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Watering Regime Effects on Sorghum Growth and Physiological Attributes of Sorghum (*Sorghum bicolor* {L.}Moench) Varieties Under Screenhouse Conditions

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ABSTRACT
Study examined responses of growth, yield and physiological attributes of sorghum varieties to watering regimes under screenhouse condition. Experiment was 3 watering levels and six sorghum varieties. Watering regimes were well watering at full field capacity, 60 and 40 % field capacity and sorghum varieties were CSR-01, Bida local, SK5912, SAMSORG 14, 17 and 44. Data were collected on root and shoot biomass, water use, stomatal conductance, proline, water soluble carbohydrate and leaf chlorophyll concentrations of cacao seedlings. Results showed that watering level affected growth and physiological characters of sorghum varieties. Among watering treatments, differences were obtained in biomass, densities of stomatal, concentrations of chlorophyll, and proline and water soluble carbohydrates. Sorghum varieties evaluated differed in their responses to watering regimes based on the measured growth and physiological attributes. Sorghum had better vigour of growth when grown under 100 and 60 % field capacity watering compared with 40 % FC. The measured variables were superior at well watering (100 % FC) compared with 40 % FC. Most of the measured growth and physiological variables appeared to play important roles for moisture deficit stress tolerance in sorghum.

Keywords: *Sorghum, rootzone moisture, growth, yield, functional traits, tolerance, climate*

Introduction

Sorghum (*Sorghum bicolor* {L.} Moench) is an important member of the family Poaceae. It is the fifth most significant world cereal crop after maize, wheat, rice, and barley (FAOSTAT, 2019). It is a staple food in drier parts of Africa, China and India (Ajeigbe et al., 2018, Akinseye et al., 2023). Total world production in 2023 was 59,920 metric million tonnes (FAO STAT, 2019). The largest sorghum producers were United State with annual grain production of 8, 175 metric million tons, Nigeria with 6,700 metric million tons, Sudan with 5,000 metric million tons, Mexico with 4,800 metric million tons and Ethiopia with 4,400 metric million tons. Nigeria is the leading sorghum producer in Africa, followed by Ethiopia in Africa in terms of total production. Sorghum is utilized for human consumption, animal feed, forage, fodder. It is also a source of fibre and feed stock for biofuel production (Bollam et al., 2021). Due to the benefits of its low glutten content, sorghum grains are recommended for consumption by people with celiac disease (Ratnavathi et al., 2016).

Due to changes in climate conditions, changing patterns of precipitation and temperatures (drought and warming events) are expected to become more frequent and severe in the near future (Sheffield *et al.*, 2012, Tombesia *et al.*, 2018, Agele, 2021). Droughts are predicted to occur more frequently, intensify, and stay longer due to changes in the global water cycle and associated drought (Trenberth *et al.*, 2014, Agele, 2021). The sub-Saharan Africa have been predicted to have lower average rainfall and higher temperatures due to global climate change (Akinseye *et al.*, 2019, Agele, 2021). According to (Barbagallo *et al.*, 2013) drought stress is a serious issue for agriculture globally (Sakhi et al., 2014). If plants do not receive adequate rainfall or irrigation, the resulting moisture deficits can reduce growth more than all other environmental stresses combined. Inadequate rootzone moisture status halts plant growth and development resulting in reduced vigour and yield (Tezara et al., 2016, Agele et al., 2018).

In plants, changes in morphological and physiological trait of annual and perennial species under drought or soil moisture deficit has been evaluated severally (Glenn et al., 2014, Tezara et al., 2016, Agele and Ajao, 2018). These reports affirmed that plants exhibit adaptive strategies to cope with rootzone moisture deficit stress and recovery following stress alleviation via rewatering (Glenn et al., 2014, Haeberle et al., 2015, Agele and Ajao, 2018). These strategies may involve complex, interacting mechanisms (e.g. desiccation tolerance and drought performance) (Li et al. 2016, Tombesia et al., 2018). Root-zone moisture deficits affect stomatal functions especially densities of stomatal on the upper and lower leaf surfaces Putra et al. (2012) found that stomatal density of newly emerging leaves plants grown in dry and warm soil increased after the treatment had been removed. The differences in stomatal densities have consequences on stomatal gas exchange (conductance of the stomatal to gases, g) (Putra et al., 2012, Glenn et al., 2014). In addition, it has been reported that the low stomatal conductance characteristic of dry root-zone environment may not be due to low stomatal density alone but also to stomatal aperture (Putra et al., 2012, Glenn et al., 2014).

