al*.,* 2021). Thus ROS, among other parameters, may indicate tolerance or susceptibility of plants to drought. These parameters include soil moisture content, relative water content and proline content of leaves. The moisture content is the main source of water available to plants and it is critical for plant growth (Abd Elhamid et al*.,* 2020). This has resulted in many practices such as mulching to preserve water content in the soil (Liu et al*.,* 2019; Zhang et al*.,* 2021). On the other hand, relative water content (RWC) is a measure of available water in any tissue of the plant at any point in time (Sinclair and Ludlaw, 1985). RWC plays a critical role in the physiological process and survival of plants (Sarabi et al*.,* 2017). Proline is an amino acid and critical for protein synthesis and plant development (Furlan et al*.,* 2020). Plants under stress accumulate proline and other osmotic balancing salts so as to lower their water potential in order to increase their water content to avoid desiccation.

Therefore, it might be necessary to determine how seed coat colour influences tolerance of cowpea seedlings to water stress. This study was therefore, designed to evaluate the drought tolerant potentials of four seed coat colours, namely *Red*, *Cream*, *Brown* and *Black* selected *V. unguiculata* L. Walp. (cowpea) varieties under different levels of controlled desiccation stress.

MATERIALS AND METHODS

Plant materials

This study was conducted with four (4) varieties of cowpea based on the colour of the seed coat namely; *Red*, *Black*, *Brown* and *Cream* cowpea varieties. The seeds of the different varieties were obtained from the seed bank of the Genetics Laboratory in the Department of Plant and Environmental Biology.

Soil

Soil was obtained from the Teaching and Research Garden at the Department of Plant and Environmental Biology, University of Ghana and sterilized using an autoclave at 15 psi and 121°C for 30 min. 50 g of soil was weighed and placed into 144 polythene bags.

Location of study

This study was conducted in screen house of Department of Plant and Environmental Biology, University of Ghana.

Experimental design

The experiment involved the testing of two factors (water stress and cowpea varieties) in a 3 × 4 factorial design laid out in Randomized Complete Block Design (RCBD). Three blocks were used and they represented normal, moderate and severe watering regimes. Each block consisted of 4 columns of seedlings corresponding to cowpea seed coat colour with each column containing 12 replicate seedlings. The variables tested were *Red*, *Brown*, *Black* and *Cream* seeds and three water regimes used in this experiment namely normal (rehydrated every other day), moderate (rehydrated every 3 days) and severe (rehydrated every 7 days). A total of one hundred and forty four (144) cowpea seedlings were used for the entire experimental set up. 150 ml of water was used in watering each seedling in polythene bag under each treatment block. Prior to application of watering regime, all the seedlings were saturated with water for the first week of planting. After the first week, chlorophyll content, relative water content, soil moisture content, free proline content of leaves. Mean number of pods, mean number of seeds and shoot to root ratio were measured 8 weeks after planting.

Determination of chlorophyll content

The spectral absorbance data (SPAD) chlorophyll content was measured in newly emerged leaves in each treatment block weekly for 9 weeks using the SPAD Chlorophyll Content Metre (CCM- 200 plus, Opti-Sciences Inc.).

Determination of leaf relative water content (RWC) of experimental seedlings

The RWC of selected leaves of each cowpea variety was measured to assess the water status of the seedlings. The Relative Turgidity Method of Barrs and Weatherley (1962) was followed for the determination of leaf RWC. On each sampling occasion, three leaves were detached from the cowpea varieties and were thoroughly cleaned. A cork borer, with diameter measuring 1 cm was used to create three (3) leaf discs and placed in a clean petri dish and covered. The three sets of leaf discs were immediately transferred to the laboratory where their fresh weight were determined together using a fine balance (AL 104; Mettler Toledo, Columbus, OH, USA). Subsequently, each set of the 3 leaf discs were floated on 10 ml of distilled water in a covered petri dish for three 3 h after which they were removed with clean pair of forceps, surface blotted between 2 layers of tissue paper and weighed in order to determine their full turgid weight (TW). The leaves were then dried in an oven at 60°C for 24 h (in order to remove only the moisture content) after which their dry weights (DW) were determined.

The RWC for each set of three leaf discs for each cowpea variety was calculated as follows;

$$\text{RWC} = \frac{Fresh\ Weight\ (FW) - Dry\ Weight(\ DW)}{Turgid\ Weight\ (TW) - Dry\ Weight\ (DW)} \times 100$$

Where, *FW*: Fresh weight of three (3) 1 cm inner diameter leaf discs.*TW*: Turgid weight of three (3) 1 cm diameter leaf discs of each cowpea genotype after floating on 10 ml of distilled water for 3 h. *DW*: Dry weight of the three 1 cm diameter leaf discs after oven drying for 24 h at 60°C.

Measurement of soil moisture content (SMC)

Soil moisture content (SMC) was determined by the Gravimetric Method and expressed as a percentage of the initial weight. On each SMC sampling occasion, soil samples were collected from each plastic container (at 5 cm) along the sides of the plastic container (to avoid breaking roots) using a 1-cm inner diameter stainless steel tube, quickly emptied into a glass Petri dish, and covered. The initial weight of the collected soil samples was determined in the laboratory with a fine balance (AL 104; Mettler Toledo, Columbus, OH, USA). The soil samples were then dried in the oven at 60°C for 24 h (to remove only the moisture). The dry weight (DW) of the soil was determined. The soil moisture content was then calculated as a percentage of the initial weight of the soil as;

$$\text{SMC} = \frac{Initial\ Weight\ (IW) - Dry\ Weight\ (DW)}{Initial\ \ Weight\ (IW)} \times 100$$

Determination of free proline content of leaves

On each sampling occasion, three leaf discs were removed from the leaf blade of selected leaves using a 1-cm inner cork-borer on a clean flat tile, avoiding midrib and major veins. The method of Singh et al. (1972) as modified by Mukherjee and Choudhuri (1983) was used. The three (3) leaf discs from the three replicates of each accession per treatment were boiled for 5 min in 80% (5 ml) ethanol and decanted.

The discs were then ground in 5 ml of the same 80% ethanol, refluxed for 15 min and decanted. The residue was further refluxed for 15 min using 40% ethanol. Further refluxing was done with distilled water for 15 min. Filtrate obtained were pooled together, reduced to 10 ml by flash evaporation on water bath and added to 5 ml phosphate buffer of pH, 8.0.

The filtrate was then extracted two times with petroleum ether (5 ml), using a separating funnel, followed by two times extraction with ethyl acetate. Subsequently, the aqueous solution (extract) was reduced to 15 ml by flash evaporation on a water bath. An acid ninhydrin reagent was prepared by warming 1.25 g of ninhydrin with 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid over a water-bath with agitation. 2 ml of the aqueous solution obtained from the extraction procedure was mixed with 2 ml of glacial acetic acid in a capped test tube in a water bath at 100°C for 1 h. The reaction was terminated in an ice bath for 5 min. The colour developed was extracted by shaking vigorously with 4 ml of toluene in a separate funnel for 15 s. The colour containing toluene phase was allowed to cool to room temperature 25°C in a test tube, transferred into a glass cuvette and its absorbance was determined at 520 nm with a spectrophotometer (Jenway 6320D Spectrophotometer) using toluene as blank. The actual proline content was expressed as µg proline per gram leaf dry using the formula:

$$\text{Proline (µg/g)} = \frac{\text{Proline}\left(\frac{\text{ug}}{\text{ml}}\right) \times \text{ml benzene}}{\text{weight of discs (g)}}$$

Measurements

Number of pods, number of seeds per pod, seed weight per pod and root to shoot ratio were determined and recorded for all the four seed coat varieties used in this study and under the three varying water regimes.

