Vol. 14(16), pp. 725-735, 18 April, 2019
DOI: 10.5897/AJAR2019.13961
Article Number: 56DA81560682
ISSN: 1991-637X
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African Journal of Agricultural Research

Full Length Research Paper

Physiological response of soybean [*Glycine max* (L) Merrill] to soil moisture stress

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Received 14 February, 2019; Accepted 22 March, 2019

This study was done to determine the effects of varying soil moisture regimes on CO_2 assimilation of soybean [*Glycine max* (L.) Merrill] in pots under greenhouse conditions during 2017 and 2018 cropping seasons. The experiment was conducted as a Randomized Complete Block Design (RCBD) in a 4 x 6 factorial treatment arrangement and replicated 3 times. Soil moisture regimes (80, 60, 40 and 20% of field capacity) and cultivars (Gazelle, Nyala, EAI 3600, DPSB 8, Hill and DPSB 19) were first and second factors, respectively. Collected data were subjected to Analysis of Variance (ANOVA) using Linear Mixed Model in GENSTAT. Significantly different treatment means were separated using Tukey's test at 0.05 significance level. Leaf relative water content, stomata conductance, photosynthesis rate and sub-stomatal CO_2 concentrations significantly ($P < 0.001$) declined with increasing soil moisture stress. Total leaf chlorophyll content increased ($P < 0.001$) with increased soil moisture stress. Cultivars DPSB 19 and DPSB 8 had relatively higher leaf relative water content and stomata conductance at reduced soil moisture regime at 20% moisture from field capacity indicating moisture stress tolerance potential of the cultivars.

Key words: Flowering stage, podding stage, seasons, soil moisture regimes, soybean cultivars.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most important legume crops with total production of 261.6 million metric tonnes worldwide (FAOSTAT, 2013). Soybean is a main source of protein, carbohydrates, vegetable oils, vitamins and minerals for human consumption and production of livestock feed. Soybean farming is also the most cost-effective ways resource-constrained smallholder farmers can use to maintain soil fertility of their lands as soybean helps to improve soil fertility through biological nitrogen fixation of soybean between 44 and 103 kg N ha^{-1} (Kananji et al., 2013; Ciampitti and Salvagiotti, 2018). The potential of soybean to significantly contribute to food and nutrition security and to generate substantial income for farmers is however constrained by low yields arising from soil moisture stress effects amongst other biotic and abiotic stresses. Soil moisture stress has become a recurring event due to unpredictable weather patterns arising from changes in climatic conditions occasioned by global warming (Abedinpour, 2012). Understanding the response of soybean to limited soil moisture stress, identification and use of moisture stress tolerant cultivars

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are options to reduce negative impacts of moisture stress and hasten soybean yield improvement (Farooq et al., 2009; Yunusa et al., 2014). This is more important considering that $^2/_3$ of global food production is through cultivation under moisture stress conditions (Madhu and Hatfield, 2015). Equally challenging to agriculture sector is the need to increase current food production levels by between 70 to 100% by the year 2050 in order to meet food requirements of the ever increasing human population (Alexandratos and Bruinsma, 2012). Optimization of soybean production and yields would therefore help narrow human food requirements and consequently help alleviate malnutrition in children and nutritional deficiencies in the elderly and people living with HIV and Aids.

For countries like Kenya, increased soybean production would help reduce huge importations of the crop and thus contribute to macroeconomic stability of the country. Apart from contributing to foreign exchange earnings through direct exports of the crop, soybean would also help provide raw materials to agro-based industries and in the process contribute to job creation in the country. Achievement of these benefits is however hampered by unavailability of information on how available soybean cultivars in Kenya respond to moisture stress. Understanding the response at physiological level is of significance considering that plant physiological processes have a direct bearing on crop yields (Liu et al*.,* 2012). Soil moisture stress interferes with key plant physiological processes like radiation use efficiency by photosynthesis, transpiration rate, level of stomata conductance, plant water status and degree of sub-stomatal carbon dioxide concentration in most crops (Ku et al., 2013; Hossain et al., 2014). It was for this reason that a study was conducted to determine the effect of varying soil moisture regimes on CO_2 assimilation of selected soybean cultivars in Kenya.

MATERIALS AND METHODS

Site description

The experiment was conducted in pots in a greenhouse at Egerton University, Njoro campus in Kenya, during 2017 and 2018 seasons. Egerton University (0° 22'S; 35°56'E) is at an altitude of 2267 meters above sea level (m.a.s.l) and had mean annual temperature of 15.9^{0}C.

Determination of moisture at field capacity

A sample of ten planting pots (18 cm in height and 22 cm in diameter giving a pot volume of 6,842 cm^3) used in the experiment were filled with soil and then saturated for several hours with water until all micro pores were filled with water. The top of the pots were then covered with black plastic sheets overnight to avoid evaporation. Moisture content at 100% field capacity (FC) was determined using IMKO-HD2 Time Domain Reflectometer (TDR) by inserting TDR probes vertically in the pot soil. The amount of moisture held by the soil at subsequent field capacities were then determined with reference to mean soil moisture level at 100% FC which was then used to come up with the following: 80, 60, 40, and 20% of FC. After sowing, moisture levels in all treatments were maintained close to 100% field capacity for 30 days after which respective soil moisture treatment regimes were initiated up to physiological maturity of the crop. After initiation of moisture regime treatments, soil moisture regimes at respective field capacities were monitored using TDR, and changes in soil moisture were corrected by supplying additional water.