Limitation of water supply has impact on photosynthesis, growth and yield of plants, and drought affect various levels of plant metabolism (Witt et al., 2012, Sheffield et al., 2012, Zhang et al., 2013, Glenn et al., 2014, Li et al., 2016). Limitation in soil moisture affects phytochemistry especially parameters such as total soluble solids, soluble sugar, organic acids and vitamin C has been reported (Khan et al., 2015, Soni et al., 2015). Drought-induced accumulation of solutes in plant tissues offer plant protection against environmental stresses (Yancy et al., 1982, Tokihiko et al.,2003, Verbruggen and Hermans, 2008, Kantar et al., 2011, Soni et al., 2015). It is reported that soluble carbohydrates (sugars), other metabolites and osmolytes concentrations increase under drought (Keller and Ludlow, 1993, Bray, 1997, Garcia-Sanchez et al., 2007, Li-Xin et al., 2009, Khan et al., 2015, Panda et al., 2021). In other studies, it has been reported that drought-enhanced fluctuations in metabolic pools of carbohydrates and amino acids in plants has implications for drought tolerance (Mafakheri et al. , 2010, Soni et al., 2015) via the activation of osmotic adjustment (Boyer et al., 2008, Scalabrin et al., 2015). Soluble carbohydrates (sugars) which may increases in proportion with the intensity of moisture deficits, protect plant against water shortage induced damage to proteins and cell membrane (Sawhney and Singh, 2002, Scalabrin et al., 2015, Panda et al., 2021), maintain leaf turgidity (Khan et al., 2015) and serves to activate protective enzymes (Nasser, 2012, Panda et al., 2021). It has been reported that proline accumulates in plant tissue under moisture deficits and may serve as a marker and stress signal influencing adaptive responses to environmental stress, particularly under drought stress (Rouley, 1966, Sanchez et al., 1998, Maggio et al. 2002, Mafakheri et al., 2010). Studies have shown that proline content increased under drought stress in maize (Soronin et al., 2019) while proline accumulation has also been obtained for plants under high temperature and poor soil fertility status (Sairam et al., 2002, Verbruggen and Hermans 2008). Severe wilting under soil drought has been reported to stimulate proline synthesis and accumulation of carbohydrate (Routley, 1966, Mafakheri et al., 2010). Proline accumulation in plant tissues has been described as a marker for environmental stress, and as an important part of stress signal influencing adaptive responses (Routley, 1966, Yancy et al., 1982, Mafakheri et al., 2010).

Results had affirmed the role of adjustments in metabolic pathways for ameliorating adverse effects of rootzone moisture deficits and improved plant performance (Soni et al., 2015, Zhang et al., 2017, Tezara et al., 2020). Changes in chlorophyll and carotenoid contents have served as an index for evaluation of plant response to drought or soil moisture deficit stress (Pastori and Trippi, 1992, Kyoarissis et al., 1995, Khayatnezha et al., 2012). Ommen et al. (1999) and Agele et al. (2018) reported that drought stress caused large decline in leaf chlorophyll a, b and total chlorophyll content in cacao. The decrease in chlorophyll under drought stress has been ascribed to damage to chloroplasts caused by active oxygen species (Smirnoff 1995, Khayatnezha et al., 2012). Whole – plant responses to soil moisture deficits involve interacting mechanisms (e.g desiccation tolerance and drought performance) which are integrated into drought tolerance strategies (Glenn et al.,2014, Tombesia et al., 2018). Inadequate (sub-optimal) water application may profoundly affect sorghum performance. Improved insight is required about adjustment of physiological processes for enhanced tolerance of drought including photochemical compounds in sorghum

Climate variability and change including extremity of weather has set new environmental boundaries) occasioned by drought, dry spells, elevated temperatures, variability of rainfall (amount, spread, intensities), increased pest pressures and the southward shift of the Sahel (Sahara desert) (Akinseye et al., 2017, Agele, 2021). Bearing in mind, the importance of sorghum to national economy, increased efforts should focus on the extension its frontiers of production beyond its current environmental boundaries. Sorghum crop therefore needs to adapt to new regimes of climate/weather and areas that were hitherto (previously) not suitable for their production. Reports of climate projection and crop simulation studies have confirmed changes in crop responses to changing environment conditions: in particular, sorghum (Msongaleli et al., 2014, Akinseye et al. 2017, Faye, et al., 2018), maize and pearl millet (Lizaso, et al., 2018, Singh et al., 2017), sorghum (Akinseye et al., 2015). In SSA, sorghum grain yields are very low (about 0.28 t/ha) which is far below the genetic potential of the crop compared with countries like the USA (4.3 t/ha), Argentina (4.9 t/ha) and China (3.2 t/ha) (FAOSTAT, 2019). Such low yields has been attributed to abiotic and biotic stresses and poor adaptation of improve and farmer's varieties. Large genetic diversity exists in sorghum (Singh *et al.*, 2017), with diverse maturity groups and potentials for adaptation (in terms of capture and use of growing season resources) to agroecologies. Such genetic diversity has relevance for taken advantage of opportunities offered by the various agroecologies and resilience building. Sorghum landraces (African sorghum varieties) have been selected by farmers over generations (Sissoko et al., 2008, Kouressy et al., 2014). These varieties are characterized by low yield but with good grain qualities and tolerance to biotic and abiotic stresses (Kouressy et al., 2014, Potgieter et al., 2016, Clarke et al., 2019).
This study aims to investigate morphological and physiological responses of cocoa provenances to watering regimes under screenhouse condition and the implications of the measured variables in the drought tolerance strategy in sorghum

Materials and Methods

Experiments were conducted at the Screenhouse of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Sorghum seeds were acquired from International Crop for Research and Agriculture Kano (ICRSAT).

Experimental design and layout

The screen house experiment were laid out in a split-plot design of 3 by 6 factorial combination of levels of watering andsorghum varieties which were replicated three times. The three watering regimes are 40 % FC (600 ml per pot) 60% FC (800 ml per pot,) and 100% FC (1000 ml) per pot capacity from two weeks after germination twice weekly. The sorghum varieties were: CSR -01, Bida local, Sk5912, SAMSORG 14, 17 and 44. Five litre capacity pots perforated at the base for drainage were filled with top soil. Sorghum seeds were sown at 2-3 seeds per pots. Weeding was done by handpicking of weeds around the potted plants.

Data collection

Data collection started from four weeks after planting. Measurements were made on sorghum growth, development, yield and yield component. Plant height was measured from the base of the plant to the flag leaf .Number of green leaves were counted. Leaf area was calculated from the relation of leaf length to the width using an equation of the form:

Leaf area = L x W x 0.75 ………………….1

where L= leaf length, W= maximum width, and constant k = 0.75.

