

Research Article

Leaf Gas Exchange and Root Nodulation Respond to Planting Density in Soybean [*Glycine max* (L) Merrill]

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Planting density influences structural characteristics and affects mineral nutrient acquisition, irradiance and photosynthesis amongst plants. An experiment was conducted to determine the effect of planting density on leaf gas exchange and nodulation of soybean (*Glycine max* (L) Merrill). The experiment was conducted as a randomized complete block design (RCBD) in a 5 by 2 factorial treatment arrangement and was replicated three times. Planting density (10, 12, 20, 40, and 80 plants m⁻²) and soybean varieties (EAI 3600 and DPSB 19) were first and second factors, respectively. Collected data were subjected to analysis of variance in GENSTAT. Significantly different treatment means were separated using Tukey's honestly significant difference test at 0.05 significance level. Higher planting density significantly increased ($p < 0.001$) interception of photosynthetically active radiation. Increasing number of plants per unit area significantly ($p < 0.001$) reduced root nodulation, stomata conductance, sub-stomatal CO₂ concentration, photosynthetic and transpiration rates. Total chlorophyll content was not responsive to planting density though concentration of chlorophyll "a" content was significantly ($p < 0.005$) higher at lower plant density than at higher plant density. Soil moisture status increased with reduction in plant density. Indeterminate variety DPSB 19 had higher rates of stomata conductance, photosynthesis and sub-stomatal CO₂ concentration compared to determinate variety EAI 3600.

1. Introduction

Planting density affects plant structural characteristics and may help improve disease avoidance, lodging resistance, adaptation to mechanical harvesting and seed yield [1]. A higher plant density has the potential to increase competition for nutrients, light and space while lower plant density may lead to inefficient use of natural resources and inputs [2, 3]. Total dry weight of leaves, leaf area index (LAI), crop growth rate (CGR) and nodulation are all dependent on plant density [2]. In soybeans, leaf gas exchange, grain quality such as protein, oil and mineral contents depend on field production conditions with planting population playing a significant role [4].

Previous plant density studies have recommended different plant populations for optimization of soybean plant growth and yield. In United States of America, optimum plant populations for soybean vary from 30 to 50 plants m⁻² [5] while in Iran and India, soybean yields were optimized at 60 plants m⁻²

[6, 7]. In Turkey, Mehmet [8] found highest soybean yields at plant density of 12.5 plants m⁻² while Rahman and Hossain [1] recommended a planting density of 80–100 plants m⁻² in Bangladesh. In Ethiopia, the recommended plant population for soybean is about 40 plants m⁻² [3]. The reported results on soybean plant population studies suggest that plant density may vary with type of variety used and that some areas, depending on geographical location, have capacity to support higher planting densities than others due to differences in soil and other environmental conditions [9]. The advent of climate change due to global warming in recent years has also brought with it various biotic and abiotic stresses which make it prudent to adjust agronomic practices in line with prevailing climatic conditions. Considering that leaf gas exchange and root nodulation may vary with planting density, this study was undertaken to determine the effect of plant density on leaf gas exchange and root nodulation of determinate and indeterminate soybean cultivars in Kenya.

TABLE 1: Description of treatments for soybean density experiment, Egerton, Kenya during 2018 season.

Treatment no.	Treatment combinations
1	Cultivar EAI 3600 at 10 plants m ⁻²
2	Cultivar EAI 3600 at 12 plants m ⁻²
3	Cultivar EAI 3600 at 20 plants m ⁻²
4	Cultivar EAI 3600 at 40 plants m ⁻²
5	Cultivar EAI 3600 at 80 plants m ⁻²
6	Cultivar DPSB 19 at 10 plants m ⁻²
7	Cultivar DPSB 19 at 12 plants m ⁻²
8	Cultivar DPSB 19 at 20 plants m ⁻²
9	Cultivar DPSB 19 at 40 plants m ⁻²
10	Cultivar DPSB 19 at 80 plants m ⁻²

2. Materials and Methods

2.1. Site Description. The experiment was conducted at Egerton University agronomy teaching and research farm in Njoro, Kenya. Egerton University teaching and research farm (0°22'S; 35°56'E) is at an altitude of 2267 m.a.s.l. The site has *mollic andosols* and pH of soils ranged from 6.85 to 6.94. The experiment was conducted over two seasons; the first season was during long rains of March to July 2018 while the second season was during short rains of September to November 2018.