Data analysis

All data was analysed with ANOVA and Turkey pairwise comparison using 2018 version of Minitab.

RESULTS

Effects of watering regimes on chlorophyll content

Generally, higher chlorophyll content values were recorded for all four cowpea varieties during the first three weeks of planting under the three treatment blocks, after which a decline in chlorophyll content was observed. One-way analysis of variance (ANOVA) indicated that cultivar had significant effects on chlorophyll content under the normal watering regime ($F = 10.81$; $p < 0.05$) with the red variety recording the highest (23.23 μmol/m^2) chlorophyll content whilst the least chlorophyll content was recorded for the cream variety (15.73 μmol/m^2) (Table 1). Significant differences in chlorophyll content were higher in week six (6) (Table 1)

Cultivars had significant effects on chlorophyll content under the moderate watering regime ($F = 9.48$; $p < 0.05$). The highest and lowest chlorophyll contents were recorded for *Red* variety (22.77 μmol/m^2) and *Cream* variety (18.46 μmol/m^2) varieties respectively (Table 1).

There was a general decreasing trend in chlorophyll content for the *Red*, *Brown*, *Black* and *Cream* varieties (Figures 1 to 4) under the three watering regimes.

The effect of water stress on chlorophyll content was indicated by a change in leaf coloration for the *Red* seed. Green leaves (Figure 5B) were observed under normal water regime whereas an increasing degree of leaf yellowing was observed with increasing drought stress (Figure 5B, C and D).

A similar decreasing trend for chlorophyll content was observed for the *Brown* cowpea variety (Figure 5E) under the three watering regimes. Chlorosis was observed in the seedlings of *Brown* and *Black* seed varieties with increasing water stress, and yellowing of leaves more pronounced under severe water stress treatment (Figure 5F, G, and H; 5I, J, K and L) respectively. Chlorophyll content for seedlings of *Cream* variety (Figure 5M) followed the decreasing trend as was observed in the *Red* and *Brown* cowpea varieties (Figure 5M, N, O and P). The decline in chlorophyll content was indicated by an intense leaf chlorosis for all the varieties with increasing desiccation stress. Intense chlorosis of leaves in the severe water stress treatment led to senescence and subsequently followed by abscission of leaves.

Effects of drought on relative water content (RWC)

Generally, all the four cowpea varieties recorded higher RWC values after two weeks of planting under the three treatment blocks. A one-way ANOVA indicated that cultivar had significant effects on RWC under the normal watering regime (*$F = 13.15$; $p \leq 0.05$*). The highest (86.57%) and lowest (77.20%) RWC under normal watering regime was recorded for the *Red* and *Cream* cowpea varieties respectively (Table 3). The effect of cultivar on RWC was more significant after five (5) weeks of planting (Table 4). Higher RWC was recorded for the four varieties under normal watering regime, however a decline in RWC was observed in week 7 for the *Brown*, *Cream* and *Red* varieties (Figure 11).

Significant effects of cultivar on RWC under moderate watering regime were confirmed by one-way ANOVA ($F = 2.84$; $p < 0.05$). It was observed under moderate stress treatment that the *Red* cowpea variety recorded the highest RWC (81.43%) while the least RWC (73.57%) was recorded for the *Cream* cowpea variety as was previously observed under normal watering regime (Table 2).

Soil moisture content (SMC)

Although the highest (88.01%) and lowest (78.67%) SMC values were recorded for the *Red* and *Cream* varieties respectively (Table 3), the effect of cultivar on SMC was not significant under the normal and moderate watering regimes (*$F = 13.81$; $p \leq 0.05$*).

However, SMC values for *Red* and *Brown* cultivars were significantly different from that of *Black* and *Cream* under both moderate and severe water stress treatments (Table 3).

Proline accumulation

Proline concentration was generally low in the first week of planting for all cowpea varieties under the normal watering regime. A peak in proline concentration was observed for all cowpea varieties between weeks 5-7 WAP (Figures 6 to 8).

Proline concentration increased in week six (6) in all the cowpea varieties and declined in 7 WAP for all varieties in the normal watering treatment (Figure 6). In the intermediate watering treatment (moderate), *Black* and *Cream* varieties maintained their increasing proline concentration whereas *Red* and *Black* proline concentrations dropped at 7 WAP (Figure 7). Proline concentration was generally low for all cowpea varieties under severe drought stress treatment in the first week of planting and followed by a general increasing trend to 7 WAP (Figure 8).

We further sought to compare the significance of mean differences in proline content of leaf cultivars under their respective water treatments using the Tukey mean difference method and observed that in the normal water

Table 1. Mean chlorophyll content of the four cowpea varieties under the three watering regimes.

Cultivar	Mean chlorophyll content (µmol/m^2)		
	Normal ± SE	Moderate ± SE	Severe ± SE
Red	23.23 ± 0.9^a	22.77 ± 0.8^a	20.77 ± 0.74^a
Brown	23.01 ± 0.9^a	22.17 ± 0.6^a	21.62 ± 0.75^b
Black	20.14 ± 0.7^b	19.56 ± 0.7^b	18.52 ± 0.5^b
Cream	15.73 ± 0.51^c	18.46 ± 0.6^b	17.51 ± 08^a

Means that do not share a letter are significantly different.