The number of days to first and 50 % flowering were noted. Number of days to 50% flowering was counted from sowing dates to the day when 50% of the plant flowered. Panicle length was measured from the base of the panicle to the tip of the panicle. Panicle weight was obtained by weighing the total numbers of panicles at harvest. 100 seed weight was obtained by weighing 100 grains using electronic balance. Destructive sampling of shoot and root biomass were oven dried (80°C for 24 hours) and weighed using a weighing balance. For the determination of root and shoot biomass, sorghum plants were gently uprooted, separated into root and shoots and subjected to oven-drying at 60 °C for 48 hours. Samples were re-weighed to obtain dry weights. The mass of each fraction was then averaged to obtain the corresponding dry mass per plant. Total biomass was a combination of root and shoot dry masses:

Stomata characters of sorghum leaves

By the by the Impression Method using clear nail polish to make an impression of the leaf surface and placed on a microscope slide. Leaf samples were harvested, placed in plastic envelopes and transported immediately to the laboratory where the upper and lower surfaces of leaves were identified as they are under normal conditions. Clear nail polish was spread as thin layer on each surface. Both the upper side and lower side of leaf surface and are left to dry. The casts of leaves were made by pressing leaf sections onto a microscope. Number of stomata of the sampled leaves was counted using a light microscope. Stomatal density was averaged per ring, and viewed under the microscope (100 or 400x magnification). The number of stomata of the sampled leaves was counted using a light microscope, and stomatal density was averaged per ring.

The proline content was determined following the free proline accumulation method of Bates et al. (1973) and Marin et al. (2009) based on proline's reaction with ninhydrin. For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. Plant samples are harvested, approximately 100 mg of fresh samples was deployed for the analysis. Plant samples were frozen in liquid nitrogen and when necessary, and stored below 80 °C. The plant materials are ground and kept in tubes and store on ice. Samples are centrifuged for 5 min at room temperature using bench top centrifuge with maximum speed. Afterwards, exactly 100 µl of 3 % sulfosalicylic acid and 200 µl glacial acetic acid are added in addition to 200 µl acidic ninhydrin to 100 µl to the supernatant of the plant extract and properly mixed while the tubes are incubated at 96 °C for 60 min. Plant samples are extracted with toluene (1 ml toluene which was added mixture in the tube). Samples are taken and vortexed for 20 seconds, and left on the bench for 5 min to allow the separation of the organic and water phases. The chromophore containing toluene is removed into a fresh tube while the absorbance of the extract is measured using Spectrophotometer at 520 nm using toluene as reference. Proline concentration was determined using a standard concentration curve and calculated on fresh weight basis (usually expressed as microgram per gram FW or micromole per gram FW: µ moles g^{-1}).

The leaf chlorophyll concentrations was determined using the acetone method. Leaf samples were collected from intact leaves still attached, and placed in vials with 10 ml 95% ethanol in the cold room for 24 hours for chlorophyll extraction. Chlorophyll determination. Leaf chlorophyll was extracted and determined using leaf sampled from the uppermost leaves of sorghum varieties from the treatments. One gramme of the fresh plant samples were cut into pieces and smashed in a mortar. The samples were put in a test tube and its chlorophyll content was repeatedly extracted with successive volume of 100 ml acetone/water (80:20 v/v) until no traces of green colour were noticed (residue became white). While adding the solvent (acetone), the test tubes containing the samples were kept boiling in hot water bath. The total volume of the extract was also recorded at end of the extraction. Three millimeter (3 ml) of the extract was taken and the absorbance of chlorophyll was determined with a spectrophotometer at two wave length of 663 nm and 645 nm that corresponds to maximum absorption of chlorophyll "a" and "b" respectively. Total chlorophyll content was calculated according to Wellburn and Lichtenthaler (1984) as follows:

Total chlorophyll content (mg/100 g tissue) = $(20.2A_{645} + 8.02A_{663})$ (V/10 w)…………2

where A645 = absorbance at 645 nm wavelength; A663 = absorbance at 663 nm wavelength; A = absorbance, C_a = chlorophyll a, C_b = chlorophyll b, C_{a+b} = total chlorophyll, V is the final volume (cm^3) of chlorophyll extract in 80% acetone and W is fresh weight (g) of tissue extracted.

Stem and leaf water soluble carbohydrate was determined using the Antrone extraction method. About 1.0 g of plant samples were ground and transferred into 250 ml test tube and 220 ml of water was added. The bottles was capped and shaken on a shaker for about an hour and filtered. The first few ml was ejected and the filtrate was retained for the determination of soluble carbohydrate using Antrone reagents. 770 ml of concentrated H_2SO_4 was added to 330 ml of distilled water, in addition to 1 g of thiourea, 1 g of antrone, stirred until dissolved and was stored in a refrigerator. Glucose stock solution, 1.0 g of anhydrous D (+) glucose in water and diluted to one litre prepared immediately before use. From the glucose working standard solutions, 10 ml of stock to 100 ml was diluted to produce100 ppm. From these, 0, 5, 10, 20, 40, 80 ml was pipetted and made up to 100 ml and these produced 0, 5, 10, 20, 40, 80 ppm. Samples of 2 ml of each glucose working standard solutions were pipetted into the glass test tube and rapidly, 10 ml of anthrone reagent was added and mixed by shaking. The test tube was loosely covered with a glass bulb stopper and placed immediately in boiling water for 20 minutes. The absorbance was measured using spectrophotometer device in a 10 mm optical cell at 620 mm. The graph of absorbance was plotted against glucose concentration in ppm and prepare standard graph with each batch of extracts examined. The glucose standard becomes 0, 0.8, 1.7, 3.3, 6.7, 13.3 ppm respectively.