Experimental design and treatments

The experiment was conducted using the Randomized Complete Block Design (RCBD) with a 4 x 6 factorial treatment arrangement with 3 replicates. Treatments consisted of two factors: factor 1 being moisture regimes and factor 2 being soybean cultivars. Soil moisture regimes were at 80%, 60%, 40% and 20% of soil moisture content at field capacity. Soybean cultivars used in the experiment were Gazelle, Nyala, EAI 3600, DPSB 8, Hill and DPSB 19. Characteristics of soybean cultivars are as follows (Table 1).

Planting and crop management

Soil growth medium was a mixture of clay loam soil and river sand in a 2:1 ratio. The growth medium was put in planting pots measuring 18 cm in height and 22 cm in diameter giving a pot volume of 6,842 cm^3. Planting pots were placed on a bench, at 100 cm above greenhouse floor. Natural lighting was used for plant growth and daily minimum and maximum temperatures were taken using a minimum and maximum bulb thermometer. Soybean seeds were inoculated with BIOFIX *(Bradyrhizobium japonicum)* inoculant strain USD 110 from Mea Limited–Kenya at the rate of 10 g kg^{-1} of seed prior to sowing. Three soybean seeds were sown in each pot and thinned to one plant per pot 14 days after emergence. Each treatment had 4 plants per replicate. Triple Super Phosphate (TSP) and Muriate of Potash (MOP) were applied as basal dressing fertilizers at the rates of 0.68 g per pot TSP (30 kg P_2O_5 ha^{-1}) and 0.27 g per pot (30 kg K_2O ha^{-1}), respectively. Hand weeding was done in pots as weeds appeared.

Determination of leaf relative water content

Leaf relative water content (LRWC) was measured on a third leaf from top of the plant at 50% flowering stage. Leaf samples were collected at midday and cut leaves were put in pre-weighed 150 milliliter tubes and sealed to avoid moisture loss. Closed tubes were put in an outdoor and indoor Marina cooler box and taken to laboratory where leaf fresh weights were measured. Equal amounts (150 milliliters) of distilled water were then added to tubes and samples placed in a refrigerator at 4^{o}C for 24 h for leaves to reach full turgor. After 24 h, leaf samples were removed from plastic containers, blotted dry with paper towel and weighed to get turgid weights. Leaf samples were then oven dried at 65^{o}C for 24 h after which dry weights were measured (Sade et al., 2015). Leaf relative water content was determined using the following formula:

LRWC (%) = [fresh leaf wt.-dry leaf wt. / leaf turgid wt.-dry leaf wt.]* 100%,

Where, LRWC is leaf relative water content.

Determination of leaf chlorophyll content

Chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll contents were

Table 1. Growth habits and phenology of soybean cultivars used in the experiment.

S/N	Cultivar name	Characteristics
1	Gazelle	Indeterminate, medium maturity
2	Nyala	Determinate, early maturity
3	EAI 3600	Determinate, early maturing
4	DPSB 8	Indeterminate, promiscuous, late maturity
5	Hill	Determinate, medium maturity
6	DPSB 19	Indeterminate, promiscuous, medium maturity

analyzed on a 3rd trifoliate leaf at 50% flowering using a procedure described by Goodwin and Britton (1988).

Measurement of stomata conductance

Stomata conductance was determined at 50% flowering and 50% podding stages of soybean growth on abaxial side of a middle leaflet of a third trifoliate leaf from top of the plant. It was measured between 12.00 - 14.00 hours on sunny days using a steady state leaf porometer (SC1, Decagon Devices, USA).

Measurement of leaf photosynthesis rate and sub-stomatal carbon dioxide concentration

Leaf photosynthesis rate and sub-stomatal CO_2 concentration were determined at 50% flowering and 50% podding stages of soybean growth on a middle leaflet of a 3rd trifoliate leaf from top of the plant. Photosynthesis rate and sub-stomatal CO_2 concentration were measured between 12.00 - 14.00 hours during sunny days using a TPS-2 portable photosynthesis system (V2.02-PP systems Inc., USA).

Statistical analysis

Data were checked for fulfilment of analysis of variance (ANOVA) assumption of normality by using Shapiro-Wilk normality test in Genstat release 18.1. Data that did not meet the aforesaid ANOVA assumption were subjected to a square root transformation before analysis. Data were then subjected to ANOVA using the linear mixed model for RCBD with factorial treatment arrangement in Genstat (Restricted Maximum Likelihood-REML) and statistically significant treatment means were separated using Tukey's test at 0.05 level of significance.