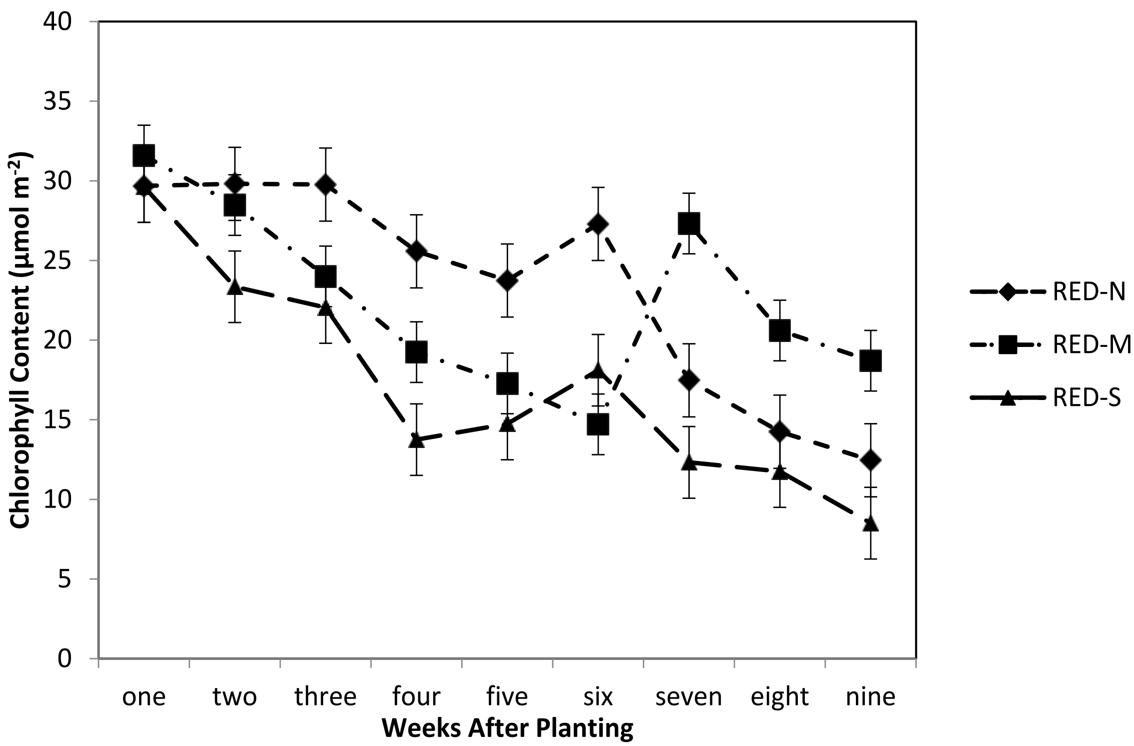

Figure 1. Mean weekly chlorophyll content of the Red cowpea variety under the three (3) watering regimes.

regime, cultivar had no significant effect on mean free proline content in the leaves of the four cowpea varieties (F = 0.55; p> 0.05). The highest (2.00 µg/g DW) and lowest (1.28 µg/g DW) concentrations of free proline were measured for the *Red* and *Cream* cowpea varieties respectively (Table 4). Similarly, no cultivar effect on mean proline content of leaves were found in the moderate water treatment (Table 4). However, when severe water stress regime was considered, cultivar had significant effect on free leaf proline content (F = 4.71; p <0.05). The highest (3.80 µg/g DW) proline concentration was measured in the *Red* variety followed by the *Black* variety whilst the lowest (2.87 µg/g DW) free proline was recorded in the leaves of the *Cream* cowpea variety (Table 4).

Effects of drought on yield

Number of pods

Cultivar had no significant effects on the mean number of harvested pods under normal watering regime (*F = 1.45; p > 0.05*). The highest (2.14) number of pods was harvested from the *Cream* cowpea variety whilst the least (1.43) number of pods were harvested from the *Black* variety. One-way ANOVA indicated no significant effects of cultivar on number of harvested pods under the

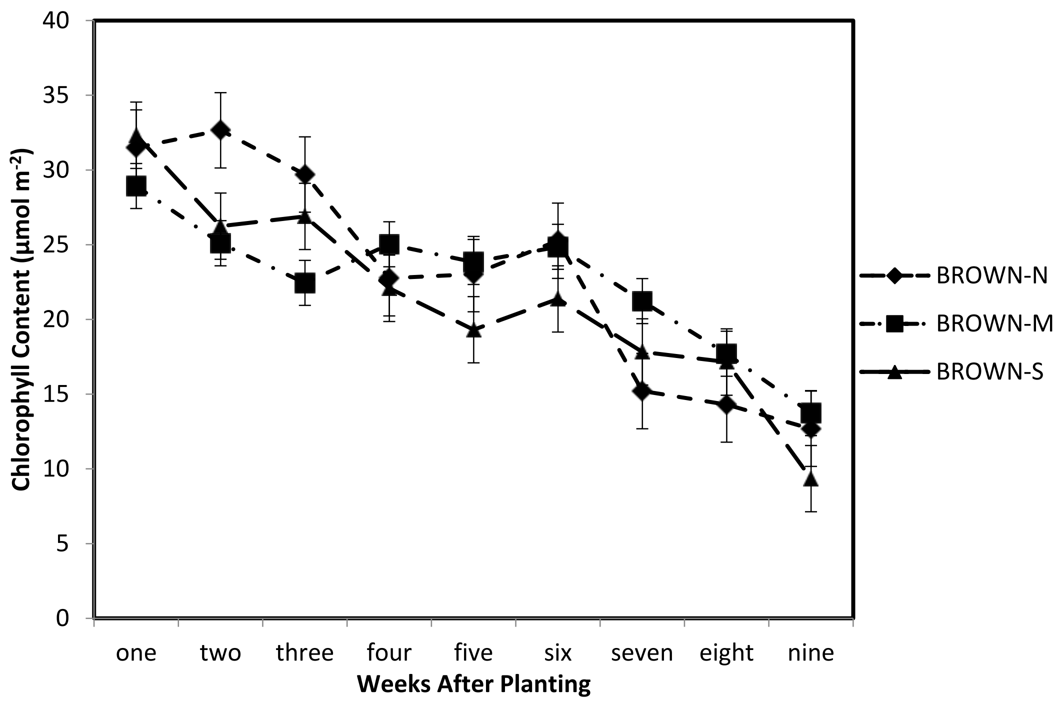

Figure 2. Mean weekly chlorophyll content of the brown cowpea variety under the three (3) watering regime.

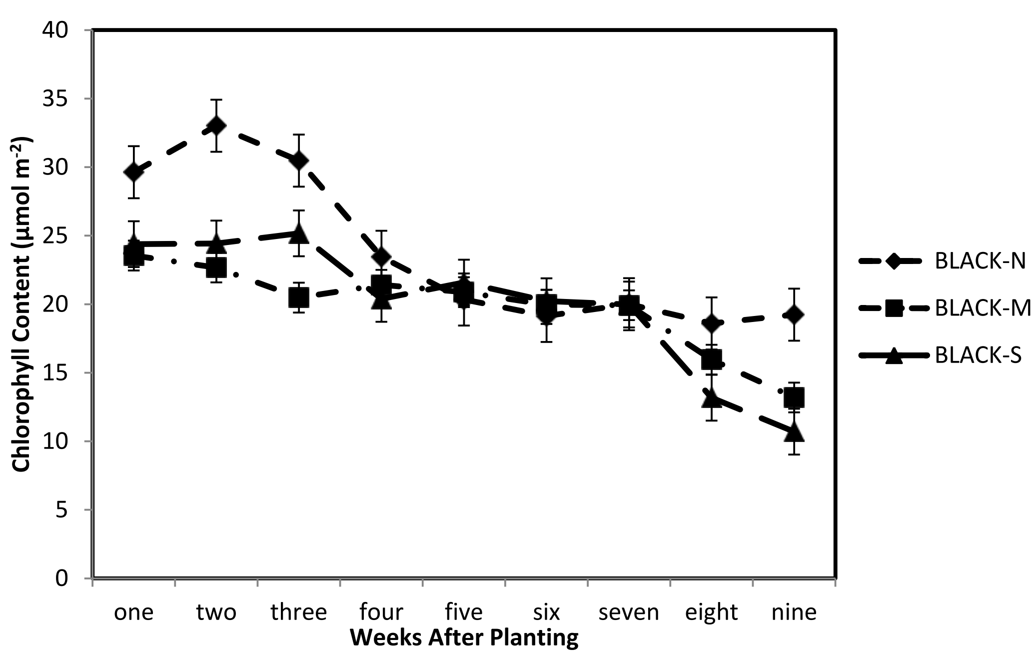

Figure 3. Mean weekly chlorophyll content of the black cowpea variety under the three (3) watering regimes.