Data were subjected to analysis of variance (ANOVA). Data analysis was carried out using the Minitab Version 17 statistical package (Minitab Inc., PA, USA) and where necessary, significance was determined at the 95% level ($\alpha = 0.05$). The means were separated using Tukey HSD Test ($P<0.05$).

RESULTS

Effect of watering regimes on the growth attributes of sorghum

The effect of watering regimes (full and fractions of field capacity moisture) on sorghum morphological attributes was presented in Table 1a and b. Watering regimes imposed was not significant difference ($P<0.05$) for most of the parameters measured (number of leaves, leaf area, first day to flowering 50 % flowering and shoot biomass). However, sorghum plant sown under 100 % FC had the tallest plant (103.94cm) and (73.44cm). The length of sorghum plant sown under water deficits decline with decrease in water application with severe water deficits plants recording the least value of plant height. The effects of watering regimes was not significant difference ($P< 0.05$) on the leaf and leaf area of sorghum plant. However, the number of functional leaves and leaf area observed were higher under full watering capacity compared to those grown under water deficits. Watering regime did not significant increased ($P<0.05$) the 1st and 50 % flowering date. The number of days from emergence to onset of 50 % flowering among watering regimes were very close.

Biomass production related to shoot and root weight observed among the differential watering regimes varies with water application. Amount of water applied led to higher shoot biomass, watering regimes of 100 % FC watering regimes gave maximum shoot biomass. Shoot biomass obtained in 60% FC (51.51g) (4.15g) were close in values to what is observed in 100 % FC(55.26g) (4.62g).Sorghum grown under 40 % FC had the least shoot weight (38.36g) and (3.06g) in both trials. Likewise, root biomass of sorghum increased with increasing water application. Significant differences ($P<0.05$) were obtained for root biomass of plant grown under differential watering regimes. Full watering of 100 % FC had the highest root biomass (5.95g) and (3.09g) per plant, while 40 % FC had the least value of root biomass (3.27g) and (1.64g) per plant in both trials.

The yield and yield components of sorghum observed under differential watering regimes was presented in Table 1. Panicle and grain yield were heaviest for sorghum sown under 100 %FC. 100 % FC had outstanding panicle weight, panicle length, 100 % FC (full watering) had better panicle and 100 seed grain weight compared to 40 % FC. Yield and yield components obtained in partial watering regimes were lower. However, 40% FC watering regimes (severe water deficit) recorded the least values of panicle weight and100 seed grains.

Sorghum varietal responses

The effect of sorghum variety on plant height, number of functional leaves, leaf area, shoot and root biomass was presented in Table 1. The result indicated that Bida local had significant higher value of plant height (122cm) in the first trial. SK5912 and SAMSORG 14 had similar values of plant height (98.23cm), (87.68cm) and (98.00cm), (87.00cm) in both trials respectively. However, SAMSORG 44 recorded the least value of plant height. The number of functional leaves observed at 50% flowering date varies among the sorghum genotypes. SK 5912, and SAMSORG 17 produced the highest number of leaves in the first trial, while in the second trial, SAMSORG 14 and Bida local produced the highest number of leaves. However, the number of leaves produced among the various genotypes are not significantly difference ($P<0.05$). Leaf area measured at 50% flowering date was significant among the variety. Bida local had outstanding leaf area (281.30cm3), (182.30) while SAMSORG 44 had the lowest value of leaf area (128.3cm3), (62.17cm3) in both trials respectively. Similarly SAMSORG 17 and SK5912 had close values of leaf area (190.90cm3,) (111.06cm3) and (197cm3) (110cm3) respectively. Longest days to first and 50% flowering was recorded in Bida local (122 days, 159.40days) and (160.9days, 124 days). Bida local had significant higher shoot dry weight compared to all the other varieties. Likewise, Bida local had the best root biomass (6.70g), (3.68 g) per plant, followed by Sk5912 (5.46g), (2.66g) in both trials. Yield and yield components of sorghum varies significantly among the sorghum varieties. Bida local, SK5912 and Samsorg 17 had outstanding panicle weight. 1000 seed weight were heaviest for Bida local and SK5912.

Effects of watering regime and variety on sorghum performance

Significant interactions were found for watering regime and variety for most of the growth and yield variables of sorghum measured. The interactions of watering regime on sorghum varieties were significant particularly for plant height, leaf area, shoot and root biomass. The result indicated that Bida local and Sk5912 that were watered at 100 % FC had the tallest plants, while SAMSORG 14 watered at 40 % FC had the shortest plant. The interaction of watering regimes and variety was also significant on the leaf area. Bida local grown under 100 % FC had the largest leaf area, while SAMSORG 14 grown under 40 % FC had the least leaf area.

The interaction between watering regimes and varieties was significant for shoot biomass (second trial) and root biomass. However, Bida local at 100 % FC watering regime had the highest number of shoot weight. Also Bida local at 100 % FC watering regime had significant higher root weight while SAMSORG 14 produced the lowest root weight under 40 % FC watering regime. The interaction of watering regime and variety was significant for yield and yield components of sorghum ($P<0.05$) in the yield parameters measured. Bida local at 60 % and 100 % FC watering regimes had the longest panicle while SAMSORG 14 at 40% FC watering regimes had the shortest panicle. Also Bida local and SAMSORG 17 at 100 % FC watering regimes had the highest panicle weight while CSR -01 at 40 % FC watering had the least panicle weight. Bida local watered at 100 % FC watering regimes had the highest number of spikelets while SK5912 at 40 % FC had the least number of spikes.