RESULTS

Leaf relative water content

Soil moisture regimes significantly influenced leaf relative water content (LRWC) in both 2017 and 2018 seasons (Figure 1). In 2017, moisture regimes at 80% FC and 60% FC registered LRWC which were significantly ($P < 0.001$) higher compared to LRWC registered at 40% FC and 20% FC. In 2018, 20% FC moisture regime significantly ($P < 0.01$) reduced LRWC while non-significant differences were observed amongst soil moisture regimes at 80% FC, 60% FC and 40% FC. While LRWC did not significantly differ amongst cultivars during 2017 season, LRWC significantly ($P \leq 0.05$) varied with cultivars during 2018 season (Figure 2). Cultivars DPSB 8 and Hill had highest and lowest LRWC during 2018 season, respectively.

Leaf chlorophyll content

Interactive effects of soil moisture regimes and cultivars on chlorophyll 'a' content was observed in both 2017 (Table 2) and 2018 seasons (Table 3). Soybean cultivars had highest ($P < 0.001$) chlorophyll 'a' content at lower soil moisture regimes of 40% FC and 20% FC during both seasons. While significant ($P < 0.001$) interactive effects of soil moisture regimes and cultivars for chlorophyll 'b' content was registered during 2017 season, soil moisture regimes, cultivars and their interactions were not significantly different for chlorophyll 'b' content during 2018 season. Overall, interaction of soil moisture regimes and soybean cultivars significantly ($P < 0.001$) influenced total chlorophyll concentration in soybean leaves in both seasons. Cultivar EAI 3600 had highest total chlorophyll content at the lowest soil moisture regime of 20% FC in both seasons.

Stomata conductance

Interaction of soil moisture regimes and cultivars significantly ($P < 0.001$) increased stomata conductance at 50% flowering and 50% podding stages of 2017 and 2018 seasons (Figures 3 to 6). All cultivars attained highest levels of stomata conductance at the least stressing moisture regime of 80% FC. During 2017 season, indeterminate cultivars DPSB 19 and DPSB 8 had highest stomata conductance at the most limiting soil moisture regime of 20% FC at 50% flowering and 50% podding stages, respectively. In 2018, highest stomata conductance levels at the lowest soil moisture regime were attained by cultivars DPSB 19 and EAI 3600 at 50% flowering stage and 50% podding stages, respectively.

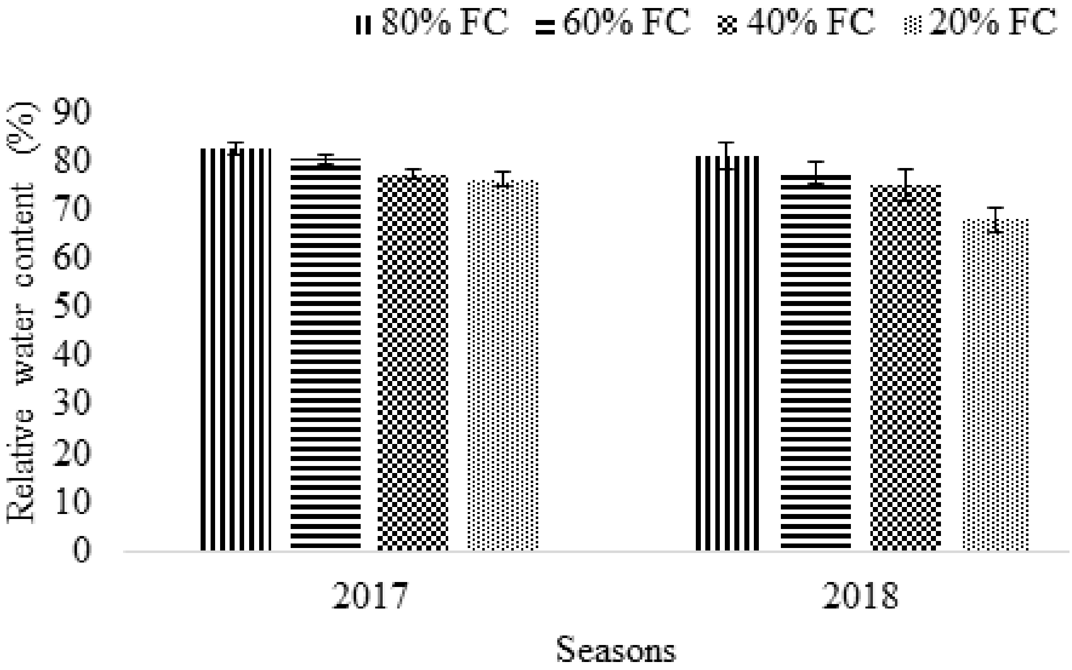

Figure 1. Effects of soil moisture regimes on leaf relative water content during 2017 and 2018 seasons (error bars represent standard error).