2.2. Experimental Design and Treatments. The experiment was conducted in a randomized complete block design (RCBD) with a 5 × 2 factorial treatment arrangement and replicated 3 times. Treatments consisted of two factors: factor 1 being planting density and factor 2 being cultivars. Treatment combinations are presented in Table 1.

2.3. Planting and Crop Management. Soybean varieties used in the study were a determinate cultivar EAI 3600 and indeterminate cultivar DPSB 19. First season experiment was planted on 29th March 2018 while a second season experiment was planted on 12th July 2018. Gross plot sizes were 4.5 m long and 4 m wide (18 m²) while net plot size were 3 m by 2.25 m (6.75 m²). Two soybean seeds were planted per hill and where both emerged, it was thinned to one plant 7 days after emergence. 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 planting. Triple super phosphate and muriate of potash were applied at 30 kg P₂O₅ ha⁻¹ and 30 kg K₂O ha⁻¹ respectively as basal dressing fertilizers.

2.4. Data Collection

2.4.1. Soil Moisture Status. Volumetric soil moisture content was determined at 50% flowering stage by using time domain reflectometer (IMKO-HD2, IMKO Micromodultechnik GmbH, Ettlingen, Germany). This was done by inserting time domain reflectometer probes vertically in the soil at four points randomly selected in a net plot.

2.4.2. Chlorophyll Content Determination. Chlorophyll “a” chlorophyll “b” and total chlorophyll contents were analyzed on a third trifoliate leaf at 50% flowering using a procedure described by Goodwin and Britton [10].

2.4.3. Intercepted Photosynthetically Active Radiation. Intercepted photosynthetically active radiation (IPAR) was measured at 50% flowering stage using an AccuPar/LAI Ceptometer (LP-80, Decagon Devices, Inc., Pullman, USA). Measurements were done above and below canopy of soybean plants. Calculation of percent IPAR used the following modified Purcell [11] formula as follows.

$$\text{IPAR (\%)} = \left[1 - \left(\frac{\text{PAR}_b}{\text{PAR}_a} \right) \right] \times 100, \quad (1)$$

where IPAR = intercepted photosynthetically active radiation (PAR); PAR_a is PAR (μmol m⁻² s⁻¹) measured above soybean canopy and PAR_b is PAR (μmol m⁻² s⁻¹) measured below soybean canopy.

2.4.4. Measurement of Stomata Conductance. Stomata conductance was determined on three plants per plot at 50% flowering stage 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 and 14.00 hours on sunny days using a steady state leaf porometer (SC1, Decagon Devices, Inc., Pullman, USA).

2.4.5. Determination of Sub-Stomatal Carbon Dioxide Concentration, Leaf Temperature Photosynthetic and Transpiration Rates. Sub-stomatal carbon dioxide concentration, leaf temperature, photosynthetic and transpiration rates were determined on two plants per plot at 50% flowering stage of soybean on a middle leaflet of a third trifoliate leaf from top of the plant. Measurements were done between 12.00 and 14.00 hours during sunny days using a TPS-2 portable photosynthesis system (V2.02-PP systems Inc., Amesbury, USA).

2.4.6. Root Nodulation. Total number of nodules per plant were determined by counting number of nodules formed on five plants randomly selected in a net plot at 50% flowering stage.

3. Data 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 were considered normally distributed when *p*-value for Shapiro-Wilk statistic was greater than the threshold *p*-value of 0.05. Data that did not meet the aforesaid ANOVA assumption were subjected to square root transformation before analysis. Data were then subjected to analysis of variance (ANOVA) using linear mixed model for factorial experiment in GENSTAT (REML) and statistically significant treatment means were separated using Tukey's honestly significant difference test at 0.05 significance level.

TABLE 2: Effect of plant density and cultivar on soil moisture content (% v/v) at 50% flowering stage.