Effects of variety and watering regime on biochemical constituents and stomatal architecture of sorghum

The effect of variety on leaf chlorophyll contents was presented in Table 3. Leaf chlorophyll contents differed significantly among the varieties. The higher chlorophyll a content were obtained in the leaf of CSR -01, Bida local and SAMSORG series (17, 44) while, the lowest value of leaf chlorophyll a was obtained in Sk5912. However no significant difference ($P<0.05$) was obtained among the varieties for the effect of chlorophyll a. Application of water significantly increased the concentration of chlorophyll a among the sorghum varieties ($P<0.05$). Leaf chlorophyll a increased in the order 60% FC > 100% FC > 40% FC. The interaction of variety and watering regime was significant. Higher chlorophyll a concentration were found on the leaf of CSR -01, Bida local and SAMSORG series (17, 14) at 60 % FC watering regime.

The data on the effects of variety on chlorophyll b on leaf of sorghum showed that chlorophyll b were higher in the leaf of CSR-01, SK5912 and SAMSORG series (14, 17) while it was least in the leaf of SAMSORG 17. Chlorophyll b concentration was higher in the leaf of sorghum varieties grown under 100 % FC watering regime compared to 60 % FC and 40 % FC watering regimes.

The data on the effects of variety on total chlorophyll of sorghum was significant difference ($P<0.05$). The concentration of total chlorophyll increased in SAMSORG 44, CSR -01 and SAMSORG 14. Application of water did not significantly increased the concentration of total chlorophyll ($P<0.05$). However total chlorophyll were higher under 100 % FC and 60 % FC compared to 40 % FC. The interaction of variety and watering regime was significant on total chlorophyll. SAMSORG 14, CSR -01 and SAMSORG 14 at 100 % and 60 % field capacity.

The effect of variety on water soluble carbohydrate was not significant ($P<0.05$). The higher value of soluble water carbohydrate content on leaf was obtained in sorghum varieties Bida local, SAMSORG 17 and CSR -01. Sorghum grown under 40 % FC partial watering was higher in leaf soluble carbohydrate compared to 60 % field capacity and 100 % field, while the least soluble carbohydrate was obtained in the leaf of sorghum sown under 60% field capacity. Significant interaction were found for Bida local, SAMSORG 17 and CSR- 01 grown under 40 % FC and 100 % FC watering regimes. The stem of sorghum sown under 40% FC recorded the highest value of soluble carbohydrate while 60% field capacity had the least value of soluble carbohydrate in stem (Table 3). Highest value of water soluble carbohydrate was found in the stem of CSR -01and SAMSORG 17, while SAMSORG 44 had the least value of soluble carbohydrate in the stem. Significant interaction was obtained CSR -01 and SAMSORG 17 sown at 40 % FC watering regimes. Plant grown under water deficit 40 % and 60 % field capacity (partial and severe) had higher proline accumulation than plant grown under full moisture 100 % field capacity. Proline accumulation differed significantly different ($P<0.05$) among the varieties (Table 3). S SAMSORG 14 had the highest proline content, followed by CSR -01. The least value of proline accumulation was found in the leaf of SK5912. However the effect of watering on proline accumulation ($P<0.05$) was not significant for sorghum varieties. The interaction of variety and watering regime was significant ($P<0.05$). SAMSORG 14 at 40% and 60 % field capacity had highest value of proline accumulation.

Stomatal apparatus of sorghum

Stomatal density showed significant difference on sorghum variety ($P<0.05$). The sorghum variety SAMSORG 17 had the highest stomatal density in the upper and lower surface of the leaves (Table 3). Significant differences ($P<0.05$) were not obtained among watering regimes on water on stomatal density. However, sorghum grown under 40 % field capacity had more dense stomata both at abaxial and adaxial compared to 100 % field capacity. The interaction of differential watering on stomatal density showed significant interaction on sorghum varieties. SAMSORG series (14, 17) and CSR-01 at 40 % FC watering regimes had the highest value of stomatal density at both at the adaxial and abaxial, while SAMSORG 14 at 100 % FC watering regimes had the least stomata counts.

DISCUSSION

Watering regime and variety affected root and shoot biomass production in sorghum. These observations which was consistent with reports of Agele et al. (2018) and Tezera et al. (2020) imply that sorghum requires consistently moist root zone environment for optimum growth. Sorghum varieties exhibited a gradual decline in shoot root biomass as the quantity of water applied decreased (100 < 60 < 40 % FC). The effects of differential watering was profound for other root parameters, tap root length was longest at 100% FC, the number of root hairs were significantly higher at 100% FC compared to other watering regimes. Khalil and El-Noemani (2012), Bahreininejad et al. (2013) and Haeberle et al. (2015) stated that, water stress reduces plant growth through inhibition of physiological and biochemical processes, including and nutrient uptake and metabolism. Sorghum varieties grown under 60 and 40 % FC soil moisture condition had decreased root and stem biomass which had been described as survival (tolerance) strategic among cacao progenies under drought (dos Santos et al., 2016, Tezera et al., 2020).

Treatment effects were significant on stomatal densities. Sorghum grown under 40 % field capacity had more dense stomata both at abaxial and adaxial compared to 100 % field capacity. The differential watering affected stomatal densities for both the upper (adaxial) and lower (abaxial) leaf surfaces. Stomatal densities also differed among sorghum varieties evaluated, SAMSORG 14 and 17) and CSR-01 at 40 % FC watering regimes had the highest value of stomatal density at both at the adaxial and abaxial surfaces. Variable root-zone moisture impinged on stomatal density (Rogiers *et al.*, 2011). It has been suggested that carbohydrate reserve status of plant may be an important endogenous determinant of stomatal density. Depleted starch reserves, elicited by long periods (several weeks) of high metabolism in dry root-zones, would require replenishment. The high stomatal densities obtained for well watered treatment would have promoted stomatal gas exchange and subsequently photosynthesis sand dry matter production (Rogiers et al., 2011).