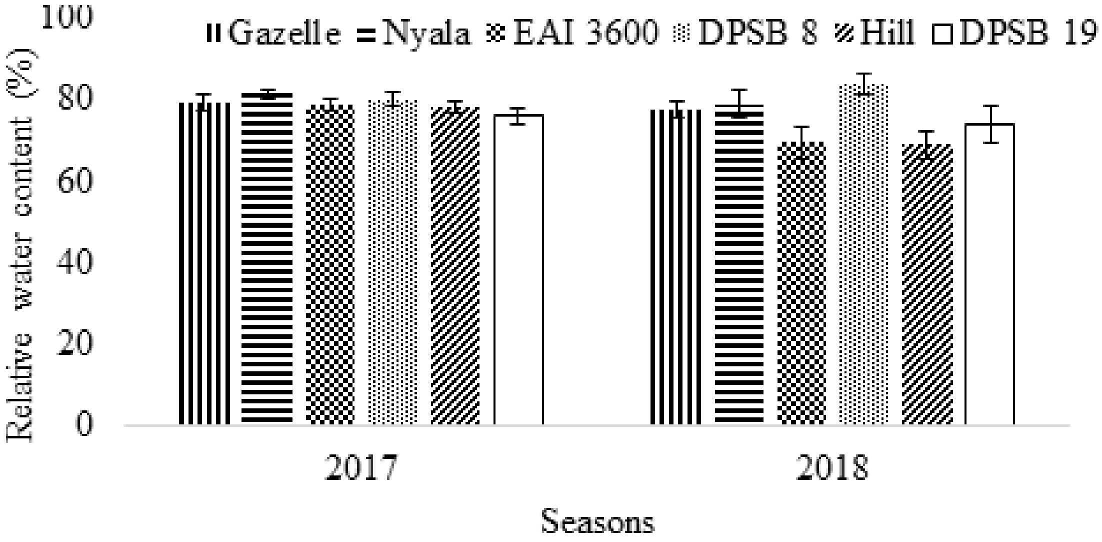

Figure 2. Effects of cultivars on leaf relative water content during 2017 and 2018 seasons (error bars represent standard error).

Sub- stomata CO_2 concentrations

Sub-stomatal CO_2 concentration at 50% flowering during 2017 season significantly ($P < 0.001$) varied with soil moisture regimes and cultivars. The highest sub-stomatal CO_2 concentration of 238.70 µmol CO_2 mol^{-1} was attained at the least limiting soil moisture level of 80% FC after which CO_2 concentrations progressively declined with increased soil moisture stress (Figure 7). Cultivar EAI 3600 had the highest sub-stomatal CO_2 concentration (242.42 µmol CO_2 mol^{-1}) though not statistically different from sub-stomatal CO_2 levels registered by cultivars

Table 2. Effects of soil moisture regimes and cultivars on soybean leaf chlorophyll content (mg g^{-1}fresh weight) at 50% flowering stage during 2017 season.

Soil moisture (% FC)	Cultivar	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
			(mg g^{-1}fresh weight)	
80	Gazelle	0.97	0.12	1.09
	Nyala	0.92	0.12	1.04
	EAI 3600	1.03	0.12	1.15
	DPSB 8	0.86	0.11	0.99
	Hill	0.85	0.12	0.97
	DPSB 19	0.85	0.11	0.96
60	Gazelle	0.87	0.12	0.99
	Nyala	1.18	0.12	1.31
	EAI 3600	0.88	0.12	0.10
	DPSB 8	0.95	0.11	1.07
	Hill	0.85	0.13	0.96
	DPSB 19	0.89	0.11	1.01
40	Gazelle	0.87	0.12	0.99
	Nyala	0.93	0.12	1.06
	EAI 3600	0.87	0.11	0.98
	DPSB 8	0.92	0.13	1.05
	Hill	1.19	0.12	1.30
	DPSB 19	2.25	0.13	2.38
20	Gazelle	0.89	0.11	1.00
	Nyala	0.88	0.11	0.99
	EAI 3600	3.79	0.03	3.82
	DPSB 8	1.68	0.10	1.79
	Hill	1.04	0.13	1.16
	DPSB 19	0.87	0.10	0.96
P-value		<0.001	0.004	<0.001
LSD $_{(0.05)}$		0.515	0.03	0.513

FC = Field Capacity; LSD = Least significant Difference.

Gazelle (178.83 µmol CO_2 mol^{-1}) and DPSB 19 (148.11 µmol CO_2 mol^{-1}). While higher soil moisture levels significantly ($P \leq 0.05$) increased sub-stomatal CO_2 concentrations at flowering stage of 2018 season (Figure 8), soybean cultivars did not have significant influence. At 50% podding stage of both seasons, soil moisture regimes significantly ($P < 0.01$) increased sub-stomatal CO_2 concentrations with the highest and lowest levels attained at 80% FC and 20% FC respectively. Type of cultivar used did not yield any significant effects.

Photosynthetic rate

Photosynthetic rate was significantly ($P \leq 0.05$) increased with reduced soil moisture stress at 50% flowering stage of both seasons. In both cases, 80% FC had highest photosynthetic rate representing 64.46% (2017) and 63.27% (2018) increase over the lowest photosynthetic rates attained at 20% FC (Figures 9 and 10). Use of different soybean cultivars did not significantly influence photosynthesis at 50% flowering stage in both seasons. At 50% podding stage, both soil moisture regimes and cultivars did not give a significant effect on the rate at which photosynthesis was taking place.

Correlations between sub-stomatal carbon dioxide concentration and photosynthesis rate

Sub-stomatal carbon dioxide concentration andphotosynthesis rate of soybean cultivars showed a positive relationship (Figures 11 and 12). A linear relationship between carbon dioxide concentration and photosynthesis at 50% flowering stage indicates that the higher the concentration of sub-stomatal carbon dioxide, the greater the photosynthesis rate. Coefficient of determination (r^2) indicates that 87.75% and 93.42% of variations in photosynthesis rates at different soil moisture regimes in 2017 and 2018, respectively may be

Table 3. Effects of soil moisture regimes and cultivars on soybean leaf chlorophyll content (mg g^{-1}fresh weight) at 50% flowering stage during 2018 season.