moderate watering regime (F = *0.94; p ≥ 0.05*). The highest (1.70) number of pods were harvested for the

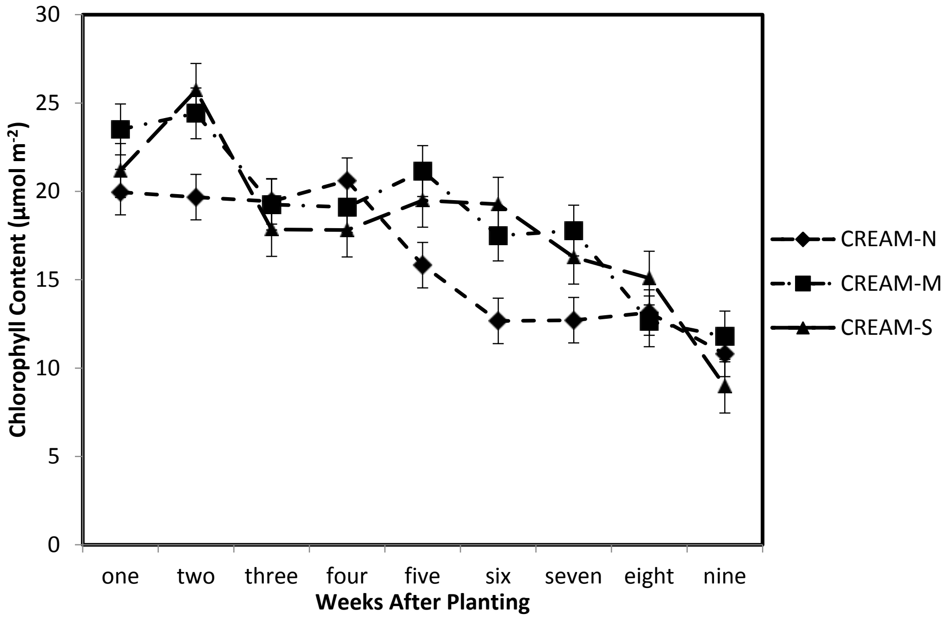

Figure 4. Mean weekly chlorophyll content of the cream cowpea variety under the three (3) watering regimes.

Black variety whilst the other cowpea varieties recorded the same mean number (1.00) of harvested pods (Figure 7). It was observed that mean number of harvested pods decreased for the *Red* cowpea variety as drought severity increased (Figure 9). The number of cowpea seeds per harvested pod was observed to decrease with increasing drought severity (Figure 10). The *Cream* variety recorded the highest percentage decrease in number of seeds (50%) whilst the least percentage decrease (35%) was observed in the *Black* cowpea variety.

Seed weight

Cultivar had significant effects on seed weight under the normal watering regime ($F = 15.12; p \leq 0.05$) with the *Red* and *Cream* cowpea varieties recording the highest (1.98 g) and lowest (0.41 g) mean seed weight respectively. One-way ANOVA indicated that cultivar had significant effects on seed weight under the moderate watering regime ($F = 2.53; p \leq 0.05$). In the severe water stress, the highest (0.48 g) mean seed weight was recorded for the *Red* variety whereas no seeds were found in *Cream*, *Black* and *Brown* seeds (Figure 11).

Root to shoot ratio

Cultivar had significant effects on root/shoot ratio of the four cowpea varieties under normal watering regime ($F = 4.54; p < 0.05$). The highest (0.23) root/shoot ratio was recorded for the *Cream* variety, followed by *Black* variety. The lowest (0.09) root/shoot ratio was measured for the *Red* and *Brown* cowpea varieties (Figure 12). The effect of cultivar on root/shoot ratio was not significant under moderate drought stress treatment. The highest (0.25) and lowest (0.07) root/shoot ratio values were measured for the *Cream* and *Brown* varieties respectively. All cowpea varieties recorded higher values for root/shoot ratio under severe drought stress as compared to their counterparts under both normal and moderate stress treatments (Figure 12).

Deep rooting was more prominent for the *Red* and *Brown* cowpea varieties under severe drought stress whilst the *Cream* cowpea variety developed deeper roots under moderate drought treatment.

DISCUSSION

Chlorophyll content

When plants are exposed to drought conditions, chloroplast content may be damaged through a process known as photoinhibition (Shao et al., 2008; Macar and Ekmekci, 2009; Nakamura and Izumi, 2018). Moreover, water stress results in a decline in total chlorophyll content which eventually results in decreased available energy for photosynthesis (Panda and Sarkar, 2013; Slattery et al., 2017; Zhao et al., 2018). These findings