There were significant ($P<0.05$) effects of watering regime and variety on proline content of sorghum. Plant grown under water deficit 40 and 60 % field capacity (severe and mild moisture stress situations) had higher proline contents compared with those t grown under 100 % field capacity. Proline accumulation differed significantly different ($P<0.05$) among the varieties, SAMSORG 14 had the highest proline content, followed by CSR -01 and least value for SK5912. Proline accumulation s an important metabolic response of plants to drought and other stresses. Under drought proline synthesized is induced in the leaves and transported to the roots to cope with water deficit. In this study, the observed increases in carbohydrate and proline contents with decline in water application is consistent with earlier reports. It has been reported that proline content increased under drought stress in maize (Anjum et al., 2011, Nikolaeva et al., 2017, Voronin et al., 2019). Other studies have shown that proline accumulation is an indicator of leaf dehydration and is associated with stress susceptibility (Szabados and Savouré, 2010, Thalmann and Santelia, 2017, Szabados and Savouré, 2010, Thalmann and Santelia, 2017, Dien et al., 2019). Proline permits osmotic adjustment, stabilizes the structure of proteins and cell membranes, acts as a protective agent for enzymes, and is a free radical scavenger and antioxidant (Kishor and Sreenivasulu, 2014, Dien et al., 2019). Stress-dependent accumulation of free proline plays various roles in stressed plants, such as balancing osmotic pressure, maintaining protein and cell membrane stability, and scavenging ROS (Kuznetsov and Shevyakova , 1999, Muhammadkhani and Heidari 2008, Kosová et al., 2018, Daryanto et al., 2020, Yadav et al., 2020)

The changes in carbohydrate metabolism under drought conditions are closely related to photosynthesis and transpiration and are of great importance for stabilization of water balance of plants (Chaves et al. 2009, Agele and Ajao, 2018). Earlier studies have reported increases in the contents of sugars and proline with resultant reduction in the rate of photosynthesis and transpiration during the adaptation of maize seedlings to drought (Nikolaeva et al. 2017). Accumulation of osmolytes in the cells is known to lead to the formation of concentration gradient between the inside and outside cell compartments. This concentration gradient might create favorable conditions for the transfer of osmolytes from the photosynthesizing cells into apoplast. Plants can protect themselves against mild drought stress by accumulating osmolytes (Yancy et al., 1982, Verbruggen and Hermans, 2008, Mafakheri et al., 2010).

Leaf chlorophyll and soluble carbohydrates (WSC) were affected by watering regime and sorghum variety. Leaf chlorophyll a increased in the order 60% FC > 100% FC > 40% FC. Total chlorophyll were higher under 100 % FC and 60 % FC compared to 40 % FC. The higher chlorophyll a contents were obtained in the leaf of CSR -01, Bida local and SAMSORG series while the lowest for SK 5912. The interaction of variety and watering regime was significant. Higher chlorophyll a concentration were found on the leaf of CSR -01, Bida local and SAMSORG series (17, 14) at 60 % FC watering regime. The interaction of variety and watering regime was significant on total chlorophyll. SAMSORG 14, CSR -01 and SAMSORG 14 at 100 % and 60 % field capacity. Sorghum grown under 40 % FC partial watering was higher in leaf soluble carbohydrate compared to 60 % field capacity and 100 % field, while the least soluble carbohydrate was obtained in the leaf of sorghum sown under 60 % FC. The effect of variety on water soluble carbohydrate was not significant ($P<0.05$). However, higher water soluble water carbohydrate contents were obtained for Bida local, SAMSORG 17 and CSR-01. Significant interaction were found for Bida local, SAMSORG 17 and CSR- 01 grown under 40 % FC and 100 % FC watering regimes.

The results showed that rootzone moisture status affected contents of total soluble sugars in plant parts with increases in intensity of soil moisture deficit stress. Increased in soil moisture deficits brought about increased accumulation of soluble sugars. These observations confirmed other reports that soil moisture deficit stress increase the content of soluble sugars in plant tissues (Sun et al., 2016) with increases in proportion with intensity of moisture deficits (Li-Xin et al., 2009, Dien et al. 2019). Soluble carbohydrates (sugars) are among metabolites and osmolytes (Bray, 1997, Voronin et al., 2019) which increased with increasing drought stress (reduced soil water content) (Garcia-Sanchez et al., 2007, Li-Xin et al., 2009, Sun et al., 2016). Considerable changes in soluble sugar accumulation s in response to drought stress have been observed both at intra-and inter-species levels in plants subjected to drought (dryness) (Verbruggen and Hermans, 2008, Mafakheri et al., 2010, Voronin et al., 2019). The increase and accumulation of soluble sugars maintain leaf turgidity under soil moisture deficits and they prevent from dehydration of proteins and cell membranes (Sawhney and Singh, 2002; Sun et al., 2016). Under drought stress or reduction of soil water content, soluble carbohydrates accumulate and would have served to activate protective enzymes in sorghum under rootzone moisture deficit. Chlorophyll a and b contents of sorghum leaves differed among varieties and watering levels. Chlorophyll contents increased on average, from well watered (FC) to deficit watering (40 and 60 % FC) showing decreases in total chlorophyll concentrations in leaves with increasing soil moisture deficit. Changes in chlorophyll and carotenoids has been associated with drought stress tolerance in plants (Lichtenthaler 1987, Agele and Ajao, 2018, Olayemi et al., 2022). In crop species, changes in chlorophyll contents during drought stress has been reported depending on the duration and severity of drought (Kpyoarissis et al., 1995, Ommen et al., 1999, Mafakheri et al., 2010). Drought stress significantly decreased chlorophyll a, chlorophyll b and total chlorophyll and increase in proline content due to drought stress in crops (Verbruggen and Hermans, 2008, Mafakheri et al., 2010, Soronin et al., 2019). The results were in agreement with earlier reports (Nyachiro et al., 2001, Mafakheri et al., 2010, Soronin et al., 2019) where significant decrease of chlorophyll a and b were obtained under water deficits. Therefore, decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting and thus photosynthesis due to degradation of light harvesting/absorbing pigments such as leaf chlorophyll.