Plant density (plants m ⁻²)	Soil moisture content		Mean
	Cultivars		
	EAI 3600	DPSB 19	
10	24.93	23.95	24.44
12	24.98	27.41	26.19
20	26.51	23.65	25.08
40	23.92	23.75	23.84
80	21.54	19.90	20.72
Mean	24.38	23.73	
<i>p</i> -value	0.043		
HSD (0.05)	2.422		
CV (%)	8.6		

Plant density (plants m ⁻²)	Season		Mean
	Long rains	Short rains	
	2018	2018	
10	33.31	8.13	
12	37.27	10.41	
20	36.29	13.87	
40	39.97	12.42	
80	37.92	10.96	
Mean	36.95	11.16	
<i>p</i> -value	0.043		
HSD (0.05)	2.422		
CV (%)	8.6		

Cultivars	Season		Mean
	Long rains	Short rains	
EAI 3600	38.14	10.62	24.38
DPSB 19	35.77	11.70	23.73
<i>p</i> -value	0.003		
HSD (0.05)	1.532		
CV (%)	8.6		

Density × season × cultivar	<i>p</i> -value
Density × season × cultivar	0.091

HSD = Tukey's honestly significant difference; CV = Coefficient of variation.

4. Results

4.1. Soil Moisture Status. Soil moisture content (Table 2) was responsive to interaction of plant density and cultivar ($p < 0.05$), density and season ($p < 0.05$) and then cultivars and seasons ($p < 0.001$). Lowest soil moisture level was attained at the highest plant density of 80 plants m⁻² by cultivar DPSB 19. Plant density and season interaction led to reduced soil moisture content during short rainy season across all plant densities. Season by cultivar interaction led to increased soil moisture during long rains by a determinate cultivar EAI 3600.

4.2. Chlorophyll Content. Total leaf chlorophyll and chlorophyll "b" contents were not responsive to variations in plant density, cultivars and seasons. However, chlorophyll "a" content significantly ($p < 0.05$) changed with plant density (Figure 1). Planting soybean at the lowest plant density of 10 plants m⁻² led to increased concentration of chlorophyll "a"

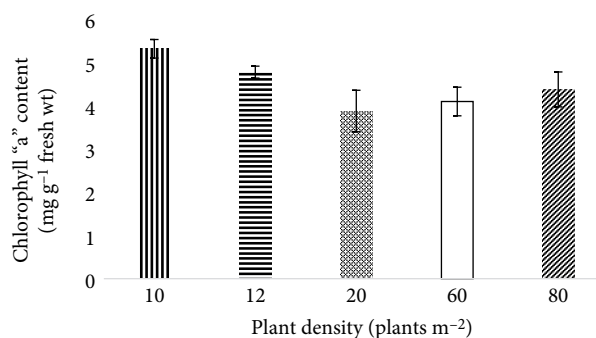


FIGURE 1: Effect of plant density on chlorophyll "a" content of soybean leaves. Vertical bars represent ± standard error. Values significantly different at $p < 0.05$.

TABLE 3: Effect of plant density and cultivar on intercepted photosynthetically active radiation (IPAR) at 50% flowering stage.

Plant density (plants m ⁻²)	Intercepted photosynthetically active radiation (% of incoming PAR)		Mean
	Cultivars		
	EAI 3600	DPSB 19	
10	86.5	60.0	73.2
12	83.1	73.8	78.5
20	87.1	67.8	77.4
40	88.7	83.1	85.9
80	92.7	90.5	91.6
Mean	87.6	75.1	
<i>p</i> -value	0.034		
HSD (0.05)	12.01		
CV (%)	12.6		

	<i>p</i> -value
Density × season	0.557
Variety × season	0.989
Density × season × cultivar	0.970

HSD = Tukey's honestly significant difference; CV = Coefficient of variation.

which was 25.38% more than the lowest chlorophyll "a" levels obtained at plant density of 20 plants m⁻².

4.3. Intercepted Photosynthetically Active Radiation (IPAR). Interception of photosynthetically active radiation significantly ($p < 0.001$) changed with interaction of plant density and cultivars (Table 3). Both cultivars had increased interception of photosynthetically active radiation at the highest plant density of 80 plants m⁻². There was an increased interception of photosynthetically active radiation by the determinate cultivar EAI 3600 compared to indeterminate cultivar DPSB 19.