Soil moisture (% FC)	Cultivar	Chlorophyll 'a'	Chlorophyll 'b'	Total Chlorophyll
		(mg g^{-1}fresh weight)		
80	Gazelle	0.82	0.10	0.92
	Nyala	0.38	0.06	0.45
	EAI 3600	0.84	0.10	0.94
	DPSB 8	0.58	0.12	0.70
	Hill	0.30	0.06	0.35
	DPSB 19	0.58	0.07	0.66
60	Gazelle	0.88	0.11	0.99
	Nyala	1.24	0.12	1.36
	EAI 3600	0.49	0.09	0.58
	DPSB 8	0.64	0.07	0.71
	Hill	0.53	0.09	0.61
	DPSB 19	0.53	0.07	0.61
40	Gazelle	0.42	0.62	0.48
	Nyala	0.49	0.07	0.56
	EAI 3600	0.73	0.10	0.83
	DPSB 8	0.89	0.08	0.98
	Hill	1.51	0.11	1.62
	DPSB 19	1.98	0.15	2.13
20	Gazelle	0.58	0.72	0.65
	Nyala	0.65	0.07	0.72
	EAI 3600	2.77	0.08	2.85
	DPSB 8	2.21	0.07	2.29
	Hill	0.54	0.06	0.60
	DPSB 19	0.65	0.08	0.72
P-value		<0.001	0.650	<0.001
LSD $_{(0.05)}$		1.186	0.090	1.228

FC = Field Capacity; LSD = Least significant Difference.

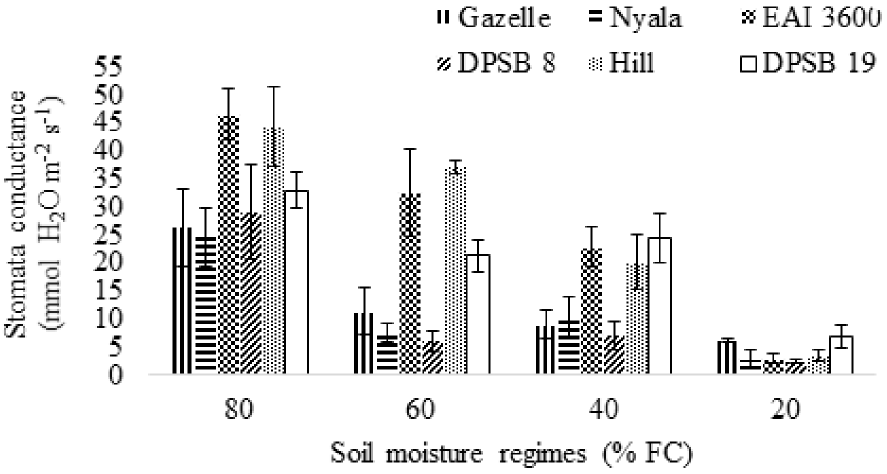

Figure 3. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% flowering stage during 2017 season (error bars represent standard error).

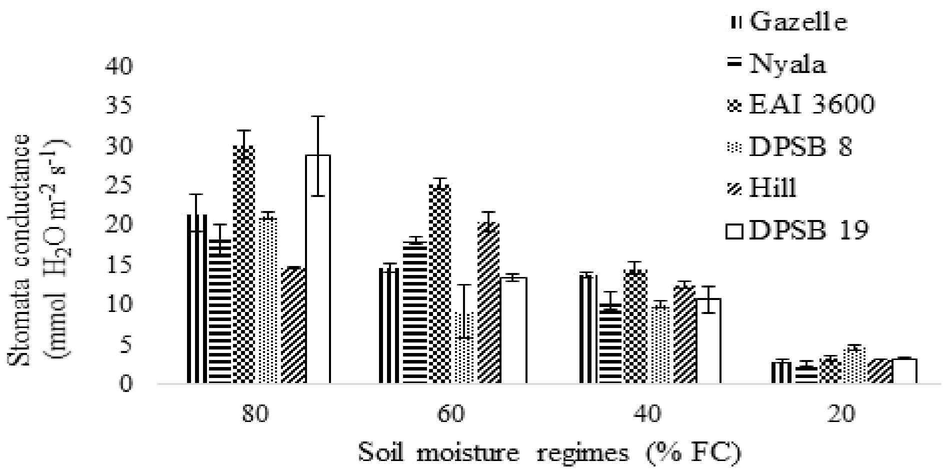

Figure 4. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% podding stage during 2017 season (error bar represent standard error).