In this study, growth and vigour of sorghum across the varieties evaluated were statistically superior under the full FC regimes compared with the 40 % FC watering. This observation supported the findings of Agele and Ajao (2018) on the effects of soil moisture deficit on rice. Our findings were also substantiated by the reports of Nikolaeva et al. (2017) and Soronin et al. (2019) on the drought stress responses of maize. Moisture deficit stress reduces leaf area and biomass accumulation which affirmed that plants which grew under water stress will end up smaller and poor in vigour (Ajeigbe et al., 2018, Agele and Ajao, 2018). The results of this study showed that the measured growth and physiological variables on sorghum varieties responded to watering regimes. This implies that sorghum though a hardy and drought tolerant cereal requires consistently moist root zone environment for optimum growth and yield

Conclusions

Root zone moisture status affected the growth and biochemical attributes of sorghum while the varieties differed in their responses to rooztzone moisture status. Sorghum varieties evaluated also differed in their responses to watering regimes and in morphological and physiological characters. The growth and physiology attributes of sorghum differed under the watering regimes imposed under screenhouse conditions. Sorghum subjected to 100 % FC watering produced taller plants, larger leaf areas and heavier weights of shoot biomass, panicle and grain compared with those grown under 60 and 40 % FC treatments. Most of the measured growth and physiological variables were driven by rootzone moisture status in sorghum. Rootzone moisture deficit stress especially as obtained under 40 % of field capacity, reduced chlorophyll and stomatal densities but increased proline contents and water soluble carbohydrate concentrations. Sorghum plants had better vigour of growth under 100 and 60 % field capacity watering compared with 40 % FC. Fewer stomatal densities, leaf chlorophyll and water soluble carbohydrate concentrations were obtained under rootzone moisture deficits (60 and 40 % FC) while proline contents were higher for water sorghum compared with full field capacity watering. The results further showed that, sorghum (independent of variety) soil moisture deficit stress (40 % FC) depressed its growth, yield and physiological functions compared with 100 % FC watering. The measured growth and physiological variables on sorghum varieties under the watering regimes imposed will help to unravels some aspects of biochemical mechanisms involved in drought tolerance of sorghum

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Table 1a: Effects of watering regime and variety on growth parameters of sorghum (2018 experiment)

Treatments		Plant height (cm)	Number of leaves per plant	Leaf area (cm^2)	Days to 1st flowering	Days to 50% flowering	Shoot weight (g)	Root weight (g)
Varieties	CSR-01	81.00	7.00	149.20	81.00	82.33	13.83	3.20
	Bida local	122.08	4.68	281.30	159.44	160.90	189.97	6.70

	Samsorg14	98.00	6.78	151.60	97.89	97.11	31.10	3.09
	Samsorg17	82.30	8.67	190.90	105.00	105.22	19.50	5.01
	Samsorg44	44.22	4.22	128.30	70.56	71.00	24.20b	4.14
	SK5912	98.22	9.89	197.80	106.33	107.00	12.44	5.46
	LSD (0.05)	24.13	2.19	51.95	4.22	4.82	12.66	1.46
Watering Regimes	40 % FC	69.92	6.06	163.00	103.78	104.67	51.51	3.27
	60 % FC	89.03	6.17	186.20	102.17	103.00	38.36	4.59
	100 % FC	103.94	7.72	200.30	103.44	104.33	55.26	5.95
	LSD (0.05)	21.17	1.95	48.48	19.65	19.68	43.43	1.10
	Var x Wr	0.01*	0.09ns	0.01*	0.00*	0.00*	0.00*	0.00*

ns (non significant,* significant at p<0.05.

Table 1b: Effects of watering regime and variety on growth parameters of sorghum (2019 experiment)

Treatments		Plant height (cm)	Number of leaves per plant	Leaf area (cm2)	Days to 1st flowering	Days to 50% flowering	Shoot weight (g)	Root weight (g)
Varieties	CSR-01	53.78	6.56	79.30	97.59	97.33	2.89	2.07
	Bida local	77.56	8.61	182.30	122.63	124.00	5.85	3.68
	Samsorg14	87.09	9.33	179.10	106.00	107.67	2.84	1.84
	Samsorg17	75.40	6.78	111.06	113.85	117.00	4.49	1.99
	Samsorg44	51.97	7.00	62.17	97.44	100.33	3.53	2.28
	SK5912	87.86	6.89	110.10	113.81	115.67	4.06	2.66
	LSD (0.05)	15.57	1.62	38.03	1.30	3.18	1.02	0.80
Watering regimes	40%FC	72.11	7.11	129.10	108.15	110.67	4.62	1.64
	60%FC	70.92	7.28	116.60	109.35	111.17	4.15	2.52
	100%FC	73.44	7.53	129.10	108.17	110.33	3.06	3.09
	LSD (0.05)	10.28	1.36	40.73	6.37	6.40	0.89	0.6
	Var x Wr	0.01*	0.00*	0.00*	0.00*	0.95ns	0.05ns	0.00*

ns (non significant,* significant at p<0.05.