4.4. Stomata Conductance. Stomata conductance at vegetative stage was significantly ($p < 0.01$) responsive to interaction of plant density and cultivar, and also plant density and season (Table 4). At 50% flowering stage, stomata conductance varied with interaction of plant density and cultivar ($p < 0.001$), plant density and season ($p < 0.01$) and also cultivar and

season ($p < 0.01$). At vegetative stage, stomata conductance was highest at the lowest plant density of 10 plants m^{-2} by cultivar DPSB 19 while cultivar EAI 3600 had highest stomata conductance at the same plant density at 50% flowering stage. Plant density by season interaction led to increased stomata conductance at the lowest plant density during long rainy season regardless of plant growth stage. Interaction of cultivar and season resulted in cultivar DPSB 19 having highest stomata conductance level during long rainy season. In general, lower stomata conductance rates were obtained during 50% flowering stage under all treatments.

4.5. Photosynthetic Rate. Photosynthetic rate at 50% flowering stage was significantly ($p < 0.001$) dependent on interaction of density and cultivar (Figure 2). Highest photosynthetic rate was attained at plant density of 20 plants m^{-2} by cultivar DPSB 19 while the lowest photosynthetic rate was achieved at the highest plant population of 80 plants m^{-2} by the same cultivar.

4.6. Sub-Stomatal Carbon Dioxide Concentration. Interaction of plant density, cultivar and season had a significant ($p < 0.001$) effect on sub-stomatal carbon dioxide concentration (Table 5). Highest concentration of carbon dioxide was achieved at the lowest plant density of 10 plants m^{-2} by variety DPSB 19 during long rains. On the other hand, the lowest level of carbon dioxide concentration was at 40 plants m^{-2} by the same cultivar.

4.7. Transpiration Rate. Transpiration rate was significantly ($p < 0.05$) responsive to interaction of plant density and seasons (Figure 3). Soybean plants had increased rate of transpiration at plant density of 20 plants m^{-2} during long rainy season while the lowest transpiration rate was achieved at the highest plant density of 80 plants m^{-2} during short rains. Overall, increasing number of plants per unit area tended to reduced transpiration rate.

4.8. Leaf Temperature. Leaf temperature significantly ($p < 0.05$) varied with interaction of plant density and seasons (Figure 4). Lower leaf temperatures were attained during long rainy seasons across all plant densities though having 10 plants m^{-2} led to a relatively lower leaf temperature compared to other treatments.

4.9. Root Nodulation. Number of nodules per plant significantly ($p < 0.05$) varied in response to interaction of plant density and seasons (Figure 5). Overall, highest number of nodules were found during short rains at all plant densities though the lowest plant density of 10 plants m^{-2} recorded the highest number of nodules per plant during both seasons.

4.10. Relationships of Some Leaf Gas Exchange Parameters and Soil Moisture Status. There were positive and linear relationships between stomata conductance and leaf sub-stomatal carbon dioxide concentration, soil moisture level and stomatal conductance, leaf sub-stomatal carbon dioxide concentration and photosynthetic rate while stomatal conductance and leaf temperature had negative linear relationship (Figure 6). Coefficient of determination (R^2) for

TABLE 4: Effect of plant density and cultivar on stomata conductance ($mmol H_2O m^{-2} s^{-1}$) at vegetative and 50% flowering stages.