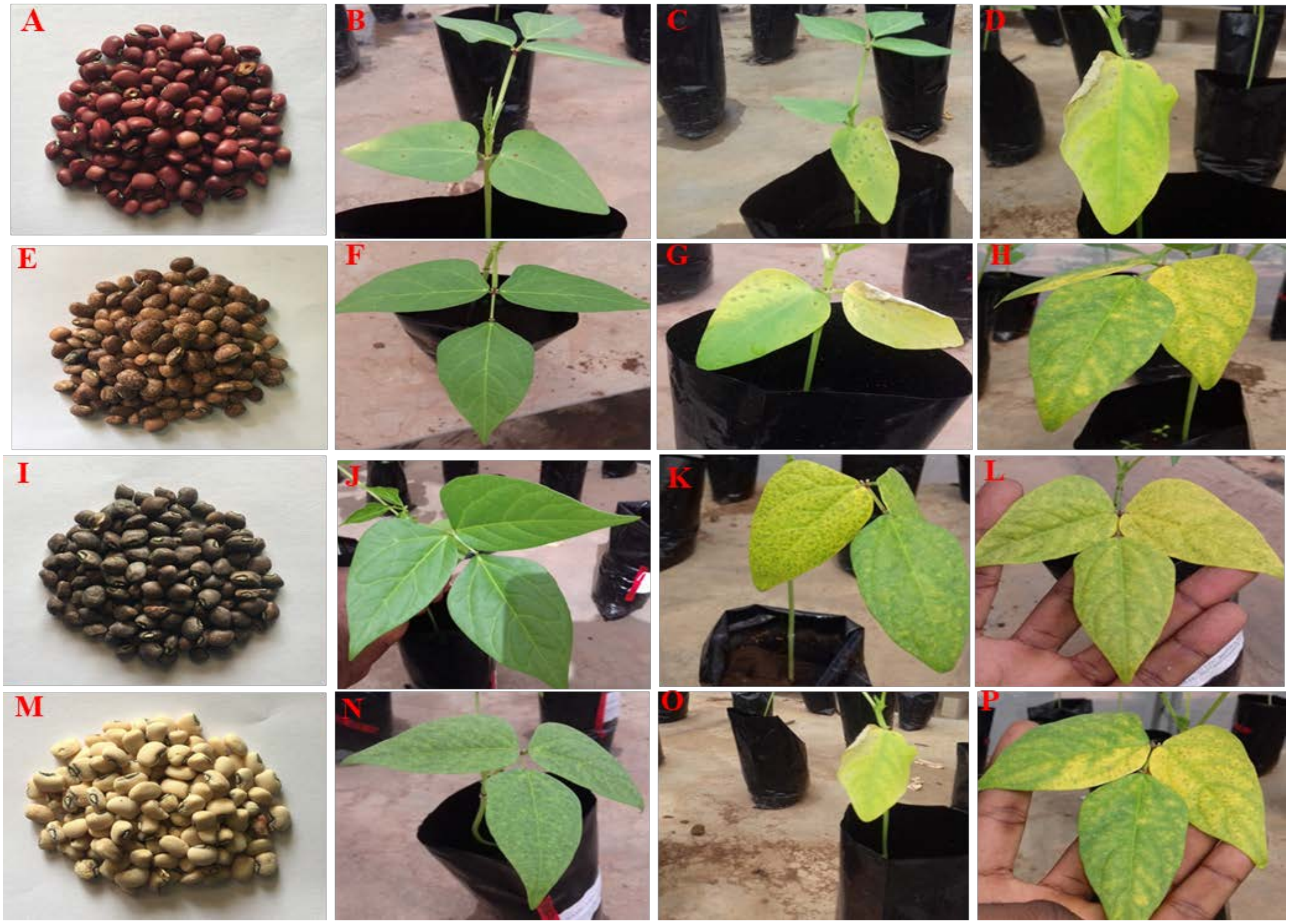

Figure 5. Seed coat colour and its corresponding seedlings in normal, moderate and severe water regimes; **A**- red seed; **B-** red-normal; **C-** red-moderate; **D-** red-severe; **E**- Brown; **F**- brown-normal; **G**- brown-moderate; **H**- brown-severe; **I-** Black; **J**- black-normal; **K**- black-moderate; **L**- black-severe; **M**- cream; **N**- cream-normal; **O**- cream-moderate; **P**- cream-severe.

reported above, were in agreement with the observations of the present study. The depth of decline in chlorophyll content was different for the four cowpea varieties under the three drought treatments. Among the four varieties, the decrease in chlorophyll in the *Red* and *Brown* cowpea varieties were lower than that in the *Black* and *Cream* cowpea varieties as observed in the severe drought conditions. The *Red* and *Brown* cowpea varieties maintained higher values of chlorophyll with the *Black* and *Cream* varieties showing lower values under moderate and severe drought treatments. These observations were consistent with the findings of Zhao et al. (2018), who reported higher chlorophyll content values for *L. barbarum* L. under severe drought conditions and reported it as drought tolerant. The removal of yellowing leaves from the main plant body cowpea varieties, subjected to severe water stress, may be explained by the fact that leaves typically senesce before they abscise when triggered by drought (Patharkar and Walker, 2019). In a related example, *Arabidopsis cauline* leaves turned from green to yellow before abscising upon drought treatment (Patharkar and Walker, 2017). The *Red* and *Brown* varieties of cowpea used in this study may therefore be potential candidates for drought resistance.

Relative water content

RWC is known to be a good tool for the measurement of dehydration tolerance in plants under abiotic stress (Abdelkhalik et al., 2019). Further, regulation of water loss by cowpea plants is dependent on whether they are well watered or exposed to drought or vapour pressure deficiency (Zegaoui et al., 2017). In addition, comparisons

Table 2. Mean relative water content (RWC) of four cowpea varieties under the three watering regimes.

Cultivar	Mean RWC content (%)		
	Normal ± SE	**Moderate ± SE**	**Severe ± SE**
Red	86.57 ± 1.1^{a}	81.43 ± 1.9^{a}	60.14 ± 2.9^{c}
Brown	85.08 ± 0.9^{a}	77.71 ± 2.5^{b}	54.14 ± 3.55^{c}
Black	78.52 ± 1.2^{b}	75.29 ± 1.7^{b}	50.71 ± 3.3^{c}
Cream	77.20 ± 1.2^{b}	73.57 ± 1.9^{b}	51.86 ± 3.5^{c}

Means that do not share a letter are significantly different.

Table 3. Mean soil moisture content (SMC) of the four cowpea varieties under the three watering regimes.

Cultivar	Mean SMC (%)		
	Normal ± SE	**Moderate ± SE**	**Severe ± SE**
Red	88.01 ± 0.1^{a}	79.72 ± 3.3^{b}	62.61 ± 3.4^{c}
Brown	86.31 ± 0.6^{a}	77.00 ± 3.3^{b}	56.56 ± 4.1^{c}
Black	80.50 ± 1.7^{a}	72.11 ± 3.6^{b}	49.78 ± 4.5^{d}
Cream	78.67 ± 1.3^{b}	70.11 ± 3.7^{b}	48.70 ± 4.2^{d}

Means that do not share a letter are significantly different.

Table 4. Mean proline concentration of the four cowpea varieties under the three watering regimes.

Cultivar	Proline (µg/g DW)		
	Normal ± SE	**Moderate ± SE**	**Severe ± SE**
Red	2.00 ± 0.39^{a}	3.54 ± 0.95^{a}	3.80 ± 1.07^{a}
Brown	1.69 ± 0.36^{a}	2.84 ± 1.11^{b}	2.97 ± 0.60^{b}
Black	1.88 ± 0.57^{a}	3.14 ± 1.49^{b}	3.57 ± 1.21^{b}
Cream	1.28 ± 0.35^{a}	2.73 ± 1.29^{b}	2.87 ± 0.86^{b}

Means that do not share a letter are significantly different.

of wild populations or cultivated varieties indicated that RWC can be used to differentiate between varieties that are susceptible or tolerant to drought. For example, in *Phaseolus vulgaris*, RWC has been used to reveal a drought susceptible cultivar with high RWC and water content (WC) indicating the ability of this cultivar to maintain water balance in water stressed conditions (Rosales et al., 2012). In the model plant *Arabidopsis,* different accessions were identified depending on variation in RWC in response to moderate drought (Zegaoui et al*.,* 2017). Drop in RWC due to water stress was observed in this study as has been previously reported for other cowpea genotypes and pigeon pea plants (Kumar et al*.,* 2011; Hayatu et al*.,* 2014). Moreover, it has been reported that RWC of cultivars that are under drought stress, may be as a result of differential abilities of more absorption of water from soil or ability of stomata to reduce the loss of water (Keyvan, 2010). The *Red* and *Brown* cowpea varieties maintained an identical RWC under moderate stress conditions seven weeks after planting. It is, therefore, possible that both varieties may be tolerant to mild drought stress that can only be distinguished in terms of RWC after severe water shortage. After seven weeks of severe drought, the *Red* variety maintained a higher RWC, thus distinguishing it from the *Brown* variety as tolerant to prolonged water shortage. It was noted among all four cowpea varieties used in this study that the *Cream* cowpea variety appeared to be more susceptible to mild to severe drought stress conditions as it showed lower RWC values.