Table 2a. Effects variety and watering regime on yield and yield components of sorghum (2018 experiment)

Treatments		Panicle Length (cm)	Panicle weight (g)	No. of spikelets per plant	100 seed weight (g)
Variety	CSR-01	24.36	2.12	11.89	3.19
	Bida local	35.94	10.84	30.44	7.86

	Samsorg 14	27.83	6.12	12.56	3.45
	Samsorg 17	25.11	5.50	8.00	3.20
	Samsorg 44	17.61	3.34	6.11	3.50
	SK5912	29.44	5.50	4.89	6.34
	LSD (0.05)	12.16	1.79	4.32	0.85
Watering regimes	40% FC	21.56	4.75	9.11	3.75
	60% FC	29.65	5.32	12.44	4.65
	100%FC	28.92	7.49	15.39	5.36
	LSD (0.05)	8.82	2.11	6.38	1.32
	Var x Wr	0.37ns	0.00*	0.00*	0.00*

Table 2b. Effects of variety and watering regime on yield and yield components (2019 experiment)

Treatments		Panicle length (cm)	Panicle weight (g)	No. of spikelets per plant	100 seed weight (g)
Variety	**CSR-01**	18.98	1.34	10.67	2.47
	Bida local	20.06	4.58	24.56	8.65
	Samsorg 14	15.65	3.76	12.44	4.04
	Samsorg 17	16.00	4.47	8.44	3.48
	Samsorg 44	5.65	3.34	5.33	3.58
	SK5912	18.44	4.24	4.56	8.66
	LSD (0.05)	3.79	1.11	3.56	0.77
Watering regimes	**40%FC**	14.46	3.32	8.78	4.54
	60%FC	15.72	3.50	11.06	5.04
	100%FC	37.11	4.04	13.17	5.86
	LSD (0.05)	4.14	1.06	5.05	2.52
	Var x Wr	0.00*	0.00*	0.02*	0.03*

Table 3a: Effects of variety and watering regime biochemical constituents and stomatal architecture of sorghum (2018 experiment)

Treatments	Chlorophyll (a) (mg/100g tissue)	Chlorophyll (b) (mg/100g tissue)	Total chlorophyll content (mg/1/100g tissue)	Leaf Soluble carbohydrate content (mg/g)	Stem Soluble carbohydrate content (mg/g)	Proline content (µg/g)	Stomatal density	
							Adaxial	Abaxial

Variety	CSR-01	0.62	0.82	1.44	15.25	18.67	2.12	85.73	81.74
	Bida local	0.59	0.59	1.18	15.92	14.50	2.11	80.88	76.40
	Samsorg14	0.52	0.59	1.11	14.59	10.90	2.04	76.30	76.30
	Samsorg17	0.61	0.11	0.72	15.82	20.02	0.92	94.07	83.60
	Samsorg44	0.59	0.92	1.51	14.66	15.27	2.61	70.37	70.14
	SK5912	0.49	0.88	1.32	14.19	15.11	0.77	93.23	72.20
	LSD (0.05)	0.04	0.31	0.31	2.35	4.42	0.34	12.48	13.04
Watering regime	40%FC	0.44	0.79	1.23	15.99	16.58	1.82	87.77	82.69
	60%FC	0.68	0.79	1.40	14.10	14.27	1.87	82.61	76.17
	100%FC	0.58	0.86	1.44	15.24	16.39	1.60	79.93	71.34
	LSD (0.05)	0.08	0.24	0.25	1.58	3.58	0.59	10.16	8.97
	Var x Wr	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

ns (non significant,* significant at p<0.05.

Table 3b: Effects of variety and watering regime biochemical constituents and stomatal architecture of sorghum (**2019 experiment)**

Treatments		Chlorophyll content (a) (mg/100g tissue)	Chlorophyll content (b) (mg/100g tissue)	Total chlorophyll content (mg/1/100g tissue)	Leaf Soluble carbohydrate content (mg/g)	Stem Soluble carbohydrate content (mg/g)	Proline content (µg/g)	Stomata Density	
								Adaxial	Abaxial
Variety	CSR-01	0.54	1.13	1.67	16.65	19.31	2.32	84.67	83.55
	Bida local	0.64	0.78	1.42	16.66	14.97	1.85	80.33	75.70
	Samsorg14	0.57	0.99	1.26	15.28	11.52	1.95	74.54	75.33
	Samsorg17	0.62	0.60	1.22	16.05	21.12	1.03	93.33	86.67
	Samsorg44	0.61	0.68	1.28	15.02	16.10	2.42	75.00	70.00
	SK5912	0.53	1.78	1.41	14.42	15.19	0.94	87.40	70.33
	LSD (0.05)	0.13	1.15	0.21	2.52	4.49	0.25	15.42	12.25
Watering regimes	40% FC	0.44	0.94	1.24	16.76	17.53	1.83	86.00	81.39
	60% FC	0.71	0.79	1.49	14.80	14.90	1.77	81.83	74.89
	100% FC	0.59	1.25	1.39	16.75	16.68	1.69	79.81	74.52
	LSD (0.05)	0.06	0.79	0.16	1.74	3.67	0.43	11.41	9.19
	Var x Wr	0.00*	0.38ns	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*