	Stomata conductance ($mmol H_2O m^{-2} s^{-1}$)	
	Vegetative stage	50% Flowering stage
<i>Cultivar and density (plants m^{-2})</i>		
Cultivar EAI 3600 at 10 plants m^{-2}	60.9	38.44
Cultivar EAI 3600 at 12 plants m^{-2}	69.6	29.33
Cultivar EAI 3600 at 20 plants m^{-2}	61.8	33.87
Cultivar EAI 3600 at 40 plants m^{-2}	50.0	33.08
Cultivar EAI 3600 at 80 plants m^{-2}	50.7	30.38
Cultivar DPSB 19 at 10 plants m^{-2}	68.7	24.09
Cultivar DPSB 19 at 12 plants m^{-2}	46.2	16.28
Cultivar DPSB 19 at 20 plants m^{-2}	60.4	26.45
Cultivar DPSB 19 at 40 plants m^{-2}	49.1	15.95
Cultivar DPSB 19 at 80 plants m^{-2}	53.1	24.12
<i>p</i> -value	0.009	< 0.001
HSD _(0.05)	11.81	5.99
<i>Density (plants m^{-2}) × season</i>		
10 × Long rains	72.7	45.11
10 × Short rains	57.8	22.67
12 × Long rains	64.2	39.52
12 × Short rains	47.6	27.43
20 × Long rains	54.3	36.36
20 × Short rains	65.1	18.12
40 × Long rains	52.6	23.96
40 × Short rains	54.0	18.77
80 × Long rains	48.6	25.30
80 × Short rains	53.6	14.77
<i>p</i> -value	0.005	0.002
HSD _(0.05)	11.81	5.99
<i>Cultivar × season</i>		
EAI 3600 × Long rains	54.7	31.58
EAI 3600 × Short rains	52.8	22.39
DPSB 19 × Long rains	62.3	36.51
DPSB 19 × Short rains	58.5	18.32
<i>p</i> -value	0.719	0.002
HSD _(0.05)	7.47	3.79
Density × cultivar × season	ns	ns

HSD = Tukey's honestly significant difference; ns = not significant.

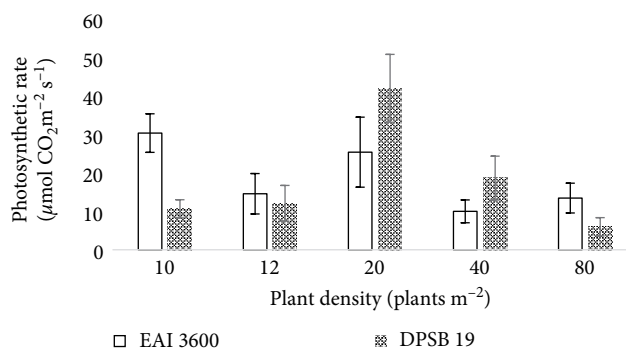


FIGURE 2: Effect of plant density and cultivar on photosynthetic rate at 50% flowering stage. Vertical bars represent \pm standard error. Values significantly different at $p < 0.001$.

TABLE 5: Effect of plant density, cultivar and season on sub-stomatal carbon dioxide concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$).

Plant density (plants m^{-2})	Sub-stomatal carbon dioxide concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)				
	EAI 3600		DPSB 19		Mean
	Long rains 2018	Short rains 2018	Long rains 2018	Short rains 2018	
10	93.12	61.78	207.65	81.90	104.86
12	154.75	79.56	58.37	107.54	96.83
20	65.57	103.43	149.57	97.42	102.41
40	107.95	40.70	27.04	70.06	57.61
80	46.92	38.93	109.41	48.16	58.06
Mean (cultivar)	75.86		89.30		
<i>p</i> -value	<0.001				
HSD _(0,05)	55.70				
CV (%)	20.0				

HSD = Tukey's honestly significant difference; CV = Coefficient of variation.

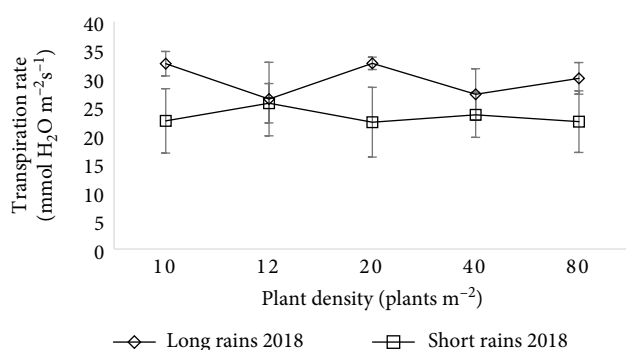


FIGURE 3: Effect of density and season on transpiration rate at 50% flowering stage. Vertical bars represent \pm standard error. Values significantly different at $p < 0.05$.