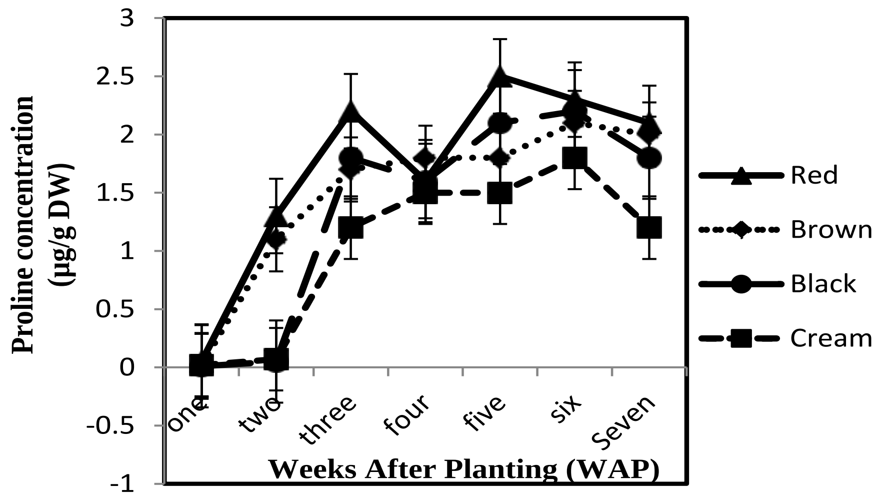

Figure 6. Free proline concentration for the four (4) cowpea varieties under normal watering regime.

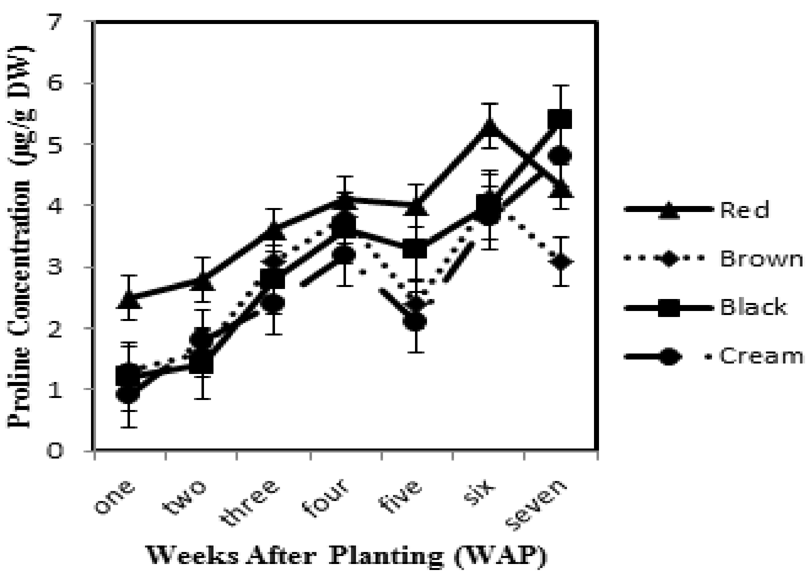

Figure 7. Free proline concentration for the four (4) cowpea varieties under moderate watering regime.

Yield

The ultimate purpose of growing crops is to increase yield (Jaleel et al*.,* 2009). In addition, water stress or deficit has been reported to reduce yield in *Sesamum indicum* L. populations with contrasting seed coat colour (Kermani et al., 2019). The findings of the present study revealed a general reduction in yield for all cowpea genotypes with increasing desiccation stress. These findings in this study seem to be consistent, for example, with the findings of Anjum et al*.* (2011), who reported a substantial reduction in yield and yield components such as grain yield/plant, biological yield/plant and harvest index in maize. Further, four varieties of cowpea used in this study, seem to show significant differences for final harvestable yield under drought stress. Drought-related reduction in yield and yield components of plants could be due to stomatal closure in response to low soil water content, which

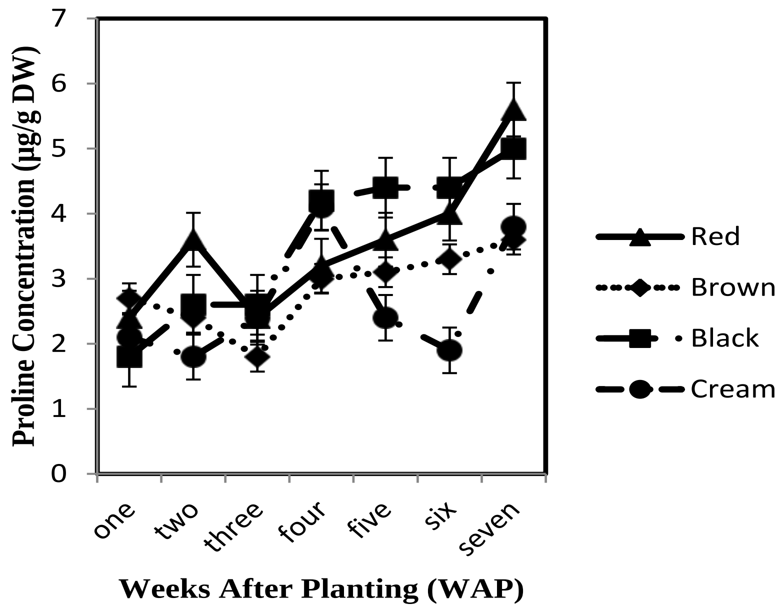

Figure 8. Free proline concentration for the four (4) cowpea varieties under severe stress treatment.

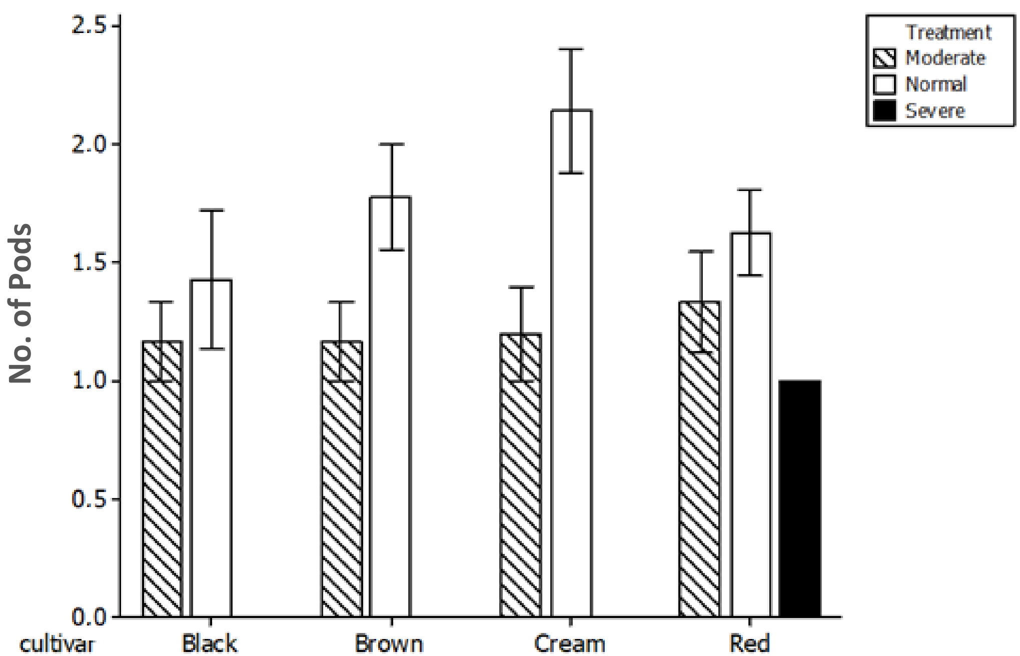

Figure 9. Mean number of harvested pods of the four (4) cowpea varieties under the three watering regimes.