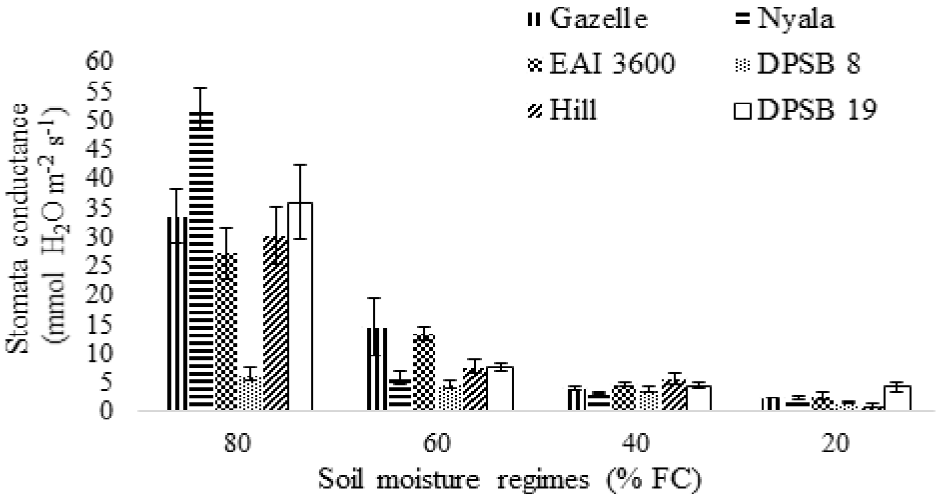

Figure 5. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% flowering stage during 2018 season (error bars represent standard error).

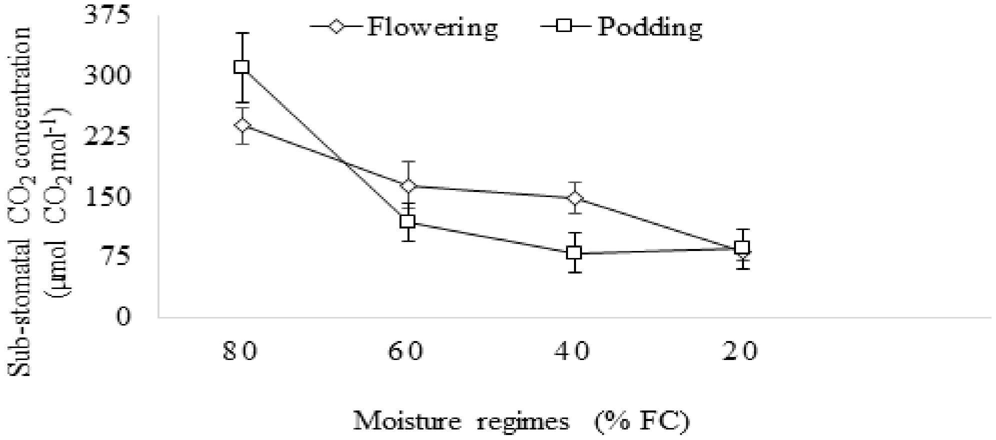

Figure 7. Response of sub-stomatal CO_2 concentrations to soil moisture regimes during 2017 season (error bars represent standard error).

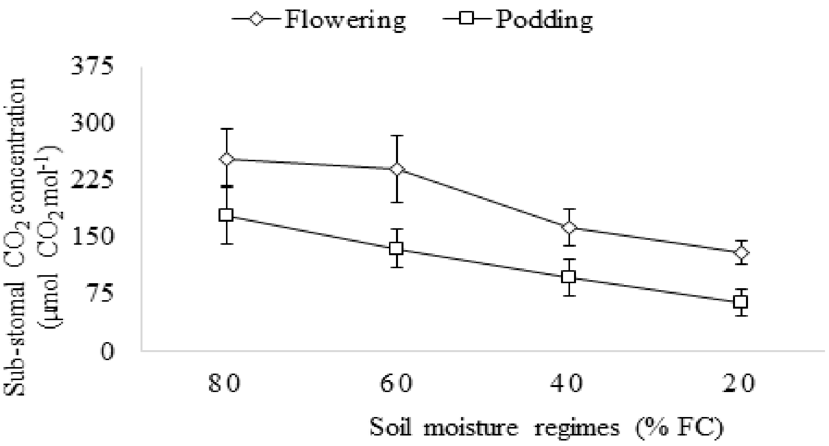

Figure 8. Response of sub-stomatal CO_2 concentrations to soil moisture regimes during 2018 season (error bars represent standard error).

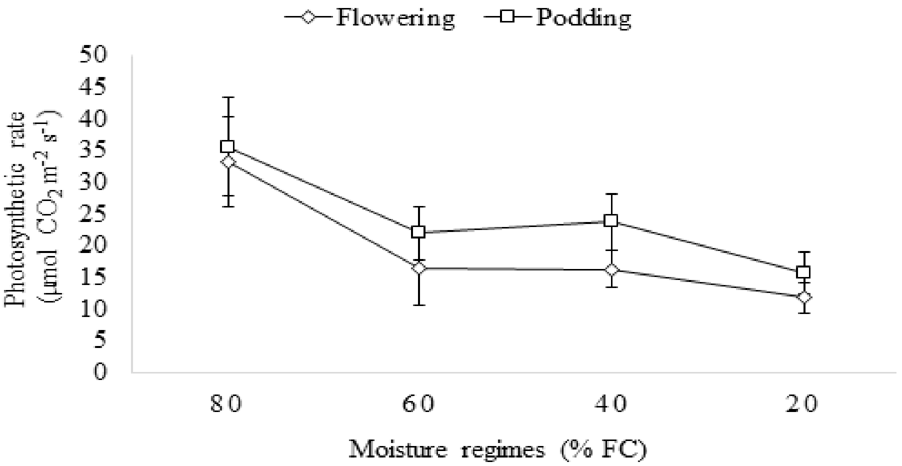

Figure 9. Effects of soil moisture regimes on soybean photosynthetic rate during 2017 season (error bars represent standard error).