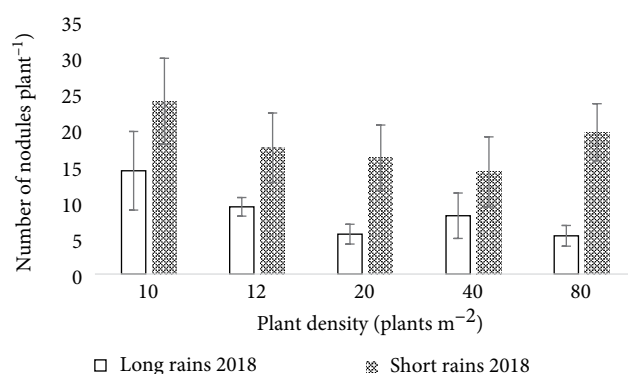


FIGURE 5: Effect of plant density and season on nodulation at 50% flowering stage. Vertical bars represent \pm standard error. Values significantly different at $p < 0.05$.

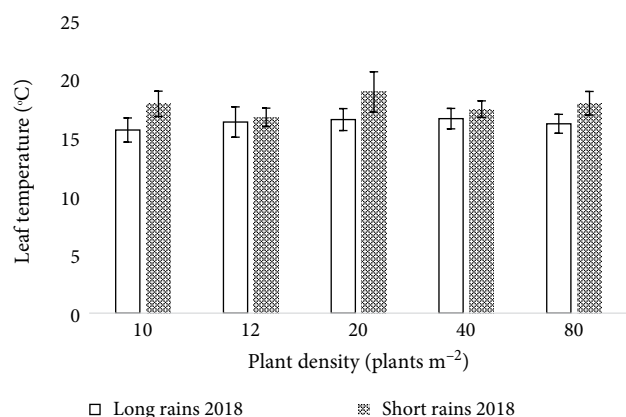


FIGURE 4: Effect of plant density on leaf temperature at 50% flowering stage. Vertical bars represent \pm standard error. Values significantly different at $p < 0.05$.

respective positive relationships imply that 72.67%, 62.49%, and 57.24% variations in sub-stomatal carbon dioxide concentration, stomatal conductance and photosynthetic rate amongst different planting density treatments may be attributed to differences in levels of stomatal conductance, soil moisture and sub-stomatal carbon dioxide concentration, respectively.

5. Discussion

Root nodulation, stomata conductance, sub-stomata carbon dioxide concentration, photosynthetic rate, transpiration and chlorophyll “a” content were higher at lower planting density. Interception of photosynthetically active radiation and leaf temperature increased with increased plant population. Determinate growth habit led to increased interception of photosynthetically active radiation (IPAR) by cultivar EAI 3600 while higher rates of stomata conductance, transpiration and sub-stomatal carbon dioxide concentration were registered with the indeterminate cultivar DPSB 19. These results are in agreement with reports by Koosmaryono et al. [12], Zhou et al. [13] and Moreira et al. [14] whose studies indicated reductions in stomata conductance, photosynthetic and transpiration rates at lower plant populations of soybean. Similarly, studies with sorghum by Li et al. [15] also reported reductions in stomata conductance and photosynthetic rate at higher planting populations relative to lower planting density. On the other hand, studies with pigeon peas by Wilson et al. [16] showed that varying planting density did not have a significant effect on stomata conductance, photosynthetic and transpiration rates which is at variance with findings of this study.