decreases the intake of CO_2 and, as a result, a decrease in photosynthesis (Massacci et al., 2008). The higher number of pods harvested for *Red* cultivar under both moderate and severe water stress conditions could be linked to the *Red* variety showing the highest chlorophyll content under both moderate and severe desiccation stress. The decline in the number of harvested pods observed for the *Cream* variety under moderate stress

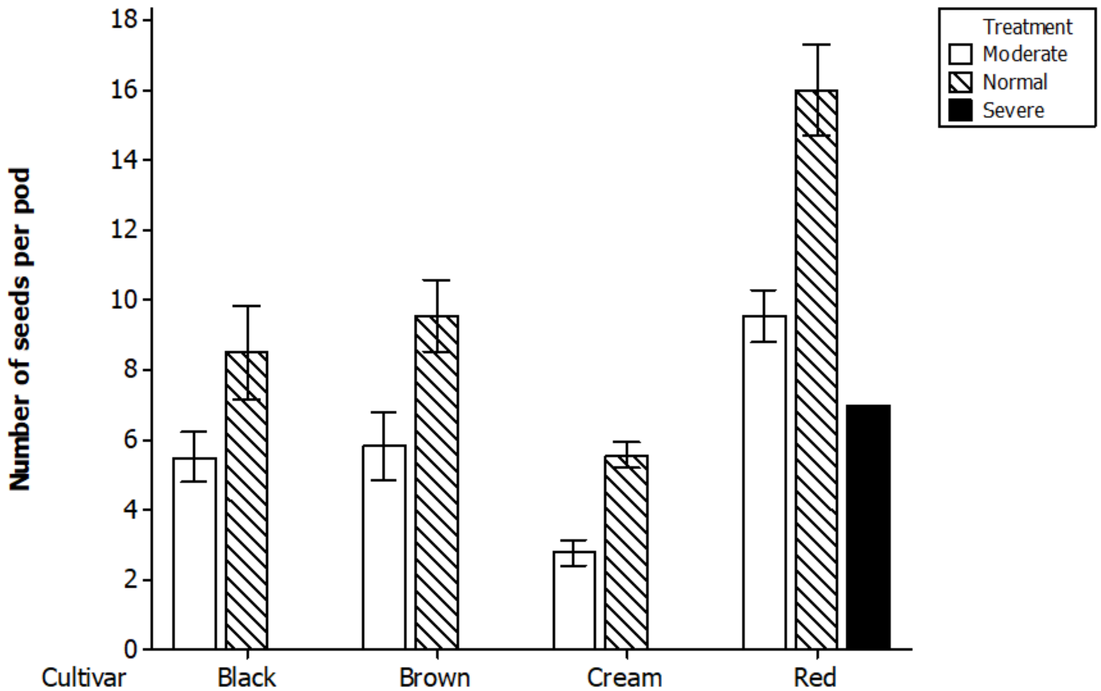

Figure 10. Mean number of seeds per harvested pods of the four (4) cowpea varieties under the three watering regimes.

conditions also corresponded with significant reduction in chlorophyll content for *Cream* variety under moderate and severe water stress. The reduction in number of pods in this study may be due to a reduction in water use resulting in reduced photosynthetic ability as reported in chickpea (Zaman-Allah et al., 2011; Sivasakthi et al., 2020) and common bean (Rezene et al., 2012).

The findings in this study indicated that drought stress had an effect on the mean number of seeds as well as seed weight for all selected cowpea genotypes. A general decline in the mean number of seeds and seed weight owing to moderate drought stress was observed for all cowpea genotypes. However, there was a lower percentage reduction in seed number as well as seed weight for the *Red* cultivar under moderate stress conditions as compared with the other cowpea varieties. These results were similar to the findings of Petropoulos et al. (2008), who reported that the accumulation of dry matter is critical in the process of yield determination in water stressed *Parsley*. Moreover, yield has been associated with an increase in both grain number and individual grain weight in water stressed sunflower (Soriano et al., 2004). Under moderate water stress, the seed yield (number of harvested pods, number of seeds and seed weight) for the *Cream* was less when compared to well-watered control plants and this is consistent with a reduction in yield components such as grain number and grain weight in drought stress treatment in wheat (Dickin and Wright, 2008). Water stress has been reported to be most damaging in reducing seed yield in *Petroselinum crispum* (Petropoulos et al., 2008) and this is consistent with the low yields observed for the *Cream* variety used in this study.

Soil moisture content

Soil moisture stress has been reported to have significant effects on total dry weight, seed number, individual seed dry weight, and seed yield in Soybean (Wijewardana et al., 2019). Our findings revealed that the reduction in soil moisture content from the normal, moderate through severe water treatments were in agreement with published data where soil water content or the relative water content of leaves affected photosynthesis leading to yield loss (Mathobo et al., 2017; Tankari et al., 2019). One of the possible links between low soil moisture content and low chlorophyll content could be that as water stress increases, stomata may remain closed for a long time, leading to damage of the chloroplast and hence affecting synthesis of chlorophyll. Low photosynthetic activity, resulting from drought stress may affect yield as seen in all the varieties subjected to severe water stress which culminated in low soil moisture content. Our results were in consistent with the previous findings of Samarah et al. (2009) in which they found that

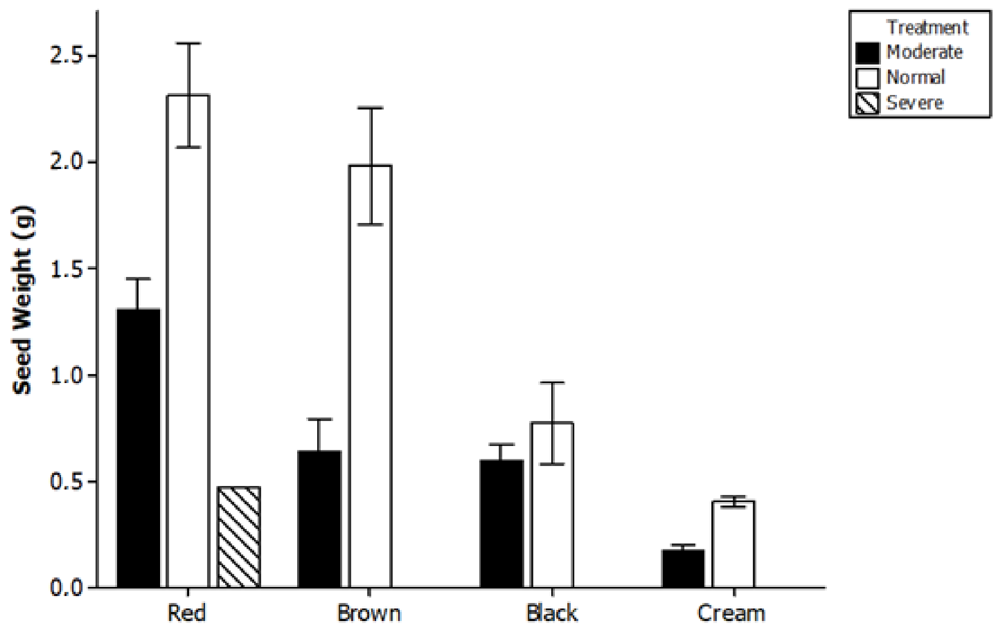

Figure 11. Mean seed weight of the four (4) cowpea varieties under the three treatment blocks.

drought stress reduced seed quality by producing small and underdeveloped seed. These results seem to confirm that *Red* and *Brown* varieties were more tolerant to drought stress compared to their more susceptible *Black* and *Cream* counterparts.