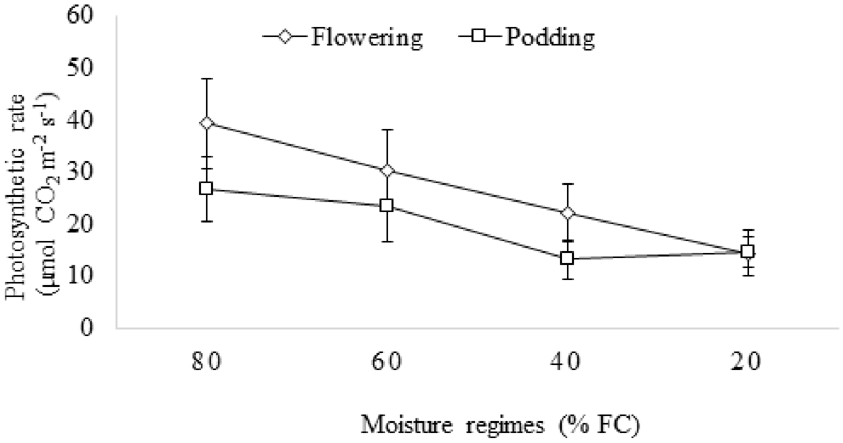

Figure 10. Effects of soil moisture regimes on soybean photosynthetic rate during 2018 season (error bars represent standard error).

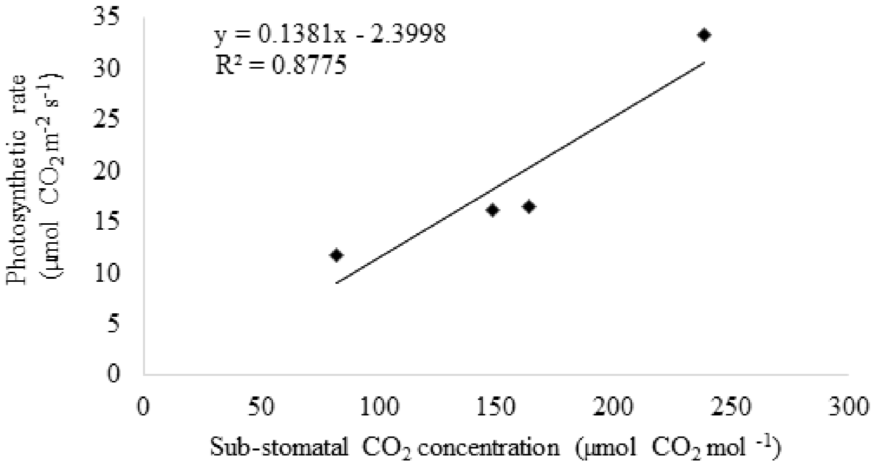

Figure 11. Correlation between sub-stomatal CO_2 concentration and photosynthetic rate at 50% flowering stage during 2017 season.

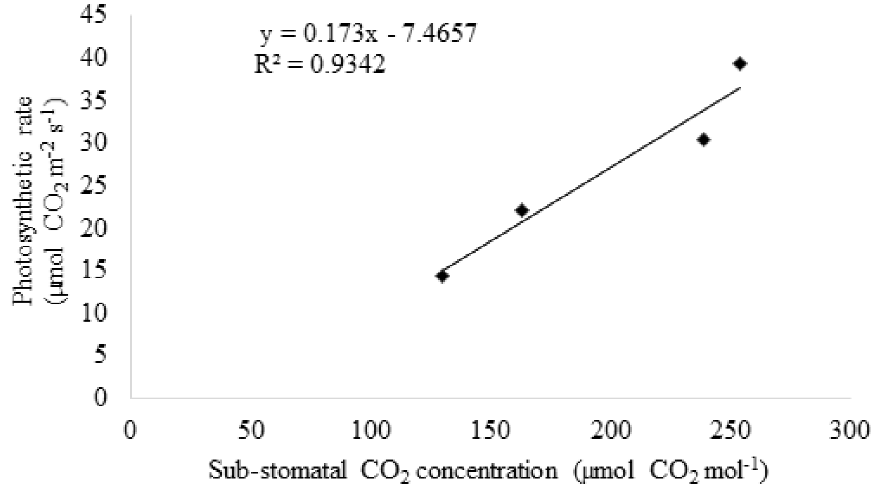

Figure 12. Correlation between sub-stomatal CO_2 concentrations and photosynthetic rate at 50% flowering stage during 2018 season.

attributed to differences in sub-stomatal carbon dioxide concentrations.