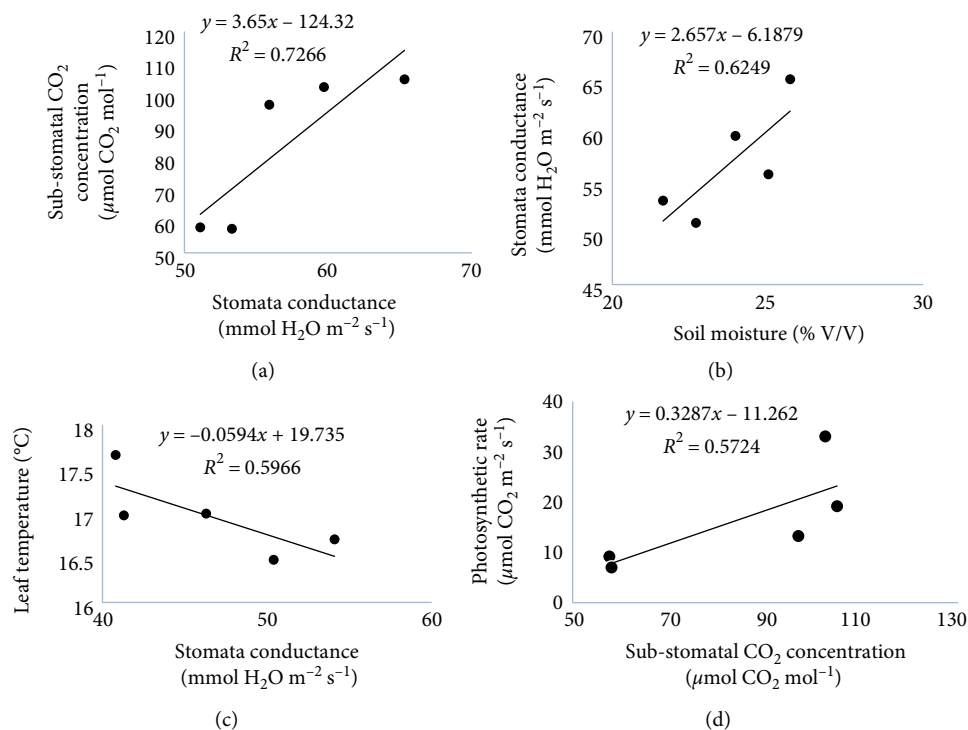


FIGURE 6: Correlations among leaf gas exchange parameters and soil moisture.

Higher number of plants per unit area led to reduced soil moisture level which could have contributed to poor root growth and related reduction in nodulation due to penetration resistance of roots from drier soils [17]. Lower soil moisture levels at higher plant density could also have contributed to both increased desiccation and death of rhizobium or reduction in the efficiency of nitrogenase enzymes to facilitate biological nitrogen fixation [18]. Overall, soybean nodulation was optimized during short rains relative to long rains. Long rainy season was characterized by lower temperatures (mean of 18.9°C) and higher amount of rainfall (total 847.9 mm) compared to short rains mean temperature of 22.03°C and total rainfall of 234.7 mm. Excessive soil moisture levels and low temperature suppress root nodulation and biological fixation in soybean [19, 20].

Results of this study, which correspond to findings from previous studies by Hailey and Higley [21], Gilbert et al. [22] and Nasaruddin and Ridwan [23], have shown a positive and linear relationship between soil moisture levels and stomata conductance, stomatal conductance and photosynthetic rate and sub-stomatal carbon dioxide concentration and photosynthetic rate. Lower soil moisture status at higher plant density could have led to reductions in stomata conductance. Reduction in stomata conductance meant reduced diffusion of carbon dioxide and water in and out of plant tissues leading to lower photosynthetic and transpiration rates. Stomata conductance, carbon dioxide concentration and transpiration were higher during long rains compared to short rains. Variations in levels stomata conductance, photosynthetic and transpiration rates between long and short rains may also be associated with differences in soil moisture levels between the two seasons considering that soil moisture level was

significantly higher during long rains compared to short rains. Soil moisture levels during long rains were 74.17% higher than during short rains. Tanaka and Shiraiwa [24] reported that indeterminate soybean varieties have higher numbers of stomata and epidermal cells per unit area compared to determinate soybean varieties. This, in addition to the fact that indeterminate soybean varieties have smaller leaflets with a potential of minimizing leaf overlaps within a plant, explains the prevalence of increased levels of stomata conductance, photosynthetic and transpiration rates by indeterminate variety DPSB 19 compared to a determinate cultivar EAI 3600. Increased leaf temperature at higher plant population could have been a result of stomata closure to limit water loss from plant tissues which meant that there was a reduction in evaporative cooling of plants offered by increased transpiration rates [25]. Higher soil moisture depletion by the determinate variety (EAI 3600) relative to indeterminate cultivar DPSB 19 could have been due to increased evaporation demand that comes with vigorous plant growth and increased leaf area which characterize determinate plant growth habit [26].

6. Conclusions

High planting density increased interception of photosynthetically active radiation but reduced root nodulation, stomata conductance, sub-stomatal carbon dioxide concentration, photosynthetic and transpiration rates. Indeterminate cultivar DPSB 19 had higher rates of stomata conductance, photosynthesis and sub-stomatal carbon dioxide concentration compared to determinate cultivar EAI 3600.

Data Availability

The data used to support findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding authorship and publication of this paper.

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