Root to shoot ratio

By breeding cowpea with deeper rooting, adaptation to drought may be enhanced in areas where rainfall is limiting (Hall, 2012). However, it has also been reported that lack of correlation between the root biomass and shoot biomass in estimating root to shoot (R: S) ratio may be due to the effect of the environment (Ordóñez et al., 2020). Generally, all cowpea varieties used in this study recorded relatively, lower root: shoot ratio values under normal watering regime owing to small length in their root systems. This corresponded to the observations of Hall (2012), who reported that deeper roots may not be of an advantage to cowpea during periods of rainfall in the soils of Sahel region. An increase in the root: shoot ratio for the cowpea varieties used in this research were observed under moderate water stress treatment as expected. Deeper rooting was observed in the *Red* cowpea variety and it corresponded to high root: shoot ratio under severe stress treatment. This implied that the *Red* variety may be better adapted to severe water shortage as compared to the other cowpea varieties. The adaptation to severe stress by the *Red* variety was supported by its high relative water content (RWC) when exposed to severe water stress conditions, indicating that their roots were able to penetrate deeper to reach available water in the dry soil.

Proline accumulation

Proline is an amino acid that performs multiple functions in the cell, plays a key role in osmotic adjustment and has the increased ability to resist cellular dehydration (Gholami-Zali and Ehsanzadeh, 2018; Ghaffari et al., 2019; Primo-Capella et al., 2021). Plants accumulate proline in response to water deficit so as to ameliorate their susceptibility levels to drought (Hasan et al., 2020; Hasegawa et al., 2000). Moreover, Merwad et al. (2018) reported increased proline content in drought induced *V. unguiculata* compared to absence of water stress in the same plant. In our study, we observed that the cowpea varieties had higher free proline in their leaves under moderate and severe stress treatment as compared to their normal water regime control counterparts and these observations were similar to what was observed by Sultan et al. (2012) in some *Triticum* L. (wheat) species. The *Red* cowpea accumulated the highest free proline under severe water stress treatments and this is consistent with high proline accumulation observed in drought resistant varieties of rice and sunflower subjected to low water potentials (Chutipaijit et al., 2009; Yousfi et al., 2010). Our findings confirmed previous reports that increase in proline is associated with response to water stress (de Mezer et al., 2014). Proline, a compatible

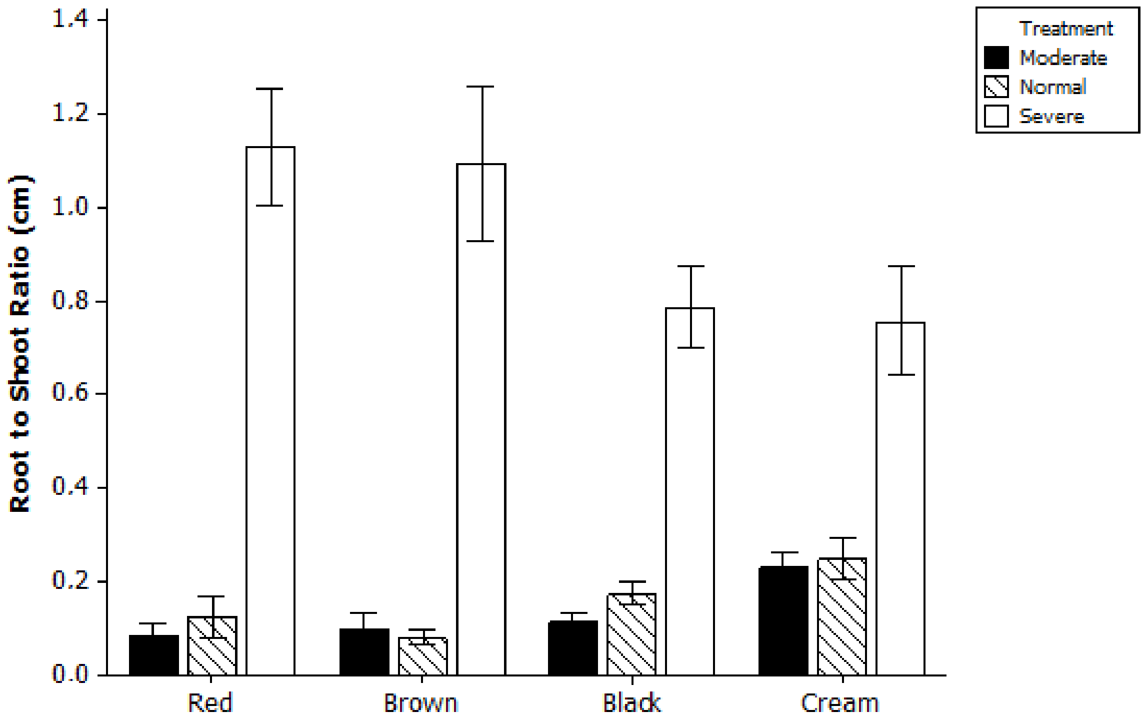

Figure 12. Root to shoot ratio of the four (4) cowpea varieties under the three watering regimes.

osmolyte, contributes to osmotic adjustment as well as stabilizing subcellular structures and in addition scavenging free radicals under low water potentials (Bandurska et al*.,* 2017). The *Red* variety showed a high drought resistance tolerance potential, followed by the *Black* variety which recorded a relatively higher free proline level under severe stress. There seemed to be a positive correlation between proline and relative water content (RWC) levels as the *Red* variety also maintained the highest RWC under severe stress treatment. However, Sperdouli and Moustakas (2012), contrary to our findings, reported lower dehydration and lower proline levels in *Arabidopsis thaliana* under severe drought conditions. In this present study, we showed that accumulation of proline in the cultivars was correlated with increase in seed weight and number of seeds. This has practical agricultural significance as demonstrated by Semida et al*.* (2020), who found that exogenous application of proline in the fields to onion (*Allium cepa*) led to accumulation of proline as an osmolyte in tissues, thereby contributing to water uptake and subsequent increase in bulb size.

Conclusion

Chlorophyll content decreased with increasing drought stress in all cowpea varieties. The *Red* and *Cream* cowpea varieties recorded the highest and lowest chlorophyll content respectively under both moderate and severe watering regimes. The *Red* cowpea variety recorded the highest RWC, SMC and proline contents compared to the *Cream*, *Brown* and *Black* seed coat varieties under severe water stress treatment. These findings give an indication that the *Red* seed coat variety may have the ability to tolerate severe water stress conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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