DISCUSSION

Leaf relative water content measures the dehydration status of plants relative to the maximum water holding capacity at full turgidity. A cultivar with the ability to minimize stress by maintaining turgid leaves under limited soil moisture conditions may be considered drought tolerant (Lugojan and Ciulca, 2011; Soltys-Kalina et al., 2016). Results of the study have shown that soil moisture stress reduced leaf relative water content with cultivars DPSB 8, Nyala, Gazelle and DPSB 19 maintaining higher percent leaf relative water content which signifies moisture stress tolerance potential of the cultivars. Previous studies on soybean have also demonstrated that soil moisture stress reduces leaf relative water content with a pronounced effect on moisture stress susceptible cultivars (Amira and Qados, 2014; Hossain et al., 2014). Under limited soil moisture conditions, there is lower cell water potential which may lead to reduced leaf relative water content in plants grown under such conditions (Cheruiyot et al., 2010., Hossain et al., 2015). In drought tolerant soybean cultivars, high leaf relative water content is maintained by the increased expression of P5CS gene resulting in increased biosynthesis of proline, which helps in cell stabilization and maintenance of cell turgidity (Hayat et al., 2012).

Chlorophyll ‘a’ is the principal photosynthesis pigment that interacts directly with light requiring processes of photosynthesis. Chlorophyll ‘b’, on the other hand, is an accessory photosynthesis pigment and it acts indirectly in photosynthesis process by transferring light it absorbs to chlorophyll ‘a’. A combination of chlorophylls ‘a’ and ‘b’ constitutes total chlorophyll content in plant leaves (Guidi et al., 2017). This study has shown that chlorophyll ‘a’ and total chlorophyll content of soybean leaves increased with increased soil moisture stress in both seasons. There was no explicit effect of soil moisture regimes on chlorophyll ‘b’ concentration considering that during 2017 season, chlorophyll ‘b’ content was significantly increased at higher soil moisture regimes while soil moisture regimes did not have a notable significant influence, despite a trend of higher chlorophyll ‘b’ content at higher soil moisture regimes in 2018. In 2017 season, chlorophylls ‘a’ and ‘b’ including total chlorophyll concentration varied with soybean cultivars used. In 2018, however, all chlorophyll components were not significantly influenced by soybean cultivars. Contradicting results on effect of soil moisture stress on leaf chlorophyll content have been reported from previous studies. Significant decreases in chlorophyll ‘a’, ‘b’ and total chlorophyll content in leaves of soybean plants grown under drought stress were reported by Atti et al. (2014) and Mannan et al. (2016). Nonetheless, a studies on corn by Rahman et al. (2004) and Muhumed et al. (2014) indicated an increase in total chlorophyll content with increase in water stress, with corn cultivars showing an inverse relationship in increases of chlorophylls ‘a’ and ‘b’. Maintaining high soil moisture regimes in this study required frequent application of water which might have led to leaching of nutrients from growth medium. This might have deprived soybean plants of the required nitrogen to sustain high chlorophyll levels. Reduced nitrogen contents in sweet corn leaves and roots as a result of increased irrigation frequencies were reported by Muhumed et al. (2014).

Highest levels of stomata conductance, sub-stomatal CO_2 concentration and photosynthesis rates were attained at highest soil moisture regime of 80% FC, with largely limited variations amongst plant growth stages and cultivars. Higher photosynthesis rate was highly correlated with higher concentration of sub-stomatal CO_2. These results are in agreement with observations by Makbul et al. (2011), Hossain et al. (2015) and Chowdhury et al. (2016) who reported reductions in stomata conductance, sub-stomatal CO_2 concentration and photosynthesis rate due to increased moisture stress in soybean plants grown under greenhouse conditions and other related growth chambers. Catuchi et al. (2011) and Fanourakis et al. (2014) indicated that most plants close stomata at limited soil moisture levels to prevent excess water loss to the environment. Closure of stomata by plants at limited soil moisture levels in the current study triggered a series of events in plant physiological processes. Reduced stomata conductance at lower soil moisture regimes might have arisen from a combination of reductions in relative water content in soybean leaves and stomata closure to prevent excess water loss to the environment. Considering that stomata conductance indicates a degree of exchange of CO_2 and water vapour between ambient and inner leaf, reduced stomata conductance due to stomata closure could have then led to minimal diffusion of CO_2 from the atmosphere to plant cells leading to low concentrations of sub-stomatal carbon dioxide (Fanourakis et al., 2014). It has been shown from this study that photosynthesis rate was strongly correlated with sub-stomatal CO_2 concentrations which implies that lower photosynthesis rate at lower soil moisture regimes could have been a result of reduced sub-stomatal CO_2 diffusion to carboxylation site of Rubisco (Xu et al., 2016).

Conclusion

Soil moisture stress reduced leaf relative water content, stomata conductance, sub-stomatal carbon dioxide concentration and photosynthesis rate, while leaf chlorophyll content increased with increased soil moisture limitation. Cultivars DPSB 19 and DPSB 8 had relatively higher leaf relative water content and stomata conductance at reduced soil moisture regime of 20%, indicating moisture stress tolerance potential of the cultivars.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The study was conducted with financial support from Government of Malawi through Agricultural Productivity Programme for Southern Africa (APPSA) under the Department of Agricultural Research Services (DARS). We thank Egerton University for all the support rendered towards implementation of the study.

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